Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science, 4 (3) 161- 168, 2009
ISSN 1816-4927

Population Genetic Structure of Pikeperch (*Sander lucioperca* Linnaeus, 1758) in the Southwest Caspian Sea Using Microsatellite Markers

[1]M. Gharibkhani, [2]M. Pourkazemi, [1]M. Soltani, [3]S. Rezvani and [2]L. Azizzadeh
[1]Department of Fisheries, Science and Research Branch,
Islamic Azad University, Tehran, Iran
[2]International Sturgeon Research Institute, P.O. Box 41635-3464, Rasht, Iran
[3]Iranian Fisheries Research Organization, P.O. Box 14155-6116, Tehran, Iran

Abstract: The aims of this study were to analysis the population genetic structure and genetic diversity among and between populations of *Sander lucioperca* based on microsatellite markers. For this purpose, 149 samples of adult pikeperch from three regions of Southwest Caspian Sea (Talesh Coasts, Anzali Wetland and Chaboksar Coasts) were collected. DNA was extracted and using 13 pairs of microsatellite primers, Polymerase Chain Reaction (PCR) was conducted. DNA bands were analysed using Biocapt and GenAlex software package. Out of 13 microsatellite primers, 11 loci were produced, in which 6 of them were polymorphic and 5 monomorphic. Analysis revealed that the average number of alleles per locus and observed heterozygosities were not statistically significant ($p>0.05$) for all 3 populations. The F_{ST} value between populations was low but significant ($p<0.01$), suggesting that the 3 populations are genetically differentiated. Deviation from Hardy-Weinberg equilibrium was obvious in most cases, mostly due to the deficiency of heterozygosities. The highest genetic distance was between Anzali Wetland and Chaboksar Coast populations. The data generated in this study provide useful information on the genetic variation and differentiation in populations of Southwest Caspian Sea pikeperch.

Key words: *Sander lucioperca*, pikeperch, population genetic, microsatellite, Southwest Caspian Sea

INTRODUCTION

The pikeperch (*Sander lucioperca*) is found in freshwater and brackish water and is a semi-anadromous, cool-water species distributed in the Caspian watershed (Ural, Volga, Kura and Sefid Roud rivers) as well as in the basins of the Black, Azov, Aral and Baltic Seas (Craig, 2000). This species seems to prefer salinities lower than 12 g L^{-1}. Pikeperch occurrence in the Caspian Sea is restricted to estuaries and costal zones (Kazancheyev, 1981). As a predator and commercially valuable species, pikeperch constitutes an important component of the Caspian ichthyofauna, both ecologically and commercially (Abdolmalaki and Psuty, 2007). Catches of pikeperch first recorded in the Caspian Sea in the late 1920s indicate that it constituted about 1-3 of the total taken from the costal zone of the Southern Caspian Sea, some 3000-4000 t annually (Razavi, 1999). Shortly thereafter, catches decreased suddenly to some 30 t annually and they have never again reached the initial level. The cause of the sudden disappearance of the pikeperch stock from the Southern Caspian Sea has not been determined conclusively but its occurance is assumed because of the excessive catching that led to overfishing and degradation of spawning grounds and habitats (Kiabi *et al*., 1999). Historically, the most important spawning area for pikeperch in Iran was the Anzali Wetland. Although the precise

Corresponding Author: M. Gharibkhani, Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran Tel: +98 131 2245185

cause is not known, natural pikeperch spawning in the Anzali Wetland stopped completely and it has not been recorded in last decade. Bearing in mind the size of historical catches of the species and assuming that the carrying capacity had not changed, the Iranian fisheries administration in 1990 initiated a pikeperch stock-enhancement programme. Since then, the Anzali Wetland had been stocked systematically with fingerlings collected from spawners held at Aras dam, a border reservoir that lies between Iran and Azerbaijan Republic (Abdolmalaki and Ghaninezhad, 1999). So, regarding the fact that this species is being reared in Aras dam and released into the Caspian Sea and Anzali Wetland for restocking, regular monitoring of genetic variability among the progenies is essential to avoid the loss of current polymorphism due to inbreeding and outbreeding problems.

