Application of microsatellite markers to study the genetic structure of stellate sturgeon populations (*Acipenser stellatus* Pallas, 1771) in the south Caspian Sea

Norouzi M.[1*] ; Pourkazemi M.[2] and Fatemi M.[3]

1- Department of Marine Biology and Fishery, Islamic Azad University, Tonekabon Branch, Zip code: 46814161167, Tonekabon, Iran

2- International Sturgeon Research Institute, P.O.Box: 41635-3464 Rasht, Iran

3- Department of Marine Biology, Science and Research Branch of Islamic Azad University, P.O.Box: 14155-775 Tehran, Iran

Received: September 2007 Accepted: June 2008

Abstract

Population genetic structure of stellate sturgeon (*Acipenser stellatus*) in 197 adult specimens from four fishery regions along the Iranian coastline of the southern Caspian Sea was investigated using microsatellite markers. Out of 15 microsatellite primers, 11 loci were produced, in which 10 of them were polymorphic and 1 was monomorph. Totally, 184 alleles were identified and on average 13.1 alleles per locus were found (ranged 8 to 18 alleles). All sampled regions contained unique alleles. Average observed and expected heterozygosity were 0.667 and 0.854, respectively and significant genetic differences between 4 regions were observed ($P \leq 0.01$). Deviations from Hardy-Weinberg equilibrium were found in most cases. Population differentiation test was modest and significant ($P \leq 0.01$). Based on F_{ST} estimate ($P \leq 0.01$), more than one population of stellate sturgeon is identified in the south Caspian Sea. Therefore, fishery management for restocking and conservation of gene pool is highly recommended.

Keywords: Stellate sturgeon, Population genetic, Microsatellite, South Caspian Sea

* Corresponding author's email: nmehrnoosh@gmail.com

Introduction

Stellate sturgeon (*Acipenser stellatus)* is one of the shared stocks of the Caspian states that is also found in the Black and Azov Seas (Holcik, 1989). Stellate sturgeon populations play an important role in biodiversity and commercial harvest of the Caspian Sea and were listed as an endangered species in IUCN Red list (IUCN, 1996) and since April 1998 its trade regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Over-fishing and increasing illegal/ unaccounted catches are the main causes in decreasing the stocks (Pourkazemi, 2006). The stock of *A. stellatus* in the Caspian Sea is maintained through natural spawning as well as artificial breeding, which necessitates the conservation of genetic diversity of this species. Therefore, identificatin of population and stocks can be considered the first measure for management and conservation of sturgeon species in the Caspian Sea (Pourkazemi *et al.,* 1999). Two different ecological or froms of stellate sturgeon have been reported, namely *A. stellatus stellatus* and *A. stellatus cynensis,* in the north and south Caspian Sea, respectively; they are reportedly identical in spawning time, growth rate and immunological characteristics (Holcik, 1989).

Pourkazemi (2001) and Shabani *et al.* (2006) studied mtDNA variation of stellate sturgeon (*Acipenser stellatus*) population in South Caspian Sea using RFLP analysis of PCR Amplified ND 5/6 gene regions and they found low genetic variation and non-significant difference in haplotype frequency. It has been concluded that mitochondrial DNA technique was not powerful techniques to detect genetic variability sufficiently population-differentiation in order to help management units of stellate sturgeon. In population genetic studies, more genetic variability would provide a better means by which to compare sturgeon collection from different localities differentiation (McQuown *et al.*, 2003).

Microsatellites are hyper-variable, co-dominant nuclear DNA markers in which variation is partitioned in one to five base pair repeat motifs (Zajc *et al.,* 1997). In recent years, microsatellite markers have been applied for many populations as well as phylogenetic studies. This is because microsatellites typically have a higher level polymorphism than nuclear loci. In addition, DNA for microsatellites analysis can be easily extracted from tissues obtained by non-lethal sampling (fins, hair, feces), which is essential when working on threatened or endangered species (McQuown *et al.,* 2003).

Several investigations on population genetic structure of various Acipenseriforme species in the Caspian Sea have been conducted (on stellate sturgeon, Pourkazemi, 2001; Shabani *et al.,* 2006; on Russian sturgeon, Pourkazemi *et al.,* 1999; Rezvani Gilkolahaei, 2000; Khoshkholgh, 2007 and on ship sturgeon Qasemi *et al.,* 2006).

