Microsatellite DNA markers for analysis of genetic population structure of stellate sturgeon (*Acipenser stellatus* Pallas, 1771) in the North (Volga and Ural Rivers) and South Caspian Sea (Sefidrud and Gorganrud Rivers)

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Abstract

This study represents a large-scale population genetic analysis of the stellate sturgeon, *Acipenser stellatus*, in the Caspian Sea. In total, 192 samples of adult stellate sturgeon were collected from four commercial catch stations in the Northern (Volga and Ural Rivers) and Southern Caspian Sea (estuary of Sefidrud and Gorganrod Rivers-Iran). Fifteen sets of microsatellite primers developed from lake sturgeon (*Acipenser fulvescens*) and shovelnose sturgeon (*Scaphirhynchus platorynchus*) were tested on genomic DNA. Ten primer sets (*LS-19, 34, 54, 68, Spl-105, 104, 163, 170, 173, 113*) revealing polymorphic loci were used to analyze the genetic variation found in adults of the stellate sturgeon populations. Analyses revealed that the average number of alleles per locus was 13.05 (range 8 to 18 alleles per locus in regions, Ne= 7.86). All sampled regions contained private alleles. The average observed and expected heterozygosities were 0.665 and 0.862, respectively. Deviations from Hardy-Weinberg equilibrium were seen in most cases. Average of Fis, Fit and Nm were 0.230, 0.261 and 7.498, respectively. Pairwise Population F_{ST} Values ranged from 0.019 to 0.035. $F_{ST,}$ $R_{ST,}$ and gene flow estimates in AMOVA indicated significant genetic differentiation among and regions, indicating that the populations were divergent. The genetic distance between populations indicates that the genetic difference among the studied populations is pronounced. These results together with highly significant R_{ST} of genotypic differences between these pairs of collections support the existence of different genetic populations along the Caspian Sea coast.

Keywords: Genetic variability, Genetic differentiation, *Acipenser stellatus*, Microsatellite markers

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Introduction

Sevruga or stellate sturgeon, *A. stellatus*, is an anadromous species, and lives mainly in the Caspian Sea while spawning in the upper Ural, Volga, Sefidrud and Gorganrud Rivers. The age structure of its spawning population is very indicative of pressure levels. Thus, sevruga may be regarded as an indicator species for all sturgeons, featuring the tendencies of sturgeon stocks change and decline, in general (Velikova *et al*., 2012). Stellate sturgeon is faced with the challenges resulting from overfishing, pollution, and habitat destruction. Stellate sturgeon is listed as a critically endangered species (IUCN, 2009).

Molecular genetics research on stellate sturgeon in the Caspian Sea was so far limited to a few studies using RFLP methods (Pourkazemi, 2001; Shabani *et al*., 2006) and low genetic variation was stated while no significant differences in haplotype frequency were found. Microsatellite genotypes are particularly helpful to detect structure in closely related populations, regardless of whether they are in an evolutionary equilibrium. Additionally, primers designed for one species can often be used with other related species (Chistiakov *et al*., 2005). In recent years, many microsatellite loci were used to investigate the genetic structure of various Acipenseriformes species including: *A. ruthenus* (Fopp-Bayat *et al*. 2015); *A. persicus* (Chakmehdouz Ghasemi *et al*., 2011; Moghim *et al*., 2009, 2012, 2013); *A. stellatus* (Norouzi *et al*., 2009); *Acipenser gueldenstaedtii* (Khoshkholgh *et al*., 2008); *Scaphirhynchus* (Ray *et al*., 2007; Schrey and Heist, 2007); *Acipenser sinensis* Gray (Zhao *et al*. 2005) and *A. fulvescens* (McQuown *et al*., 2003).

So, the objectives of the present study were to investigate the genetic structure of the stellate sturgeon in northern and southern Caspian Sea and also to test the hypothesis that stellate sturgeon has identical populations in each original spawning river in the Caspian Sea.