Microsatellites are hyper variable, codominant nuclear DNA markers in which variation is partitioned in one to five base pair repeat motifs (Zajc *et al*., 1997; Goldstein and Schlotterer, 1999). In recent years, microsatellite markers as a reliable method, have been applied for many population as well as phylogenetic studies, this is because microsatellites typically have a higher level polymorphism than traditional nuclear loci. In addition, DNA for microsatellites analysis can be easily extracted from tissues obtained by non-lethal sampling (fins, hairs, faces), which is essential when working with threatened or endangered species (McQuown *et al*., 2003). It has also proved that microsatellite genotyping is a powerful tool for accurate genetic assessment and for sustainable use of wild resources (Barroso *et al*., 2005; Li *et al*., 2006; Memis and Kohlmann, 2006).

Several studies have been carried out on the population genetics of Percids species: On *Perca favesens* (Li *et al*., 2007; Leclerc *et al*., 2000; Kapuscinski and Miller, 2000); *Stizostedion vitreum* (Wirth *et al*., 1999; Zipfel, 2006) and *Sander lucioperca* (Kohlmann and Kersten, 2008; Bjorklund *et al*., 2007; Poulet *et al*., 2009).

Despite the commercial and conversation importance of this species, information on genetic relationship and diversities of *Sander lucioperca* at the molecular level in Caspian Sea basin is scarce. The development of management plants and implementation of actions to restore pikeperch 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 source of reintroduction and in formulating restoration goals regarding population structure. Therefore in this study, the population structure of pikeperch from three regions in Southwest of Caspian Sea basin was investigated. The main objectives of this study were to analysis the population genetic structure and genetic diversity among and between populations of *Sander lucioperca* based on microsatellite markers.

MATERIALS AND METHODS

Samples Collection

Totally 149 samples of adult *Sander lucioperca* were caught from 3 regions including 50 samples from the Coasts of Talesh, 50 samples from Anzali Wetland and 49 samples from the Coasts of Chaboksar in 2007-2008 from Southwest Caspian Sea in Iran (Fig. 1). For each sample, 2-3 g dorsal fin tissues was collected and conserved in absolute alcohol for subsequent DNA extraction and amplification.

DNA Extraction

Total genomic DNA was extracted from fin tissue using proteinase-K digestion, phenol: chloroform; isoamylalchol precipitation as described by Pourkazemi *et al*. (1999). The quality and concentration of DNA were assessed using agarose gel electrophoresis and spectrophotometry (model Nano Drop, ND-1000). Finally, DNA was stored at -20°C until use.

PCR Profiles and Primer Sequences

Nuclear DNA was amplified using 13 microsatellites primers (YP13, YP17, YP41, YP60, YP68, YP78, YP110, YP111, YP113, P*fla* L2, P*fla* L3, P*fla* L8, P*fla* L9) designed for *Perca flavescens*

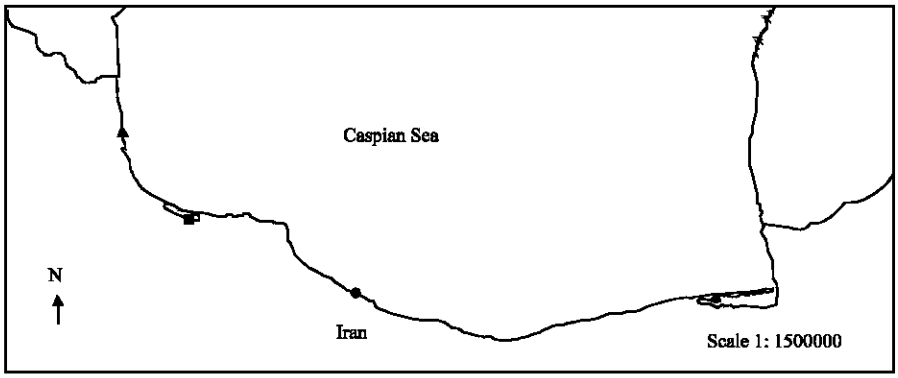

Fig. 1: Map showing sampling regions of three populations of *Sander lucioperca*: Talesh Coasts (▲), Anzali Wetland (■) and Chaboksar Coasts (●)