The development of management plans and implementation of actions to restore

stellate sturgeon within its native stocks can be useful from an understanding of the genetic diversity of its populations. This information is helpful in choosing donor populations to use as sources of reintroduction, and in formulating restoration goals regarding population structure. Therefore, in this study the population structure of stellate sturgeon from four regions in Iranian coastline of the Caspian Sea was investigated. The objectives of the study were to analyse the population genetic structure and genetic diversity and to identify individual populations of stellate sturgeon using microsatellite markers.

Materials and methods

Totally 197 samples (2-3g dorsal fin tissue) of adult stellate sturgeon were collected from four regions along the Iranian coasts of the Caspian Sea, including 52 samples from region one (Astara-Anzali), 43 samples, from region two (Kiyashahr-Ramsar), 50 samples from region three (Nowshahr-Babolsar), 52 samples from region four (Miyankale-Gomishan) (Fig. 1).

Genomic DNA was extracted from fin tissue following the method as described by Pourkazemi *et al.* (1999). The quality and concentration of DNA were assessed by agarose gel electrophoresis and spectrophotometry (model CECIL CE2040) stored at -20°C until use.

Nuclear DNA was amplified using 15 microsatellites primers designed for genera *Acipenser* and *Scaphirhynchus* (LS-19, 34, 39, 54, 57, 62, 68, 69 May *et al.*, 1997; Sp1-104, 105, 113, 163, 168, 170, 173; McQuown *et al.*, 2000). Polymerase Chain Reaction (PCR) condition for each primer set was optimized for stellate sturgeon. Experimental condition tested included varying annealing temperature (49-61.2°C), $MgCl_2$ concentration (1-2.5mM), dinucleotide triphosphate concentration (200µM), 1U *Taq* DNA polymerase and primer concentration (0.5-1pµ) and approximately 100ng of template DNA. All reactions were performed in 20µl (Table 1). PCR products were separated on 6% polyacrylamid gel (29:1 acrylamid: bis-acrylamid; 1X TBE buffer) and followed by silver-staining. Gels were run at 170 W for 2h and 30min. Alleles were sized using BioCapt software, and each gel contained an allelic ladder (50bp) to assist in consistent scoring of alleles.

Allelic frequencies, observed and expected heterozygosities, genetic distance and genetic identity (Nei, 1978) were computed in Gean Alex 6.0 software (Peakall & Smouse, 2005). This package was also used to calculate F_{ST} value, Nm, Hardy-Weinberg (HW) tests of equilibrium, AMOVA (Analysis of Molecular Variance). The dendrograms of genetic distance (Nei, 1972,1978) UPGMA computed in TFPGA (version 1.3).

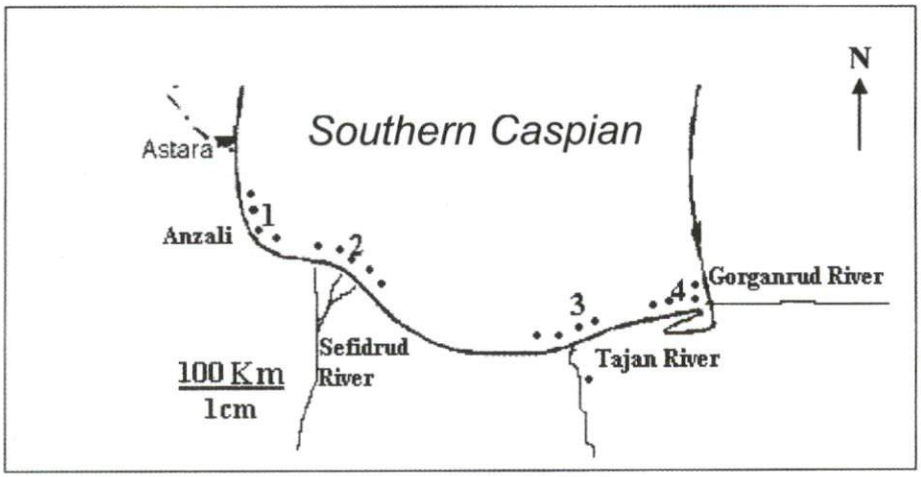

Figure 1: Map shows the sampling sites of stellate sturgeon where 1, 2, 3 and 4 represent each region and shows sturgeon fishing stations

