Study of genetic diversity of wild Caspian trout *Salmo trutta caspius* in the Sardabrud and Astara Rivers, using D- Loop region sequencing

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Abstract

In this study the genetic diversity of wild Caspian trout (*Salmo trutta caspius*) in the Sardabroud and Astara Rivers was evaluated using D- Loop region sequencing. For this purpose, 35 specimens of adult Caspian brown trout were collected from these rivers in the Mazandarn and Gilan Provinces in fall and winter 2011. Approximately 3-5 g of soft and fresh fin tissue was isolated and fixed in ethanol 96% and then transferred to the Caspian Sea Ecology Research Center Genetics Laboratory in Sari, Iran. Genomic DNA from the samples was extracted using Ammonium Acetate Method. The quality and quantity of the extracted DNA were assessed by spectrophotometer and agarose gel (1%) electrophoresis. Polymerase Chain Reaction (PCR) was performed on the target DNA using a primers sequence D- Loop region of mtDNA molecule. Then the product was purified and DNA sequencing was carried out using chain termination method. The D- Loop region of Caspian trout contained 654 bp. Data were analyzed using Bio-Edit, DnaSP, Arlequin and Mega software. 20 and 15 haplotypes was observed in Sardabrud and Astara River. Tthe DNA sequence of one of them was recorded in Gene Bank with numbers KC991027 and KF015727. 223 and 240 polymorphic loci were detected in Sardabrud and Astara River that all of them were out of Hardy- Weinberg equilibrium (p<0.05). Average nucleotide and haplotype diversity were 0.127±0.067, 1.000±0.005 in Sardabrud River and 0.118±0.063 and 1.000±0.005 in Astara River.

Keywords: Genetic diversity, *Salmo trutta caspius*, Sardabrud, Astara, Sequencing

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Introduction

Caspian trout, *S. trutta caspius* Kessler, 1870 is one of the nine subspecies of brown trout *Salmo trutta* in the world (Quillet *et al.,* 1992) and is an anadromous form and endemic subspecies of the Caspian basin. The most important Iranian rivers for their spawning consist of, Karganrud, Navrud, Astarachay in Gilan Province and Tonekabon (Cheshmehkileh), Chalus, Sardabrud in Mazandaran Province. A loss of intra and inter population genetic diversity through exploitation of brown trout populations, stocking of hatchery bred fish, transfer of fish from other localities, pollution, alteration and degradation of habitats are considered to be the main threats to wild brown trout populations (Laikre, 1999). In Iran, Caspian trout populations that has been considered for a biological conservation program in the southern part of the Caspian Sea (Hasanzadeh Kiabi *et al*., 1999) have experienced a strong decline during the past two decades as a result of population growth, development of fishing equipment, overfishing, habitat pollution and reduction in spawning areas and similar to other brown trout populations is at risk of extinction and was listed as threatened in the Red List of International Union for Conservation of Nature (IUCN)

An understanding of the genetic diversity in aquatic organisms can be useful in stock conservation. Genetic diversity is important in both natural and cultural populations because it provides the necessary spectrum of genotypes for adaptive response to changing conditions and heterozygous individuals usually are superior to less heterozygous individuals in many economically important characteristics like growth, fertility and disease resistance (Beardmore *et al.,* 1997).

Wild populations and the preservation of their genetic purity play a key role in the conservation of Caspian trout genetic resources. As a first step, the remaining wild populations need to be identified and genetically characterized (Liu and Cordes, 2004). mtDNA has a number of characteristics that makes it a valuable molecular marker for evolutionary and population-genetic structure studies (Zhang and Hewitt, 2003). mtDNA is inherited maternally without intermolecular recombination and it has a higher mutation rate (Avise, 2000), which is one of the reasons for its use in the majority of phylogeographic studies (Bernatchez, 2001; Cortey *et al.,* 2004; Maric *et al.,* 2006; Vera *et al.*, 2010a; Kohout *et al.*, 2013). A high copy number of mitochondrial genome by a factor of up to 10,000 (1000 mitochondria per cell, each 10 copies of the genome) is advantageous (Alberts *et al.,* 1990). The mtDNA evolves much faster than nuclear DNA and thus contains more sequence diversity compared to ncDNA (Brown *et al*., 1979; Brown *et al*., 1982; Vawter and Brown, 1986; Bavornlak *et al*., 2009). The nucleotide sequence of D- Loop region is considered to be variable and

