Genetic diversity in the Persian sturgeon, *Acipenser percicus*, from the south Caspian Sea based on mitochondrial DNA sequences of the control region

M. Khoshkholgh1*, M. Pourkazemi2, S. Nazari1, L. Azizzadeh Pormehr2

1. Dept. of Fisheries, Faculty of Natural Resources, University of Guilan, P.O. Box 1144, Someh Sara, Iran
2. International Sturgeon Research Institute, P.O. Box 41635-3464, Rasht, Iran
* Corresponding author’s E-mail: majidrezagu@yahoo.com

ABSTRACT

The Persian sturgeon, *Acipenser persicus* (Borodin, 1897), is an economically important species, which mainly inhabits the Caspian Sea. However, little is known about its population genetic structure. In this study, variation in nucleotide sequences of the mitochondrial DNA (mtDNA) control region of wild stock Persian sturgeon was determined to assess the genetic diversity among different natural populations of this species. The fish (*n* = 46) were collected from four sites (Astara, Sefidrood, Noshahr and Bandare-Turkaman) in the south Caspian Sea. As a result 6 haplotypes and 44 variable sites were found. The average haplotype diversity (*h*) and nucleotide diversity (*π*) were 0.640±0.028 and 0.0442±0.011, respectively. Analysis of molecular variance (AMOVA) demonstrated that most variations occurred within samples, and the difference between the populations from Astara and Noshahr or Bandare-Turkaman was not significant (*p* < 0.001). Estimates of gene flow indicated reproductive isolation between the Sefidrood River population and the other collections. The divergence might be related to geographical isolation. The results are consistent with the findings from PCR-RFLP analysis (PCR-RFLP) and suggest considerable genetic diversity of the population from Sefidrood River.

Keywords: Persian sturgeon; Acipenser persicus; mitochondrial DNA; genetic variation.

INTRODUCTION

The Persian sturgeon, *Acipenser persicus* is an economically important species mainly observed in the Iranian rivers, Sefidrood and Gorgan-chaii, flowing into the Caspian Sea. It also enters to a smaller degree into the Terek, Suli and Tamur rivers in the Russian Federation and Republics, respectively. A small group of individuals live in the Volga, Kura, and Ural Rivers (Birstein *et al.*, 1997). The Persian sturgeon is concentrated in Iranian waters where sea fishing is permitted. (Vlasenko *et al.*, 1989; Birstein *et al.*, 1997). The decline in Persian sturgeon populations in the last decade is largely due to environmental changes such as the loss of spawning grounds and rearing habitats. Commercial fishing of The Persian sturgeon has diminished in most of its range, but it continues for certain populations because of the value of its caviar (Vecsei and Artyukhin, 2001; Pikitch *et al.*, 2005; Moghim *et al.*, 2006; Pourkazemi, 2006).

Molecular genetic studies using allozyme and DNA markers have provided useful insights into a number of areas of biology of some sturgeon species in the Caspian Sea, including their evolution, taxonomy, and fishery management. For example, the identification of genetically distinct populations of stellate sturgeon *A. stellatus* (Pourkazemi, 1996; Shabani *et al.*, 2003; Norouzi *et al.*, 2008), Russian sturgeon *A. gueldenstaedtii* (Pourkazemi, 1996, Pourkazemi *et al.*, 1999; Rezvani-Gilkolaei, 2000; Khoshkholgh *et al.*, 2008 and Vodolazhskii *et al.*, 2008) Beluga *Huso huso* (Rezvani-Gilkolaei, 1997) and Ship *A. nudiventris* (Pourkazemi, 1996; Qasemi *et al.*, 2004 and Safari *et al.*, 2008) are of significant value to the development of management strategies of these genetic resources in the Caspian Sea.