Table 1: PCR condition and reaction, locus and allel size (bp) on stellate sturgeon

Locus	Actual size (bp)	Components	Cycling condition
LS-19	132-213	200µM each dNTPs; 1pM each primer; 1.6mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s;56 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
LS-34	132-180	200µM each dNTPs; 1pM each primer; 2mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 58 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
LS-39	120	200µM each dNTPs; 1pM each primer; 2mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 58 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
LS-54	152-224	200µM each dNTPs; 1pM each primer; 2mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 59 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
LS-69	No amplification	200µM each dNTPs; 1pM each primer; 1mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 49 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
LS-57	Multiple bands & smear	200µM each dNTPs; 1pM each primer; 1mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 61°C/30s; 72 °C/ 30]^{35} 72 °C/5m
LS-62	No amplification	200µM each dNTPs; 1pM each primer; 2mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 59.5 °C/30s; 72 °C/ 30]^{30} 72 °C/5m
LS-68	104-160	200µM each dNTPs; 1pM each primer; 1mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 61.2 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
Spl-104	184-248	200µM each dNTPs; 1pM each primer; 2.5mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 57°C/30s; 72 °C/ 30]^{25} 72 °C/5m
Spl-105	104-180	200µM each dNTPs; 1pM each primer; 1.25mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 58 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
Spl-113	160-212, 260-348	200µM each dNTPs; 1pM each primer; 2.5mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 59 °C/30s; 72 °C/ 30]^{25} 72 °C/5m
Spl-163	160-244	200µM each dNTPs; 1pM each primer; 2.5mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 56 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
Spl-168	No amplification	200µM each dNTPs; 1pM each primer; 2.5mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 49 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
Spl-170	200-264	200µM each dNTPs; 1pM each primer; 2.5mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 58 °C/30s; 72 °C/ 30]^{35} 72 °C/5m
Spl-173	176-296	200µM each dNTPs; 1pM each primer; 2.5mM $MgCl_2$; 2 U/Taq	94°C/3m[94°C/30s; 58.5 °C/30s; 72 °C/ 30]^{35} 72 °C/5m

Results

Out of 15 sets of microsatellite primers, four sets (LS-69, LS-57, LS-62 and Spl-168) did not show any flanking sites on stellate sturgeon genome. Eleven sets of primers were successfully amplified and one set (LS-39) showed monomorphic pattern. Therefore, totally 10 loci were investigated in the present study. All microsatellite primers producing DNA bands displayed a characteristic disomic banding pattern, where heterozygous individuals showed two bands of equal intensity on the gels and sometimes with one band darker than the others. These banding patterns may be a result of the presence of a homologous locus that is detected under certain PCR conditions.

The average number of alleles found at each locus was 13.1 and ranged from 8 (Spl-105) to 18 (Spl-113, 163) alleles (Table 2). The total number of alleles found in each region ranged from 113 (region two) to 140 (region four). Low frequency ($P<0.05$) alleles were observed in all ten loci. Out of 184 observed alleles, 116 alleles occurred at frequencies of $P<0.05$ in all samples. Spl-173 showed the highest allele number (30 allele) of which 24 alleles at frequencies of $P<0.05$ and LS-34 showed the maximum variability ranging in frequency (0.058-0.567).

All sampled populations contained unique alleles (Table 3), neither of which was found in other regions. For example, loci LS-19, Spl-105, LS-19 and Spl-173 were unique marker for population differentiation from region one to four, respectively.

Average heterozygosity over the loci was 0.667 and ranged from 0.650 in region one to 0.677 in region four (Table 4).

Deviation from Hardy-Weinberg equilibrium was there in all cases ($P\leq0.01$) except Spl-173 in region one and Spl-68 in region two and Spl-113 in region three (Table 2).

Genetic distance calculated between each pair of collections ranged from 0.272 (between regions one and four) to 0.513 (between regions two and four). In general, similarities in more geographically close areas were higher than more apart ones (Fig. 1, Table 5).

Population differentiation was modest, especially among regions one and four (0.028). The population differentiation (F_{ST}) between regions two and four was the highest (0.058) and significant among the regions pair ($P\leq0.01$). The estimated gene flow (Nm) value between regions one and four across all the studied loci was the highest (8.528), while the Nm value between regions two and four was the lowest (4.092), (Table 6).