with no effect on transcription and replication. In fact D- Loop is the most variable region of mtDNA. Substantial genetic variation is found in the D-Loop region, even among individuals within a given species. Haplotype analysis of the D- Loop region is a useful tool for revealing genetic diversity, which is essential for the preservation of species. Nowadays decreases in populations lead to reduced genetic diversity, which can cause a population survival crisis (Cecconi *et al.,* 1995). The PCR technique is used to amplify a specific region of a DNA strand and then the target gene can be recognized from the other genes by electrophoresis. Rows of nucleic acids and bases will be cleared by standard techniques for DNA sequencing (Newton and Graham, 1997).

Molecular markers, such as mtDNA D- Loop and cytochrome b, microsatellites, RFLP (Restriction Fragments Length Polymorphism) and AFLP (Amplified Fragments Length Polymorphism), were recently used in a variety of investigations regarding brown trout (Tosic *et al.,* 2014). Rezvani Gilkholahi *et al.* (2006) conducted PCR-RFLP analysis of mitochondrial DNA for identification of Caspian roach (*R. rutilus caspius*) populations in the southern coast of the Caspian Sea, Iran. Atabeyoglu (2007) determined genetic differences between mtDNA D- Loop F1 and 12S1-H region of native salmons (*Salmo trutta sp*.) caught in the rivers of Aras, Kapasu and Coruh using PCR- RFLP and microsatellite method. Vera *et al*. (2010b) evaluated the population and family structure of brown trout, *S. trutta*, in a Mediterranean stream. Apostolidis *et al*. (2011) Genetic divergence among native trout *S. trutta* populations from southern Balkans based on mitochondrial DNA and microsatellite variation. Nematzadeh *et al*. (2012) determined genetic differences and phylogenic relationships among six Mugilidae species (*Mugil cephalus, M. capito, Liza subviridis, L. saliens, L, aurata, Valmugil buchanani*) using PCR-equencing. Kohout *et al*. (2013) assessed genetic diversity and phylogenetic origin of brown trout *S. trutta* populations in eastern Balkans. Saeidi *et al*. (2014) studied population genetic studies of golden mullet (*L. aurata*) using D- Loop sequencing in the southeast and southwest coasts of the Caspian Sea. Tosic *et al*. (2014) evaluated new mtDNA Haplotype of Brown Trout *S. trutta* l. from Crni Timok Drainage Area in Serbia.

Despite the economic importance of the Caspian trout, study on its genetic and population structure in the rivers of south Caspian Sea is scarce and more studies are necessary. This study was conducted to determine the causes and the extent of decline in brown trout fishery, and to protect indigenous Caspian trout populations in the Southern Caspian Sea and provide conservational management strategies to regulatory agencies. It also provides

basic information on effective and sustainable brood stock management and conservation of this valuable endemic fish of the Caspian Sea and can be applied for future genetic improvement and assessment of this species in hatcheries and to design suitable management guidelines for artificial breeding activities.

Material and methods

Sample collection

A total of 35 caudal fin samples of wild Caspian trout were collected from Sardabrud River in Mazandaran Province and Astara River in Gilan Province by small beach seine and cast net in fall and winter 2011 (Table 1).

Table 1: The Geographical location of rivers for Caspian trout sampling.

River	Latitude	Longitude	S. No.
Sardabrud	36° 41	51° 23	20
Astara	38° 24'	48° 27'	15

Samples were kept in 96% ethanol (Barber *et al.,* 2000) and then transferred to the Genetics Laboratory located at the Caspian Sea Ecology Research Center, Sari, Iran. The extracted DNA was stored at -4ºC until use.

Genomic DNA extraction

Total DNA was extracted from 50 mg of fin sample by ammonium acetate method (McQuown *et al*., 2000). The quality and quantity of the extracted DNA were assessed by spectrophotometer (Bio photometer, Eppendorf) and agarose gel (1%) electrophoresis (Tsoi *et al.,* 2005). DNA absorption was measured by spectrophotometer at 260 and 280 nm wavelengths. Samples with a ratio of 1.8 to 2 were selected and DNA was then re-extracted from unsuitable samples. Purified DNA was stored at -20ºC until use.

