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Microsatellite genetic differentiation between two populations of European catfish (*Silurus glanis*) in Iran

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Abstract

In the present study the population genetic structure of European catfish in the Anzali Lagoon and Aras Lake were examined using microsatellite markers. Sixty fin clip samples of *Silurus glanis* from two regions were collected and for genetic analysis 6 microsatellite loci were used to assess the population genetic structure of *S. glanis*. There were significant differences based on average number of alleles per locus and heterozygosity between two populations (p<0.01). The observed heterozygosity (H_o) for each population (H_e) per locus was from 0.297 to 0.733 in Aras lake and Anzali lagoon samples, respectively.The Analysis of molecular variance (AMOVA) indicated that the proportion of the genetic variation attributed to differences among populations of the *S. glanis* was highly significant for both F_{ST} and R_{ST} ($F_{ST} = 0.165$, $R_{ST} = 0.38$, p<0.001). Excess or lacks of heterozygosity was observed but most of used microsatellite loci in selected areas were at Hardy-Weinberg equilibrium. Our finding showed the two populations are genetically separated, therefore fisheries management and restocking program of this species especially in Anzali Lagoon is recommended.

Keywords: European Catfish, Genetic population, microsatellite markers

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Introduction

Diversity plays important roles in populations due to provide necessary spectrum of genotypes for adaptive response to change environmental conditions (Webster *et al*., 2018). Heterozygous individuals usually are superior to less heterozygous individuals in characteristics such as growth, fertility, and disease resistance (Beardmore *et al*., 1997). Microsatellites are known as a simple sequence repeats (SSRs), simple sequence length polymorphisms (SSLPs), or short tandem repeats (STR), are regions of DNA that exhibit short repetitive sequence motifs. These motifs are often composed of 1-6 base pair repeat sequences. Microsatellites have been used as genetic markers to assess population genetic structure, broodstock identify for management in hatcheries, evolutionary biology, conservation biology and genetic mapping (Triantafyllidist *et al*., 2002).

European catfish has global distribution as a native or an introduced species (Cucherousset *et al*., 2018). *Silurus glanis* is well known as an economic species and an important for aquaculture industry (Rees *et al*., 2017). There are large number of studies have been conducted on reproductive cycles, genome manipulation and biology of gametes of *S. glanis* (Legendre *et al*., 1996; Smitherman *et al*., 1996; Varkonyi *et al*., 1998; Brzuska and Adamek, 1999; Bolliet *et al*., 2001; Kumar *et al*., 2017; Zibiene and Zibas, 2019; Linhart *et al*., 2020) but study of population genetic structure is rare. Although, allozyme (Triantafyllidis *et al*., 1999a) and mitochondrial DNA (mtDNA) (Triantafyllidis *et al*., 1999b; Krieg *et al*., 2000) were examined to study population genetic of *S. glanis*, neither of them were able to reveal high levels of genetic variability to separate wild and hatchery populations. Krieg *et al*. (1999) reported population differentiation among different species of Siluridae family using microsatellite markers. Triantafyllidis *et al*. (2002) showed that the genetic diversity was much higher than previous allozyme and mtDNA studied on this species by using 10 microsatellite markers in different areas. Quan *et al*. (2006) examined population structure and genetic variation of northern sheatfish (*S. soldatovi*) in wild and farmed populations using microsatellite markers. Results showed that H_o and H_e and other heterozygosity parameters were not different between populations. Bahrami Kamangar *et al*. (2015) investigated the genetic diversity and genetic structure of four populations of *Silurus glanis* by microsatellite DNA markers.

S. glanis is one of the native species of Iran where mainly distributed in Anzali Lagoon and Aras Lake, north and northwest of Iran. Due to overfishing and poor fisheries management, the capture production has been declined over the last decades. Thus, the present study aimed to assess genetic diversity of Iranian populations of *S. glanis* in Anzali Lagoon and Aras Lake regions using microsatellite DNA markers.