The dendrogram based on genetic distance Nei (1972,1978) revealed three major cluster. One cluster consists of region two alone, a second consists of region three, while the third consists of region one and Four together (Fig. 2). Region Two in all three dendrogram showed a separate cluster while other 3 regions (one, three and four) were genetically closer stocks.

Table 2:Absolute number of allelles observed within 4 sampling sites (region) using 10 sets of microsatellite primers

Locus	Region 1	Region 2	Region 3	Region 4	N
LS-19	17	12	14	15	14.5
LS-68	11	12*	12	11	11.5
LS-34	9	9	9	11	9.5
LS-54	12	10	10	14	11.5
Spl-105	11	8	12	12	10.7
Spl-104	14	14	14	13	13.7
Spl-163	18	11	17	15	15.2
Spl-170	16	13	17	15	15.2
Spl-173	17*	13	14	16	15
Spl-113	14	11	16*	18	14.7
Total	139	113	135	140	
Average	13.9	11.3	13.5	14	13.1

N= mean number of allelles observed per locus.
***Loci= loci in accordance with H-W equilibrium (P≤0.01)**

Table 3: Sample size and number of unique alleles associated with each stellate sturgeon regions

	Region 1	Region 2	Region 3	Region 4
Sample size	52	43	50	52
Observed number of unique alleles	3	6	5	8
The most allele frequency	0.106	0.276	0.120	0.221

Table 4: Observed (*Ho*) and expected (*He*) heterozygosities at 10 loci in four sampling regions

	Locus	Region 1	Region 2	Region 3	Region 4	Average
Ho(He)	LS-19	0.865 (0.900)	1.000 (0.888)	0.860(0.908)	0.923(0.879)	0.912
	LS-34	0.442 (0.713)	0.767 (0.812)	0.620 (0.796)	0.635 (0.653)	0.616
	LS-54	0.615 (0.881)	0.605 (0.783)	0.540 (0.843)	0.596 (0.839)	0.589
	LS-68	0.673(0.862)	0.581 (0.801)	0.640 (0.865)	0.654 (0.862)	0.637
	Spl-104	0.904 (0.885)	0.860 (0.905)	0.700 (0.888)	0.808 (0.880)	0.818
	Spl-105	0.538 (0.841)	0.349 (0.785)	0.720 (0.882)	0.635 (0.849)	0.560
	Spl-113	0.365 (0.830)	0.500 (0.759)	0.490 (0.788)	0.510 (0.875)	0.466
	Spl-163	0.462 (0.899)	0.535 (0.833)	0.460 (0.887)	0.577 (0.898)	0.508
	Spl-170	0.769 (0.898)	0.977 (0.888)	1.000 (0.902)	0.865 (0.896)	0.903
	Spl-173	0.692 (0.895)	0.581 (0.857)	0.400 (0.878)	0.577 (0.875)	0.563
	Average	0.650(0.866)	0.672(0.824)	0.670(0.876)	0.677(0.852)	0.667(0.854)

Table 5: Pairwise Genetic Distance (below diagonal) and Genetic Identity (above diagonal) (Nei, 1972) detected at 10 loci in stellate sturgeon samples

		Genetic Identity			
	Samples	**Region 1**	**Region 2**	**Region 3**	**Region 4**
Genetic Distance	Region 1	-	0.612	0.720	0.762
	Region 2	0.491	-	0.670	0.599
	Region 3	0.328	0.401	-	0.719
	Region 4	0.272	0.513	0.329	-

Table 6: Pairwise estimates of genetic differentiation detected at 10 loci in stellate sturgeon samples, using *Nm* (below diagonal) and F_{ST} values (above diagonal). Probabilities of *Nm* and F_{ST} determined by AMOVA tests, P≤ 0.01.

