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Population Structure of Persian Sturgeon (*Acipenser persicus* Borodin, 1897) in the southern part of Caspian Sea

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We estimated the genetic diversity and population structure of Persian Sturgeon across six regions of Caspian Sea along the coast of Iran using eight DNA microsatellite loci. Full genotypes were obtained for 195 individuals of Persian Sturgeon. Significant differences were detected between *A. persicus* specimens collected from Sefidrud River ($P \leq 0.0001$) and those collected from the southern fishery zones. According to the neighbor-joining tree *A. persicus* specimens collected from the Sefidrud River nested in one cluster and all other specimens placed in the second cluster. Mean diversity was greatest in the Sefidrud River (14 alleles per locus) collections, and lowest in the Kiashahr-Chaboksar (Zone 2) (8 alleles per locus). The average observed and expected heterozygosity indicated a high level of genetic variability in where regions. Pairwise F_{ST} values revealed genetic differentiation among the populations. Based on the results it might be concluded that two independent populations of *A. persicus* exist in the south coast of Caspian Sea which include the Sefidrud River population and zone 3 in which this calls for additional investigations on the genetic structure. These results and significant F_{ST} of genotypic differences between these pairs of collections support the existence of genetic structuring in this species along the south coast of Caspian Sea.

Key words: *Acipenser persicus,* Microsatellite DNA, Population structure, Caspian Sea

INTRODUCTION

Sturgeons (family Acipenseridae) represent one of the most ancient families of bony fishes (Gardiner, 1984). The fossil record dates the family to at least the upper Cretaceous period (> 100 Mya), and the order Acipensiformes is believed to have existed for at least 200 million years (Patterson, 1982; Bemis et al., 1997). Sturgeons are long-lived (> 50 years), characterized by relatively late ages at first maturity (> 5–10 years), and include the largest freshwater fish (Moyle and Cech, 1996). Six sturgeon species, belonging to two genera (*Huso* and *Acipenser),* are found in the Caspian Sea and its drainage basin which provide today the bulk of the world's caviar yield (Pourkazmi, 2006). Sturgeons are currently classified as endangered species because of anthropogenic influences, such as over-exploitation, habitat alteration and pollution, loss of spawning habitat and barriers to migration (Birstein et al., 1997; Pikitch et al., 2005; Pourkazmi, 2006). All these factors caused the decrease in the last past decades of all sturgeon stocks, including Persian sturgeon, *Acipenser persicus*, in the Caspian Sea and this fact requires the development of conservation programs (Pourkazemi, 1996; Khoshkholgh et al., 2011) and studies of the genetic diversity and gene flow among geographic populations.

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Persian Sturgeon are distributed both in the northern and southern parts of the Caspian Sea and is one of the large sturgeons, with a maximum length of ≈230 cm and weight of 70 kg. Age at maturity varies latitudinally; females mature at 12 years in Volga River, 10-11 years in the Kura River and 12–18 years in Ural River (Putilina, 1985; Vlasenko et al., 1989). Maximum life expectancy is 60 –70 years (Putilina and Artyukhin, 1985). As with other Acipenserids, female Persian sturgeon does not reproduce annually. Afraei et al. (2006) stated that Sefidrud River females spawn every 3–5 years. In the southern Caspian basin, the Persian Sturgeon spawns in April-September, but reproduction is interrupted from June to August when temperature rises above 25°C. Most individuals migrate upriver in April-May, but some may enter rivers at other times of the year. In the southern Caspian basin, there is a second run in September-October. Juveniles migrate to the sea during their first summer and remain there until maturity. At sea, the Persian Sturgeon feeds on a wide variety of benthic molluscs, crustaceans and small fish (Moghim et al., 2006). Previous studies by Moghim et al. (2006) and Tavakoli et al. (2010) revealed that population sizes of all sturgeon species have declined, with some estimates suggesting 80–90% decreases in the last 30–40 years. However, decreases in many Persian sturgeon populations have led to conservation concerns; since 2000 the Persian sturgeon has been classified under endangered Appendix II of CITES and listed as endangered species with IUCN (Vecsei and Artyukhin, 2001; Kottelat et al., 2009). As all sturgeon species inhabiting the Black and Caspian seas, the Persian sturgeon is at serious risk of extinction. Reduced natural spawning and deterioration in water quality has contributed to the decline of Persian sturgeon (Pourkazmi, 2006). In response to population declines, the Islamic Republic of Iran has banned netting for sturgeon along their shores of the south Caspian Sea. Furthermore, Iranian productions of *A. persicus* has increased in recent decades and in 1987, over a million juveniles were released into the Caspian Sea (Abdolhay and Baradaran Tahori, 2006).

