J. Agr. Sci. Tech. (2012) Vol. 14: 327-337

A Study of Genetic Structure of *Rutilus frisii kutum* in Anzali Lagoon, Using Microsatellite Markers

S. Rezvani Gilkolaei[1], S. L. Kavan[2], and R. Safari[3]*

ABSTRACT

Kutum (*Rutilus frisii kutum*) is regarded as one of the valuable aquatic species in Southern Caspian Sea. Previous reports have indicated that there are two different forms of this fish in the Caspian Sea, the spring-run and the autumn-run. Despite high importance of availability of knowledge around the subject, there is yet no genetic study carried out on the fish's population structure. A number of nine microsatellite loci were employed in the present study to investigate the genetic variation and differentiation between spring- and autumn-run of the species in Anzali Lagoon. For the purpose, genomic DNA from 105 specimens from spring- and autumn-run kutum from Anzali Lagoon and as well from Khoshkrud spring-run fish were extracted, and PCR amplification performed. A total of 149 alleles were detected at the 9 loci across the 3 populations (Anzali spring- and autumn-runs as well as Khoshkrud spring-run). Khoshkrud population exhibited a lower allelic and genetic variation (A= 5, H_o= 0.406 and H_e= 0.612) than the populations at Anzali Lagoon, and in spite of the higher number of alleles per locus (5.8) as well as higher observed heterozygosities (0.606) in the autumn-run in comparison with the spring-run (5.7, 0.571) in Anzali Lagoon, the differences weren't found as significant (P≥ 0.05). Both F_{is} value and significant deviation from Hardy–Weinberg Equilibrium (HWE) in all the cases (9 loci×3 populations) indicated a deficit in heterozygosity. The highest population differentiation was found between Anzali spring-run and Khoshkrud run (F_{st}= 0.119, P≤ 0.01) and the lowest (F_{st}= 0.07, P≤ 0.01) between Anzali spring- *vs.* autumn-run. The highest genetic distance (D= 0.337) was observed between Anzali autumn-run and Khoshkrud whereas it was found to be the lowest (D= 0.25) between the spring- and the autumn-runs in Anzali. The obtained data suggested that the spring and autumn-runs of kutum in Anzali Lagoon should be considered in the studies and enhancement programmes of this species in the Caspian sea.

Keywords: Caspian Sea kutum, Genetic structure, Heterozygosity, Microsatellite.

INTRODUCTION

Rutilus frisii kutum (Kamensky, 1901), considered as an economically high value species, are mainly distributed along the south and southwest coast of the Caspian Sea from Atrek river located in the Caucasus region (Western coasts of the central Caspian region) into the southern coasts of Turkmenistan (Valipour and Khanipour, 2008). Nearly 60% of catch of bony fish in the southern part of Caspian Sea goes to this species. The catch was over 17,000 tons in 2008 (Abdolmaleki and Ghaninezhad, 2008). Today, Anzali Lagoon in Iran and Ghazel Aghaj Lagoon in Azerbaijan, considered as the main spawning grounds for kutum (Emadi, 1979) in the past have

[1] Iranian Fisheries Research Organization, Tehran, Islamic Republic of Iran.
[2] Department of Biology, Faculty of Science, Savadkoh Branch, Islamic Azad University, Savadkoh, Islamic Republic of Iran.
[3] Agricultural Sciences and Natural Resources University of Gourgan, Department of Fisheries, Gourgan, Islamic Republic of Iran.
* Corresponding author, e-mail: roghi_safari@yahoo.com

lost their significance and are no more able to well support kutum spawning (Valipour and Khanipour, 2008). Also, only a few rivers including Lemir, Khoshkrud, Sefidrud, Shirud are used as the main spawning grounds for the spring migration and for the artificial breeding of this species in the Iranian Coast of the Caspian Sea. Kavan *et al.* (2009) listed overfishing, illegal catch, water pollution along with the deterioration of habitats, and lack of natural spawning grounds as important factors in the decline of this species in Anzali Lagoon and in rivers in the southern shores of the Caspian Sea. Temperature and probably river flow are the factors determining the entrance of the fish into the rivers in the course of spawning migration. This is considered as the main migration, but there also exists a second, a much weaker run, which has been observed during the autumn (Razavi Sayad, 1995). Molecular markers provide a good estimate of the genetic diversity, since they are almost unlimited in number and are not influenced by the environment. Various such DNA markers as RFLP, AFLP, RAPD and microsatellites have been developed which can be employed either separately or in combination, to evaluate genetic diversity (Naghavi *et al*., 2010). Microsatellites are highly divers nuclear genetic markers, which are inherited co-dominantly in a Mendelian inheritance (Liu and Cordes, 2004). Microsatellites have been found suitable for a variety of applications in fisheries and aquaculture research, particularly where genetic differentiation within and among populations may be limited. Potential applications in aquaculture include monitoring change in genetic variation as a consequence of different breeding strategies, the investigation of interactions between wild and cultured populations, parentage assignment and estimation of relatedness between potential breeding pairs (Cross *et al*., 2005). The high level of polymorphism, relatively small size and rapid detection protocols make them especially suitable for stock identification in species previously exhibiting low levels of detectable variation, using allozymes or mtDNA (Bentzen *et al*., 1991).