Materials and methods

Sample collection and DNA isolation

Totally 192 tissue samples including fin clips of adult stellate sturgeon were collected from four regions including 54 samples from Volga (Russia, 45°30′N, 47°47'E), 43 samples from Ural (Kazakhestan, 46°50′N, 51°32' E) the North Caspian Sea, 43 samples from Sefidrud River (Iran, 37°28'N, 49°56'E), 52 samples from Gorganrud drainage (Iran, 36°58'N, 54° 0'E) the South Caspian Sea in Iran (Fig.1) and preserved in 95% ethanol stored at room temperature.

DNA extraction microsatellites data set

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 1% agarose gel electrophoresis and spectrophotometry (CECIL model CE2040 Germany) and then stored at -20°C until use.

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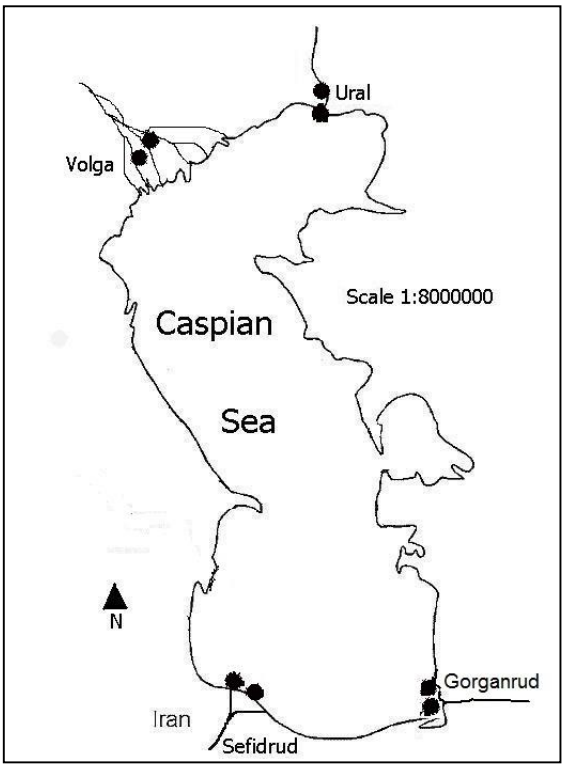


Figure 1: Map shows the sampling sites of stellate sturgeon in Volga, Ural and Sefidrud and Gorganrud Rivers.

PCR profiles and primer sequences

Genomic DNA was used as a template to amplify microsatellite loci by touchdown polymerase chain reaction (PCR). Totally 15 primer pairs were designed for *Acipenser* and *Scaphirhynchus* (*LS-19, 34, 39, 54, 57, 62, 68, 69,* May *et al*., 1997 ; *Spl-104, 105, 113, 163, 168, 170, 173,* McQuown *et al*., 2000). For all primer sets, amplification was performed in a reaction volume of 20µL containing 0.2 mM of dNTPs, 0.5-1pµ each primer, 100 ng of template DNA; 1 units of *Taq* DNA polymerase and 1-2 mM Mg Cl2. Microsatellites were amplified (see Table 1 for specific annealing temperatures) using a Eppendorf thermocycler (Master cycle rep gradient, 96 plus, Eppendorf, Germany). An initial denaturing step of 10 minutes at 95°C was followed by amplification for 25-35 cycles under the following conditions: 30 seconds at 95°C, 30–60 seconds at 49-61.2°C and 45–120 seconds at 70–72°C. A final 5-minute extension at 72°C completed the protocol (See Table 1).

PCR products were electrophoresed on 6% polyacrylamide gels (29:1 acrylamide: bis-acrylamide; 1X TBE buffer) and followed by silver-staining. Gels were run at 170 W for 2h and 30min (BIO-RAD sequi Gen, GT, 38 ×30 cm/ Power PAC5000, USA). Alleles were sized using BioCapt

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software (http:// biocapt. software. informer.com/), and each gel contained an allelic ladder (50bp) to assist in consistent scoring of alleles.