Earlier studies demonstrated wide variability of morphological characters and genetic diversity in Persian sturgeon (Ataei et al., 2004; Rezvani-Gilkolaii, 2000; Khoshkholgh et al., 2011; Chakmehdouz et al., 2011; Pourkazmi et al., 2012), along with high polymorphism of serum proteins (Bikdorudi, 1997). Some authors subdivided the species into three or four geographically isolated subspecies based on ecological and meristic characters (Putilina and Artyukhin, 1985; Parvaneh, 1994). Estimates of population structure within and among regions of Caspian Sea will provide an important genetic foundation on which to develop and evaluate conservation plans to assure Persian sturgeon populations remain healthy.

An understanding of genetic variability and population structure are critical for management and conservation of exploited sturgeon species (Liu and Cordes, 2004; Ludwig, 2006). Molecular genetic data provide fishery managers with essential information regarding the existence and distribution of discrete populations and/or management units (Billington and Hebert, 1991; Wirgin et al., 1997). Over the last decade, microsatellites have become the most commonly used nuclear markers in population genetic studies for a number of reasons (Avise, 1994). Microsatellites are biparentally inherited and most accumulate neutral mutations rapidly (Jarne and Lagoda, 1996; Liu and Cordes, 2004). Polymorphic microsatellites typically have high information content and are therefore expected to provide stronger discriminatory power for resolving population structure than mtDNA, (Avise, 1994; Balloux and Lugon-Moulin, 2002). However, for the Persian sturgeon, genetic information would also provide useful insight into the establishment of baseline stocks for development of broodstock management strategies (Pourkazemi, 2006). In this study, the genetic variation of Persian Sturgeon in the southern portion of Caspian Sea was analyzed based on eight polyploid microsatellite DNA loci.

MATERIAL AND METHODS

Sample collections and DNA Extraction

Fin clip samples of the Persian sturgeon were obtained from five sites along the Iranian coast in the south Caspian Sea as follows: Astara-Anzali (Zone1), Kiashahr-Chaboksar (Zone 2), Noshahr-Sari (Zone 3), Miankaleh-Bandare Torkaman (Zone 4), and Chaboksar-Noshahr (Zone 5). Sefidroud River was considered as sixth site. All zones are depicted in Figure 1. The fisheries zones are relative sampling area that defined by Iranian fisheries organization to develop further management. We collected and analyzed between 20 and 45 individuals from each locality (Table 1). The position of sampling sites is shown in Figure 1. All were obtained during the reproductive times. The samples (2-3 g dorsal fin tissue) first were kept in 96% ethanol and then at -20° C until DNA extraction. Total DNA was isolated as described by Hillis and Moritz (1990) with some modifications (Pourkazemi, 1996). The DNA was re-suspended in TE Buffer (10 mM Tris, 10 mM EDTA, pH 8.0). Further isolation steps were the same as in the procedure described in Pourkazemi (1996). The quality and quantity of total DNA were determined by agarose gel electrophoresis and spectrophotometery.

Microsatellite genotyping

In this study, eight microsatellite loci reported by May et al. (1997), McQuown et al. (2002) and Moghim et al. (2009) were used (Table 2). A total of 27 primer pairs from Shovelnose sturgeon *Scaphirhynchus platorynchus* (McQuown et al., 2000) were tested by Moghim et al. (2009) on Persian sturgeon individuals to evaluate polymorphism and the quality of amplification. The PCR conditions and mixtures were optimized for each microsatellite system (Table 2).

PCR amplification was carried out in 20µl reaction volumes with 50 ng of template DNA (Table 2). Optimization included varying annealing temperature (51- 60° C), MgCl2 concentration (1-2.5 mM), dNTP concentration (150-200 µm), 0.75 -1.2U of Taq DNA polymerase (Vio Taq^{Tm} VT1001, Fermentase) and 1µl (10-20 pmol) each of forward and reverse primers (Table 2). The PCR amplification protocols are given in Table 2. PCR products were analysed by electrophoresis on 6% non-denaturing polyacrylamide gels in 1× TBE buffer and visualized with silver staining. The images obtained were scanned with an Epson Perfection 2480 scanner and analysed using BioCapt software (version 2.0) (Pourkazemi, 1996).

TABLE 1. Persian sturgeon samples collected for population genetic analysis. Sampling localities (Fisheries Zone 1- 5) are from south Caspian Sea along the Iranian coast.