There are several reports, suggesting differentiations among fish of one species or population, according to their migratory behavior, *viz*. those which enter a location in different times, and at different ages of migration, those which enter sea after their first year of life and as well, those that spend two years in freshwater (Razavi Sayad, 1995; Riazi, 1996). Holcik (1995) stated that Iranian young from the Anzali Lagoon never entered the Sea but remained in fresh or brackish water for 1-2 years. Riazi (1996) reported that this species migrates into the Siah-Keshim, a protected region of the Anzali Lagoon.

Several studies have been made regarding the genetic build up of *Rutilus frisii kutum* in the rivers running along the Iranian coast of the Caspian Sea (Kavan *et al.,* 2009; Abdolhay, 2010, Rezvani Gilkolahi *et al*., 2010), but there are only a limited number of reports addressing the different forms of this species in the Caspian Sea and Anzali Lagoon. Reports by fishermen recount that two spawning migrations (spring and autumn populations), exist into Anzali Lagoon. Valipour and Khanipour (2008) reported two forms or stocks of this species in the Iranian waters of the Caspian Sea because of two spawning migrations, spring- and autumn-runs. Electrophoretic studies of blood proteins have revealed three stocks (Shilat, 1996). Despite the capability of molecular markers and the high importance of an identification of different forms (populations) of this economically important species for Fisheries Organization, yet there is no substantial study available on the subject. So, the aim of this study was to obtain information about genetic variation and differentiation of *Rutilus frisii kutum* in Anzali Lagoon spring and autumn runs to be employed in the future enhancement programmes of this species in the Caspian Sea.

MATERIALS AND METHODS

Fish Sampling and DNA Extraction

Samplings were made from Anzali Lagoon (latitude: 37° 26́ 46.63˝; altitude: 49° 22́ 23.02˝) in spring and autumn 2006 and as well from Khoshkrud (latitude: 36° 49́ 26.35˝; altitude: 53° 45́ 45.32˝) in spring (Figure 1). Khoshkrud choosing was to better elaborate the differentiations. Fin tissue samples were prepared from 35 fish in each time of sampling from Anzali and Koshkrud, then stored in 96% ethanol for subsequent DNA extraction and amplification. Genomic DNA was extracted from pieces of fin clips using the phenol-chloroform procedure described by Hillis and Moritz (1990). The quality and concentration of DNA from samples were assessed through 1% agarose gel electrophoresis. The samples were then stored at -20°C until use.

PCR Amplifications and Electrophoresis

PCR amplifications were done using nine microsatellite loci analyzed: Ca1, Ca2, Ca3, Ca4 (Dimsoski *et al*., 2000), Lco1, Lco2, Lco3, Lco4 (Turner *et al*., 2004), and MFW2 (Crooijmans *et al*., 1997), with GeneBank Accession number are AF277573, AF277574, AF277575, AF277576, AY318777, AY318778, AY318779, AY318780 and EF144125 respectively. The Polymerase Chain Reaction (PCR) conditions, especially the annealing temperatures, were optimized for the 9 microsatellite primers as necessary to produce amplification products. Annealing temperatures were 55°C for Ca1, 58°C for Ca2 and Ca3, and 61°C for Ca4, 60°C for Lco1, 62°C for Lco2, 53°C for Lco3, 57°C for Lco4, and 66°C for MFW2. Amplification was performed in PCR system (Gradient Eppendorf) using a 25 µl total volume containing 5 µl of 10X reaction buffer, dNTPs 10 mM, $MgCl_2$ 50 mM, primer 20 pmol of each (Foward and Reverse) Table1, genomic DNA 100ng and 1.5-2 unit of Taq polymerase. Initial denaturation was achieved at 94°C for 3 min followed by 30 cycles of denaturation in 30 seconds at 94°C, 30 seconds at the respective annealing temperatures, and extension to 72°C for 1 minute. The final step was extended to 5 minutes at 72°C. PCR products were separated using 8% polyacrylamide gels stained with silver nitrate.

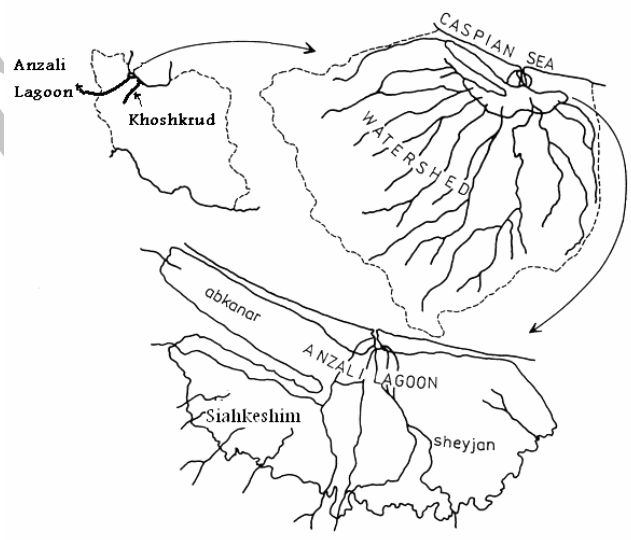

Figure1. Map of sampling locations: Anzali Lagoon and Khoshkrud.