Data analysis

Estimate of allele frequencies and frequency-based analyses include *F*-statistics and Nei's genetic distance. The total genetic diversity (heterozygosity) can be divided into within and among populations as follows: *Ho*= Observed heterozygosity, *He*= Expected heterozygosity. Hardy-Weinberg (HW) tests of equilibrium estimated.

Wright's F-statistics (Wright, 1965) is as follows: F_{IS}= The inbreeding coefficient within individuals relative to the subpopulation for each locus and stellate sturgeon sampling site were assessed; Fit =the inbreeding coefficient within individuals relative to the total. F_{ST}= the inbreeding coefficient within subpopulations relative to the total. F_{ST} *and* R_{ST} based on AMOVA (Analysis of Molecular Variance) to estimate genetic variation among populations and regions. AMOVA calculations and allelic richness (A_R) were performed on Arlequin 3.5 (Excoffier and Lischer, 2010) using 10,000 permutations in each case. Nei's genetic identity and distance were determined using a pairwise, individual-by-individual genetic distance, with all codominant data computed in Gean Alex 6 software (Peakall and Smouse, 2005). The Cornuet and Luikart (1996) programme BOTTLENECK ver. 1.2.02 was used to detect recent effective population size reduction (to assess the impact of population decline) using data from the microsatellites under the more suitable two-phased model (TPM).

Results

Amplification and banding patterns

Out of 15 sets of microsatellite primers, 4 sets have not shown any flanking sites in stellate sturgeon genome. Ten sets of primers (*LS-19, 34, 54, 68, Spl-105, 104, 163, 170, 173, 113*) were successfully amplified and showed polymorphic pattern in the 192 individuals assayed and one set (LS-39) showed monomorphic pattern (Table 1). All 10 microsatellite primers were able to produce DNA bands displaying a characteristic disomic banding pattern.

Genetic variation within sampling

A total of 140 alleles were identified in 192 individuals, 67 alleles in Sefidrud, 70 alleles Ural, 76 alleles in Volga and 63 alleles in Gorganrud drainage, with frequencies >0.05 in all samples. LS-34 showed the maximum variability ranging in frequency from 0.056 to 0.567. Allele sizes ranged from 104 to 296 bp (Table 1).

The Na per locus ranged from 8 to 18, with an average of 13.05 (±0.397).

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Table 1.Characteristics of 11 polymorphic microsatellite loci isolated from stellate sturgeon

Locuse/ GenBank Accession No.	Primer sequence (5'→3')	Ta (°C)	Mg+2	Repeat motif	Size range (bp)	Primer sources
LS-19/ U72730	F-CATCTTAGCCGTCTGTGGTAC R-CAGGTCCCTAATACAATGGC	56	1.6	$(TTG)_9$	132-213	May *et al.*(1997)
LS-34/ U72733	F-TACATACCTTCTGCAACG R-GATCCCTTCTGTTATCAAC	58	2	$(GTT)_{10}$	132-180	
LS-39/ U72734	F-TTCTGAAGTTCACACATTG R-ATGGAGCATTATTGGAAGG	58	2	$(GTT)_{10}$	120	
LS-54/ U72735	F-CTCTAGTCTTTGTTGATTACAG R-CAAAGGACTTGAAACTAGG	59	2	$(GATA)_6$ $(GACA)_7$	152-224	
LS-68/ U72739	F-TTATTGCATGGTGTAGCTAAAC R-AGCCCAACACAGACAATATC	61.2	1	$(TATC)_{13}$	104-160	
Spl-104/ AF276173	F-TTATATGGGTGGGGTGGATG R-TCCTCTTTGGCATTTGTTCC	57	1	$(TCTR)_{12}$	184-248	McQuown *et al.*, (2000)
Spl-105/ AF276174	F-GCGATTTGATTGGCTCTTGT R-GGCACTGAATAAATGGACCG	58	2	$(TAGA)_{12}$	104-180	
Spl-113/ AF276182	F-TCCCACATGGCTTGTATTGA R-ACCACACCATGCGTCATAAG	59	1	$(AGAT)_{14}$	160-212, 260-348	
Spl-163/ AF276205	F-TGCTTGTAAACTGCCCCACT R-CCACATGCAGTTTGAGCTGC	56	2.5	$(GATA)_{17}$	160-244	
Spl-170/ AF276213	F-GGACGCACTAGACAGGCTTT R-CACCAAACACAGCAGATTTCA	58	1.25	$(GAT)_5$ $(ATAG)_{11}$	200-264	
Spl-173/ AF276216	F-GGCTTTTGTCTGAAACGTCC R-TGGTGTGTCATTTTGAAGGC	58.5	2.5	$(TCTA)_{10}$	176-296	