Table 1: Loci, repeat motif, primers sequence, gene bank number and primer source used at present study

Loci	Repeat motif	Primer sequence	Gene bank No.	Primer source
YP13	$(GTA)_{11}$	F-GGCACCCAAACTACCACT	DQ826683	Li *et al.* (2007)
		R-CAGTCGGGCGTCATCATC		
		AAACAAGCCCCATACA		
YP17	$(TAG)_{10}$	F- CAGTCGGGCGTCATCAC	DQ826686	
	$TANGTG(TAG)_2$	AGCGTTTCCACAGTATTGACC		
		R- GGGTTTTACACTGTTGATGGGAT		
YP41	$(TCTT)_{11}$	F-CGCTCCCTCCCTCTATCC	DQ826692	
		R- CAGTCGGGCGTCATC		
		ATTGCTGTGCTGCCATTTC		
YP60	$(AGAA)_{10}$	F- ATGTGTTATTGCTTTGCGTA	DQ826697	
		GTTCCTGTAATGTGTTG		
		R- CAGTCGGGCGTCATCAGCT	DQ826701	
YP68	$(AC)_5GCACGC$	F- GACAGAAAGCAAGAAGGGAA		
	$(AC)_5AT(AC)_9$	R- CAGTCGGGCGTCATCAATCC		
		TTTTCTCCAATCCTGA		
YP78	$(GTA)_{13}$	F- GCAGCCCCTACAATGGTT	DQ826705	
		R- CAGTCGGGCGTCATCAGC		
		CTTCTTCTGTTATTTTTCC		
YP110	$(TTG)_{18}$	F- CAGTCGGGCGTCATCATTC	DQ826719	
		AGACCCCTTCACTTTTG		
		R- ATCAGAGCAATGACCAAGCC		
YP111	$(CTA)16(ATA)_{18}$	F- CAGTCGGGCGTCATCATGT	DQ826720	
		GTATGGCTATTGTGCTC		
		R- TTTGTTCAGTGTTTTTTCGC		
YP113	$(GT)_{17}$	F- CAGTCGGGCGTCATCACG	DQ826721	
		GTTGGGACACAGAGACAC		
		R- TGGTGTGGATTGGGGCAT		
*Pfla*L2	$(CA)_{27}$	F-GTAAAGGAGAAAGCCTTAAC	AF211827	Leclerc *et al.* (2000)
		R-TAGCATGACTGGCAAATG		
Pfla L3	$(TG)_{18}$	F-GCCGAATGTGATTGAATG	AF211828	
		R-CGCTAAAGCCAACTTAATG		
Pfla L8	$(TG)_{39}$	F-GCCTTATTGTGTGACTTATCG	AF211833	
		R-GGATCTTTCACTTTTTCTTTCAG		
Pfla L9	$(TG)_{24}$	F-GTTAGTGTGAAAGAAGCATCTGC	AF211834	
		R-TGGGAAATGTGGTCAGCGGC		

(Li *et al*., 2007; Leclerc *et al*., 2000) (Table 1). The Polymerase Chain Reaction (PCR) conditions, especially annealing temperatures, were optimized for the microsatellite loci as necessary to produce scorable amplification products. Polymerase chain reaction was performed in a 20 μL reaction volume

Table 2: Polymorphic amplified locus, allele size (bp) and annealing temperature on *Sander lucioperca*