Table1. Microsatellite Loci, GenBank acc no., Primer sequence, PCR product size (bp) and Polymorphism Information Content (PIC) of nine microsatellite markers from *Rutilius frisii kutum*.

Microsatellite Loci	GenBank acc no.	Primer sequence	PCR product size range (bp)	PIC
Lco3	AY318779	F:GCAGGAGCGAAACCATAAAT R:AAACAGGCAGGACACAAAGG	160-268	0.88
Lco1	AY318777	F:CACGGGACAATTTGGATGTTTTAT R:AGGGGGCAGCATACAAGAGACAAC	184-280	0.87
Lco2	AY318778	F:ATTTTTAGGAGTGATGTTCAGCAT R:CAAGTGTGTCATTGAGGAAGTGAG	132-196	0.756
Lco4	AY318780	F:ATCAGGTCAGGGGTGTCACG R:TGTTTATTTGGGGTCTGTGT	104-116	0.603
MFW2	EF144125	F:CACACCGGGCTACTGCAGAG R:GTGCAGTGCAGGAGTTTGC	208-224	0.678
Ca1	AF277573	F:AAGACGATGCTGGATGTTTAC R:CTATAGCTTATCCCGGCAGTA	104-116	0.645
Ca2	AF277574	F:GGACAGTGAGGGACGCAGAC R:TCTAGCCCCCAAATTTTACGG	232-276	0.829
Ca3	AF277575	F:TTGAGTGGATGGTGCTTGTA R:GCATTGCCAAAAGTTACCTAA	136-164	0.714
Ca4	AF277576	F:GTGAAGCATGGCATAGCACA R:CAGGAAAGTGCCAGCATACAC	138-160	0.643

Microsatellite Analysis

The presence of null alleles was tested using Microcheker version 2.2.3 (Van Oosterhout *et al.,* 2004). The recorded microsatellite genotypes were applied as input data for the GeneAlex software version 6 package (Peakall and Smouse, 2006) to calculate allelic and genotypic frequencies, observed (H_o) and (H_e), expected heterozygosities and to test for deviations from Hardy-Weinberg Equilibrium (HWE). For each marker allelic variation was estimated by the polymorphism information content (PIC) value first described by Botstein *et al*. (1980) and modified by Anderson *et al.* (1993). Polymorphism Information Content was calculated as follows:

$$\mathbf{PIC=1-(}\sum_{i=1}^{n} pi^2\mathbf{)-}\sum_{i=1}^{k-1}\sum_{j=i+1}^{n} 2\,pi^2\,pj^2$$

Where *pi* and *pj* are frequencies of *i* and *j* the alleles for a given microsatellite and *k* is the total number of alleles detected for that microsatellite. Genetic distance among populations (spring- and autumn-runs in Anzali Lagoon and Khoshkrud spring-run) was estimated from *Nei* standard genetic distance and genetic similarity index (Nei, 1972). Genetic differentiation among populations was evaluated through an assessment of pair wise estimates of F_{st} values (F_{st}= H_T-$\hat{H}_e/H_T$). The number of migrants *Nm* (Nm= [(1/ F_{st})-1]/4 Wright, 1969; Slatkin, 1987) was also calculated employing GeneAlex software (Peakall and Smouse, 2006).

RESULT

The displayed fragment in PCR presented different lengths in nine microsatellitic loci, the minimum fragment size being observed at Ca1 with 104-116 bp length and the maximum at Ca2 with 232-276 bp (Table 1). All the nine employed microsatellitic loci exhibited polymorphism (Table 1).

A total of 149 alleles were detected at the 9 loci and across 3 populations (Anzali spring-, Anzali autumn-runs and Khoshkrud spring-run) (Table 2) Polymorphism Information Content (PIC) of these nine microsatellite markers ranged 0.603-0.88 in this species.

The number of alleles ranged from 3 at Lco2, Lco4 and Ca4 in Khoshkrud to 13 at Lco3 in Anzali spring-run. Among the

Table 2. Variability of nine microsatellite loci in *Rutilus frisii kutum* populations from Anzali Lagoon and Khoshkrud (A, alleles number; H_o, observed heterozygosity; H_e, expected heterozygosity; P, P-values of x^2 tests for Hardy-Weinberg Equilibrium); F_{is}, Fixation index).