The number of alleles in LS-34 ranged from 8 to 11 (A_R=13), in LS-19 from 12 to 15 (A_R=17.25), in LS-54 from 10 to 15 (A_R=16.25), in LS-68 from 11 to 15 (A_R=13.5), in Spl-105 from 8 to 12 (A_R=12.25), in Spl-104 from 13 to 15 (A_R=15.75), in Spl-163 from 11 to 15 (A_R=18.5), in Spl-170 from 13 to 16 (A_R=23.5), in Spl-173 from 13 to 17 (A_R=29.75), and in Spl-113 from 11 to 18 (A_R=20.25), with a tendency toward being fewer in the Sefidrud drainage samples (Table 2). All sampled populations contained a significant number of private alleles (unique alleles) except Volga samples. In total, 10 alleles were found, with the number of private alleles being Ural 5 alleles, Sefidrud 3 alleles and Gorganrud 2 alleles, none of which were found in other regions. Bottleneck analysis of stellate sturgeon was 0.03490 in Ural, 0.04296 in Volga, 0. 03596 in Sefidrud and 0.03996 in Gorganrud.

The Ho and He per locus ranged from 0.394 to 1 and from 0.653 to 0.910, with an average of 0.665 (±0.031) and 0.862 (±0.008), respectively (Table 2). The LS-19 loci had the highest level of heterozygosity, and lower heterozygosities were consistently observed in most samples screened, which may be due to the presence of null alleles or small sample sizes.

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Table 2: Absolute numbers of allelles observed within 4 sampling sites using 10 sets of microsatellite primers. Observed (*Ho*) and expected (*He*) heterozygosities, number of effective alleles (*Ne*), allelic richness (A_R), the inbreeding coefficient (F_{IS}), Hardy-Weinberg (HW) tests of equilibriumat 10 loci in four sampling regions.