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Materials and methods

Sample collections and DNA extraction

Sixty individuals of *S. glanis* were obtained from two selected sites, Anzali Lagoon (37°28′22″N 49°27′44″E) and Aras Lake (39°05′28″N 45°24′08″E) through trap and fin clips were taken and placed in 100% ethanol immediately. Total DNA was extracted from each individual using standard SDS proteinase-K digestion; phenol: chloroform: isoamylalcohol extraction and ethanol precipitation as described in Hillis *et al*. (1996). The quality and quantity of extracted DNA and Polymerase chain reaction (PCR) was examined by 1% agarose gel electrophoresis and nanodrop spectrophotometery (ND 1000, USA) respectively.

PCR profiles and primer sequences

The PCR amplifications were done according to Krieg *et al*. (1999) with some modifies in regents and annealing temperature. Six microsatellite loci for *S. glanis* were used and all of the loci have been described by Krieg *et al*. (1999). Detailed information of these microsatellite loci such reference, forward and reverse primers, fragment size and annealing temperature was described in Table 1. The PCR amplification was performed in a final volume of 25 µL, containing 100 ng template DNA, 10 mM forward and reverse primers, 200 µm of dNTPs, 0.5 u/µL of *taq* DNA polymerase (Cinagen, Iran), 50 mM $MgCl_2$, 10X PCR buffer and distilled water using BIOER thermal cycler (XP cycler gradient, 96 plus, Bioer, China) under the following conditions: initial denaturation of 4 min at 94ºC (primary denaturation) followed by 30 cycles of 30 s denaturation at 94ºC, 60 s at the respective annealing temperature, and 60 s extension at 72ºC, finishing with 10 min at 72°C as the elongation period.

Table 1: Information of 6 polymorphic microsatellite loci used in the present study.

Locus	Primer (5'-3')	Size (bp)	Annealing temp. (ºC)	Reference
Sgl33INRA	F-CCACTTATGCACCTGAAGG R-GGCCAATTAAACAGGTACAG	150-200	58	Krieg *et al*. (1999)
Sgl5fINRA	F-CCAATTTACCTCAGACTACTTCTG R-GCACGTGCAAAGTCCTG	125-210	55	
Sgl695INRA	F-CTTTGGTGAGTCAGAAACACG R-GCACTACTGGTAGATGCT	180-250	56	
Sgl7159INRA	F-CTGCTCAATCAAAGTTGGTTC R-CAAACTAAGTTCAGCCAGGC	220-300	55	
Sgl7eINRA	F-GTGAATGTGCTTTAAGGGC R-GTTCATGGTGTCACTGCG	200-300	59	
Sgl7fINRA	F-GGCTGTATGTTAAGTTATTTTCAG R-CTGAGCAGTGGCCAGAATG	220-300	60	

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Gel electrophoresis and statistical analyses

The PCR products were separated by electrophoresis through 6% (w/v) polyacrylamide gel (29:1 acrylamide: bis acrylamide; 1x TBE buffer) using a Cleaver gel electrophoresis system (Cleaver Scientific Ltd, UK). Gel run was carried out at 120 V until the loading buffer reached the bottom of the plate. After electrophoresis, the gel was stained with a silver nitrate protocol (Fig. 1).

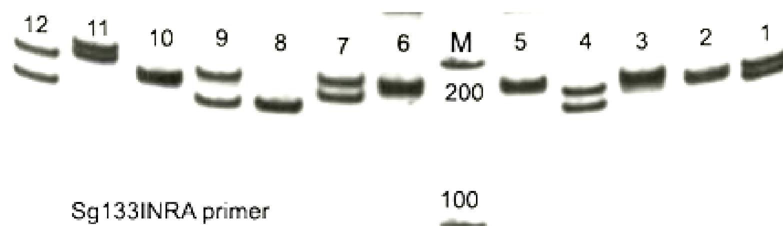

Figure 1: Polyacrylamide electrophoresis patterns of *Silurus glanis* in the locus Sg133INRA.