PCR

PCR was used for amplification of target DNA. PCR was performed in an Auto-Qselver Thermal Cycler (Quanta biotech Company, England) using primers D- Loop region F1 (5'−TGGCATTTGGTTCCTACTTCAGG−3', 12S1-H Reverse (- 5'−TGCGGAGACTTGCATGTGTAAGT−3') (Atabeyoglu, 2007) under the following conditions: Initial denaturation (94ºC, 3 min) followed by 30 cycles of strand denaturation (94ºC, 30 sec), primers annealing (48ºC, 45 sec) and DNA extension (72ºC, 45 sec); the last extension prolonged to 5 min) in the Thermo Cycler. PCR was performed in 25 µL reaction volumes. Each reaction contained 1X PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTP, 1.2 nM of each primer 1U of *Taq* DNA polymerase (CinnaGen Company) and 5-10 ng of genomic DNA.

Gel electrophoresis and staining

Amplified DNA fragments were run on 1.5% agarose gel at 90 V for 45 min using horizontal electrophoresis and stained with ethidium bromide for visualization and DNA ladder 50bp (MBI Fermentase Company) was used to calculate the fragment length. The

PCR products were separated for visualization on 1.5% agarose gels containing ethidium bromide at 90 V for 45 min. Photographs of the gels.

DNA sequencing

The fragment length of D- Loop sequencing in the Caspian trout was evaluated to be 654 bp. Single-pass sequencing was performed on each template using forward (D- Loop) primer. PCR products were purified and sequenced at BIONEER Company using modified Sanger sequencing method (Tosic *et al.,* 2014).

Statistical analysis

Data were analyzed by Bio-Edit (Ver. 7.1.3.0) (Hall, 1999), DnaSP (Ver. 5.10.01) (Rozas *et al.,* 2003), Arlequin (Ver. 3.5.1.2) (Excoffier *et al.,* 2005) and Mega (Ver. 5.05) (Tamura *et al.,* 2007) software. All sequences were aligned with Clustal X multiple-alignment program (Thomson *et al*., 1997) in Bio-Edit software. Haplotype frequencies among populations (Excoffier, 2004), population pairwise Fst_s and their significance (Reynolds *et al.,* 1983), the polymorphic genetic loci, the number of gene copies, the number of alleles and the expected heterozygosity (Nei, 1987), the real and expected number of alleles (Slatkin, 1995), the gene diversity, nucleotide composition and the number of transition and transversion (Tajima, 1993), nucleotide diversity (P) for each population and mean number of pairwise differences (Excoffier, 2004), divergence time (Tajima, 1996) were estimated using Arlequin software. Genetic distance within samples was estimated using Kimura 2-parameter 1980 software (Kumar *et al.,* 2004) by Mega. The haplotype diversity (*h*), fixation index (*F*st) and Gene flow (N_m) were calculated using DnaSP software. The mean difference of paired nucleotide within and among samples of regions was constructed using Mega. The partitioning of genetic diversity among and within populations was examined using analysis of molecular variance (AMOVA) (Excoffier *et al.,* 2005). The Φ statistics generated by AMOVA were used to assess population genetic differentiation.

Results

The sequence length of the samples determined was 654 bp. There are 35 haplotypes in the Sardabrud and Astara Rivers. Haplotypes were specific to each river and were not seen in other rivers. The two sequences have been deposited in database (NCBI) under the following accession numbers: KC991027 and KF015727.

The number of polymorphic loci was 208 and 201 in the Sardabrud and Astara Rivers.

The average of real allele was 1.422±0.651 and 1.410±0.575 in the Sardabrud and Astara Rivers.

The gene diversity was calculated as 1.000±0.039 and 1.000±0.045 in Sardabrud and Astara Rivers, respectively according to Nie (1987).

Haplotype frequencies in the Sardabrud and Astara Rivers were 0.09% and 0.10%, respectively and it was significantly different between these rivers (p<0.05).

