Iranian Journal of Animal Biosystematics (IJAB)
Vol.8, No.1, 15-26, 2012
ISSN: 1735-434X

Population genetic structure of Mahi Sefid (*Rutilus frisii kutum*) in the of South Caspian Sea: Implications for fishery management

Abdolhay, H. A.[a*], Daud, S. K.[b], Rezvani, S.[a], Pourkazemi, M.[c], Siraj, S. S.[d], Laloei, F.[a], Javanmard, A.[d], Hassanzadeh Saber M.[c]

[a] *Iranian Fisheries Research Organization, Tehran-Karaj Highway, Iran Khodro Exit, Azadshar, Sarv- Naz St. sarv-Azad. P.O. Box.14155-6116, Tehran, Iran.*
[b] *Department of Biology, Faculty of Science, University of Putra Malaysia 43400, Serdang, Selangor, Malaysia.*
[c] *Sturgeon International Research Institute. P.O. Box. 41633-3464, Rasht, Iran.*
[d] *Department of Aquaculture, Faculty of Agriculture, University Putra Malaysia 43400, Serdang, Selangor, Malaysia.*

Mahi sefid (Cyprinidae) is an economically important fish in the south Caspian Sea. Specimens was collected from the Lamir River, Sefid Rud, Shir Rud and Tajan Rivers and analyzed at 38 microsatellite DNA loci. Eight primers out of the thirty analyzed loci were polymorphic in all sampling regions. The highest observed heterozygosity (1) was in locus Ca1 in Sefid Rud and lowest was 0.07 in locus SYP5 in Lamir River. The highest expected heterozygosity was 0.92 in locus SYP4 in Shir Rud and lowest was 0.24 in locus SYP6 in Tajan River. The average allele per locus was 7.22, maximum allele per locus was 15 and minimum allele per locus was two. The highest genetic distance was between Shir Rud and Tajan River (0.07) and lowest genetic distance was between Sefid Rud and Tajan River (0.05). All loci and all rivers deviated from Hardy-Weinberg Equilibrium expectations ($P<0.01$) except at the SPY6 locus. Populations clustered in three groups corresponding to Shir Rud, Lamir Rivers and Tajan-Sefid Rud.

Key words: Caspian Sea, genetic variation, Mahi Sefid, microsatellite markers, *Rutilus frisii kutum*

INTRODUCTION

Mahi Sefid (family Cyprinidae) plays an important role in the fisheries and economy of the southern part of Caspian Sea. Due to continuing population decline of the fish since 1975, the Iranian government started the enhancement programs of this species in early 1983. The Iranian Fisheries Organization stock enhancement program has produced and released more than 200 million of fingerlings every year (Abdolhay, 1997; Abdolhay et al., 2011). The annual fisheries production had stabilized at around 17,196 t/yr until 2007 and 14,834 in 2008, 12,495 in 2009 and 11,573 in 2010.
Although stock enhancement programs have been successful in many countries such as Japan, the United States of America, Norway and Iran, there is a growing concern over the genetic effects of these activities on natural populations (Gonzalez et al., 2011; Kitada et al., 2011; Russell et al., 2011) such as reduced fitness or increased susceptibility to diseases. This is especially true when the genetic material of hatchery populations is very different from those of the wild populations because of inbreeding, selective breeding and domestication (Taniguchi and Sugama, 1990). Therefore, genetic

*Corresponding Author: hossein_abdolhay@yahoo.com

characters of the stocking materials used in the enhancement programs should be assessed for the purpose of monitoring their effects on wild stocks (Bartley and Leber, 2004).

Microsatellites have been isolated and characterized in a large number of fish species and have been used in a wide range of applications in evolutionary biology, population genetics and ecology (Skaala et al., 2004; Chistiakov et al., 2006; Thai et al., 2007; Liu et al., 2011; Messmer et al., 2011). They have been successfully used in uncovering cryptic population structure in many organisms and for monitoring changes in genetic materials of farmed stocks, parentage assignment and fine-scale studies of population structure of many fish species (Tong et al., 2002; Lal et al., 2004; Remmy et al., 2005; Thai et al., 2007; Kasapidis and Magoulas, 2008; Somridhivej et al., 2008; Golda et al., 2010; Hsu et al., 2010; Nan et al., 2010; Tripp et al., 2010; Gonzalez et al., 2011).