Microsatellite Loci	Parameter	Anzali spring	Anzali autumn	Khoshkrud
Lco3	A	13	10	9
	H_o	0.457	0.429	0.229
	He	0.87	0.858	0.827
	P	0.000***	0.000***	0.000***
	F_{is}	0.471	0.5	0.724
Lco1	A	8	7	7
	H_o	0.429	0.171	0.114
	He	0.822	0.831	0.785
	P	0.000***	0.000***	0.000***
	F_{is}	0.478	0.79	0.854
Lco2	A	6	5	3
	H_o	0.714	0.857	0.714
	He	0.779	0.749	0.561
	P	0.000***	0.000***	0.038*
	F_{is}	0.083	-0.145	-0.273
Lco4	A	4	4	3
	H_o	0.371	0.857	0.314
	He	0.622	0.646	0.355
	P	0.000***	0.003**	0.000***
	F_{is}	0.402	-0.32	0.114
MFW2	A	4	5	4
	H_o	0.457	0.457	0.571
	He	0.55	0.684	0.692
	P	0.000***	0.000***	0.000***
	F_{is}	0.159	0.332	0.175
Ca1	A	4	4	4
	H_o	1	0.971	1
	He	0.59	0.693	0.6
	P	0.000***	0.001**	0.000***
	F_{is}	-0.698	-0.402	-0.576
Ca2	A	4	8	7
	H_o	0.714	0.629	0.286
	He	0.7	0.832	0.744
	P	0.000***	0.000***	0.000***
	F_{is}	-0.024	0.244	0.616
Ca3	A	4	4	5
	H_o	0.286	0.686	0.343
	He	0.75	0.684	0.749
	P	0.000***	0.001**	0.000***
	F_{is}	0.478	-0.003	0.542
Ca4	A	5	5	3
	H_o	0.714	0.4	0.086
	He	0.72	0.652	0.16
	P	0.001***	0.000***	0.000***
	F_{is}	0.01	0.386	0.466
Average number of alleles per locus		5.7	5.8	5
Avarage H_o		0.571	0.606	0.406
Avarage *H*e		0.687	0.736	0.612

studied populations and, with a consideration of all the loci, Khoshkrud population revealed lower allelic and genetic variations (A= 5, Ho= 0.406 and He= 0.612) than the population of Anzali Lagoon. In spite of higher number of alleles per locus (5.8) and observed heterozygosity (0.606) in the autumn run in comparison with the spring run (5.7, 0.571) in Anzali Lagoon, the differences between them weren't found as significant (P≥ 0.05) (Table 2).

Both F_{is} value and significant deviation from Hardy–Weinberg Equilibrium (HWE) in all the cases (9 loci×3 populations) indicated deficit in heterozygosity (Table 2). The population differentiation was found highest between Anzali spring-run and Khoshkrud (*Fst*= 0.119, P≤ 0.01) while the lowest (*Fst*= 0.07, P≤ 0.01) between Anzali spring and autumn-runs (Table 3). Principle Coordinates Analysis (PCA) (Figure 2) also, revealed the differences among studied populations. The estimated number of migrant (N_m) between the spring and autumn runs in Anzali Lagoon across all the studied loci was the highest (N_m =3.23) while it was the lowest (N_m= 1.85) between the spring run population in Anzali Lagoon and Khoshkrud (Table 3). Genetic distance (*D*) and genetic similarity index (*I*) among the three populations are presented in Table 4. The highest genetic distance (D= 0.337) was found between Anzali autumn- run and Khoshkrud, while the lowest one (D= 0.25) between the spring- and the autumn-runs in Anzali. Mantel Test indicated that the estimated standard genetic distance according to Nei (1972) is positively correlated with *Fst* (Y = 0.3936x -0.0557, R^2= 0.9747) (Figure 3).

Table 3. Multilocus N_m (above diagonal) and F_{st} values (below diagonal) between pairs of *Rutilus frisii kutum* populations across all loci.

Populations	Anzali Lagoon spring	Anzali Lagoon autumn	Khoshkrud River
Anzali Lagoon spring	****	3.32	1.85
Anzali Lagoon autumn	0.07*	****	1.95
Khoshkrud River	0.119*	0.114*	****

Statistically significant values are marked with asterisks.*≤0.01

DISCUSSION

Genetic diversity is important for ecological and evolutionary processes ranging from individual fitness to ecosystem function. Heterozygosity serves as an indicator of evolutionary potential and is important in determining population dynamics as well as population viability (Reed, 2009). The results of the study indicated that the average number of alleles per locus and the observed heterozygosity in Anzali lagoon autumn-run (5.8 and 0.606)

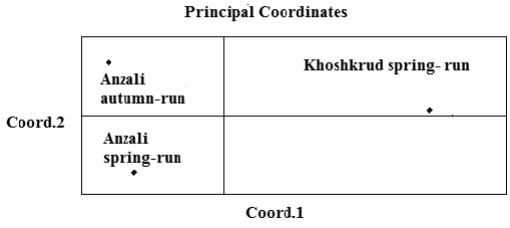

Figure2. PCA (Principle Coordinates Analysis).

Table 4. Genetic distance (D) (above diagonal) and genetic similarity (below diagonal) between pairs of *Rutilus frisii kutum* Populations.