Mitochondrial DNA (mtDNA) has been widely used to identify both population structure and genetic variability because of
its rapid evolutionary rate (Avise, 1994; Brown, 2008). Within mtDNA, the non-coding control region has been shown to evolve five times faster than the coding region, and often has higher variability (Brown, 1985; Billington & Hebert, 1991). Therefore, the control region has been recommended for assessing intraspecific genetic variation in sturgeons (Brown et al., 1993; Onge et al., 1996; Wirgin et al., 2000). Nowadays, analysis of the mtDNA control region (D-loop) is the frequently used method to resolve genetic differentiation, population structure, and intraspecific phylogenesis in sturgeon species (Pourkazemi et al., 1999; Pourkazemi et al., 2000; Ludwig et al., 2000; Wirgin et al., 2000; Grunwald et al., 2002; Wirgin et al., 2002; Waldman et al., 2002; Wirgin et al., 2005; Mugue et al., 2008).

Two preliminary studies on population genetics of the Persian sturgeon based on the analysis of mtDNA variation have been published. Ataei et al. (2004) found extensive genetic variability among three populations of A. persicus from south Caspian Sea by using PCR-RFLP technique. Rezvani-Gilkolaii (1997) investigated the genetic diversity of two wild populations of A. persicus from the western and eastern of Caspian Sea using partial sequence analysis of MtDNA NADH 5 gene but this study has suffered very limited geographic sampling. They found its genetic diversity was considerable in its sampled area of distribution, western and eastern part of the Caspian Sea. Ataei et al. (2004) suggested that the conservation of genetic diversity of this species in the Sefidrood River should be considered.

In this study, the genetic variation among A. persicus populations from different locations of south Caspian Sea was analyzed based on the sequences of mtDNA control region. The aim of this study was to characterize the geographical patterns of genetic diversity among different wild populations of Persian sturgeon using analysis sequences of the mitochondrial DNA (mtDNA) control region.

Materials and Methods
Sampling and DNA Extractions
A total of 46 specimens of the Persian sturgeon, Acipenser persicus, were collected from 4 different locations in the south Caspian Sea: Astara (11), Sefidrood River (13), Noshahr (10) and Bandare Turkaman (12), (Figure 1). The position of sampling sites is shown in figure 1. All were obtained during the years 2000–2003 by the International Sturgeon Research Institute. The samples (2-3 g dorsal fin tissue) were first kept in 96% ethanol and then at -20° C until DNA extraction. Total DNA was isolated from fin tissues by standard phenol–chloroform extractions and ethanol precipitations following the method 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). The quality and quantity of total DNA were determined by agarose gel electrophoresis ethidium bromide staining and spectrophotometry, respectively.

PCR amplification and sequencing
Amplification of the mitochondrial control region was performed using the oligonucleotide primers: D loop F (5'-GCTCAACCCTCCTAATCATTT-3') and D loop R (5'-AGTGTGATGAGGAGGATTGA-3'). The primers were designed based on mtDNA control region sequences of the Persian sturgeon A. persicus available at GenBank (Pourkazemi et al., 1999; Accession No. EU714033). PCR amplification was conducted according to Pourkazemi (1996). A total volume of 25 µl containing 2 µl of template DNA (at a final concentration of 50 ng µl⁻¹), 2.5 µl 10×PCR buffer (Fermentase), 1.5 µl MgCl₂ (25 mM),1 µl D loop F (0.4 mM), 1 µl D loop R (0.4 mM), 0.25 U Taq DNA Polymerase (Vio Taq™ VT1001, Fermentase) and 1 µl dNTPs (2.5 mM). The step programs for PCR amplification were as follows: a denaturation step at 94° C for 3 min, followed by 35 cycles consisting of 94° C for 30 s, 51° C for 60 s, 72° C for 70 s and a final extension at 72° C for 10 min. The reaction products of the PCR were assessed on 0.8% agarose gel in 0.5× TBE buffer. PCR products were sequenced on an ABI autosequencing machine (MegaBACE™) using a DYEnamic™ ET dye terminator cycle sequencing kit (MegaBACE™). The sequencing was performed bi-directionally and checked twice for every site of the sequence. Partial sequences of mtDNA D
loop were deposited in GenBank (GenBank accession numbers: Acipenser persicus, FJ364156–FJ364162).