Recently microsatellite loci have been widely used in fisheries management and aquaculture (Castro et al., 2006; Castro et al., 2007; Launey et al., 2007; Chang et al., 2008; Dixon et al., 2008; Ha et al., 2009; Gonzalez et al., 2011). The application of these markers has become common in investigating population subdivision via significant differences in allele frequencies, tracking gene flow between populations, and identifying origin of fish stocks (Ferguson and Danzmann, 1998; Vandeputte et al., 2008; Ha et al., 2009). The hyper-variability of microsatellite loci make them an excellent choice for forensic applications, such as the identification of parents, estimation of sibling groups, and individual identification (Gheyas et al., 2009). The accurate rebuilding of pedigrees for which such information has been lost make this marker useful in managing small populations, such as hatchery populations where pedigree information can be used to avoid inbreeding. The use of genetic markers for parentage testing and pedigree reconstruction in aquaculture situations has been suggested by many authors (Ferguson and Danzmann, 1998; Cunningham, 1999; Davis and Hetzel, 2000; Shikano et al., 2008; Haynes et al., 2009; Hsu et al., 2010; Tripp et al., 2010).

MATERIAL AND METHODS

Sample collection

Samples were collected from four rivers: the Lamir River, Sefid Rud (in Farsi River is called Rud), Shir Rud and Tajan River, during the adult migration for spawning in the spring of 2005. The rivers are located within 140 to 310 km from one another in the southern coast of Caspian Sea (Fig. 1). Thirty samples from each river were collected; 2-3 grams of caudal fin were taken and placed in sampling tubes with 96% ethanol at 4°C and transferred to the laboratory for genetic analysis.

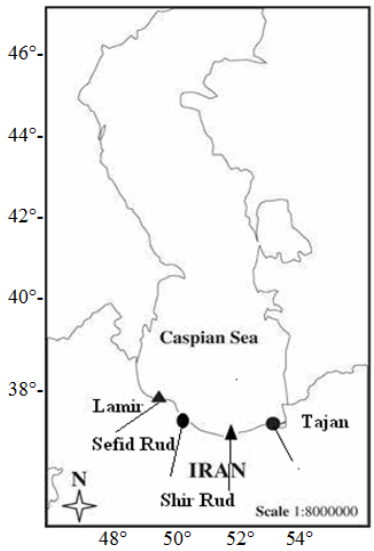

FIGURE 1. Sampling localities of Mahi Sefid in the south Caspian Sea.

TABLE 1. Polymorphic Primers used in this study

Primer sequence	Locus	Size (bp)	Annealing temperature (°C)	Repeat motif	Number of Allele	Gene bank Accession number
F: 5'-CAC GGG ACA ATT TGG ATG TTT TAT- 3' **R:** 5' -AGG GGG CAG CAT ACA AGA GAC AAC- 3'	SYP4	196-296	60	$[GATA]_8GGC$ 60 1.5 $TA[GATA]_2$	19	AF277573
F: 5' -GCA GGA GCG AAA CCA TAA AT- 3' **R:** 5'-AAA CAG GCA GGA CAC AAA GG- 3'	SYP2	196-284	58	[TG]9	18	AF277574
F: 5' -TTA CAC AGC CAA GAC TAT GT- 3' **R:** 5' -CAA GTG ATT TTG CTT ACT GC- 3'	SYP6	122-132	57	$[CAGA]_2[CA]_{14}$	3	AF277575
F: 5' -ATT TTT AGG AGT GAT GTT CAG CAT- 3' **R:** 5' -CAA GTG TGT CAT TGA GGA AGT GAG- 3'	SYP5	156-192	53	$[TGTC]_6[TATC]_3$ TATA TG(A)TC]16	4	AF277576
F: 5' -GTG AAG CAT GGC ATA GCA CA-3' **R:** 5' -CAG GAA AGT GCC AGC ATA CAC- 3'	CA12	124-152	61	$(TAGA)_{10}$ $(CAGA)_4$ $(TAGA)_2$	7	AY318777
F: 5' -TTG AGT GGA TGG TGC TTG TA- 3' **R:** 5' -GCA TTG CCA AAA GTT ACC TAA- 3'	CA5	136-168	55	$(TAGA)_{15}$	8	AY318778
F: 5' -GGA CAG TGA GGG ACG CAG AC- 3' **R:** 5' -TCT AGC CCC CAA ATT TTA CGG- 3'	CA3	232-276	61	$(TAGA)_{14}$	12	AY318779
F: 5' -AAG ACG ATG CTG GAT GTT TAC- 3' **R:** 5' -CTA TAG CTT ATC CCG GCA GTA- 3'	CA1	104-124	51	$(CA)_{24}$	6	AY318780

DNA extraction and PCR

DNA was extracted from the caudal fin of individuals following standard methods of Hillis et al. (1996) and modified by Pourkazemi (1996). Approximately 2-3g of tissue was digested in 500 µl of extraction buffer (0.1 M Tris HCl, 10 mM EDTA, 0.1 M NaCl, 2% SDS and 0.1 mg Proteinase K ml–1) for 3 h. The cellular debris was removed using phenol/chloroform extraction, and the DNA was precipitated with cold absolute ethanol and then washed twice in 70% ethanol. After air drying, DNA was resuspended in 100 µl of double distilled water and stored at –20°C.