Populations	Anzali Lagoon spring	Anzali Lagoon autumn	Khoshkrud River
Anzali Lagoon spring	****	0.25	0.323
Anzali Lagoon autumn	0.779	****	0.337
Khoshkrud River	0.724	0.714	****

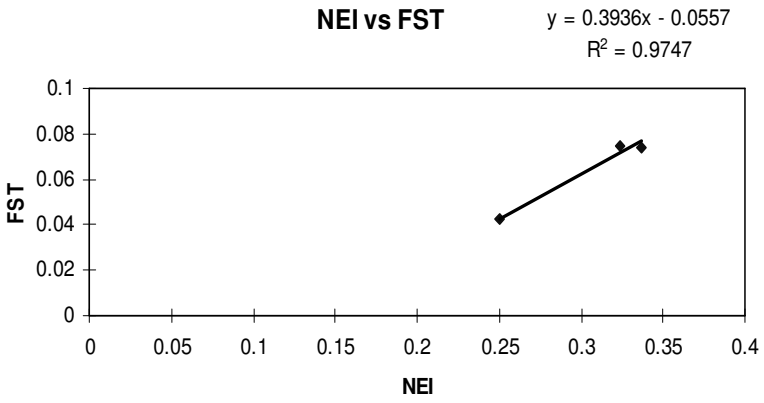

Figure3. Correlation between Nei's genetic distance and *Fst* revealed by the Mantel test

were higher than those in the spring-run (5.7 and 0.571 respectively), but the differences between the two weren't found as significant, both being higher than Khoshkrud (5 and 0.4) and all being lower than those (A= 11.3, H= 0.68) reported by DeWoody and Avise (2000) for anadromous fish. Loss of genetic variation in hatchery stocks is a common phenomenon reported in many species [*Salmo trutta* (Was and Wenne, 2002); *Rutilus rutilus caspicus* (Keyvanshokoh *et al.,* 2007); *Abramis brama* (Ghasemi *et al*., 2007); *Cyprinus carpio* (Thai *et al.,* 2007); *Orechromis niloticus* (Nyingi *et al*., 2009); *Ctenopharyngodon idella* (Liu *et al.,* 2009)]. This is mainly due to a reduction in the effective population size (Ne), inbreeding or combinations of these events (Falconer, 1998). As natural spawning grounds of this species have been destroyed due to pollution and its stock being decreased, annually, millions of fries (average weight 1 g) have been produced and released into the Caspian Sea by Iranian Fisheries Organization hatcharies (Kavan *et al*., 2009). This species is highly fecund (Absolute fecundity, on the average, is 74,774 eggs) (Valipour and Khanipour, 2008), and the tendency of keeping a small number of fish as broodstock to reduce the cost of production, coupled with mass spawning practiced by many hatcheries has promoted random genetic drift, resulting in reduction in genetic diversity in hatchery stocks. Higher number of alleles per locus and observed heterozygosity in Anzali lagoon autumn-run should be considered in restocking of this species in Caspian Sea.

Significant deviations from HWE were detected at all loci in all the populations ($P \leq 0.05$). Several such possible alternative reasoning as null alleles, heterozygosity deficiency may explain these observations. Where heterozygosity deficiencies were detected, such deviations would generally indicate that such factors as non-random mating, reduction in effective breeding population or selection pressure at a specific locus are the causes for the observed instances (Garcia de Leon *et al*., 1997). By using Microchecker, null alleles were found in some loci. This would have been likely; primers had been designed for the other species and failed to amplify some alleles (produce nulls) in this species. Genetic structure of populations may vary considerably among species depending on the relative importance of drift, gene flow as well as selection (Slatkin, 1985) along with such long-term historical events, as postglacial recolonization from different glacial refuges (Taberlet *et al*., 1998). Pairwise genetic differentiation (F_{st}) was employed to assess genetic differentiation, which is the acquisition of allele frequencies that differ among populations (Hartl and Clark 1997). F_{st} analysis revealed significant

genetic differentiation (P≤0.01) among the spring-run and the autumn's, and both with Khoshkrud. PCA test also, verified differentiation among them. The population differentiation between Khoshkrud-Anzali spring run (0.119) and Khoshkrud-Anzali autumn's (0.114) were considerable. This moderate genetic differentiation between Khoshkrud and Anzali (spring and autumn), despite low geographic distance could be indicative of the high conservation and permanence of Khoshkrud population. Khoshkrud is one of the rivers that is still used as the main spawning grounds for migration and it is usually utilized as the progeny origin of brood stock from that locality for restocking purposes in recent years, therefore, it appears that Khoshkrud population has conserved its stock, to some extent.