Region	Map no.	Sample size	Life history stage
Astara-Anzali (Zone 1)	1	45	Adult
Kiashahr-Chaboksar (Zone 2)	2	32	Adult
Sefidroud River	6	36	Adult
Chaboksar-Noshahr (Zone 5)	5	20	Adult
Noshahr-Sari (Zone 3)	3	24	Adult
Miankaleh-Bandare Torkaman (Zone 4)	4	38	Adult
Total		**195**	

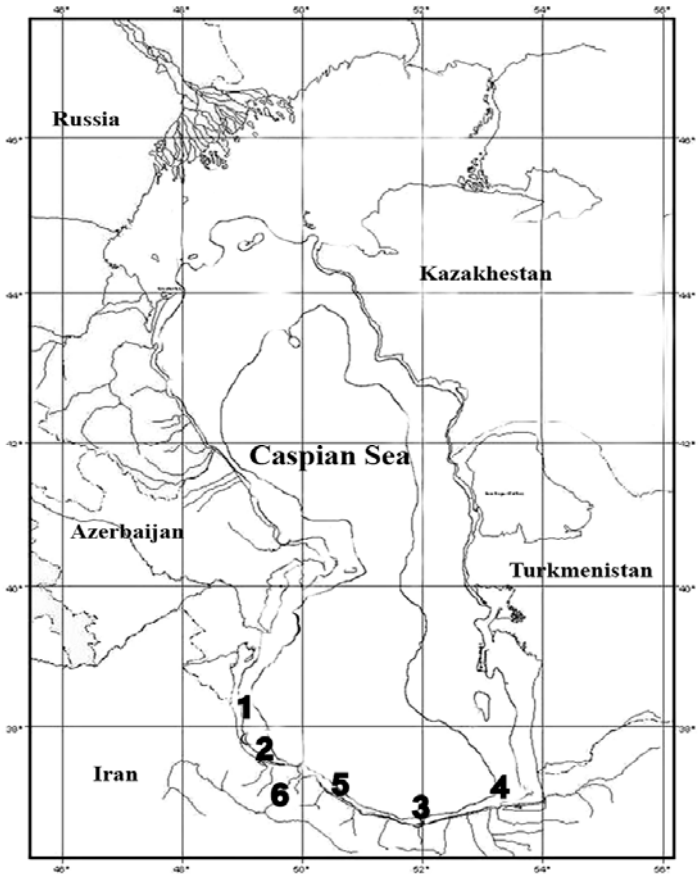

FIGURE 1. Map showing the locations of the Persian sturgeon sampling sites in the Caspian Sea. Sampling localities: 1, Astara-Anzali (Zone1); 2, Kiashahr-Chaboksar (Zone 2); 3, Noshahr-Sari (Zone 3); 4, Miankaleh-Bandare Torkaman (Zone 4); 5, Chaboksar-Noshahr (Zone 5); 6, Sefidroud River; from south Caspian Sea along the Iranian coast.

TABLE 2. PCR condition and reaction, locus, product sizes and repeat motifs on Persian sturgeon.

Locus	Product size(bp)	components	Cycling condition	Repeat motif	Primer source(s)
AfuG 19	126-198	1.3mM MgCl2, 150µ MdNTPs, 10pmol each primer and 1.2UTaq DNApolymeraz	94/3min[94/30sec, 60/25sec and 72/30sec]^{30},72/5min	$(TTG)_9$	May et al., 1997
AfuG 34	138-180	1mM MgCl2, 200 µMdNTPs, 20pmol each primer and 1UTaq DNApolymerase	94/3min[94/30sec, 51/25sec and 72/30sec]^{30},72/5min	$(GTT)_{10}$	May et al., 1997
AfuG 39	78-216	1.5mM MgCl2, 150µMdNTPs, 15pmol each primer and 0.75 UTaq DNApolymerase	94/3min[94/30sec, 57/25sec and 72/30sec]^{30},72/5min	$(GTT)_{10}$	May et al., 1997
AfuG 68	184-260	1mM MgCl2, 200 µMdNTPs, 20pmol each primer and 1UTaq DNApolymerase	94/3min[94/30sec, 60/25sec and 72/30sec]^{30},72/5min	$(GATA)_{13}$	May et al., 1997
Spl 35	274-307	1mM MgCl2, 150 MdNTPs, 10pmol each primer and 1.2UTaq DNApolymerase	94/3min[94/30sec, 57/25sec and 72/30sec]^{30},72/5min	$(TG)_{13}$	McQuown et al., 2000
Spl 49	154-197	1mM MgCl2, 200 µMdNTPs, 10pmol each primer and 0.85UTaq DNApolymerase	94/3min[94/30sec, 58/25sec and 72/30sec]^{30},72/5min	$(TC)_{15}$	McQuown et al., 2000
Spl 53	201-231	1.5mM MgCl2, 150µMdNTPs, 15pmol each primer and 0.75 UTaq DNApolymerase	94/3min[94/30sec, 55/25sec and 72/30sec]^{30},72/5min	$(GA)_{16}$	McQuown et al., 2000
Spl 106	184-224	1.2mM MgCl2, 200 µMdNTPs, 20pmol each primer and 1UTaq DNApolymerase	94/3min[94/30sec, 58/25sec and 72/30sec]^{30},72/5min	$(CTAT)_{12}$	McQuown et al., 2000