Microsatellite Primer pairs

Thirty microsatellites primers developed by Dimsoski (2000) and Turner et al. (2004) were used in this study, but only eight of these were polymorphic even after optimization (Table 1).

PCR conditions were further optimized for the eight microsatellite loci. Annealing temperatures ranged from 51°C to 61°C at 2 mm MgCl2 and 0.2 µM primer concentrations. The concentrations of Taq polymerase (1 U/25 µl) and nucleotides (0.4 mM) were the same for all loci. Thermal profile for PCR was 94 °C 5 min, (94°C 1 min, 52-54°C 30 sec) 72°C 1 min and 30 cycle, extension 72°C 5 min and 1 cycle. PCR was followed by electrophoresis of products in 6% polyacrylamide.

Statistical analysis

Genetic diversity was quantified in terms of expected heterozygosity (H_e), observed heterozygosity (H_o), number of alleles per locus and allele frequencies observed in the samples for each population. The data were analyzed using GenAlex (Peakall and Smouse, 2006). Exact tests were also used to perform pairwise comparison of allele frequencies at individual locus between samples of given populations.

TABLE 2. Allelic variation at eight microsatellite loci in four Regions for *R. frisii kutum*

Sampling Regions	Locus	N_a	N_e	I	H_o	H_e	F_{is}
Lamir River (N=30)	SYP4	12	7.06	2.15	0.47	0.86	0.46
	SYP2	10	4.42	1.84	0.47	0.77	0.4
	SYP6	3	1.35	0.48	0.3	0.26	-0.16
	SYP5	2	1.72	0.61	0.07	0.42	0.84
	Ca12	6	4.05	1.55	0.47	0.75	0.38
	Ca5	6	4.12	1.56	0.47	0.76	0.38
	Ca3	11	6.59	2.12	0.3	0.85	0.65
	Ca1	6	3.4	1.44	0.9	0.71	-0.28
	$\overline{X}$	**7.00**	**4.09**	**1.47**	**0.43**	**0.67**	**0.33**
Sefid Rud (N=30)	SYP4	13	8.78	2.34	0.53	0.89	0.4
	SYP2	14	4.71	2.04	0.67	0.79	0.15
	SYP6	3	1.41	0.57	0.33	0.29	-0.14
	SYP5	3	1.84	0.7	0.13	0.46	0.71
	Ca12	6	3.09	1.37	0.4	0.68	0.41
	Ca5	8	5.13	1.77	0.53	0.81	0.34
	Ca3	10	7.86	2.17	0.3	0.87	0.66
	Ca1	5	2.82	1.22	1	0.65	-0.55
	$\overline{X}$	**7.75**	**4.46**	**1.52**	**0.49**	**0.68**	**0.25**
Shir Rud (N=30)	SYP4	15	12.68	2.61	0.83	0.92	0.1
	SYP2	12	4.35	1.92	0.57	0.77	0.26
	SYP6	3	1.46	0.6	0.37	0.32	-0.16
	SYP5	4	2.03	0.85	0.17	0.51	0.67
	Ca12	5	2.77	1.28	0.3	0.64	0.53
	Ca5	6	4.68	1.66	0.53	0.79	0.32
	Ca3	8	4.89	1.77	0.37	0.8	0.54
	Ca1	6	3.68	1.45	0.77	0.73	-0.05
	$\overline{X}$	**7.38**	**4.57**	**1.52**	**0.49**	**0.69**	**0.28**
Tajan River (N=30)	SYP4	14	10.71	2.47	0.53	0.91	0.41
	SYP2	10	3.45	1.68	0.33	0.71	0.53
	SYP6	3	1.31	0.47	0.27	0.24	-0.12
	SYP5	2	1.84	0.65	0.17	0.46	0.63
	Ca12	4	2.61	1.07	0.43	0.62	0.3
	Ca5	7	4.65	1.69	0.3	0.79	0.62
	Ca3	9	7.6	2.1	0.4	0.87	0.54
	Ca1	5	2.92	1.26	0.97	0.66	-0.47
	$\overline{X}$	**6.75**	**4.39**	**1.42**	**0.43**	**0.66**	**0.31**
average	$\overline{X}$	7.22	4.37	1.48	0.46	0.67	0.29

N=Sample sizes, N_a=No. alleles, N_e= No. effective alleles, I= Information index, H_o =Observed Heterozygosity, H_e= Expected Heterozygosity, and F_{is}= Inbreeding Coefficient

TABLE 3. Summary of Chi-Square Tests for Hardy-Weinberg Equilibrium in different sampling Regions