The estimated F_{st} between Anzali spring and autumn runs, in this survey, was about 0.07, which is lower than the F_{st} estimated (mean of 0.1) by reviewing 7 anadramous fish species (Ward *et al*. 1994). On the other hand, it is slightly higher than (0.05) that of Balloux and Lugon-Moulin (2002) and Tevfik Dorak, (2005) considered as a single population. Estimates of $Nm > 1$ suggest that gene flow among populations could be accounted for as one of the main factors in genetic diversity (Li *et al*., 2007). Estimated gene flows also indicate lower levels of migration between Khoshkrud and Anzali spring (1.85) and Khoshkrud and Anzali autumn (1.95) than between Anzali spring- and autumn-runs (3.32). The lower value of *Nm* again reflects the higher value of F_{st}. Emadi (1979) pointed out that intensive sea fishing and fishing in rivers hindered spawning of the autumn form, and led to merely the spring form spawning. Reports of fishermen indicate that two spawning migrations (spring and autumn populations) into the Anzali Lagoon still exist. Razavi Sayad (1995) found three populations in Iran, one autumn and two spring populations. There may be three stocks based on electrophoretic studies of blood proteins associated with the two spawning migrations (Shilat, 1996). According to the catch records of the fishermen throughout the year, only about 1% of kutum enters the lagoon in autumn, and more than 98% in spring (Razavi Sayad, 1995). This could be attributed to use of spring run for restocking by Fisheries Organization.

The lowest genetic distance was observed between the spring-run and the autumn-run in Anzali, suggesting that they had originated from a common ancestor. The genetic distance between populations in this research (0.25-0.337) fall within the range of (0.03-0.61) for congeneric (Shaklee *et al*., 1982; Thorpe and Solé-Cava, 1994) species, suggesting their genetic divergence.

In conclusion, this study provides one with useful insight into the genetic variability and differentiation of *Rutilus frisii kutum* populations (forms) in the Caspian Sea and Anzali Lagoon, suggesting that there is need for development of management and conservation plans for different forms of this species in Anzali Lagoon and in Caspian Sea.

ACKNOWLEDGEMENTS

This work was funded by Iranian Fisheries Research Institute. Sincere gratitude is forwarded to personnel at the Ecology Research Institute of the Caspian Sea for their collaboration.

REFERENCES

1. Abdolhay, H., 2010. Investigation on Population Genetic Structure of *Rutilus frisii kutum* (Kamensky 1901) from Iranian Coastline of Caspian Sea Using mt-DNA. Ph.D. Thesis**,** Biotechnology Department, UPM University, 200 PP.
2. Abdolmaleki, S. H. and Ghaninezhad, D. 2008. Rehabilitation of Kutum Fingerlings and Its Role on the Stock of This Fish along Southern Part of the Caspian Sea. *Abzyan*, **86**: 8-13. (In Persian).
3. Anderson J. A., Churchill G. A., Autrique J. E., Tanksley S. D. and Sorrells M. E. 1993.

Optimizing Parental Selection for Genetic. Linkage Maps. *Genome.* **36**: 81–86**.**