Statistical analysis

Given the tetraploid condition of the Persian sturgeon, gene dosages for the eight tetrasomic microsatellite DNA markers were interpreted by analysis of relative peak intensity from the genotyping electropherograms, similar to other sturgeon studies with polysomic markers (McQuown et al., 2000; Pyatskowit et al., 2001; Israel et al., 2009). We performed analyses based only on the presence or absence of different alleles. For these analyses, genotypic data were transformed into pseudodominant "allele phenotypes" (Rodzen and May, 2002; De Silva et al., 2005; Israel et al., 2009) by scoring an allele as present or absent, regardless of dosage. This presence–absence matrix combined all of the alleles at the eight loci.

Spawning populations were assessed using the Weir and Cockerham (1984) estimator of the genetic differentiation index F_{ST} estimated in Arlequin software (Schneider et al., 2000). Visual analyses of the genetic relationships among spawning populations were conducted through the construction of a neighbor-joining (NJ) tree based on the Cavalli-Sforza and Edwards (1967) chord distance in PHYLIP software (Felsenstein, 2005). This distance measure was selected because similar studies have shown that it is one of the most accurate genetic distance measures for microsatellite analyses (Takezaki and Nei, 1996). Bootstrap values were calculated based on 1000 replicates.

RESULTS

The average number of alleles observed at each locus ranged from 7.8 to 14 (Table 3). Average expected heterozygosity of each population ranged from 0.66 (Zone 5) to 0.85 (Sefidrud River), and the overall average was 0.76.

Table 4 presents the results of using F-statistics methods to estimate the genetic differentiation of the examined populations. Pairwise estimates of F_{ST} values ranged between 0.003 and 0.052 (Table 4). The lowest F_{ST} occurred between samples from Zone 1 and Zone 2. Thus, two distinct genetic groups were identified (Sefidroud River and all others). Figure 2 shows that the bootstrap values range 43 to 98 and supports the existence of these two groups. Population comparisons with nonsignificant F_{ST} values included (1) Zone1 versus Zones 2, 3 and 5; (2) Zones 2 versus Zones 5; and (3) Zones 5 versus Zones 4. Mean F_{ST} for all eight populations was 0.023 (Table 4).

TABLE 3. Estimates of observed (*Ho*) and expected heterozygosity (*He*) and number of alleles per locus (*A*) in each collection.

	AfuG 19			*AfuG 34*			*AfuG 39*			*AfuG 68*		
	Ho	***He***	***A***	***Ho***	***He***	***A***	***Ho***	***He***	***A***	***Ho***	***He***	***A***
Zone 1	0.62	0.63	9	0.61	0.74	6	0.79	0.86	10	0.67	0.86	8
Zone 2	0.51	0.65	8	0.65	0.67	5	0.70	0.74	11	0.60	0.83	9
Sefidroud	0.79	0.87	16	0.76	0.78	11	0.84	0.93	24	0.74	0.84	13
Zone 5	0.54	0.56	7	0.55	0.61	6	0.64	0.71	14	0.50	0.86	9
Zone 3	0.78	0.88	10	0.58	0.77	10	0.81	0.82	16	0.79	0.89	11
Zone 4	0.52	0.78	9	0.41	0.84	6	0.76	0.77	16	0.62	0.88	11

	SPL 35			*SPL 49*			*SPL 53*			*SPL 106*		
	HO	***HE***	***A***	***HO***	***HE***	***A***	***HO***	***HE***	***A***	***HO***	***HE***	***A***
ZONE 1	0.65	0.74	10	0.67	0.76	3	0.61	0.64	10	0.53	0.67	6
ZONE 2	0.53	0.58	11	0.62	0.63	6	0.64	0.65	7	0.57	0.63	7
SEFIDROUD	0.75	0.83	18	0.85	0.83	9	0.76	0.81	14	0.82	0.86	11
ZONE 5	0.39	0.53	9	0.66	0.65	8	0.59	0.65	9	0.66	0.64	10
ZONE 3	0.78	0.79	13	0.72	0.75	6	0.68	0.71	12	0.59	0.66	12
ZONE 4	0.69	0.76	10	0.54	0.68	3	0.79	0.85	10	0.73	0.74	14

TABLE 4. Pairwise F_{ST} estimates for spawning population and summer aggregation collections used in this study. Bold values indicate significant.