Locus	Observed allele size (bp)	Annealing temperature (°C)
YP13	274-307	60
YP60	205-245	59
YP110	461-473	59
Pfla L3	211-230	53
Pfla L8	170-198	57
Pfla L9	188-244	61

containing 100-150 ng of template DNA, 10 pmol of each primer, 200 μM each of the dNTPs, 1U of Taq DNA polymerase (Cinnagen, Tehran, Iran), 1.5 mM $MgCl_2$ and 1x PCR buffer. The temperature profile consisted of 2 min initial denaturation at 94°C, followed by 35 cycles of 30 sec denaturation at 94°C, 30 sec annealing at a locus-specific temperature (Table 2), 30 sec extention at 72°C and a final 5 min extension at 72°C for the first 9 microsatellite primers (YP13, YP17, YP41, YP60, YP68, YP78, YP110, YP111 and YP113). For the rest microsatellite primers (P*fla* L2, P*fla* L3, P*fla* L8 and P*fla* L9) the temperature profile consisted of 3 min initial denaturation at 96°C, 30 sec annealing at a locus-specific temperature (Table 2), 1 min extention at 72°C and a final 5 min extension at 72°C. Polymerase chain reaction products were separated on 6% polyacrylamide gels and stained by silver nitrate. Alleles were sized using BioCapt software and each gel contained an allelic ladder (100 bp) to assist in consistent scoring of alleles.

DNA Analysis

Allelic frequency, observed and expected hetrozigosities, genetic distance (Nei, 1972) and genetic identity (Nei, 1972) were computed in GenAlex 6.0 software (Peakall and Smouse, 2005). This package was also used to calculate F_{ST} and R_{ST}, Nm, Hardy-Weinberg (HW) tests of equilibrium and AMOVA (Analysis or Molecular Variance).

RESULTS

Amplification and Banding Patterns

Out of 13 sets of microsatellite primers, two sets (YP78 and YP113) have not shown any flanking sites on pikeperch genome. Eleven sets of primers were successfully amplified where 5 sets (YP17, YP41, YP68, YP111 and P*fla* L2) showed monomorphic pattern in all samples. Therefore, totally 6 sets of primers produce polymorphic bands. Loci YP13, YP60 and P*fla* L8 had the highest numbers of alleles (4), while the locus YP110 had the lowest (2).

Genetic Variation Within Sampling Regions

The average number of alleles found per locus in Talesh Coasts, Anzali Wetland and Chaboksar Coasts samples were 3.5, 2.7 and 3, respectively. The average observed hetrozigosities in these 3 regions were 0.540, 0.570 and 0.517, respectively (Table 3). The observed heterozigosity of all 3 regions at YP13 and P*fla* L9 were significantly higher than the corresponding expected hetrozygosity ($p<0.01$). The differences between all three sampling area were not statistically significant ($p>0.05$), neither for the average number of alleles nor for the observed heterozygosities.

Significant deviations from Hardy-Weinberg equilibrium at the locus level are shown in Table 3. Talesh Coasts, Anzali Wetland and Chaboksar Coasts regions deviated at 4, 3 and 3 loci, respectively, mostly due to the deficiency of heterozygosities.

Genetic Variation Among Sampling Regions

Genetic distance calculated between each pair of collections ranged from 0.035 (between Talesh and Chaboksar Coasts) to 0.068 (between Anzali Wetland and Chaboksar Coasts, Table 4). The

Table 3: Variability of six microsatellite loci in three populations of *Sander lucioperca* from Caspian Sea