		Fst			
	Samples	**Region 1**	**Region 2**	**Region 3**	**Region 4**
Nm	Region 1	-	0.052	0.030	0.028
	Region 2	4.529	-	0.042	0.058
	Region 3	7.99	5.751	-	0.033
	Region 4	8.528	4.092	7.252	-

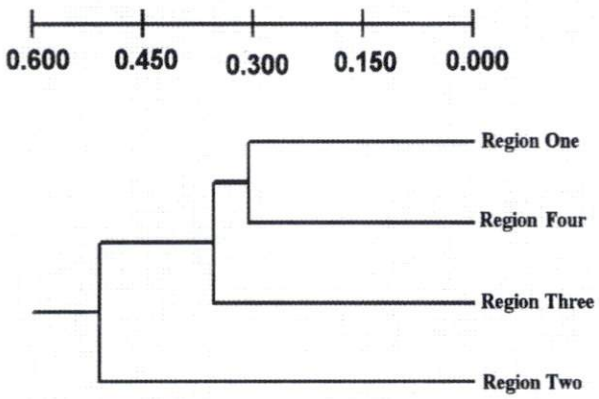

Figure 2: UPGMA dendrogram based on the genetic distance computed by Nei's (1972) between *A. stellatus* population, according to microsatellite DNA analysis.

Discussion

Out of 15 sets of microsatellite primers developed from genera *Acipenser* and *Scaphirhynchus*, ten were amplified in stellate sturgeon. These results suggest that there is evolutionary conservation of the flanking regions for these loci among related taxa. Four pairs were not amplified in the PCR reaction (Table1), which may be due to the lack of flanking sites in these primers due to high genetic distance of primers source species and the stellate sturgeon.

The long-term persistence of an endangered fish species can be investigated by allelic diversity, gene diversity, effective population size and population structure (Yue *et al.*, 2004). The degree of genetic diversity index is commonly measured by heterozygosity and it is used as a general index of population diversity at the genetic (Beardmore *et al.*, 1997).

The average number of alleles per locus and observed hetero-zygosities were compare-able in the Caspian Sea's populations as reported earlier in the RFLP analysis of the same populations by Shabani *et al.* (2006). As expected, levels of genetic variability revealed by microsatellite loci stand in stark contrast

to previous study used mtDNA. Low levels of variation were detected and little differentiation among sampling site was detected over a comparable geographic range (Pourkazemi, 2001; Shabani *et al.*, 2006). This may result from the more rapid rate of mutational change at microsatellite loci compared with that at even the most rapidly evolving area of the mitochondrial genome (Wirgin *et al.*, 2002).

In fact, although the populations do not differ in the amount of genetic variation expressed as heterozygosity or alleles per locus, they are very different in the nature of the genetic variation, which depends on the unique alleles and genotypes. Given that this species is released into the Caspian Sea for restocking, regular monitoring of genetic variability among the progenies is essential to avoid the loss of current polymorphism due to inbreeding and out-breeding problems.

Heterozygosity is important in populations because it provides a large spectrum of genotypes for adaptive response to changing conditions, and many economically important characteristics like growth, fertility and disease resistance (Beardmore *et al.*, 1997).

In the present study, deviation from the Hardy-Weinberg equilibrium was observed in most loci (Table 2), which could be due to presence of null alleles. Similarly, the null alleles were reported in inheritance of microsatellite loci in lake and white sturgeons (Pyatskowit *et al.*, 2001; Rodzen & May, 2002; McQuown *et al.*, 2003; Zhao *et al.*, 2005; Welsh & May, 2006). It may also be related to not using species specific primers and the most important reason sampling from mixtures of migrating population and sampling methodology. To detect such a population structure, samples must be collected from spawning sites. Samples from non-spawning adults may reflect mixtures of migrating population (McQuown *et al.*, 2003).

Shaklee *et al.* (1982) and Thorpe and Sol-Cave (1994) showed that genetic distance values for conspecific populations averaged 0.05 (ranged: 0.002-0.07) and for congeneric species averaged 0.30 (ranged: 0.03-0.61). As shown in Table 5, the distance value obtained in the present study falls within the average value of congenerics, indicating that the genetic difference among the studied populations is pronounced.

The results show pair-wise F_{ST} estimates between all sampling sites were low and significant ($P \leq 0.01$). Overall, these tests suggest that each of the four samples analyzed represent genetically definable four populations. Low F_{ST} would be possible to effect of polymorphism due to mutations drastically deflates F_{ST} expectations (Balloux & Lugon-Moulin, 2002), mixing and high gene flow between populations. Also, negative correlation between F_{ST} values and dispersal capability (Waples, 1987), due to high dispersal capability presumably to absence of physical or ecological barriers to individuals may take into account.