Data Analysis
All nucleotide sequences were aligned with Clustal X 1.8 multiple-alignment program (Thompson et al., 1997) with subsequent refinement by means of the Chromas 2.23 program (Technelysium, Tewantin, Australia). Sequence polymorphisms and genetic distances within and between the populations were estimated. A Neighbor-Joining (NJ) tree was constructed for all haplotypes according to Kimura 2-parameter model (Kimura, 1980) using Mega Version 3.1 (Kumar et al., 2004). Haplotype (h) and nucleotide diversity (π) (Nei, 1987) were estimated using DnaSP 4.0 (Rozas et al., 2003). Population structure was evaluated using the analysis of molecular variance model (AMOVA) (Excoffier et al., 1992) by using Arlequin Version 3.00 software package (Excoffier et al., 2005). Fixation indices (Fst), (Hudson et al., 1992), were also calculated to assess genetic divergence overall and between paired populations. Estimates of gene flow were derived using the equation: Nm = [(1/Fst)-1]/2 (Weir and Cockerham, 1984). The statistical significance of the total and pairwise fixation indices was estimated by comparing the observed distribution with a null distribution generated by 10,000 permutations. Statistical significance was at P = 0.05.

Fig 1. Sampling sites in the south Caspian Sea from which the Persian sturgeon Acipenser persicus specimens were obtained. The four sampling sites are indicated by black dots for the Persian sturgeon.
Results
The aligned mtDNA sequence consisted of part of the control region containing 390 base pairs (bp). Forty four variable sites were observed in the sequences and all substitutions were transitions, and no insertions or deletions were observed. Six control-region haplotypes were found among the sequences (Table 1). The haplotypes tend to be restricted to separate populations and regions. All the individuals of *A. persicus* in the south Caspian Sea shared a common haplotype (Ha4). The haplotype (Ha6) was only seen in one individual from Sefidrood River. Four haplotypes were observed in the populations from Sefidrood river (Ha1, Ha3, Ha4 and Ha6), one of which (Ha4) was shared with Astara, Noshahr and Bandare Turkaman populations (Table 1). The Bandare Turkaman and Noshahr regions shared two haplotypes (Ha4, Ha5) that differed by only one transition (Table 1).

Table 1. Distribution of mitochondrial haplotypes in the Persian sturgeon collections analyzed in this study

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Astara</th>
<th>Sefidrood river</th>
<th>Noshahr</th>
<th>Bandare Turkaman</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ha1</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ha2</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ha3</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ha4</td>
<td>8</td>
<td>1</td>
<td>9</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Ha5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Ha6</td>
<td>1</td>
<td></td>
<td>10</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td>Σ</td>
<td>11</td>
<td>13</td>
<td>10</td>
<td>12</td>
<td>46</td>
</tr>
</tbody>
</table>

Genetic structure analysis among and within regions
The haplotype diversity (h) of the control region within the regions Astara, Bandare Turkaman, Noshahr and Sefidrood River were 0.559±0.027, 0.449±0.014, 0.598±0.015 and 0.955±0.057 respectively. Whereas the nucleotide diversity (π) was 0.0413±0.013, 0.0221±0.017, 0.0324±0.006 and 0.0810±0.009, respectively (Table 2).

Table 2. Levels of genetic diversity within the four samples of the Persian sturgeon (n sample size; h haplotype diversity; π nucleotide diversity). Data shown as mean± standard error

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>Number of haplotypes</th>
<th>Molecular diversity indices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astara</td>
<td>11</td>
<td>1</td>
<td>0.0413±0.013</td>
</tr>
<tr>
<td>Sefidrood river</td>
<td>13</td>
<td>3</td>
<td>0.0810±0.009</td>
</tr>
<tr>
<td>Noshahr</td>
<td>10</td>
<td>1</td>
<td>0.0324±0.006</td>
</tr>
<tr>
<td>Bandare Turkaman</td>
<td>12</td>
<td>1</td>
<td>0.0221±0.017</td>
</tr>
<tr>
<td>All samples</td>
<td>46</td>
<td>6</td>
<td>0.0442±0.011</td>
</tr>
</tbody>
</table>

The AMOVA indicated significant differences (P<0.05) and low gene flow (N_a) among four regions. The AMOVA also partitioned of totally 58.03% genetic variation among the regions and 41.97% of the total within the regions (Table 4), indicating that much of the variation was between the regions.