The mean number of pairwise differences was 82.782±38.658 and 77.111±36.355 in the Sardabrud and Astara Rivers, respectively.

The average haplotype diversity in the Caspian trout caught was 1.000±0.005 in the Sardabrud and Astara Rivers and the highest average nucleotide diversity (0.127±0.067) was observed in the Sardabrud River (Table 2).

AMOVA analysis revealed that the majority of genetic variation (89%) occurred within populations (p<0.01) (Table 3).

Table 2: Number of Alleles (A), Real allele (N_A), Effective allele (N_E), Observed heterozygosity (H_O), Expected heterozygosity (H_E), Haplotype diversity (h), Nucleotide diversity (p), Tajima′s D and Hardy-Weinberg equilibrium (HWE) of Caspian trout samples.

River	*A*	N_A	N_E	H_O	H_E	*H*	*p*	HWE	Tajima′D
Sardabrud	20	1.422±0.651	2.238±0.485	0.126±0.195	0.371±0.145	1.000±0.005	0.127±0.067	0.033	0.338
Astara	15	1.410±0.575	2.117±0.334	0.118±0.177	0.321±0.141	1.000±0.005	0.118±0.063	0.021	-0.512

Table 3: Analysis of molecular variance (AMOVA) for sources (within populations, among populations) geographic scales: attributed to the different levels in the spatial hierarchy of *Salmo trutta caspius*.

Source	df	SS	MS	Est. Var.	% Var.	Φ	*p*
Among populations	114	692.145	7.718	6.21	11		
Within populations	3	69.523	31.128	0.412	89	0.845	<0.01

Phi (Φ) statistics are analogous to Wright's F-statistics

p-values are based on 99 permutations

The genetic distance between Sardabrud and Astara River was calculated as 0.02. Based on the Nei (1978) model, the rate of gene flow was 2.78.

The pairwise F_{ST}s was calculated (0.02) between Sardabrud and Astara River which indicated there was genetic differentiation among populations in these rivers (p<0.001).

Transition number was 90 and 71 and transversion number was 149 and 137 in the Sardabrud and Astara Rivers, respectively and the relative degree was calculated as 0.34 and -0.51 in the Sardabrud and Astara Rivers, respectively.

Discussion

The first step to protect biological diversity and genetic structure of fish populations being exploited is a sustainable harvest management strategy. This strategy should be based on accurate and robust methods such as

molecular data to maximize the uptake and utilization to the conservation of biodiversity (Thai *et al.,* 2006). Genetic markers and identification of diversity at the DNA level provide the opportunity to investigate the correct genetic differences between individuals. mtDNA is applied to identify fish stocks and determine stock contribution in mixed catches. mtDNA also provides useful information to study the genetic differences in fish (Murgia *et al.,* 2002). D- Loop, a displacement loop in mitochondrial DNA, is applied as a mediator at the beginning of replication. Nucleotide sequence from D- Loop region reveals diversity occurring without any effects on translation and replication. Nucleotide sequence in mtDNA occurs 10 times faster than nuclear DNA and D- Loop is the most changeable region of mtDNA (Cecconi *et al.,* 1995). Genetic diversity is one of the three levels of biodiversity, proposed by IUCN for conservation reserves (Lucentini *et al.,* 2006). Therefore, it is essential to study the genetic diversity of Caspian trout as a highly endangered species (Hasanzadeh Kiabi *et al.,* 1999).

There are several ways to assess the genetic diversity of biological communities but the allele frequency measurement is a useful tool for detecting expression and evolutionary relationships of close populations (Takezaki and Nei, 1996). In this study, Haplotypes were specific to each river and were not seen in other rivers. Also haplotype frequencies were significantly different between the rivers studied (p<0.05), which indicate the separation hypothesis of Caspian salmon populations in the rivers studied.

The real number of alleles (N_A) and effective allels (N_E) are the criteria to determine the polymorphic locus (Ferguson *et al.,* 1995). To determine the degree of heterozygosity is the most common measure of a population's gene diversity (Fei *et al.,* 2007).

The average number of alleles was less than the number reported for anadromous fish (11.3) (Dewoody and Avise, 2000) which can be attributed to several factors such as differences in temperature, salinity and nutrients in different habitats of the Caspian Sea.