Locus	Lamir River			Sefid Rud			Shir Rud			Tajan River		
	Df	X^2	**P value**	**Df**	X^2	**P value**	**Df**	X^2	**P value**	**Df**	X^2	**P value**
SYP4	66	127.00	0.000***	78	147.90	0.000***	105	160.50	0.000***	91	199.84	0.000***
SYP2	45	136.16	0.000***	91	150.62	0.000***	66	170.57	0.000***	45	153.27	0.000***
SYP6	3	0.93	0.820ns	3	1.20	0.75 ns	3	1.51	0.68 ns	3	0.71	0.87 ns
SYP5	1	21.23	0.000***	3	17.79	0.000***	6	46.76	0.000***	1	12.05	0.000***
CA12	15	70.88	0.000***	15	52.63	0.000***	10	49.58	0.000***	6	30.37	0.000***
CA5	15	43.18	0.000***	28	41.78	0.050***	15	67.36	0.000***	21	111.66	0.000***
CA3	55	187.97	0.000***	45	180.05	0.000***	28	79.32	0.000***	36	127.98	0.000***
CA1	15	29.45	0.010*	10	30.00	0.000***	15	31.71	0.010**	10	69.26	0.000***

TABLE 4. Analysis of genetic differentiation between pairs of populations across all loci based on estimates of F_{st} values above and R_{st} values (above the diagonal) and R_{st} values below diagonal).

	Tajan	Shir Rud	Sefid Rud	Lamir
Tajan	****	0.01*	0.05**	0.04*
Shir Rud	0.12*	****	0.03**	0.04*
Sefid Rud	0.34**	0.40**	****	0.08ns
Lamir	0.09*	0.01*	0.04*	****

*P<0.05 **P<0.01 ns= not significant

TABLE 5. The F_{is} for *R. frisii kutum* in four rivers of the south Caspian Sea

Population Locus	Tajan River	Shir Rud	Sefid Rud	Lamir River
SYP4	0.41	0.10	0.40	0.46
SYP2	0.53	0.26	0.15	0.40
SYP6	-0.12	-0.16	-0.14	-0.16
SYP5	0.63	0.67	0.71	0.84
CA12	0.30	0.53	0.41	0.38
CA5	0.62	0.32	0.34	0.38
CA3	0.54	0.54	0.66	0.65
CA1	-0.47	-0.05	-0.55	-0.28

TABLE 6. AMOVA analysis of microsatellite loci, considering variation among and within all 4 populations (Rst)

Comparison	df	Sum of squares	Variance components	percentage of variation	Fixation indices
Among Regions	1	19.675	0.123	4%	F_{rt} 0.0416
Among Populations within regions	2	9.783	0.035	1%	F_{sr} 0.0123
Within Populations	236	661.367	2.802	95%	F_{st} 0.0534
Total	239	690.825	2.96		

RESULTS

Locus SYP4 had the highest numbers of alleles (19), while the locus SYP6 had the lowest (3) (Table 2). The average (and range) for the number of alleles in the four rivers was 7.22 (6.75-7.75), the effective number of alleles 4.37 (4.09-4.57), the observed heterozygosity was 0.46 (0.43-0.49) less than the expected heterozygosity of 0.67 (0.66-0.69).

A large variation in H was observed among loci, and ranged from 0.07 at SYP5 at Lamir River to 1.00 at Ca 1 at Tajan (Table 2). For a given locus, Ho varied greatly among the samples. For example, at locus SPY4, Tajan River and Sefid Rud are the same and (0.53) and Shir Rud is 0.83 and Lamir River is 0.47.

The maximum average H_o was 0.91 in locus Ca1 and minimum was 0.13 in locus SYP5 and maximum average of H_e was 0.89 in locus SYP41 and minimum was 0.28 in locus SYP6 (Table 2). The highest H_e was 0.92 in locus SYP4 in Shir Rud and lowest was 0.24 in locus SYP6 in Tajan River (Table 2). The average allele per locus was 7.22, maximum allele per locus was 15 and minimum allele per locus was 2.

All rivers deviated from Hardy-Weinberg Equilibrium expectations ($P<0.01$) except at the SPY6 locus (Table 3).

The highest inbreeding coefficient (Fis), was in Lamir River (0.84) at locus SPY5 and in Sefid Rud at locus SPY5 (0.71), at locus Ca12 (0.67) at Shir Rud and SPY5 (0.63) in Tajan River. The lowest Fis is at locus Ca1 (-0.55) in Sefid Rud, in locus Ca1 (-0.47) in Tajan River, in locus Ca1 (-0.28) in Lamir River and in locus SPY6 (0.16) in Shir Rud (Table 5).

AMOVA analysis showed that most genetic variation was concentrated among individuals within the same population (95%), that among regions was 4%, and that among populations within regions was 1% and significant (Table 6).