	Zone 1	Zone 2	Sefidroud	Zone 5	Zone 3	Zone 4
Zone 1	-					
Zone 2	0.003	-				
Sefidroud	**0.052**	**0.046**	-			
Zone 5	0.006	0.008	**0.039**	-		
Zone 3	**0.017**	**0.012**	**0.027**	**0.013**	-	
Zone 4	0.008	**0.010**	**0.049**	0.011	0.006	-

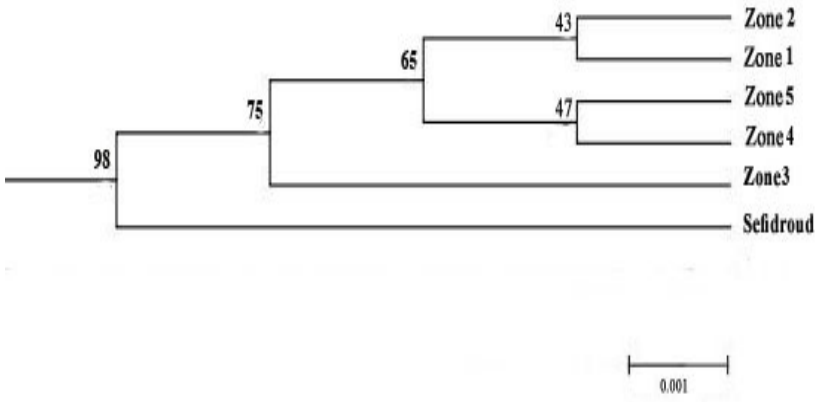

FIGURE 2. Unrooted neighbor-joining cluster analysis diagrams based on Cavalli-Sforza and Edwards' (1967) chord distance for microsatellite markers in the south Caspian Sea.

The NJ tree corroborated significant pairwise F_{ST} values by spawning Persian sturgeon from the Sefidrud River with high bootstrap support and the Sefidrud River also had the higher branch lengths. The tree showed that the Zone 1 and Zone 2 consistently grouped together (Fig. 2) and also the Zone 4 and Zone 5 grouped together, albeit with less support.

DISCUSSION

The results indicate that there is a genetic structure in the Persian sturgeon populations along the southern portion of Caspian Sea in which in consistent with our previous study using sequence analysis of mtDNA control region (Khoshkholgh et al., 2011), PCR-RFLP analysis of ND5 gene (Pourkazemi et al., 2012) and also a work conducted by Chakmehdouz et al. (2011) using four microsatellite loci. Chakmehdouz et al. (2011) reported high levels of genetic variability for populations from two region including Sefidrud and Gorganrud rivers watershed with observed heterozygosity value at 0.67 ± 0.21. In our experiments, tetrasomic inheritance of microsatellite loci of Persian sturgeon was demonstrated, which was typical of tetraploids. Species with increased ploidy are characterized by low population divergence due to weak selection and gene drift, caused by a low number of homozygotes (Ludwig et al., 2001). Nevertheless, the use of microsatellites succeeds in demonstrating moderate albeit statistically significant interpopulation differences of Persian sturgeon over eight microsatellite loci. It should be noted that the highest values of genetic distances were observed between the Sefidrud River and populations from Zone 1. These findings also contrast with the patterns of control region variation reported by Ataei et al., (2004), who observed no significant differences in mtDNA control region among three populations. Rezvani-Gilkolaii (2000) in their study suggested that ND5 gene might not be useful for population genetic analysis, but it would be useful for phylogenetic analysis of sturgeon fishes. Nevertheless, the estimates of the genetic differentiation slightly differed between the two molecular makers. This study reinforced the finding that the genetic diversity of these six wild populations of Persian

sturgeon (Khoshkholgh et al., 2011; Pourkazemi et al., 2012) and two collections from Sefidrud and Gorganrud rivers were considerable (Chakmehdouz et al., 2011). Significant variance in microsatellite allele frequency (mean F_{ST} = 0.023) provide evidence that Persian sturgeon populations in the Caspian Sea are spatially genetically structured. Genetic affinities among populations revealed in the NJ tree showed high bootstrap support for two distinct population segments, generally corresponding to location of origin (Sefidrud River, Zone 3) (Fig. 2). In general, longer branch lengths (indicating greater genetic) separated the spawning populations of Sefidrud River from those of the other location, and spawning populations of Zone 3 in south did not group with that of in the south shore, indicating substantial population genetic structure in this portion of Caspian Sea.