The average observed heterozygosity was higher than that reported for anadromous fish (0.68) (Dewoody and Avise, 2000). Also the rate of observed heterozygosity was higher than the expected heterozygosity and there were also significant differences between the observed and expected heterozygosity (p<0.05), because genetic diversity of species that live in unstable and stressful environments is greater than that of the same species in a sustainable environment (Welch *et al.,* 2010).

*F*st is a common method for estimating genetic differentiation in genetic studies that is directly or indirectly related to the degree of gene flow or effective migration (N_m) between populations (Rousset, 2004). Population differentiation refers to the

degree to which populations are genetically distinct from one another (Toro and Caballero, 2005). In this study, the genetic differentiation between the rivers was low (Dorak, 2005).

High levels of gene flow between populations show the evolution of these groups and if it is low, it indicates that the evolution of populations is almost independent of each other (Slatkin, 1993). When N_m>1, the gene is the major factor in the creation of genetic differentiation and when N_m<1, genetic drift is the main factor to differentiate genetically (Li *et al.,* 2007). In this study, gene flow is the main factor to differentiate genetically and shows the evolution of populations of Caspian trout in the rivers of the study group.

Homozygosity increased, presence of null alleles, genetic drift, the intercourse between closely related species, mooring limited number of alleles, selection, mixing of populations, non-random mating, insufficient sampling and sampling error can cause a deviation from Hardy-Weinberg equilibrium (Callen *et al.,* 1993; McQuown *et al.,* 2003; Skaala *et al.,* 2004; Liu *et al.,* 2005; Zhao *et al.,* 2005; Dahle *et al.,* 2006; Chauhan *et al.,* 2007; Li *et al.,* 2007). In this study, samples of both rivers were out of Hardy-Weinberg equilibrium (p<0.05) which could be due to the presence of null alleles, fusion of kinship, non-random mating and mixing.

Shaklee *et al*. (1982) and Thorpe *et al*. (1994) showed that the average genetic distance of Nei (1978) for conspecific populations is 0.05 (range: 0.002-0.07) and that for congeneric species is 0.30 (range: 0.03-0.61). Therefore based on the genetic distance obtained in this study, the Caspian trout populations migrating to these rivers are conspecific populations.

Haplotypes are good indicators to determine the genetic differences and the level of genetic variation, or haplotypes that can vary from zero (all members of the population have the same haplotype) to one (all members of the population have different Haplotypes) (Aboim *et al.,* 2005). In this study, the highest average nucleotide diversity was observed in Sardabrud River and there were significant differences in nucleotide diversity between these rivers. Also average haplotype diversity (h) in both rivers was 1.000 and shows that all members of the population have different haplotypes.

Analysis of molecular variance is the appropriate test to determine the population structure and degree of genetic differentiation between populations (Grassi *et al.,* 2004). The results of the molecular variance in this study showed that there is genetic diversity within populations of the rivers and differences in genetic variation within and between populations of Caspian trout were significant in the rivers (p<0.01) which is indicative of the populations ability to respond to natural selection (Kalinowski, 2005).

Transition and transversion are the molecular diversity indices (Tamura *et al.*, 2004) and in this study, the highest level of molecular variation was observed in the Sardabrud River.

The problem of classification is determined by the degree of kinship. If $p<0.05$, reject the null hypothesis (equality between the tree and the rate of evolution) and if $p>0.05$ it shows the evolution of exchange rates between the trees (Tajima, 1993) and the Caspian trout degree of kinship in this study shows that equality between the tree and the rate of evolution.

Overall, the results showed that there are two different genetic groups of Caspian trout in these rivers.

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References

Aboim, M.A., Menezez, G.M., Schlitt, T. and Rogers, A.D., 2005. Genetic structures and history of populations of the deep- Sea fish *Helicolenus dactylopterus* inferred from mtDNA sequence analysis. *Molecular Ecology*, 14, 1343-1354.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J.D., 1990. Molekularbiologie der zelle. VCH Verlagsgesellschaft, Weinheim.