Microsatellite alleles were identified by their size in base pairs (Peakall and Smouse, 2006). Allele lengths were estimated by comparison with a 100 bp DNA marker ladder (Fermentas GmbH, Germany). Measures of genetic diversity were determined for each population including, number of alleles per locus, observed heterozygosity (H_o), expected heterozygosity (H_e) and deviations from Hardy-Weinberg equilibrium (HWE) between pairwise loci. The genetic distance between populations of two areas was estimated using the Nei's standard genetic distance index (Nei, 1972). The pairwise differentiation between populations of the two areas was also characterized using pairwise estimates of θ values of *Fst* were tested for significant departure. All calculations were conducted using GENALEX version 6. Analysis of molecular variance (AMOVA) was used to examine the partition of variance between and within two populations.

Results

PCR amplification

Eight microsatellite loci were successfully amplified where two sets (Sgl310INRA and Sgl325INRA) showed monomorphic bands in two populations and six sets produced polymorphic bands (Table 2). Locus Sgl5fINRA in samples of Anzali Lagoon showed highest number of alleles (12) and locus Sgl7eINRA in samples of Aras Lake presented the lowest (4) (Table 2).

Genetic variations of microsatellite loci within populations

A total of 88 unique alleles were found across the six loci in the two populations. Number of alleles (N_A), the observed (H_o) and expected (H_e) heterozygosity of 6 polymorphic primers per population are shown in

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Table 3. These parameters were in the range of 4-12, 0.197-0.807 and 0.226-0.733, respectively. The mean of N_A (9.3), H_o (0.639) and H_e (0.589) in Anzali Lagoon were significantly higher than Aras Lake (p<0.01).

Table 2: The genetic polymorphism of Anzali Lagoon and Aras Lake population. (N_A, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity)

Locus	Parameters	Anzali lagoon	Aras lake
Sgl33INRA	N_A	10	6
	H_o	0.795	0.289
	H_e	0.702	0.425
Sgl5fINRA	N_A	12	6
	H_o	0.486	0.350
	H_e	0.385	0.318
Sgl695INRA	N_A	8	5
	H_o	0.807	0.197
	H_e	0.733	0.380
Sgl7159INRA	N_A	9	5
	H_o	0.621	0.395
	H_e	0.640	0.226
Sgl7eINRA	N_A	8	4
	H_o	0.610	0.274
	He	0.505	0.297
Sgl7fINRA	A	9	6
	H_o	0.515	0.201
	H_e	0.569	0.436
Mean N_A		9.3 ± 3.28	5.3 ± 2.18
Mean H_o		0.639 ± 0.055	0.284 ± 0.155
Mean H_e		0.589 ± 0.031	0.324 ± 0.098

Table 3: The computed genetic differentiation parameters.

Populations	Genetic difference (F_{st})	Genetic distance
Anzali Lagoon and Aras Lake	0.165	0.38

Population genetic differentiation

The results of AMOVA indicated significant differentiation in pair-wise F_{ST} between two populations. The F_{st} was 0.165 which showed significant differences between populations. Genetic distance calculated based on Nei (1972). Both computed parameters presented significantly differences between populations (p<0.01).

Hardy–Weinberg equilibrium tests (HWE)

All 6 loci used in this study were tested for deviation from Hardy–Weinberg equilibrium. The results showed that significant departures from HWE were detected using the probability test (p<0.05). Of the six markers assessed for HWE, the loci Sgl695INRA and Sgl7eINRA in Anzali Lagoon and Sgl5fINRA locus in Aras Lake showed excess heterozygosity. Sgl7159INRA in Anzali Lagoon and Sgl7eINRA and

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Sgl7fINRA loci in Aras Lake presented lack of heterozygosity. The other loci in two populations were at HWE test (Table 4).

Table 4: The genetic deviation test (HWE) of *S. glanis* population.

