Feasibility of Identification of Fall and Spring Migrating Caspian trout (*Salmo trutta caspius*) by Using AFLP Molecular Marker

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Abstract

Salmo trutta caspius is a valuable endemic species in the Caspian Sea. There are two migrating forms of spring and fall run with different appearance characteristics. AFLP molecular markers were used to evaluate the possibility of identification of fall and spring migrating Caspian trout. 12 different *EcoRI/ MseI* primer combinations were used, from which a total of 807 bands were produced that 227 of them were polymorphic loci. Percentage of polymorphism loci in spring-run (85.02%) was higher than fall-run (76.21%). The obtained results regarding mean heterozygosity did not show significant differences between fall-run and spring-run Caspian trout. Nei's genetic distance was 0.034% and the variance between and within two populations were 6% and 94%, respectively. The results show that the genetic variation between fall and spring migrating Caspian trout is not significant. Therefore, spring forms are probably a part of Fall-run population and the difference could be attributed to physiology, environment or other related factors.

Keywords: The Caspian Sea, Fall-run, Genetic variation, Spring-run.

Introduction

Caspian trout (*Salmo trutta caspius*) is an anadromous and endemic subspecies in Caspian Sea which reaches the greatest weight, length and growth rate inside Salmo trutta complex (Sedgwick, 1995). As a result of river pollution, destruction of natural spawning regions, poaching and over-fishing, natural populations of Caspian trout have decreased dramatically and is considered critically endangered in the southern part of the Caspian Sea according to IUCN criteria (Abdoli, 2000; Coad, 2000; Barannik *et al.*, 2004; Niksirat and Abdoli, 2009). Its distribution occurs commonly at the western and southern coasts of the Caspian Sea. Based on its reproductive life cycle, Caspian trout has two immigrant forms named fall-run and spring-run. Spring forms migrate to rivers at the end of winter and beginning of spring with immature gonads and remain in rivers until next fall to reach sexual maturity for spawning. Fall forms migrations occur between summer and the middle of fall with rather mature gonads which are ready for spawning. Furthermore, spring forms have more fusiform and silver body than fall forms (Kiabi *et al.*, 1999). Therefore, there is a hypothesis that these two immigrant forms may be genetically different and

probably belongs to different populations. It should be regarded that in recent years as activity of propagation and stock restoration center in Iran has been just limited to fall-run propagation, fall forms proportion has been increased. Unfortunately, the efforts have not been successful for keeping of spring breeds, artificial propagation and spring fries releasing in the propagation center.

Genetic diversity and its measurement have great importance in effective management of populations and individuals (Okumus and Ciftci, 2003). The level of genetic diversity may indicate obvious information about historical population sizes and structure (Sivasundar and Hey, 2003). Range of PCR based DNA fingerprinting methods such as SSR, RAPD, RFLP and AFLP are used for detection of genetic diversity (Money *et al.*, 1996). Molecular markers are useful for population genetic studies aiming on evaluating the effect of different factors on the genetic diversity and population structure (Englbrecht *et al.*, 2000; Whitehead *et al.*, 2003). Among different types of molecular markers, amplified fragment length polymorphism (AFLP) has been demonstrated as a useful tool for population genetic studies of fish (Liu *et al.*, 2003). The advantage of this method is that it does not need

probe or primary sequence information which is necessary for RFLP (Okumus and Ciftci, 2003; Liu, 2007). Because each AFLP primer combination can produce a lot of polymorphism markers ~~in a gel~~ and requires only a small amount of DNA, comparatively it is an economical choice for population genetic studies. Also, since the PCR for AFLP requires higher annealing temperature than in RAPD, it allows AFLP to yield more PCR product (Blears *et al.*, 1998). This technique has been widely applied to study species, strain and hybrid identification, gene mapping, linkage and genetic diversity of species and populations in a wide variety of organisms including fishes and shrimp (Liu and Cordes, 2004; Wang *et al.*, 2004; Kakehi *et al.*, 2005; Sonstebo *et al.*, 2007; Kassam *et al.*, 2005).

In Iran the maintenance of the critical endangered Caspian trout populations depends on artificial breeding followed by releasing the juveniles to the sea, which makes it very important to accurately identify the spring and fall immigrant forms for further stock restoration program. However, with limited genetic diversity information for this valuable species, it is very difficult to separate the two forms. Considering the difficulties in maintenance of Caspian trout, AFLP is ~~among~~ ideal technique~~s~~ that can be used in genetic diversity studies of Caspian trout.

Materials and Methods

Fish Sampling

Shahid Bahonar propagation center located in Kelardasht is the only trout propagation center in Iran. This center receives the brood stock collections of the Caspian trout which have been caught from different rivers in the south Caspian basin. Taking into consideration limitations of collecting samples from Caspian trout- particularly spring forms- due to its decreased population, in this study 30 fall-run and 15 spring-run were collected during the autumn of 2007, late winter and spring of 2008.