Apostolidis, A.P., Stoumboudi, M. Th., Kalogianni, E., Cote, G. and Bernatchez, L., 2011. Genetic divergence among native trout *Salmo trutta* populations from southern Balkans based on mitochondrial DNA and microsatellite variation. *Journal of Fish Biology*, 79(**7**), 1950–1960.

Atabeyoglu, K., 2007. Determination of genetic differences between mtDNA D- Loop F1 and 12S1-H region of native Salmons (*Salmo trutta sp.*) caught in the Rivers of Aras, Karasu and Coruh in our district using PCR- RFLP and microsatellite methods. Ms Thesis, Department of Fisheries, Institution of Natural and Applied Sciences, Ataturk University, 62P.

Avise, J.C., 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge.

Barber, P.H. and Erdman, M.V., 2000. Molecular systematic of the Gonodactylidae (*stomapoda*) using mitochondrial cytochrome oxidase C (submit) DNA sequencing data. *Journal of Crustacean Biology*, 20(**2**), 20-36.

Bavornlak, K., Sirawut, K. and Piamsak, M., 2009. Genetic diversity and geographic differentiation of the giant tiger shrimp (*Penaeus monodon)* in Thailand analyzed by mitochondrial COI sequences. *Biochemical Genetics*, 47, 42-55.

Beardmore, A.L., Mair, C.G. and Lewis, C.G., 1997. Biodiversity in aquatic systems in relation to aquaculture. *Aquaculture Research*, 28, 829-839.

Bernatchez, L., 2001. The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution*, 55, 351–379.

Brown, W.M., George, M. and Wilson, A.C., 1979. Rapid evolution of animal mitochondrial DNA. Proceeding of the National Academy of Sciences of the United State of America, 76, 1967-1971.

Brown, W.M., Prager, E.M., Wang, A. and Wilson, A.C., 1982. Mitochondrial DNA sequences of primers: tempo and mode of evolution. *Journal of Molecular Evolution*, 18, 225-239.

Callen, D.F., Thompson, A.D., Shen, Y., Philips, H.A., Richards, R.I., Mulley, J.C. and Sutherland, G. R., 1993. Incidence and origin of null alleles in the $(AC)_n$ microsatellite markers. *American Journal of Human Genetics*, 52, 922-927.

Cecconi, F., Giorgi, M. and Mariottini, P., 1995. Unique features in the mitochondrial D-Loop region of the European seabass *Dicentrarchus labrax*. *Gene*, 160(**2**), 149-155.

Chauhan,T., Lal, K.K., Mohinra, V., Singh, R., Punia, P., Gopalakrishnan Prakash, C.S. and Lakra, W.S., 2007. Evaluating genetic differentiation in wild populations of Indian major carp, *cirrhinus mirgala* evidence from allozyme and microsatellite. *Aquaculture*, 269, 135-149.

Cortey,M., and García- Marín, J.L., 2002. Evidence for hylogeographically informative sequence variation in the mitochondrial control region of Atlantic brown trout. *Journal of Fish Biology*, 60, 1058–1063.

Dahle, G., Jorstad, K.E., Rusas, H.E. and Ottera, H., 2006. Genetic characteristics of brood stock collected from four Norwegian coastal cod (*Gadus morhua*) populations. *ICES Journal of Marine Science*, 63, 209-215.

Dewoody, J.A. and Avise, J.C., 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology*, 56, 461-473.

Dorak, T., 2005. Basic population genetics. WWW. Dorak. Info/ genetics/popgen. Html.

Excoffier, L., 2004. Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. *Molecular Ecology*, 13, 853–864.

Excoffier, L., Laval, G. and Schneider, S., 2005. Arlequin Ver. 3.5: An integrated software package for population genetics data analysis. *Evolution Bioinformatics*, 1, 47-50.

Fei, Ch., Wei, Y. and Fu- Liana, Y., 2007. Isolation of DNA microsatellites and preliminary genomic analysis of Mud crab (*Cirrohina moliterolla*). *Zoological Research*, 28(**2**), 119-125.

Ferguson, A., Taggart, J.B., Prodohl, P.A., McMeel, O., Thompson, C., Stone, C.E., Mcginnity, Ph. and Hynes, R., 1995. The application of molecular marker to the study and conservation of fish populations, with special reference to *Salmo*. *Journal of Fish Biology*, 47 (Supplement A), 103-126.