Locus	Anzali Lagoon	Aras Lake
Sgl33INRA	0.0	0.0
Sgl5fINRA	0.0	-0.02
Sgl695INRA	-0.1	0.0
Sgl7159INRA	0.205	0.0
Sgl7eINRA	-0.018	0.155
Sgl7fINRA	0.0	0.035

Discussion

Microsatellites are well known as suitable genetic markers and powerful tools for population genetic studies by their high level of allelic variability (Krieg *et al*., 1999; Dudu *et al*., 2008). *S. glanis* is an economic species which become popular fish with high consumption in most countries. In the present study we used six specific microsatellite loci to assess the population differentiation in two areas, Anzali Lagoon and Aras Lake from north part of Iran and all of these loci were polymorphic.

The present study found that the number of alleles per locus was in the range of 4-12 and Anzali Lagoon presented higher number of alleles (9.3 in average) compared to Aras Lake (5.3 in average). Low number of alleles in Aras lake samples may indicate bottleneck effect due to reduce of stocks, water pollution and destruction or degradation of habitat (Selkoe *et al*., 2006). The computed heterozygosity for Anzali Lagoon was higher than Aras Lake and the level of genetic diversity was significant higher ($p< 0.01$) between two populations. High level of heterozygosity in samples of Anzali Lagoon can be evidence of obtained alleles. It is worth mentioned that numerous rivers reach Anzali Lagoon and high heterozygosity could be mating between probable populations in these rivers.The present and previous studies (Bahrami Kamangar and Rostamzadeh, 2015) on the population genetic of *S. glanis* corroborate that there was genetic differentiation among two populations of *S. glanis*. Obtained alleles and heterozygosity parameters in the present study were similar to the previous studies, so that these microsatellite loci could be used as genetic markers to identify other populations of this family.

Based on the AMOVA analyses, significant genetic differentiation was found among both populations of Anzali Lagoon and Aras Lake ($p<0.01$). The F_{st} index based on AMOVA test is a useful relative measurement of genetic differentiation among populations, changes from 0 to 1 (Ballox and Lugan-Moulin, 2002; Quan *et al*., 2006). High value of F_{st} indicates larger discrepancy and vice versa. It is worth to mention that there are large number of reports in different species using F_{st} parameter that all others found various values of F_{st} and significant differentiations between studied populations (Norouzi *et al*., 2008; Chakmehdouz *et al*., 2011). In the other study between four populations of

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S.glanis in different part of Iran, Anazali Lagoon population showed a low level of diversity (Bahrami Kamangar and Rostamzadeh, 2015). In the present study the computed F_{st} value between two populations was remarkable. Sampling areas are completely isolated basins and there is no migration. This level of differentiation among *S. glanis* populations could be due to a mutation rate and gaps between allele sizes. Since geographical distance of selected area and significant values of F_{st} and genetic distance, it is suggested that two populations are genetically differentiated.

It is worth to mention that when the value of the genetic deviation index is closer to zero, the population is close to Hardy–Weinberg equilibrium (Quan *et al*., 2006). Our results were similar to those observed in other species of catfish, in which significant amounts of population structure were documented (Triantafyllidis *et al*., 2002). In the present study three loci in both populations were at HWE as well as excess or deficient of heterozygous (departure from HWE). There are several possible causes of deviation from Hardy–Weinberg equilibrium test such as shortage of samples, using of nonspecific primers, population sub-structuring, low level of polymorphism and the most probable is presence of null alleles in inheritance of microsatellite loci (Paetkau and Strobeck., 1995; Sekino *et al*., 2003). Quan *et al*. (2006) reported that environmental degradation and overfishing could be the reasons of deviation from HWE. In recent years habitat deterioration and overfishing of this species in these areas are increased that could be a reasonable explanation of observed deviation from HWE in this study. In the future, these factors could be inbreeding and diminishing the population size in selected areas.

In conclusion, the findings of this study indicated that there are genetic differentiations in Anzali Lagoon and Aras lake *S. glanis*. Significant variance in microsatellite allele frequency provide evidence that Anzali Lagoon populations in the south Caspian Sea basin is genetically structured. Thus the protection of habitat areas and prevent of harvest for conservation of gene pools should be applied.

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