Locus	Parameters	Talesh coasts	Anzali Wetland	Chaboksar coasts
YP13	A	4.00	3.00	4.00
	H_o	0.58	0.52	0.49
	H_e	0.678	0.625	0.573
	P	0.001*	0.000*	0.006*
YP60	A	4.00	2.00	2.00
	H_o	0.38	0.48	0.469
	H_e	0.608	0.461	0.38
	P	0.000*	0.768^{ns}	0.100^{ns}
YP110	A	3.00	2.00	2.00
	H_o	0.26	0.30	0.327
	H_e	0.233	0.255	0.273
	P	0.773^{ns}	0.212^{ns}	0.172^{ns}
Pfla L3	A	3.00	3.00	3.00
	H_o	0.50	0.66	0.571
	H_e	0.499	0.503	0.506
	P	0.706^{ns}	0.029*	0.507^{ns}
Pfla L8	A	4.00	3.00	4.00
	H_o	0.64	0.66	0.327
	H_e	0.62	0.552	0.653
	P	0.000*	0.267^{ns}	0.000*
Pfla L9	A	3.00	3.00	3.00
	H_o	0.88	0.80	0.918
	H_e	0.648	0.631	0.622
	P	0.000*	0.000*	0.000*
Average number of alleles per locus		3.50	2.70	3.00
Average H_o		0.54	0.57	0.517
Average H_e		0.548	0.504	0.501

*Significant at $p<0.05$, A: Number of alleles; H_o: Observed heterozigosity; H_e: Expected heterozigosity; P: p-value of χ^2 tests for Hardy-Weinberg equilibrium; ^{ns}: Not signifocent

Table 4: Pairwise population of genetic distance (below diagonal) a nd genetic identity (above diagonal) (Nei, 1972) detected at 6 loci in pikeperch samples

		Genetic identity		
Parameter	Samples	Talesh coasts	Anzali Wetland	Chaboksar coasts
Genetic distance	Talesh Coasts	-	0.954	0.966
	Anzali Wetland	0.047	-	0.934
	Chaboksar Coasts	0.035	0.068	-

Table 5: Pairwise estimates of genetic differentiation detected at 6 loci in pikeperch samples, using unbiased RST (below diagonal) and F_{ST} values (above diagonal)

		F_{ST} (N_m)		
Parameter	Samples	Talesh coasts	Anzali Wetland	Chaboksar coasts
RST (N_m)	Talesh Coasts	-	0.031 (7.791)	0.021 (11.445)
	Anzali Wetland	0.019 (13.137)	-	0.051 (4.622)
	Chaboksar Coasts	0.036 (6.710)	0.088 (2.602)	-

Probabilities of R_{ST} or F_{ST} Determined by AMOVA tests at $p \leq 0.01$

highest range of genetic difference were observed between Chaboksar Coasts and Anzali Wetland ($F_{ST} = 0.051$, $R_{ST} = 0.088$, $p \leq 0.01$) and the lowest between Talesh and Chaboksar Coasts ($F_{ST} = 0.021$, $R_{ST} = 0.036$, $p \leq 0.01$). However, F_{ST} and R_{ST} estimates often differ in a pronounced manner (Balloux and Lugon- Moulin, 2002). Values of pairwise R_{ST} among samples were consistently much higher (as much as an order of magnitude) than equivalent F_{ST} values (Table 5).

DISCUSSION

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). Despite the

importance of pikeperch as a highly economic fish in Iran, natural populations of this fish are declined. Information about these populations is pivotal for their conservation and sustainable use. Unfortunately, the knowledge on the molecular population genetic structure of this species has not conducted yet.