In the southwest of Caspian Sea, microsatellite analysis revealed a deficit of heterozygotes. However, the highly migratory nature of Persian sturgeon introduces the potential for straying which leads to significant gene flow, especially among populations in proximal spawning systems. Similar to Persian sturgeon, stellate sturgeon also disperse over great distances during non-spawning periods (Norouzi et al., 2009), yet show a high degree of fidelity to natal rivers for spawning. Although sturgeons are known to have strong homing capabilities and spawning site fidelity (Bemis et al., 1997), but the natal rivers of non-spawning Persian sturgeon aggregations remain unknown. Our lack of knowledge regarding the homing fidelity of Persian sturgeon, or any sturgeon species in the south Caspian Sea makes it difficult to assess the likelihood of this event. Limited results from genetic analysis of Persian sturgeon suggest higher homing fidelity for this species (Pourkazemi et al., 2012; Chakmehdouz et al., 2011).

Results from genetic analyses indicate that Persian sturgeon populations from Sefidrud River are genetically distinct. The population of the Persian sturgeon in the Sefidrud River has declined dramatically in the face of anthropogenic and environmental problems (Pourkazemi, 2006). Despite this reduction, the genetic variability of the current population is sufficient to survive from a genetic viewpoint (Pourkazemi et al., 2012). The lowest heterozygosity was in the Zone 1. Heterozygosity was also low in the Zone 2 and Zone 5, two reservoirs with populations that might have been effected via stocking. It seems likely that deficit of heterozygotes in Zone 1 population was associated with a dramatic decrease of its number during the last decade, as well as by inbreeding, as a result of the limited broodstock for artificial breeding (Tavakoli et al., 2010; Khoshkholgh et al., 2011; Pourkazemi et al., 2012). On the other hand, in the tetraploid genome, the deficit of heterozygotes can be explained in terms of asymmetric structure of homologous chromosome quadruplets during the first meiotic division, associated with the allotetraploid nature of multichromosomal sturgeon species (Hallerman, 2003). We found that the detection of population structure is possible with a minimum number of microsatellites and we recommend additional genotyping of different local populations using these markers for future monitoring of Persian sturgeon genetic diversity.

It is important to consider population structure when making decisions regarding stocking of hatchery-reared fishes. Evidence of fundamental genetic differences among collections presented in this study (e.g., Sefidrud River versus other populations) suggested that, when possible, selection of source populations for supplementation activities should consider genetic relationships among populations (Pourkazemi, 2006). Sampling of additional spawning locations in Caspian Sea and genetic data incorporation with other laboratories will further clarify genetic relationships that are useful for identifying units for management and conservation.

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LITERATURE CITED

Abdolhay, H.A., Baradaran Tahori, H., 2006. Fingerling production and Release for Stock Enhancement of Sturgeon in the Southern Caspian Sea: an overview. *Journal of Applied Ichthyology* 22 (Suppllement 1), 125-131.

Afraei, M.A., Safari, R., Salmani, A., 2006. Distribution and density of juvenile *Acipenser persicus* at the lower 10 meter depth of the southern Caspian Sea. *Journal of Applied Ichthyology* 22(supplement), 108-110.

Ataei, M., Pourkazemi, M., Hasanzadeh Kiabi, B., Shedaei, M., 2004. Analysis of mtDNA control region polymorphism of Persian sturgeon, *Acipenser persicus,* in Sefidrud river and South-east region of Caspian Sea. *Journal of Environmental of Shahid Beheshti University* 2(1), 27-31. (In Persian)

Avise, J.C., 1994. *Molecular Markers, Natural History, and Evolution*. Chapman and Hall, New York, NY. 511 pp.

Balloux, F., Lugon-Moulin, N., 2002. The estimation of population differentiation with microsatellite markers. *Molecular Ecology* 11, 155-165.

Bemis, W.E., Findeis, E.K., Grande, L., 1997. An overview of Acipenseriformes. *Environmental Biology of Fishes* 48, 25–71.

Bikdoroudi, B., 1997. Identification of blood serum proteins of *Acipenser persicus* using electrophosis. Unpublished MSc. Thesis. University of Tarbiat Modares, Nour, Iran. 106p.