The UPGMA dendogram based on the genetic distance computed by Nei (1972) (Fig. 2) showed differences between all four sampling regions but not as defined clusters:

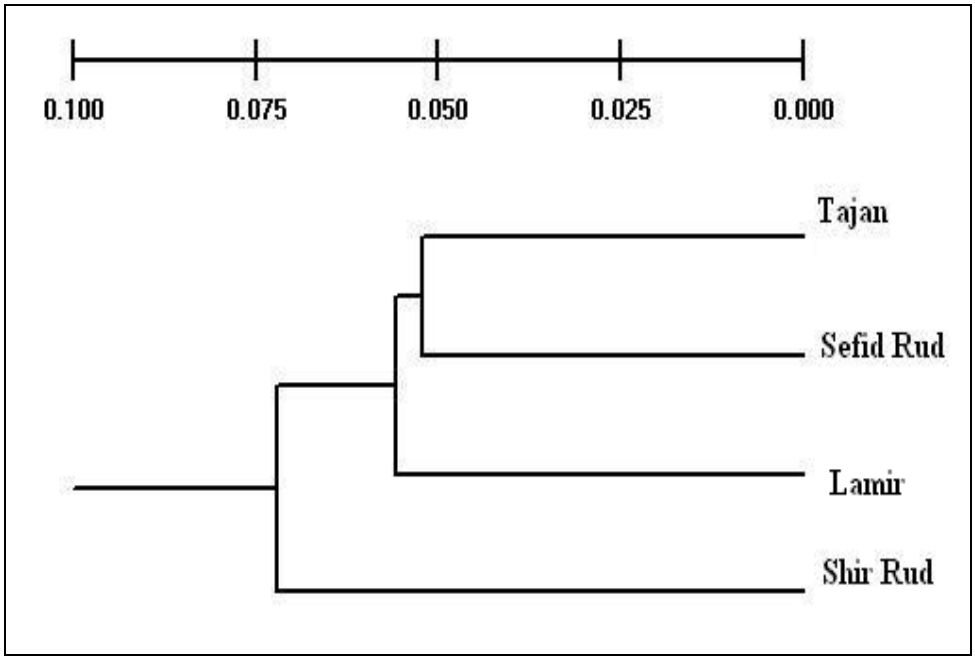

FIGURE 2. UPGMA dendrogram based on the Nei (1978) genetic distance between *Rutilus frisii kutum* populations, based on microsatellite DNA analysis.

DISCUSSION

This is the first report of using microsatellite marker to study genetic variation of Mahi sefid populations in the south Caspian Sea, which provides valuable information on genetic variation and population structure of species. Despite using a majority of Mahi sefid stocks from artificial propagation (Abdolhay et al., 2011), the knowledge on molecular genetics structure of this unique species has not been available to date.

Significant deviations from Hardy-Weinberg equilibrium were found at all except the SYP6 locus, and at more loci than described in Azerbaijan populations of *R. frisii kutum* (Kavan et al., 2009). It suggested that this may be the result of the fact that many fish in the Iranian populations have been derived from artificial reproduction.

The significant deviations from Hardy-Weinberg equilibrium could be explained by either sample size or the presence of null alleles in populations. The null alleles have been reported in *Rutilus rutilus* (Keyvanshokooh et al., 2007). 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 (Keyvanshokooh et al., 2007). Qasemi et al. (2006) stated that inbreeding of *Acipenser nudiventris* occurred in hatcheries and could lead to deviations from Hardy-Weinberg equilibrium as was observed also in *Acipenser siensis* (Zhao et al., 2005).

Genetic variability estimates for *Rutilus frisii kutum* revealed that the observed heterozygosity of 0.46 and the average alleles per locus of 7.22 were lower than those reported by De Woody and Avise (2000) for most anadromous fish (heterozygosity 0.68; alleles per locus 11.3). The study by Kavan et al. (2009) on *Rutilus frisii kutum* between Iran and Azerbaijan reported the observed heterozygosity in the Tonkabon River (0.62) was higher than those of the populations in Azerbaijan (0.47) and alleles per locus was 5.22 in Iran and 5.77 in Azerbaijan.

Percentage of AMOVA variation in this study between regions was 4%, between populations within region was 1 % and within population was 95%. This pattern of limited regional variation and large

levels of local population diversity is similar to reports in other studies. Chakmehduoz et al. (2009) studied two migrations form of Mahi sefid, autumn and spring races and found 86% of genetic diversity within autumn race and spring races and 14% between groups. The AMOVA analyses on *Rutilus frisii kutum*, from two sampling regions in Iran and Azerbaijan Republic, and four sampling locations (Iran: Khoskrud River, Tonkabon River, Gorganrud River and Azerbaijan: Kura River) revealed that the average variance was 86% within locations, 12% among locations and 2% among regions (Kavan et al., 2009). Indeed, the AMOVA for autumn and spring race revealed more sub-division than our study. Chenghui et al. (2010) studied three subspecies of common carp in China. AMOVA showed low population differentiation, with 11.60% of the molecular variance found among river drainages. Pairwise F_{ST} values between river drainages were moderate (0.033–0.261). A study by Nan et al. (2010) revealed 14 microsatellite loci in Chinese sucker and number of alleles per locus ranged from 6 to 14 with the observed (H_O) and expected (H_e) heterozygosities ranging from 0.120 to 1.000 and from 0.767 to 0.952, respectively. Five loci displayed significant departures from Hardy-Weinberg expectations. A study by Swatdipong et al. (2010) revealed 13 microsatellite in brown trout, *Salmo trutta*, populations were clustered into three separate groups, largely corresponding to geographic regions, with between-region F_{ST} values ranging from 0.11 to 016.