4. Balloux, F. and Lugon-Moulin, N. 2002. The Estimation of Population Differentiation with Microsatellite Markers. *Mol. Ecol*., **11**: 155–165.
5. Botstein D., White R. L., Skolnick M. and Davis R. W. 1980. Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms. *Am. J. Hum. Genet*., **32**: 314–31.
6. Bentzen, P., Harris, A. and Wright, J. M. 1991. Cloning of Hypervariable Minisatellite and Simple Sequence Microsatellite Repeat for DNA Fingerprinting of Important Aquacultural Species. In: "*DNA Fingerprinting: Approach and Applications*", (Eds.): Burke, T., Dolf, G. A. and Wolf, R.. Birkhauser, Basel, PP. 243-262.
7. Crooijmans, R. P. M. A., Bierbooms, V. A. F., Komen, J., Van der Poel, J. J. and Groenen, M. A. M. 1997. Microsatellite Markers in Common Carp (*Cyprinus carpio* L.). *Anim Genet.*, **28**: 129-134.
8. Cross, T. F., Coughlan, J., Burnell, G. M. C., Dillane, E., Stefansson, M. O. and Wilkins, N. P. 2005. Utility of Microsatellite Loci for Detecting Reduction of Variation in Reared Aquaculture Strains Compared with Wild Progenitors and also as Genetic "Tag" in Breeding Programmes: Evidences from Abalone, Halibut and Salmon. *Aquaculture*, **247**: 9-10.
9. DeWoody J. A. and Avise J. C. 2000. Microsatellite Variation in Marine, Freshwater and Anadromous Fishes Compared with Other Animals. *J. Fish Biol*., **56**: 461-473.
10. 9. Dimsoski, P., Toth, G. P. and Bagley, M. J. 2000. Microsatellite Characterization in Central Stoneroller *Campostoma anomalum* (Pisces: Cyprinidae). *Mol. Ecol*., **9**: 2187-2189.
11. Emadi, H.1979. The State of the Fishing and Reproduction of the Kutum, *Rutilus frisii kutum*, in the Caspian Sea of Iran. *J. Ichth*., **19**: 151-154.
12. Falconer, D. S. 1998. *Introduction to Quantitative Genetics*. 3th Edition, Longman Scientific and Technical, 438 PP.
13. Garcia de Leon, F. J., Chikhi, L. and Bonnhomme, F.1997. Microsatellite Polymorphism and Population Subdivision in Natural Populations of European Seabass *Dicentrarchus labrax* (Linnaeus 1758). *Mol. Ecol*., **6**: 51-62.
14. Ghasemi, A., Keyvanshokooh, S., Shahriari-Moghadam, M. and Khara, H. 2007 Genetic Comparison of Iranian and Azeri Populations of the Oriental Bream *Abramis brama* orientalis (Berg) Using Microsatellites . *Aquacult. Res*., **38**: 1-5.
15. Hartl, D. L. and Clark, A. G. 1997. *Principles of Population Genetics*. Sinauer Associates Sunderland, MA, USA. PP.542.
16. Hillis, D. M. and Moritz, C. 1990. *Molecular Systematics*. Sinauer Associates, Sunderland, MA, USA. PP.502-510.
17. Holcik, J. 1995. New Data on the Ecology of Kutum, *Rutilus frisii* from the Caspian Sea. *Ecol. Freshwater Fish*, **4**: 175-179.
18. Kavan, S. L., Rezvani Gilkolahi., S., Vossoughi, H., Fatemi, S. M. R., Safari, R. and Jamili, S. H. 2009. Population Genetic Study of *Rutilus frisii kutum* (Kamensky 1901) from the Caspian Sea; Iran and Azerbaijan Regions, Using Microsatellite Markers. *J. Fish. Aquat. Sci*., **4(6):** 316-322.
19. Keyvanshokoh, S., Ghasemi, A., Shahriary Moqadam, M. and Nazari, R. M. 2007. Genetic Analysis of *Rutilus rutilus caspicus* (Jakowlew 1870) Population in Iran by Microsatellite Marker. *Aquacult. Res*., **38**: 953-956.
20. Li, D., Kang, D., Yin, Q., Sun, Z. and Liang, L., 2007. Microsatellite DNA Marker Analysis of Genetic Diversity in Wild Common Carp (*Cyprinus carpio*) Populations. *Genet Genom.*, **34**: 984-993.
21. Liu, Z. J. and Cordes, F. J. 2004. DNA Marker Technologies and Their Applications in Aquaculture Genetics. *Aquaculture,* **238**: 1-37.
22. Liu, F., Xia, J. H., Bai, Z. H., Fu, J. J., Li, J. L. and Yue, G. H. 2009. High Genetic Diversity and Substantial Population Differentiation in Grass Carp (*Ctenopharyngodon idella*) Revealed by Microsatellite Analysis. *Aquaculture*, **297**: 51–56
23. Naghavi, M. R., Malaki, M., Alizadeh, H., Pirseiedi, M. and Mardi, M. 2009. An Assessment of Genetic Diversity in Wild Diploid Wheat *Triticum boeoticum* from West of Iran Using RAPD, AFLP and SSR Markers. *J. Agr. Sci. Tech.*, **11**: 585-598
24. Nei, M. 1972. Genetic Distance between Populations. *Am. Natt*., **106**: 28.