Grassi, F., Imazio, S., Gomarasca, S., Citterio, S., Aina, R., Sgorbati, S., Skala, F., Patrignani, G. and Labra, M., 2004. Population structure and genetic variation within *Valeriana wallrothii* Kreyer in relation to different ecological locations. *Plant Science*, 166, 1437-1441.

Hall, T.A., 1999. BIOEDIT: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Hasanzadeh Kiabi, B., Abdoli, A. and Naderi, M., 1999. Status of the fish fauna in the South Caspian basin of Iran. *Journal of Zoology*, 18, 57-65.

Kalinowski, S.T., 2005. Polymorphic loci require large sample size or estimate genetic distance. *Heredity*, 94, 33-36.

Kohout, J., Sediva, A., Apostolou, A., Stefanov, T., Maric, S., Gaffaroglu, M. and Slechta, V., 2013. Genetic diversity and phylogenetic origin of brown trout *Salmo trutta* populations in eastern Balkans. *Biologia, Section Zoology*, 68(**6**), 1229-1237.

Kumar, S, Tamura, K. and Nei, M., 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform*, 5, 150–163.

Laikre, L., 1999. Conservation Genetic Management of Brown Trout (*Salmo trutta*) in Europe. Division in Population Genetics, Stockholm University, Sweden, pp. 5-50.

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* L.) populations. *Genetics and Genomics*, 34, 984-993.

Liu, Z.J. and Cordes, J.F., 2004. DNA marker technologies and their application in aquaculture genetics. *Aquaculture*, 238, 1-37.

Liu, Y., Chen, S., Li, J. and Li, B., 2005. Assessing the genetic structure of three Japanese flounder (*Paralichthys olivaceus*) stocks by microsatellite markers. *Aquaculture*, 243 (Supplement A), 103-111.

Lucentini, L., Palomba, A., Lancioni, H., Gigliarelli, L., Natali, M. and Panara, F., 2006. Microsatellite polymorphism in Italian population of northern pike (*Esox Lucius* L.). *Fisheries Research*, 80, 251-262.

Maric, S., Susnik, S., Simonovic, P. and Snoj, A., 2006.

Phylogeographic study of brown trout from Serbia, based on mitochondrial DNA control region analysis. *Genetics Selection Evolution*, 38, 411-430.

McQuown, E.C., Sloss, B.l., Sheehan, R.J., Rodzen, J., Tranah, G. and May, B., 2000. Microsatellite and analysis of genetic variation in sturgeon: new sturgeon primer sequences for *Scaphirhynchus* and *Acipenser*. *Transactions of the American Fisheries Society*, 139, 1380-1388.

McQuown, E.C., Krueger, C.C., Kincaid, H.L., Gall, G.A.E. and May, B., 2003. Genetic comparison of lake sturgeon population: differentiation based on allelic frequencies at seven microsatellite Loci. *Great Lakes Research*, 29, 3-13.

Murgia, R., Tola, G., Archer, S.N., Vallerga, S. and Hirano, J., 2002. Genetic identification of grey mullet species (Mugilidae) by analysis of mitochondrial DNA sequence: application to identify the origin of processed ovary products (bottarga). *Marine Biotechnology*, 4(**2**), 119-126.

Nei, M., 1978. Estimation of average heterozygosity and Genetic distance from a small number of individuals. *Genetics*, 89, 583-590.

Nei, M., 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Nematzadeh, M., Rezvani, S., Khalesi, M. K., Laloei, F. and Fahim, A., 2012. A phylogeny analysis on six mullet species (Teleosti: Mugillidae) using PCR-sequencing method. *Iranian Journal of Fisheries Sciences*, 12(**3**), 669-679.

Newton, C.R. and Graham, A., 1997. PCR: second edition. Springer-Verlag, New York.

Quillet, E., Faure, A., Chevassus, B., Kreig, F., Harache, Y., Arzel, J., Metailler, R. and Boeuf, G., 1992. The potential of trout (*Salmo trutta L.*) for mariculture in temperate waters. *Icelandic Agricultural Sciences*, 6, 63-76.