Results from the AMOVA analysis were supported by the low and mostly insignificant Fst values for overall population differences (P<0.05) (Table 4). The only significant Fst values for overall genetic structuring were observed for the Sefidrood river (Table 4). After Bonferroni correction for multiple independent tests (adjusted P = 0.003), some of the differences were no longer significant. However, differences between the sample collected in Sefidrood River and several other samples remained significant (P<0.05). No significant differences between any of the other samples were detected, either before or after Bonferroni correction.
Table 3. Pairwise F\textsubscript{ST} values between collections of \textit{A. persicus} examined in the present study.

<table>
<thead>
<tr>
<th>Location</th>
<th>Astara</th>
<th>Sefidrood River</th>
<th>Noshahr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astara</td>
<td></td>
<td>0.732</td>
<td>-</td>
</tr>
<tr>
<td>Sefidrood river</td>
<td>0.097</td>
<td>0.825</td>
<td>-</td>
</tr>
<tr>
<td>Noshahr</td>
<td></td>
<td>0.966</td>
<td>0.082</td>
</tr>
<tr>
<td>Bandare Turkaman</td>
<td>0.054</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Haplotype Ha4 was the most common and widespread, being detected in all the studied sites (Table 1). Haplotype Ha5 was the only one that was found in both sites located of the Western Caspian Sea (i.e. in both the Astara region and Sefidrood River). The haplotype neighbor-joining tree (Figures 2) reveals two distinct clades. Individuals in clade 2 were widespread and occurred in three regions Bandare Turkaman, Noshahr and Astara, while clade 1 was totally restricted to the Sefidrood River.

The average number of pairwise F\textsubscript{ST} values are shown in Table 3. Pairwise genetic differences ranged from 0.054 (between Bandare Turkaman and Astara) to 0.966 (between Bandare Turkaman and Sefidrood River). Significant differences between Sefidrood river and all other collections pairwise F\textsubscript{ST} values (0.732 to 0.966, \( p \leq 0.05 \)) and significant probabilities (\( p \leq 0.05 \)) based on 10,000 permutations of haplotype frequencies after sequential Bonferroni correction were observed.

Discussion

Data from the present study indicate that within the south Caspian Sea, there is little evidence to suggest that \textit{A. persicus} is divided into discrete populations. Only the samples from Sefidrood River showed some differences between the samples collected in Southwest and Southeast Caspian Sea.

The mtDNA control region sequences of the Persian sturgeon revealed 6 haplotypes based on the nucleotide variation. The total haplotype diversity and nucleotide diversity were 0.640±0.028 and 0.0442±0.011, respectively. Grunwald et al. (2002) suggested that the nucleotide diversities of the control region were low to moderate for shortnose sturgeon, \textit{Acipenser brevirostrum} (ranging from 0.0022 to 0.0057), and that the diversity of haplotypes was moderate to high (ranging from 0.641 to 0.817). According to these results, the haplotype and nucleotide diversity of the
control region were moderate to high in the studied Persian sturgeon. Nevertheless, the haplotype and nucleotide diversity obtained in this study were higher than those resulted in Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* (Wirgin et al., 2000). This could be due to the differences in the sequence length analyzed. In the present study, 390 bp of control region were sequenced and analyzed, which could reveal more variable sites than previous study based on 203 bp in Atlantic sturgeon (Wirgin et al., 2000). Also, the limited population size examined in this study, compared to the larger population size in Atlantic sturgeon, *A. brevirrostrum* (Grunwald et al., 2002) might have affected the estimates for haplotype and nucleotide diversity.