Natural spawning grounds of Mahi sefid have been destroyed due to damming on rivers, harvest of adults for brood stock during their migration to rivers, and pollution, all of which has resulted in decreasing in number of adults in coastal waters of Iran. The most of restocking of Mahi Sefid, under Iranian Fisheries Organization programs, are carried out by releasing fingerlings. To restock this valuable species in the Caspian Sea, annually, more than 200 millions fingerlings (average weight 1 g) were produced and released into the Caspian Sea. The hatchery populations used for restocking are mixed populations from various rivers, which may cause loss of alleles. Reduction in genetic heterozygosity may also be explained by inbreeding and genetic drift. Genetic variability loss recovers very slowly by mutation or migration (Avise, 1994). Therefore, suitable genetic management of hatchery fish is an important step to increase the success of stocked fish into the aquatic habitat. Significant deviations from Hardy-Weinberg equilibrium were found at several loci in these four rivers. This may be explained by the presence of null alleles and/or by, heterozygote deficiency due to inbreeding between related individuals in the Iranian populations. Generally such deviations indicate that factors such as non-random mating, reduction in effective breeding population or specific locus could be under selection pressure and were the cause for observed deviations (Garcia et al., 1998; Rezvani-Gilkolaei, 2000). Pairwise genetic differentiation (F_{st}) was used to assess population subdivision. Ward et al. 1994 and Yue et al. 2009 reviewed seven anadromous fish species and observed the F_{st} estimates with a mean of 10 %. In this survey, the F_{st} value between the four rivers was significantly different ($P<0.05$), suggesting that all of these four populations were significantly differentiated that can be explained by geographical distance as found by Kavan et al. (2009). Genetic differentiation can be influenced by a number of evolutionary forces and their interaction that act on natural populations. Examples of such forces include migration, random genetic drift and mutation (Hartl and Clark, 1997).

Reduction in genetic variation through inbreeding and genetic drift is very common in a hatchery population. Loss of genetic variation is considered the loss of genetic potential for stock improvement and adaptation to environmental changes. Therefore, according to Alam and Islam (2005), It is essential to monitor any change in the genetic structure of the hatchery population with respect to a base population or wild populations.

The study suggested that there was a low genetic variability in four populations of Mahi Sefid in the south Caspian Sea but inbreeding happened. It showed the high inbreeding happened in Mahi Sefid population (because the positive of Fis). The is possibly related to artificial production of fingerling in last 20 years ago.

ACKNOWLEDGMENTS
The authors wish to thank the lab. members of Mazandaran Research Center, especially Mr. Laloei and Mr Taghvai. We are also grateful to Miss Safari. This study was funded by Iranian Fisheries Research Organization.

LITERATURE CITED

Abdolhay, H.A., 1997. Artificial reproduction of fish for stock enhancement in south of the Caspian Sea, Seventh Conference of Shilat, Responsible Fisheries. Iranian Fisheries Organization PP.187-207 (In Persian) 17-18 February 1997, Tehran, Iran.

Abdolhay, H.A., Daud, S.K., Rezvani, S., Pourkazemi, M., Siraj, S.S., Abdul-Satar, M.K., 2011. Fingerling production and stock enhancement of Mahisefid *(Rutilus frisii kutum)* lessons for others in the south of Caspian Sea. *Review Fish Biology and Fisheries* 21, 247-257.

Alam, M.S., Islam, M.S., 2005. Population genetic structure of *Catla catla* (Hamilton) revealed by microsatellite DNA markers. *Aquaculture* 246, 151-160.

Avise , J.C., 1994. *Molecular Markers. Natural History and Evolution*. Chapman and Hall, New York.

Bartley, D.M., Leber, K.M., 2004. *Marine Ranching*. Fishery Resources Division FAO Fisheries Department. FAO Fisheries Technical Paper, No. 429. FAO Rome.

Castro, J., Pino, A., Hermida, M., Bouza, C., Chavarrías, D., Merino, P., Sánchez, L., Martínez, P., 2007. A microsatellite marker tool for parentage assessment in gilthead sea bream *(Sparus aurata)*. *Aquaculture* 272, 210-216.