In our study, F_{ST} in all sampling site was low but significant ($P \leq 0.01$), sugges-

ting that more than one population are genetically differentiated and do not represent a single panmictic population. In fact, in the great majority of cases, F_{ST} is low, because the effect of polymorphism (due to mutations) drastically deflates F_{ST} expectations (Balloux & Lugon-Moulin, 2002). In fish, negative correlation has been demonstrated between F_{ST} values and dispersal capability (Waples, 1987). According to this, *A. stellatus* might present high dispersal capability presumably due to the absence of physical or ecological barriers to individuals. Feeding and spawning migrations are defined as continuous movements of fish from one part of the sea to another (Keyvan, 2003). However, the loss of genetic variability also might be caused by sampling error and releasing fingerlings with hatchery-origin returning to rivers to spawn may also contribute to the loss of regional genetic differentiation (Vasemägi *et al.*, 2005).

Genetic dendrogram of stellate sturgeon in four regions of south Caspian Sea showed three clarified clusters (Fig. 2). The samples of the region two showed a separate cluster while other regions were genetically more closely stocks.

In comparison with other molecular methods performed on population differentiation of stellate sturgeon, micro-satellite studies showed the higher genetic variation and heterozygosity than mtDNA. Since microsatellite technique has higher capacity to show the genetic variation, we recommend conducting a joint comprehensive sturgeon population studies in the Caspian Sea.

Overfishing has caused dramatic reduction in broodstocks in the rivers and has limited the number of spawners. Therefore, a genetic based program is highly recommended to maintain the genetic variability of the remaining stellate sturgeon resources.

In conclusion, this study provides preliminary evidence for the existence of different populations of stellate sturgeon in the Caspian Sea. Probably in the river-going stocks (Ural, Volga, Sefidrud, Gorganrud), more than one population exist (such as spring migration and fall migration) and more samples from these rivers should be investigated to find out the exact population numbers. Information on the levels and differentiation of genetic variability should comprise a key component of the stellate sturgeon conservation plan. Characterizing the genetic structure of *A. stellatus* currently being used in the aquaculture industry will aid in for future broodstock development and improve management plans that aim to conserve diversity and minimize inbreeding in artificial propagation. Therefore, a special consideration on conservation policy and restocking program of this species in artificial reproduction centers is highly recommended.

Acknowledgement

We are grateful to the Iranian Fishery Research Organization for the financial support (Project code 82-0710441000-53). This research was performed in the Molecular

Genetic Laboratory of the International Sturgeon Research Institute, Rasht. We would like to extend our appreciation to Mr. Noroz Fashkhami, Head of Genetic Department. Also, special thanks to Mr. Ghasemi and Mr. Chakmeduz for their great assistance.

References

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

Beardmore, J.A., Mair, G.C. and Lewis, R.I., 1997. Biodiversity in aquatic systems in relation to aquaculture. Aquaculture Research. **28:**829–839.

Holcik, J., 1989. The fresh water fishes of Europe. General introduction to fishes, Acipenseriformes, AULA-Verlarg, Wiesbaden. 1**(2):** 469.

IUCN, 1996. IUCN Red List of Threatened Animals. Gland, Switzerland: IUCN. **70:** 235-236.

Keyvan, A., 2003. [Iranian sturgeons in the Caspian Sea]. Iranian Fisheries Company. pp.101-102. (in Persian)

Khoshkholgh, M.R., Pourkazemi, M., Kamali, A. and Rezvani, S., 2007. [Genetic population structure of Russian sturgeon (*Acipenser guldenstadti*) in South of the Caspian Sea using microsatellite markers]. Iranian Scientific Fisheries Journal. 16**(4):**69-80. (in Persian).

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

McQuown, E.C., Sloss, B.L., Sheehen, R.J., Rodzen, J., Tranah, G.J. and May, B., 2000. Microsatellite analysis of genetic variation in sturgeon: New primer sequences for *Scaphyrhinchus* and *Acipenser*. Transactions of the American Fisheries Society. **129:**1380-1388.

McQuown, E., Krueger C.C., Kincaid, H.L., Gall, A.E. and May, B., 2003. Genetic comparison of lake sturgeon (*Acipenser fulvescens*) population: Differentiation based on allelic frequencies at seven microsatellite loci. Journal of Great Lake Research. 29**(1):**3-13.