Sample size / Locus	North Caspian Sea		South Caspian Sea		
	Ural	Volga	Sefidrud	Gorganrud	Average
	43	54	43	52	
LS-19					
Na	14	14	12	15	13.750
Ne	8.405	8.627	8.954	8.282	8.567
private alleles	-	-	-	-	
AR	17	18	15	19	
Ho	0.977	0.963	1.000	0.923	0.966
He	0.881	0.884	0.888	0.879	0.883
Fis	-0.109	-0.089	-0.126	-0.050	-0.093
HW	0.001	0.000	0.000	0.000	
LS-34					
Na	8	11	9	11	9.750
Ne	5.994	6.388	5.329	2.881	5.148
private alleles	-	-	1	-	
AR	11	15	12	14	
Ho	0.442	0.778	0.767	0.635	0.655
He	0.833	0.843	0.812	0.653	0.785
Fis	0.470	0.078	0.055	0.028	0.166
HW	0.000	0.000	0.000	0.001	
LS-54					
Na	11	11	10	15	11.750
Ne	5.115	7.043	4.611	6.202	5.743
private alleles	-	-	-	-	
AR	15	17	14	19	
Ho	0.488	0.593	0.605	0.596	0.570
He	0.804	0.858	0.783	0.839	0.821
Fis	0.393	0. 309	0.228	0.0289	0.305
HW	0.000	0.000	0.000	0.000	
LS-68					
Na	12	15	12	11	12.500
Ne	9.153	8.863	5.031	7.230	7.569
private alleles	-	-	-	-	
AR	13	16	13	12	
Ho	0.698	0.815	0.581	0.654	0.687
He	0.891	0.887	0.801	0.862	0.860
Fis	0.217	0.082	0.274	0.241	0.201
HW	0.000	0.231	0.281	0.001	
Spl104					
Na	14	15	14	13	14.000
Ne	9.995	11.066	10.506	8.359	9.981
private alleles	-	-	-	-	
AR	15	18	16	14	
Ho	0.744	0.907	0.860	0.808	0.830
He	0.900	0.910	0.905	0.880	0.899
Fis	0.173	0.002	0.049	0.083	0.077
HW	0.068	0.056	0.000	0.024	
Spl105					
Na	11	10	8	12	10.250
Ne	7.337	6.538	4.657	6.603	6.284
private alleles	-	-	2	-	
AR	13	11	10	15	
Ho	0.372	0.370	0.349	0.635	0.431
He	0.864	0.847	0.785	0.849	0.836
Fis	0.569	0.563	0.556	0.252	0.484
HW	0.000	0.000	0.000	0.003	

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Continued Table 2:

Sample size	North Caspian Sea		South Caspian Sea		
Locus	**Ural**	**Volga**	**Sefidrud**	**Gorganrud**	**Average**
	43	**54**	**43**	**52**	
Spl113					
Na	17	18	11	18	16.00
Ne	9.506	11.087	7.625	8.543	9.191
private alleles	-	-	-	-	
AR	21	22	15	23	
Ho	0.488	0.537	0.465	0.481	0.493
He	0.895	0.910	0.869	0.883	0.889
Fis	0.454	0.410	0.465	0.455	0.446
HW	0.000	0.000	0.000	0.000	
Spl163					
Na	14	13	11	15	13.250
Ne	8.701	7.967	5.994	9.833	8.124
private alleles	2	-	-	-	
AR	18	17	15	24	
Ho	0.953	0.481	0.535	0.577	0.637
He	0.885	0.874	0.833	0.898	0.873
Fis	-0.077	0.449	0.358	0.358	0.270
HW	0.000	0.000	0.000	0.000	
Spl170					
Na	14	16	13	15	14.500
Ne	10.359	10.760	8.911	9.657	9.922
private alleles	3	-	-	-	
AR	23	26	20	25	
Ho	0.465	1.000	0.977	0.865	0.827
He	0.903	0.907	0.888	0.896	0.899
Fis	0.485	-0.102	-0.100	0.035	0.080
HW	0.000	0.000	0.000	0.001	
Spl173					
Na	17	23	16	18	14.750
Ne	7.672	9.986	7.004	7.988	8.163
private alleles	-	-	-	2	
AR	25	31	29	34	
Ho	0.442	0.611	0.581	0.577	0.553
He	0.870	0.900	0.857	0.875	0.875
Fis	0.492	0.321	0.322	0.341	0.368
HW	0.000	0.000	0.000	0.000	
Total of alleles(overall)	129	140	113	140	
private alleles (overall)	5		3	2	
Average					
Na	13.75	15.91	12.41	15.25	
Ne	8.82	9.71	7.20	8.82	
Ho	0.59	0.66	0.652	0.65	0.665
He	0.873	0.882	0.842	0.861	0.862
Fis	0.307	0.202	0.208	0.203	0.230

Estimates of inbreeding coefficient or F_{IS} values of five microsatellites were positive and between -0.093 at LS-19 and 0.484 at Spl-105 (mean F_{IS} = 0.230 ±0.042, Table 2; mean F_{IT} = 0.261 ±0.057), and positive F_{IS} values a relative dearth of heterozygote. However, LS-19 had lower F_{IS} and higher heterozygosity than all loci in the populations assayed.