The Fst-value in the three populations of the Persian sturgeon was 0.0837, which compared to that estimated by RFLP markers (Ataei, 2004), indicates a low genetic differentiation. Besides, the overall Fst of the four collections inferred from the mtDNA control region was much higher than that from RFLP analysis, indicating a higher level of genetic differentiation at the mtDNA sequences. Pairwise population comparisons of Fst-values between the populations from Sefidrood River and Bandare Turkaman (0.966), and Sefidrood River and Noshahr region (0.825) indicated strong population structure. The results inferred from mtDNA data should be integrated with those obtained from other types of markers such as RFLP or microsatellites and occurrence of gender biased dispersal of genetic structure should be examined.

The results inferred from mtDNA control region sequences were mostly in accordance with those from RFLP analysis but in contrast with partial sequence analysis of mtDNA ND5 gene. Rezvani Gilkolaei (1997) in his 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 four wild populations of Persian sturgeon was considerable (Ataei, 2004). This study suggests that the genetic coservation of populations in the Sefidrood River is still possible despite many anthropogenic disturbances imposed on this river system. In the same manner, the genetic homogeneity revealed among southeast and southwest Caspian Sea populations suggests that these populations could be designated as one unique unit for conservation. However, the use of other molecular markers such as microsatellites could further resolve the genetic structure of these geographically distinct populations.

In conclusion, the present study clarified that there were some genetically distinct Persian sturgeon populations in the south Caspian Sea. These results provide useful information for identifying populations and for determining their management and conservation units, leading to the useful application of molecular genetics in investigating conservation biology of the Persian sturgeon.

**Acknowledgements**

We are grateful to the Stock Assessment Department of the International Sturgeon Research Institute for their kind provision of samples and help with sampling. We also thank members of the Laboratory of Genetics of the Institute. The work was supported by the University of Guilan and the International Sturgeon Research Institute.

**References**

Khoshkholgh et al., persicus, in Sefidrood river using PCR-RFLP technique. Thesis in Master of Science. The University of Guilan, Iran, 156 pp. (In Persian).


(Received: Jul. 15-2010, Accepted: Nov. 23-2010)
بررسی تنوع زننیکی جمعیت تاس ماهی ایرانی (Acipenser persicus)\n
جنسی دریای خزر با استفاده از روش توالی یابی منطقه کنترل DNA (D loop) میتوکندریایی

م. خوش خلق، م. پورکاظمی، س. نظری، ل. عزیززاده

چکیده:
تاس ماهی ایرانی (Acipenser persicus) یکی از گونه‌های با ارزش تاس ماهیان است که در دریای خزر پراکن

شده‌است. در این مطالعه به منظور تعیین ساختار زننیکی جمعیت تاس ماهی ایرانی در مناطق مختلف حوضه

جنوبی دریای خزر، روش توالی یابی منطقه DNA میتوکندریایی با کار گرفته شد. در مجموع تعداد 46 نمونه به طور مداوم در منطقه‌های باستاندارد (آسترا، آسیای ساحلی، ترکمن بند و نوشهر و بند تراکم) حوضه جنوبی دریای خزر جمع‌آوری گردید. توالی یابی با استفاده از روش استاندارد انجام گرفت و در مجموع بین 6 هاپلوپت متفاوت و 44 جایگاه متغیر بدست آمد. تنوع هاپلوپت و

نورکلئوتیدی در نمونه‌های گروهی که ترتیب برای با 028/0 ± 640/0 و 011/0 ± 0442/0 بدست آمد. نتایج آنالیز

بر اساس روش دوباره‌گرایی و آنالیز واریانس مولکولی (AMOVA) تشکیل داده شدند. تنوع درون نمونه‌ها

می‌باشد و اختلاف بین نمونه‌های منطقه آستانا، نوشهر و بند تراکم معنی‌دار نبود. برآورد جریان زننی‌نشان داد که

بین نمونه‌های رودخانه سفیدرود و دیگر مناطق جداگانه تولید می‌شود که وجود دارد که ممکن است در نتیجه جدایی

جغرافیایی باشد. نتایج این بررسی موافق مطالعه PCR-RFLP بر روی این گونه بوده و همچنین پیشنهاد می‌دهد

که تنوع زننیکی جمعیت تاس ماهی ایرانی رودخانه سفیدرود مورد توجه قرار گیرد.