Castro, J., Pino, A., Hermida, M., Bouza, C., Riaza, A., Ferreiro, I., Sanchez, L., Martinez, P., 2006. A microsatellite marker tool for parentage analysis in Senegal sole *(Solea senegalensis)*: Genotyping errors, null alleles and conformance to theoretical assumptions. *Aquaculture* 261, 1194-1203.

Chang, Y., Liang, L., Ma, H., He, J., Sun, X., 2008. Microsatellite analysis of genetic diversity and population structure of Chinese mitten crab *(Eriocheir sinensis)*. *Journal of Genetics and Genomic*s 35, 171-176.

Chistiakov, D.A., Hellemans, B., Volckaert, F.A.M., 2006. Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. *Aquaculture* 255, 1-29.

Cunningham, E.P., 1999. The application of biotechnologies to enhance animal production in different farming systems. *Livestock Production Science* 58, 1-24.

Davis, G.P., Hetzel, D.J.S., 2000. Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species. *Aquaculture Research* 31, 3-10.

Dixon, T.J., Coman, G.J., Arnold, S.J., Sellars, M.J., Lyons, R.E., Dierens, L., Preston, N.P., Li, Y., 2008. Shifts in genetic diversity during domestication of Black Tiger shrimp, *Penaeus monodon,* monitored using two multiplexed microsatellite systems. *Aquaculture* 283, 1-6.

Ferguson, M.M., Danzmann, R.G., 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? *Canadian Journal* of *Fisheries and Aquatic Science* 55, 1553-1563.

Garcia, F.J., Canonne, M., Quillet, E., Bonhomme, F., Chatain, B., 1998. The application of microsatellite markers to breeding programmes in sea bass*, Dicentrarchus labrax*. *Aquaculture* 159, 303-306.

Gheyas, A.A., Woolliams, J.A., Taggart, J.B., Sattar, M.A., Das, T.K., McAndrew, B.J., Penman, D.J., 2009. Heritability estimation of silver carp *(Hypophthalmichthys molitrix)* harvest traits using microsatellite based parentage assignment. *Aquaculture* 294, 187-193.

Golda, J.R., Jobity, A.M.C., Saillant, E., Renshawa, M.A., 2010. Population structure of carite (*Scomberomorus brasiliensis*) in waters offshore of Trinidad and northern Venezuela. *Fisheries Research* 103, 30-39.

Gonzalez, B., Masaki, A., Nobuhiko, T., 2011. Genetic interactions between wild and hatchery Red Sea Bream confirmed by microsatellite genetic markers, 4th International Symposium on Stock Enhancement and Sea Ranching, Shanghai Ocean University, 21-23 April.

Ha, H.P., Nguyen, T.T.T., Poompuang, S., Na-Nakorn, U., 2009. Microsatellites revealed no genetic differentiation between hatchery and contemporary wild populations of striped catfish, *Pangasianodon hypophthalmus* (Sauvage 1878) in Vietnam. *Aquaculture* 291, 154-160.

Hartl, D.L., Clark, A.G., 1997. *Principles of Population Genetics*. 3 ed. Sinauer Associates, Sunderland, Massachusetts.

Haynes, G.D., Gongora, J., Nicholas, F.W., Zenger, K.R., 2009. Rapid identification of maternal lineages in common carp (*Cyprinus carpio* L.) using real-time PCR and high resolution melt-curve analysis. *Aquaculture* 287, 59-66.

Hsu, T.-H., Wang, Z.-Y., Takata, K., OnozatoH, i., Hara, T., Gwo, J.-C., 2010. Use of microsatellite DNA and amplified fragment length polymorphism for Cherry salmon *(Oncorhynchus masou)* complex identification. *Aquaculture Research* 41, 316-325.

Kasapidis, P., Magoulas, A., 2008. Development and application of microsatellite markers to address the population structure of the horse mackerel *Trachurus trachurus*. *Fisheries Research* 89, 132-135.

Kavan, L.S., Rezvani-Gilkolaei, S., Vossoughi, G., Fatemi, S.M.R., Safari, R., Jamili, S., 2009. Population genetic study of *Rutilus frisii kutum* (Kamensky 1901) from the Caspian Sea, Azerbaijan regions, using microsatellite markers *Journal of Fisheries and Aquatic Science* 4, 316-322.

Keyvanshokooh, S., Ghasemi, A., Shahriari-Moghadam, M., Nazari, R.M., Rahimpour, M., 2007. Genetic analysis of *Rutilus rutilus caspicus* (Jakowlew 1870) populations in Iran by microsatellite markers. *Aquaculture Research* 38, 953-956.

Kitada, S., Hamasaki, K., Nakajima, K., Miyakoshi, Y., Hirohisa, K., 2011. Rearing and genetic effects on fitness of artificial produced animals in the wild: empirical evaluation of large scale fishery stock enhancement programs, 4th International Symposium on Stock Enhancement and Sea Ranching, Shanghai Ocean University, April 21 to 23 2011.