In all cases, significant deviations from Hardy-Weinberg equilibrium ($p \leq$ 0.01) were only found at one locus, which was in Hardy-Weinberg equilibrium in LS-68 in Volga and Sefidrud and Spl-104 in Ural and Volga samples (Table 2).

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Pairwise population F_{ST} values and estimates of Nm

The *Nm* and F_{ST} via frequency ranged from 3.3 to 19.1 and from 0.013 to 0.069, with an average of 7.49 and 0.042, respectively (Tables 3, 4). In practice, F_{ST} is rarely larger than 0.5 and often very much less. F_{ST}, R_{ST} and gene flow estimates in AMOVA indicated significant genetic differentiation among regions ($p \leq 0.01$), indicating that the populations were divergent from each other. Values of pairwise R_{ST} among samples were consistently much higher (as much as an order of magnitude) than equivalent F_{ST} values (Table 4) but differences were not significant. Results of AMOVA of genetic variation, among population in regions was 0.042 and individual within groups was 0.044 (p=0.01). Nei's genetic identity ranged from 0.593 to 0.723. Consequently, Nei's Genetic Distance ranges from 0.324 to 0.514 (Nei, 1972; Table 3).

Table 3: Pairwise estimates of genetic differentiation detected at 10 loci in stellate sturgeon samples, using FST values (above diagonal) and Nm (below diagonal).

		Fst			
	Samples	**Ural**	**Volga**	**Sefidrud**	**Gorganrud**
	Ural	-	0.019	0.033	0.029
Nm	Volga	12.9	-	0.024	0.028
	Sefidrud	7.3	10.06	-	0.035
	Gorganrud	7.292	8.79	6.88	-

Table 4: Genetic Distance (Nei, 1972) detected at 10 loci in stellate sturgeon samples .

	Samples	**Ural**	**Volga**	**Sefidrud**	**Gorganrud**
	Ural	0.000			
Genetic	Volga	0.324	0.000		
Distance	Sefidrud	0.522	0.356	0.000	
	Gorganrud	0.475	0.446	0.514	0.000

Discussion

Although DNA depended methodology such as Microsatellite loci is an important tool in fisheries management and aquaculture, the application of population genetic data to manage the Caspian Sea sturgeon is in its early stage and little information exists about the genetic population structure subdivision.

Ten out of fifteen primer sets designed originally from lake sturgeon (*A. fulvescens*) and shovelnose sturgeon (*S. platorynchus*) DNA sequences (see table 1) amplified in *A. stellatus* indicate a high degree of conservation of primer sites between two species of *Acipenser* and *Scaphirhynchus*. These results suggest that there is evolutionary conservation of the flanking regions for these loci among related taxa. The cross-amplification between lake sturgeon, shovelnose sturgeon and the stellate sturgeon is consistent with

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earlier findings closely related (May *et al*., 1997; McQuown *et al*., 2000).

Four sets of primers were not amplified in the PCR reaction. There is a significant and negative relationship between microsatellite performance and evolutionary distance between the species. The proportion of polymorphic loci among those markers that amplified decreased with relatively high genetic distance (Cui *et al*., 2005).

Compared with the heterozygosity between sites, the heterozygosity in samples from Volga was higher than other samples sites (Table 2). The genetic diversity was relatively low, and the population structure and resource have significantly declined, especially in Sefidrud samples. The average number of alleles per locus and observed heterozygosities were comparable in North and South Caspian Sea's populations as reported earlier in the RFLP analysis of the same populations by Shabani *et al*. (2006). 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 private alleles and genotypes. Unfortunately, most commercially caught adults are being used for caviar production (Abdolhay and BaradaranTahori, 2006).