Reynolds, J., Weir, B.S. and Cockerham, C.C., 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, 105, 767-79.

Rezvani Gilkolaei, S., Eimanifar, A., Aghili, R. and Laloei, F., 2006. PCR-RFLP analysis of mitochondrial DNA for identification of *Rutilus rutilus caspicus* populations on the southern coast of the Caspian Sea, Iran. *Journal of Marine Biology*, 86, 1463-1467.

Rousset, F., 2004. Genetic structure and selection in subdivided populations Princeton. Princeton University Press.

Rozas, J., Sanchez, J.C., Delbarrio, X. and Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19, 2496-2497.

Saeidi, Z., Rezvani Gilkolaei, S., Soltani, M. and Laloei, F., 2014. Population genetic studies of *Liza aurata* using D- Loop sequencing in the southeast and southwest coasts of the Caspian Sea**.** *Iranian Journal of Fisheries Sciences*, 13(**1**), 216-227.

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

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

Slatkin, M., 1993. Isolation by distance in equilibrium and non- equilibrium population. *Evolution*, 47, 264-279.

Slatkin, M., 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, 139(**1**), 457–462.

Tajima, F., 1993. Simple methods for testing molecular clock hypothesis. *Genetics*, 135, 599-607.

Tajima, F., 1996. The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. *Genetics*, 143, 1457-1465.

Takezaki, N. and Nei, M., 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, 144, 389-399.

Tamura, K., Nei, M. and Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, 101, 11030-11035.

Tamura, K., Dudley, J., Nei, M. and Kumar, S., 2007. Mega 4: Molecular evolution genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596-1599.

Thai, B.T., Pham, T.A. and Austin, G.M., 2006. Genetic diversity of common carp in Vietnam using direct sequencing and SSCP analysis of the mitochondrial DNA control region. *Aquaculture*, 258, 228-240.

Thomson, J.M., 1997. The Mugilidae of the World. *Memoirs of the Queensland Museum*, 41, 457-62.

Thorpe, J.P. and Sol-Cave, A.M., 1994. The use of allozyme electrophoresis vertebrate systematics. *Zoologica Scripta*, 23, 8-18.

Toro, M.A. and Caballero, A., 2005. Characterization and conservation of genetic diversity in subdivided populations. *Philosophical Transactions of the Royal Society B: Biological Science*, 360(**1459**), 1367–1378.

Tosic, A., Dubravka, S., Vera, N., Danilo, M. and Predrag, S., 2014. New mitochondrial DNA haplotype of brown trout *Salmo trutta* l. from Crni Timok Drainage area in Serbia.

Turkish Journal of Fisheries and Aquatic Sciences, 14, 37-42.

Tsoi, K.H., Wang, Z.Y. and Chu, K.H., 2005. Genetic divergence between two morphological similar varieties of the kurma shrimp *Penaeus japonicas*. *Marine Biology*, 147, 367-379.

Vawter, L. and Brown, W.M., 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. *Science*, 234, 194-196.

Vera, M., Cortey, M., Sanz, N. and Garcia-Marin, J.L., 2010a. Maintenance of an endemic lineage of brown trout (*Salmo trutta*) within the Duero river basin. *Journal of Zoological Systematics and Evolutionary Research*, 48(**2**), 181–187.

Vera, M., Sanz, N., Hansen, M.M., Almodovar, A., Garcia- Marin, J.L., 2010b. Population and family structure of brown trout, *Salmo trutta*, in a Mediterranean stream. *Marine and Freshwater Research*, 61, 676–685.

Welch, D.J., Balagh, A., Newman, S.J., Lester, R.J., Moore, B., Herwerden, L., Horne, J., Allsop, Q., Saunders, T., Stapley, J. and Gribble, N.A.,2010. Defining the stock structure of northern Australia, S threadfin salmon species. FRDC project, NO. 2007/032.

Zhang, D.X. and Hewitt, G.M., 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology*, 12, 563-584.

Zhao, N., Ai, W., Shao, Z., Zhu, B., Brosse, S. and Chang, J., 2005. Microsatellites assessment of Chinese sturgeon (*Acipencer sinensis* Gray) genetic variability. *Journal of Applied Ichthyology*, 21(**1**), 7-13.