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

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. **89:**583-590.

Peakall, R. and Smouse, P.E., 2005. GenAlEx 6: Genetic Analysis in Excel. Population genetic software for teaching and research. The Australian National University, Canberra, Australia. www.anu.edu.au.

Pourkazemi, M., Skibinski, D.O.F. and Beardmore, A., 2000. A preliminarly study on phylogenetic relationship between five sturgeon species in the Iranian coastline of the Caspian Sea. Iranian Journal of Fisheries Sciences. 2**(1):**1-12.

Pourkazemi, M. and Skibinski, D.O.F., 2001. Allozyme and mtDNA study on population structure of stellate sturgeon (*Acipenser stellatus*) in Iranian coastlines of the south Caspian Sea. 4th International Symposium on Sturgeon, Oshkosh, Wisconsin, USA. 8-13 July, 2001. Extended Abstract Book. General Biology: 52.

Pourkazemi, M., 2006. Caspian Sea sturgeon conversation and fisheries past, present and future. Journal of Applied Ichthyology. 22**(s1):**1-4.

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

Qasemi, A., Pourkazemi, M. and Kalbassi, M.R., 2006. [Comparison of genetic variation of ship sturgeon (*Acipenser nudiventris*) in the southern Caspian Sea and Ural River using PCR-RFLP]. Iranian Scientific Fisheries Journal. 14**(4):**151-164. (in Persian).

Rezvani Gilkolahaei, S., 2000. Study of mtDNA variation of Russian sturgeon population from the south Caspian Sea using RFLP analysis of PCR amplified ND5/6 gene regions. Iranian Journal of Fisheries Sciences. 2**(1):**13-36.

Rodzen, J.A. and May, B., 2002. Inheritance of microsatellite loci in the polyploidy white sturgeon (*Acipenser transmontanus*). Genome. **54:**1064-1076.

Shabani, A., Pourkazemi, M. and Rezvani, S., 2006. [Study of mtDNA variation of stellate sturgeon (*Acipenser stellatus*) population from the north (Volga River) and the south (Sefidrud River) Caspian Sea using RFLP analysis of PCR Amplified ND 5/6 gene regions]. Journal of Agriculture Sciences and Natural Resources. 12**(6):**195-204. (in Persian).

Shaklee, J.B., Tamaru, C.S. and Waples, R.S., 1982. Speciation and evolution of marine fishes studied by electrophoretic analysis of proteins. Pacific Science. **36:**141-157.

Thorpe, J.P. and Sole-Cava, A.M., 1994. The use of allozyme electrophoresis in invertebrate systematics. Zoologica Scripta. **23:**3-18.

Vasemägi, A., Gross, R., Paaver, T., Koljonen, M.L. and Nilsson, J., 2005. Extensive immigration from compensatory hatchery releases into wild Atlantic salmon populations in the Baltic Sea: Spatio-temporal analysis over 18 years, Heredity. **95:**76-83.

Waples, R.S., 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution. **41:**385– 400.

Welsh, A. and May, B., 2006. Development and standardization of disomic microsatellite markers for lake sturgeon genetic studies. Journal of Applied Ichthyology. **22:**337–344.

Wirgin, I., Waldman, J., Stabile, J., Lubinski, B. and King T., 2002. Comparison of mitochondrial DNA control region sequence and microsatellite DNA analysis in estimating population structure and gene flow rates in Atlanic sturgeon (*Acipenser oxyrinchus).* Journal of Applied Ichthyology. **18:**313-319.

Yue, G.H., Li, Y., Lim, L.C. and Orba, L., 2004. Monitoring the genetic diversity of three Asian arowana (*Scleropages formosus*) captive stocks using AFLP and microsatellites. Aquaculture. **237:**89–102.

Zajc, I., Mellersh, C.S. and Sampson J., 1997. Variability of canine microsatellites within and between different dog breeds. Mammalian Genome. **8:**182-185.

Zhao, N., Ai, W., Shao, Z., Zhu, B., Brosse, S. and Chang, J., 2005. Microsatellites assessment of Chinese sturgeon (*Acipenser sinensis Gray*) genetic variability. Journal of Applied Ichthyology. **21:**7-13.