The losses of alleles and heterozygosity may increase with bottlenecking and inbreeding. Positive inbreeding coefficient values are implied by a relative dearth of heterozygotes if the explanation for decreased levels of heterozygosity is biological. On the other hand, reduced genetic diversity may increase the susceptibility to disease and other selective factors, resulting in further declines in population size (Shen and Gong, 2004). A heterozygote deficiency can also be attributable to other phenomena including inbreeding, or population admixture, Wahlund effect[1].

In the present study, deviation from the H-W equilibrium was observed for most loci (p<0.001).The significant deviations from H-W equilibrium could be explained either by sample bias, the Wahlund effect, not using species specific primers or as the result of null alleles occurring in the studied populations. Heterozygotes with a null allele could be erroneously recorded as homozygotes for the variant allele. Similar results have been reported in lake and white sturgeons (Rodzen and May 2002; McQuown *et al*. 2003; Welsh and May 2006), Chinese sturgeon (Zhao *et al.,* 2005) and it may also be related to sampling from mixtures of migrating populations.

F_{ST} represents the degree of population genetic differentiation, that is, the proportion of the total genetic diversity (~heterozygosity) that separates the populations. The range of F_{ST} via frequency for codominant data was 0.019 - 0.035. In fact, in the great majority of cases, F_{ST} is low, because the effect of polymorphism (due to

[1]theWahlund effect refers to reduction of heterozygosity (that is when an organism has two different alleles at a locus) in a population caused by subpopulation structure).

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mutations) drastically deflates FST expectations (Balloux *et al*., 2002). In fish, negative correlations have been demonstrated between F_{ST} values and dispersal capability. On this basis, *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 errors contributing to the loss of regional genetic differentiation. Additionally, released fingerlings of hatchery origin that return to rivers to spawn may contribute to the loss of regional genetic differentiation (Vasema¨gi et al., 2005). $F_{ST}$and R_{ST} via AMOVA for co-dominant data in all sampling site were significant ($p \leq 0.01$),suggesting that at least four populations are genetically differentiated and do not represent a single panmictic population.

The most important finding of the present study was the degree of genetic structuring found populations of the South and north Caspian Sea areas. All tests showed these samples are genetically identical. The range of genetic distance between populations was 0.324 to 0.522 (Table 3). Haklee *et al.* (1982) and Thorpe and Sol-Cave (1994) showed that genetic distance values Nei (1972) for conspecific populations averaged 0.05 (range: 0.002–0.07) and that for congeneric species averaged 0.30 (range: 0.03–0.61). The distance values obtained in the present study are above the average value of congenerics, indicating that the genetic difference among the studied populations is pronounced.

Our data support that the Volga population is influenced by non-controlled stocking or historical pop-admixture and the nonexistence of any private allele confirms that. This may be the result of being used for spawning by fish from all other populations without previous genetic testing or checking. Based on the indication one should strongly recommend that rehabilitation and sturgeon fingerling release programs (enhancement, restoration) should be built entirely on management schemes that handle each river system as a separate entity. Not to mix populations from nearby rivers as they seem to have specific adapted behavioral and reproductive as well as migratory traits. The stellate sturgeon from Volga River seems to provide a warning for the admixture which may have lack of fitness in the long-run.

In summary, this study provides preliminary evidence for the existence of at least four differentiated populations in the Caspian Sea. The existence of private alleles and significant F_{ST} and R_{ST} confirm Ural, Volga, Sefidrud and Gorganrud populations. Probably, extra populations are present in the Caspian Sea; therefore, a comprehensive investigation using more samples from the entire Caspian Sea may confirm this

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hypothesis. Characterizing the genetic structure of *A. stellatus* in the fishery industry will help and improve the future management and conservation of this unique species. The loss of genetic diversity may increase with bottlenecking, so we should protect the genetic diversity by reducing pollution and minimizing inbreeding in artificial propagation. In addition, possible drivers of the stellate sturgeon stock decline could be of natural origin and/or various anthropogenic activities related to overfishing, pollution and habitat loss.

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