In this study we have employed 6 polymorphic microsatellite loci to assess the genetic relationship among populations of pikeperch from three regions in Iran along the Southwest Coasts of Caspian Sea. According to the results, all of three sampling regions had low number of alleles but Anzali Wetland samples showed the lowest number of alleles (2.7 in average). From the 6 primers used in this study, the first 3 primers have not been used for other pikeperch populations yet. But these primers have shown high number of alleles in the studied species (Li *et al*., 2007). So, these primers were used in this study because of their polymorphic character. The rest of primers were used in a few individuals (6-8) of *Sander lucioperca* (Leclerc *et al*., 2000). The results showed that the average number of alleles per locus similar to present study was low, but the number of individuals was insufficient for population genetic statistical analysis and the results were not comparable with present study. In another study of genetic structure of pikeperch in Rhone delta (Poulet *et al*., 2009), 5 alleles were produced in P*fla* L3 locus, which is higher than present produced alleles (3 in average) for this locus. As a conclusion, the low number of alleles per locus in the present study in comparison to earlier studies for used primers indicates the occurrence of a bottleneck effect in the progeny of selective breeding stocks in Aras dam that are transferred to Caspian Sea and Anzali Wetland. This may also occure due to the founder effect in the Caspian Sea and Anzali Wetland pikeperch populations.

The value of F_{ST} is a useful measure of genetic differentiation among populations (Peakall and Smouse, 2005). At present study the F_{ST} value in all 3 sampling regions was low but significant ($p<0.01$), suggesting that the 3 populations are genetically differentiated and don't represent a single panmictic population. The lowest of F_{ST} value on AMOVA was between Talesh and Chaboksar Coasts populations with the high gene flow. However, there is no information about the main origin of the Talesh and Chaboksar Coasts populations. Perhaps these populations are related to other rivers (e.g., Ural, Volga, Kura, and Sefid Roud) or neighboring Coasts.

The genetic distance between these 3 populations were in the range of 0.035-0.068. Shaklee *et al.* (1982) and Thorpe and Sol-Cava (1994) showed that genetic distance values (Nei, 1972) for conspecific populations averaged 0.05 (range: 0.002-0.07) and for congeneric species averaged 0.30 (range: 0.03-0.61). The distance value obtained in the present study falls within the average value of conspecifics, which indicate that the genetic difference among the studied populations is not pronounced. Regarding the fishery returning, it is possible that the caught samples are a combination of the various generation and different birth places which have formed a gathering to feed.

Although significant deviations from Hardy-Weinberg equilibrium were found at more loci in the Talesh Coast population than in the Anzali Wetland and Chaboksar Coast populations, there were no significant differences in the average expected and observed heterozygosities among all three populations ($p>0.05$). The significant deviations from Hardy-Weinberg equilibrium could be explained either by sample bias or the present of null alleles. In the presence of null alleles heterozygotes possessing a null allele could be erroneously recorded as homozygotes for the variant allele leading to a deficiency of heterozygotes in the respective population.

The data generated in this study showed that there are 3 different populations of pikeperch in the studied regions of Southwest Caspian Sea. This information can be applied for future genetic improvement by selective breeding and to design suitable management guidelines for the genetic materials. However, in order to have better conservational policy and restocking programs, further studies are recommended on determining the other different populations of this important species in other regions of Caspian Sea.

ACKNOWLEDGMENTS

This research was performed in the Molecular Genetic Laboratory of the Dr. Dadman International Sturgeon Research Institute, Rasht, Iran. The authors express their sincere gratitude to all staffmembers especially, Mr. Nuruzfashkhami, head of Genetic Department, Mr. Hassanzadeh, Mr. Chakmedouz, Mr. Bagheri and Mrs. Haghighi for their great co-operation and support.

REFERENCES

Abdolmalaki, S. and D. Ghaninezhad, 1999. A survey on releasing fingerlings and catching pikeperch (*Stizostedion lucioperca*) in Iranian costal waters of the Caspian Sea. Proceedings of the 1st National Congress on Caspian Sea Bony Fish, Jan. 21-23, Bandar Anzali, Guilan, Iran.

Abdolmalaki, S. and L. Psuty, 2007. The effect of stock enhancement of pikeperch (*Sander lucioperca*) in Iranian coastal waters of the Caspian Sea. ICES J. Mar. Sci., 64: 973-980.

Balloux, F. and N. Lugon-Moulin, 2002. The estimate of population differentiation with microsatellite markets. Mol. Ecol., 11: 155-165.