Billington, N., Hebert, D.N., 1991. Mitochondrial DNA diversity in fishes and its implications for introductions. *Canadian Journal of Fisheries and Aquatic Science*s 48 (Supplement 1), 80–94.

Birstein, V.I, Bemis, W.E., Waddman, J.R., 1997. The threatened status of Acipenseriform species: a summary. *Environmental Biology of Fishes* 48, 427-435.

Cavalli-Sforza, L.L., Edwards, A.W.F., 1967. Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics* 19, 233–257.

Chakmehdouz Ghasemi, F., Pourkazemi, M., Tavakolli, M., Yarmohammadi, M., Hassanzadeh Saber, M., Baradaran Noveiri, S., 2011. Application of microsatellite markers to determine populations of the Persian sturgeon (*Acipenser persicus*) in the South of Caspian Sea. *Iranian Journal of Fisheries Sciences* 10(4), 596-606.

De Silva, H.N., Hall, A.J., Rikkerink, E., McNeilage, M.A., Fraser, L.G., 2005. Estimation of allele frequencies in polyploids under certain patterns of inheritance. *Heredity* 95, 327–334.

Felsenstein, J., 2005. PHYLIP, Phylogeny inference package, version 3.57c. Department of Genetics, SK-50, University of Washington, Seattle.

Gardiner, B.G., 1984. Sturgeons as living fossils. In: *Living Fossils* (eds. Eldredge N, Stanley SM), pp. 148–152. Springer-Verlag, New York.

Halerman, E.M., 2003. Coadaptation and outbreeding depression. Pages 239–259 in E. M. Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Hillis, D., Moritz, M.C., 1990. *Molecular taxonomic*. Sinauer associate, Inc. Publishers. Massachusetts.

Israeil, J.A., Bando, K.J., Anderson, E.C., May, B., 2009. Polyploid microsatellite data reveal stock complexity among estuarine North American green sturgeon (*Acipenser medirostris*). *Canadian Journal of Fisheries and Aquatic Sciences* 66, 1491–1504.

Jarne, P., Lagoda, P.J.L., 1996. Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* 11, 424–429.

Moyle, P.B, Céch, JJR., 1996. *Fishes: An Introduction to Ichthyology*, 3rd edn. Prentice Hall, Upper Saddle River, New Jersey.

Khoshkholgh, MR., Pourkazmi, M., Nazari, S., Azizzadeh, L., 2011. Genetic diversity in the Persian sturgeon (*Acipenser persicus*) in the south Caspian Sea based on mitochondrial DNA sequence analysis. *Caspian Journal of Environmental Sciences* 9(2), 27-36.

Kottelat, M., Genser, J., Freyhof, J., 2009. *Acipenser persicus*. *In*: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. http://www.iucnredlist.org.

Liu, A.Z.J., Cordes., J.F., 2004. DNA marker technologies and their applications in aquaculture genetics. *Aquaculture* 238, 1 –37.

Ludwig, A., Belfiore, N.M., Pitra, C., Svirsky, V., Jenneckens, I., 2001. Genome duplication events and functional reduction of ploidy levels in sturgeon (*Acipenser*, *Huso* and *Scaphirhynchus*).*Genetics* 158(3), 1203–1215.

Ludwig, A., 2006. A sturgeon view on conservation genetics. *European Journal of Wildlife Research* 52(1), 3-8.

Luo, Z.W., Zhang, Z., Zhang, R.M., Pandey, M., Gailing, O., Hattemer, H.H., Finkeldey, R., 2005. Modeling population genetic data in autotetraploid species. *Genetics* 172(1), 639–646.

May, B., Krueger, C.C., Kincaid, H.L., 1997. Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus*. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 1542–1547

Miller, L.M., Kapuscinski, A.R., 2003. Genetic guidelines for hatchery supplementation programs. Pages 329–355 in E. M Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

McQuown, E.C., Sloss, B.L., Sheehan, R.J., Rodzen, J., Tranah, G.J., May, B., 2000. Microsatellite analysis of genetic variation in sturgeon (Acipenseridae): new primer sequences for *Scaphirhynchus* and *Acipenser*. *Transactions of American Fisheries Society* 129, 1380–1388.

McQuown, E., Gall, G.A.E., May, B., 2002. Characterization and inheritance of six microsatellite loci in lake sturgeon (*Acipenser fulvescens*). *Transactions of American Fisheries Society* 131, 299–307.