Lal, K.K., Chauhan, T., Mandal, A., Singh, R.K., Khulbe, L., Ponniah, A.G., Mohindra, V., 2004. Identification of microsatellite DNA markers for population structure analysis in Indian major carp, *Cirrhinus mrigala* (Hamilton-Buchanan, 1882). *Journal Applied Ichthyology* 20, 87-91.

Launey, S., Morin, J., Minery, S., Laroche, J., 2007. Microsatellite genetic variation reveals extensive introgression between wild and introduced stocks, and a new evolutionary unit in French pike (*Esox lucius* L.). *Aquaculture* 272, S282-S283.

Liu, Y.-G., Guo, Y.-H., Hao, J., Liu, L.-X., 2011. Genetic diversity of swimming crab *(Portunus trituberculatus)* populations from Shandong peninsula as assessed by microsatellite markers. *Biochemical Systematic and Ecology* 41, 91-97.

Messmer, A.M., Rondeau, E.B., Jantzen, S.G., Lubieniecki, K.P., Davidson, W.S., Koop, B.F., 2011. Assessment of population structure in Pacific *Lepeophtheirus salmonis* using single nucleotide polymorphism and microsatellite genetic markers. *Aquaculture* 320, 183-192.

Nan, C., Huan, L.W., Wei, M.W., Vibeke, S., 2010. Isolation of polymorphic microsatellite loci from an endangered freshwater species Chinese sucker, *Myxocyprinus asiaticus*. *Conservation Genetic Resources* 2, 73-75.

Peakall, P., Smouse, P.E., 2006. GENALEX 6*:* genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288-295.

Remmy, K.B.P ,.Siraj, S.S., Daud, S.K., 2005. *Population structure of Ikan Kelah* Tor tambrodies*, based on newly developed DNA microsatellite markers.* , In: Arshad, A.D., S.K, Siraj, S. S and Qunah,S.C (Eds.), 8th Symposium Applied Biology. University Putra Malaysia.

Rezvani-Gilkolaei, S., 2000. Study of mtDNA variation of Russian sturgeon population from the South Caspian Sea using RFLP analysis of PCR amplified ND5/6 gene regions. *Iranian Journal of Fisheries Sciences* 2, 13-36.

Russell, J., Jerry, D., Thuesen, P.A., Thomson, F.E., Smith-Keune, C., 2011 ,Ecological and genetic impacts of Barramundi (*Lates Calcarifer*) stocking in Northern Australia, 4th International Symposium of Stock Enhancement and Sea Ranching, Shanghai Ocean University, 21-23 April.

Shikano, T., Shimada, Y., Suzuki, H., 2008. Comparison of genetic diversity at microsatellite loci and quantitative traits in hatchery populations of Japanese flounder *(Paralichthys olivaceus)*. *Journal of Fish Biology* 72, 386-399.

Skaala, O., Hoyheim, B., Glover, K., Dahle, G., 2004. Microsatellite analysis in domesticated and wild Atlantic salmon (*Salmo salar* L.): allelic diversity and identification of individuals. *Aquaculture* 240, 131-143.

Somridhivej, B., Wang, S., Sha, Z., Liu, H., Quilang, J., Xu, P., Li, P., Hu, Z., Liu, Z., 2008. Characterization, polymorphism assessment, and database construction for microsatellites from BAC end sequences of channel catfish (*Ictalurus punctatus*): A resource for integration of linkage and physical maps. *Aquaculture* 275, 76-80.

Taniguchi, N., Sugama, K., 1990. Genetic variation and population structure of red sea bream in the coastal waters of Japan and the East China Sea. *Nippon Suisan Gakkaishi* 56, 1069-1077.

Thai, B.T., Burridge, C.P., Austin, C.M., 2007. Genetic diversity of common carp (*Cyprinus carpio* L.) in Vietnam using four microsatellite loci. *Aquaculture* 269, 174-186.

Tong, J., Wang, Z., Yu, X.W., Q., Chu, K.H., 2002. Cross-species amplification in silver carp and bighead carp with microsatellite primers of common carp. *Molecular Ecology Notes* 2, 245-247.

Tripp, V.M.A., García de León, F.J., Ortega-García, S., Lluch-Cota, D., López-Martínez, J., Cruz, P., 2010. Population genetic structure of dolphin fish (*Coryphaena hippurus*) in the Gulf of California, using microsatellite loci. *Fisheries Research* 105, 172-177.

Vandeputte, M., Kocour, M., Mauger, S., Rodina, M., Launay, A., Gela, D., Dupont-Nivet, M., Hulak, M., Linhart, O., 2008. Genetic variation for growth at one and two summers of age in the common carp (*Cyprinus carpio* L.): Heritability estimates and response to selection. *Aquaculture* 277, 7-13.