25. Nyingi, D., Vos, L. D., Aman, R. and Agnese, J. F. 2009. Genetic Characterization of an Unknown and Endangered Native Population of the Nile Tilapia (*Oreochromis niloticus*) (Linnaeus, 1758) (Cichlidae; Teleostei) in the Loboi Swamp (Kenya). *Aquaculture*, **297**: 57–63
26. Peakall R. and Smouse, P. E. 2006. GENALEX 6: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. *Mol. Ecol. Notes*, **6**: 288-295.
27. Razavi Sayad, B. 1995. *Kutum Fish*. Iranian Fisheries Research Organization, Tehran, 165 PP. (In Persian)
28. Reed, D. H. 2009. When It Comes to Inbreeding: Slower Is Better. *Mol. Ecol*., **18**: 4521–4522.
29. Rezvani Gilkolahi, S., Abdolhay, H., S., Shojaee, L., Safari, R., Laloei, F. and Taqavi, M. J. 2010. Population Structure of *Rutilus frisii kutum* (Kamenskii, 1901) in Iranian Coastline of Caspian Sea Using Microsatellite. *The 1st National-Regional Conference on Ecology of the Caspian Sea*. 1-2 June 2010, Sari, Iran, 170 PP.
30. Riazi, B. 1996. *Siah-Keshim: The Protected Area of Anzali Wetland*. Department of Environment, Tehran, 101 PP. (In Persian)
31. Shaklee J. B., Tamaru C. S. and Waples R. S. 1982. Speciation and Evolution of Marine Fishes Studied by Electrophoretic Analysis of Proteins. *Pacific. Sci*., **36**: 141-157.
32. Shilat, Tehran. 1996. *Annual Report of Aquaculture Production in Iran*. Aquaculture Department, Shilat (Fisheries), Tehran. 41 PP. (In Persian)
33. Slatkins, M. 1985. Gene Flow in Natural Populations. *Ann. review Ecol. Sys.*, **16**: 393-430
34. Slatkin, M. 1987. Gene Flow and the Geographic Structure of Natural Populations. *Science*, **236**: 787–792.
35. Taberlet, P., Fumagalli, L., Wust-Saucy, A. G. and Cosson, J. F. 1998. Comparative Phylogeography and Postglacial Colonization Rutes in Europe. *Mol. Ecol.,* **7**: 453-464.
36. Tevfic Dorak, M. 2005. *Basic Population Genetics*. http:// www.dorak. info/genetics/popgene.html
37. 37. Thai, T. B., Burridge, C. P. and Austin, C. M. 2007. Genetic Diversity of Common Carp (*Cyprinus carpio L.*) in Vietnam Using Four Microsatellite Loci. *Aquaculture,* **269**: 174–186.
38. Thorpe J. P. and Solé-Cava A. M. 1994. The Use of Allozyme Electrophoresis in Vertebrate Systematics. *Zoologica Scripta*, **23**: 3-18.
39. Turner, T. F., Dowling, T. E., Broughton, R. E. and Gold, J. R. 2004. Variable Microsatellite Markers Amplify across Divergent Lineages of Cyprinid Fishes (Subfamily Leusicinae). *Conser. Genet.*, **5**: 279-281.
40. Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. and Shipley, P. 2004. MICRO-CHECKER: Software for Identifying and Correcting Genotyping Errors in Microsatellite Data. *Mol. Ecol. Notes*., **4**: 535–538.
41. Valipour, A. and Khanipour, A. 2008. *Rutilus frissi kutum: Jewel of the Caspian Sea.* Iranian Fisheries Research Organization, 97 PP.
42. Ward, R. D., Woodwark, M. and Skinbinski, D. O. F. 1994. A Comparison of Genetic Diversity Levels in Marine, Fresh Water and Anadromous Fishes. *J. Fish Biol*., **44**: 213-232
43. Was, A. and Wenne, R. 2002. Genetic Differentiation in Hatchery and Wild Sea Trout (*Salmo trutta*) in the Southern Baltic at Microsatellite Loci. *Aquaculture*, **204**: 493-506.
44. Wright, S. 1969. *Evolution and the Genetics of Populations*. Vol 2. The Theory of Gene Frequencies. Chicago University Press, Chicago, PP.520.

مطالعه ساختار ژنتیکی ماهی سفید *Rutilus frisii kutum* در تالاب انزلی با استفاده از روش میکروساتلایت

س. رضوانی گیل کولایی، س. ل. کاوان، و ر. صفری

چکیده

ماهی سفید یکی ازگونه های با ارزش سواحل جنوبی دریای خزر می باشد، گزارشات قبلی حاکی از وجود دو فرم متفاوت این ماهی (بهاره و پاییزه) در دریای خزر است. علی رغم اهمیت بالای دانستن این موضوع مطالعه ژنتیکی در مورد ساختار جمعیتی آن موجود نیست . درمطالعه حاضر ۹ لوکوس میکروساتلایتی برای بررسی تنوع ژنتیکی و تمایز بین فرم های مختلف بهاره و پاییزه این ماهی درتالاب انزلی به کار برده شد. برای این منظور 105 نمونه ماهی سفید در بهار و پاییز از تالاب انزلی و رودخانه خشکرود جمع آوری شد. واکنش زنجیره ای پلیمراز انجام شد و 149 الل در سه جمعیت مشاهده گردید. جمعیت خشکرود تعداد الل و تنوع ژنتیکی کمتری (A= 5, Ho= 0.406) را نسبت به نمونه های بهار و پاییز در تالاب انزلی نشان داد و با وجود بالاتر بودن تعداد الل و هتروزیگوسیتی در نمونه های پاییزه تالاب انزلی (A= 5.8, Ho= 0.606) نسبت به نمونه های بهاره (A= 5.7, Ho= 0.571) اختلاف معنی داری بین آنها مشاهده نشد($P\geq 0.05$) . دو شاخص F_{is} و انحراف از تعادل در همه لوکوسها به ازای تمام جمعیتها حاکی از کمبود هتروزیگوسیتی بود. تمایز ژنتیکی بین همه نمونه ها معنی دار بود($P\leq 0.01$) بیشترین تمایز ژنتیکی بین نمونه های بهار ه تالاب انزلی و خشکرود ($Fst = 0.119, P\leq 0.01$) و کمترین بین نمونه های بهاره و پاییزه تالاب انزلی ($Fst = 0.07, P\leq 0.01$) مشاهده شد. بیشترین فاصله ژنتیکی بین نمونه های پاییز تالاب انزلی و خشکرود(D= 0.337) و کمترین بین نمونه های بهاره و پاییز ه تالاب انزلی (D= 0.25) مشاهده شد. داده های حاصل از این بررسی نشان می دهد که فرم های بهاره و پاییز ه تالاب انزلی باید در برنامه های باز سازی دخایر این گونه در دریای خزر مورد توجه قرارگیرد.