Bjorklund, M., T. Aho and L.C. Larsson, 2007. Genetic differentiation in pikeperch (*Sander lucioperca*): The relative importance of gene flow, drift and common history. J. Fish Biol., 71: 264-278.

Borroso, R.M., A.W.S. Hisdorf, H.L.M. Moreira, P.H. Cabello and Y.M. Traub-Cseko, 2005. Genetic diversity of wild and cultured populations of *Brycon opalinus* (Cuvier, 1819) (Characiforme, Characidae, Bryconiae) using microsatellites. Aquaculture, 247: 51-65.

Craig, J.F., 2000. Percid Fishes. Systematics, Ecology and Exploitation. 3rd Edn., Blackwell Science, Oxford, UK., ISBN: 0-632-05616-9, pp: 352.

Goldstein, D.B. and Schlotterer, 1999. Microsatellites Evolution and Applications. 1st Edn., Oxford University Press, Oxford, UK., ISBN: 0-19-850407-1, pp: 370.

Kapuscinski, A. and L. Miller, 2000. DNA-based markers for the assessment of genetic population structure in yellow perch. Great Lakes Fishery Commission, 2000 Project Completion Report 1., pp: 13.

Kazancheyev, E.N., 1981. Fishes of the Caspian Sea. 1st Edn., Food Industry Publications, Moscow, pp: 166.

Kiabi, B.H., A. Abdoli and M. Naderi, 1999. Status of the fish fauna in the South Caspian Basin of Iran. J. Zool. Mid. East, 18: 57-65.

Kohlmann, K. and P. Kersten, 2008. Isolation and characterization of nine microsatellite loci from the pike-perch, *Sander lucioperca* (Linnaeus, 1758). Mol. Ecol. Resourc., 8: 1085-1087.

Leclerc, D., T. Wirth and L. Bernatchez, 2000. Isolation and characterization of microsatellites in yellow perch (*Perca favesens*) and cross-species amplification within the family percidae. Mol. Ecol. Notes, 9: 995-997.

Li, L., P. Wang, C. Givens, S. Czesny and B. Brown, 2007. Isolation and characterization of microsatellites in yellow perch (*Perca favesens*). Mol. Ecol. Notes, 7: 600-603.

Li, Q., H. Yu and R. Yu, 2006. Genetic variability assessed by microsatellite in cultured populations of the Pacific oyster (*Crassostrea gigas*) in China. Aquaculture, 259: 95-102.

McQuown, E., C.C. Krueger, H.L. Kincaid, A.E. Gall and B. May, 2003. Genetic comparision of Lake sturgeon population: Differentiation based on allelic frequencies at seven microsatellite loci. J. Great Lake Res. 29: 3-13.

Memis, D. and K. Kohlmann, 2006. Genetic characterization of wild common carp (*Cyprinus carpio*) from Turkey. Aquacukture, 258: 257-262.

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

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

Poulet, N., P. Balaresque, T. Aho and M. Bjorklund, 2008. Genetic structure and dynamics of a small introduced population: The pikeperch, *Sander lucioperca*, in the Rhone delta. Genetica, 135: 77-86.

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

Razavi, B.A., 1999. Introduction to the Ecology of the Caspian Sea. 1st Edn., Iranian Fisheries Organization, Tehran, pp: 110.

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

Thorpe, J.P. and A.M. Sol-Cava, 1994. The use of allozyme electrophoresis in vertebrate systematics. Zool. Scripta, 23: 3-18.

Wirth, T., R. Saint-Laurent and L. Bernatchez, 1999. Isolation and characterization of microsatellites in walleye (*Stizostedion vitreum*) and cross-species amplification within the family percidae. Mol. Ecol., 8: 1960-1962.

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

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

Zipfel, K.J., 2006. The distribution and status of native walleye (*Sander vitreus*) stocks in West Virginia. M.Sc. Thesis. Ohio University, USA.