Moghim, M., Kor, D., Tavakoli, M., Khoshghalb M.B., 2006. Stock status of Persian sturgeon (*Acipenser persicus*, Borodin 1897) along the Iranian coast of the Caspian Sea. *Journal of Applied Ichthyology* 22, 99-107.

Moghim, M., Heist, E.J., Tan, S.G., Pourkazemi, M., Siraj, S.S., Panadam, J.M., 2009. Amplification of microsatellites in Persian Sturgeon, *Acipenser persicus*. *Iranian journal of Fisheries Sciences* 8(1), 97-102.

Norouzi, M., Pourkazemi, M., Fatemi, M., 2009. Application of microsatellite markers to study genetic structure of Stellate sturgeon populations (*Acipenser stellatus*) in the south Caspian Sea. *Iranian journal of Fisheries Sciences* 8(1), 73-84.

Patterson, C., 1982. Morphology and interrelationships of primitive Actinopterygian fishes. *American Zoologist* 22, 241–259.

Parvaneh, A., 1994. Determination of different races of *Acipenser persicus* using electrophosis of blood serum proteins. Unpublished Msc thesis. Islamic University. Tehran, Iran. 114 p.

Pikitch, E.K., Doukakis P., Lauck, L., Chakrabarty, P., Erickson, D.L., 2005. Status, trends and management of sturgeon and paddlefish fisheries. *Fish and Fisheries* 6, 233–265.

Pourkazemi, M., 1996. Molecular and biochemical genetic analysis of sturgeon stocks from the South Caspian Sea. Ph.D. thesis.University of Wales, Swansea.

Pourkazemi, M., Skibinski, D.O.F., Beardmore, J.A., 1999. Application of mtDNA d-loop region for the study of Russian sturgeon population structure from Iranian coastline of the Caspian Sea. *Journal of Applied Ichthyology* 15, 23–28.

Pourkazemi, M., 2006. Caspian Sea sturgeon Conservation and Fisheries: Past present and Future. *Journal of Applied Ichthyology* 22(Suppllement 1), 12-16.

Pourkazemi, M., Nazari, S., Khoshkholgh, MR., Azizzadeh, L., 2012. Genetic Relationships among Populations of the Persian sturgeon, *Acipenser percicus*, in the south Caspian Sea Detected by Mitochondrial DNA–Restriction Fragment Length Polymorphisms. *Caspian Journal of Environmental Sciences* 10(2), 215-226.

Putilina, L.A., 1985. Quantitative and qualitative assessment of spawning population of Persian sturgeon in the Volga river. *Fishing industry* 10, 71-72.

Putilina, L.A., Artyukhin, E.N., 1985. Some features of Persian sturgeon migration in the lower Volga river. Ibid. pp. 32-34.

Pyatskowit, J.D., Krueger, C.C., Kincaid, H.L., May, B., 2001. Inheritance of microsatellite loci in the polyploid derivative lake sturgeon (*Acipenser fulvescens*). *Genome* 44, 185–191.

Rezvani Gilkolaei, S., 2000. Study of mtDNA variation of Russian sturgeon population from the south Caspian Sea using RFPL analysis of PCR amplified ND5/6 gene regiens. *Iranian journal of fisheries sciences* 2, 87-98.

Rodzen, J.A., May, B., 2002. Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*). *Genome* 45(6), 1064–1076.

Schneid, E.R.S., Roesseli, D., Excoffier, L., 2000. Arlequin: a software for population genetics data analysis, version 2.0. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva, Switzerland.

Takezaki, N., Nei., M., 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* 144, 389–399.

Tavakoli, M., Parafkandeh, F., Khoshghalb, M.B., Keymaram, F., Taghavi, A., 2010. Stock status of Persian sturgeon (*Acipenser persicus,* Borodin 1897) along the Iranian coast of the Caspian Sea. *Iranian scientific fisheries journal* 1, 1-8. (In Persian)

Vesei, P., Artyukhin, E., 2001. Threatened fishes of the world: *Acipenser persicus* Borodin, 1897 (Acipenseridae) *Environmental Biology of Fishes* 61, 160.

Vlasenko, A.D., Pavlov, A.V., Vasil'ev, V.P., 1989. *Acipenser persicus* Borodin, 1897. In: The Freshwater Fishes of Europe. Vol. 1. Pt. II. General Introduction to Fishes. Acipenseriformes (ed. J. Holcik). AULA-Verlag, Wiesbaden, pp. 345–366.

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

Wirgin, I., Stabile, J.E., Waldman, J.R., 1997. Molecular analysis in the conservation of sturgeons and paddlefish. *Environmental Biology of Fishes* 48, 385-398.