DNA Extraction

DNA was purified from fin-clips in 96% ethanol by proteinase K digestion followed by ammonium acetate method. Extracted DNA was precipitated by 100% ethanol, and suspended in ddH$_2$O after washing with 70% ethanol. DNA concentration was measured with an UV spectrophotometer. The extracted DNA quality was assessed by 0.7% agarose gels electrophoresis with ethidium bromide.

AFLP Protocol

Procedure of AFLP analysis was essentially based on Vos *et al.* (1995) with some modification. Polyacrylamide gel by using of silver staining method was applied. DNA templates for AFLP reaction were generated by restrictions digestion and ligation. Initially, about 250 ng of total DNA was digested with 10 U of *EcoRI* and *MseI* enzymes (volume of 0.25 µl for each enzyme) in 10 X PCR buffers (2 µl) with final volume of 20 µl at 37°C for 2 h, then at 65°C for 24 h. In order to generate DNA template for subsequent PCR amplification, the sample were digested.

DNA fragment was ligated by 5µl of ligation solution contained 5 µM of *EcoRI* (0.5µl) and 50 µM *MseI* adaptors (0.5 µl) in a reaction containing 1 U T$_4$ DNA ligase (0.4 µl), 10 mM ATPs (0.5 µl), 10X T$_4$ DNA ligase Buffer (2 µl) at 22°C for 24 min. The ligated sample was diluted 4 times.

The preselective PCR reaction was performed with primers (E01=5´-GACTGCGTACCAATTCA-3´, M02=5´GATGAGTCCTGAGTAAC-3´) that complete to adaptors sequence, but with one selected base in 3ό. Each PCR reaction was performed in 20 µl final reaction volume by using 5 µl diluted (1:4 ddH$_2$O) ligated product, primer concentration 10 µM (volume of 1 µl for each primer), 2.5 mM dNTP mix (1 µl), 50 mM mgcl$_2$ (1 µl), 10X PCR Buffer (2 µl) and 5 U Tag DNA polymerase (0.3 µl). AFLP reaction was performed for 25 cycles with the following cycle profile: 30 s DNA denaturation step at 94°C, 1 min annealing step at 56°C, and a 1 min extension step at 72°C. The quality of the pre selective PCR products was determined on 0.7% agarose gel with10 time dilution.

The selective amplification was performed using twelve combinations of primers with two selected bases more in the 3´ end (Table 1). Each PCR reaction was performed in a 20 µl reaction volume using 5 µl diluted pre amplification product, primer concentration 10 µM (volume of 1 µl for each primer), 2.5 mM dNTP mix (1 µl), 50 mM mg Cl$_2$ (1

Table 1. *MseI\EcoRI* primer sequence with 2 selective nucleotides in selective PCR

Type of primer	Sequence	Type of primer	Sequence
1EcoR I +3	GACTGCGTACCAATTCA AC	2Mse I +3	GATGAGTCCTGAGTAAC AC
2EcoR I +3	GACTGCGTACCAATTCA AG	3Mse I +3	GATGAGTCCTGAGTAAC AG
3EcoR I +3	GACTGCGTACCAATTCA CC	4Mse I +3	GATGAGTCCTGAGTAAC AT
4EcoR I +3	GACTGCGTACCAATTCA CT	5Mse I +3	GATGAGTCCTGAGTAAC CT
5EcoR I +3	GACTGCGTACCAATTCA GC	6Mse I +3	GATGAGTCCTGAGTAAC GT
6EcoR I +3	GACTGCGTACCAATTCA CA	7Mse I +3	GATGAGTCCTGAGTAAC CG
1Mse I +3	GATGAGTCCTGAGTAAC AA	8Mse I +3	GATGAGTCCTGAGTAAC CC

μl), 10X PCR Buffer (2 μl) and 5 U Tag DNA polymerase (0.3 μl). AFLP reaction was performed for 30 cycle with the following cycle profile: 30 s DNA denaturation step at 94°C, 1 min annealing step (the annealing temperature in the first cycle was 65°C, was subsequently reduced each cycle by 1°C for the next 11 cycle then continues in 54°C) and a 1 min extension step at 72°C.

Electrophoresis and Silver Staining

The PCR products were mixed with 8 μl formamide dye (98% formamide, xylene cyanol, bromo phenol blue and 10mM EDTA pH 8.0). The product mixture was denaturated at 95°C for 6 min, and quickly cooled on ice bath after denaturation. A 6% denaturating polyacrylamide gel (40% acrylamide, urea, 10X TBE) was prerun at 75 w for 45 min, each well was loaded with 8-10 μl of product mixture. The gel was electrophoresed for 1.5 h in (Bio Rad) DNA sequencing gel (38×30 cm) at 50°C.

After electrophoresis, the gel was fixed in acetic acid for 20 min and then stained with silver nitrate for 30 min; the stained gel was rinsed again with distilled water and immersed in developing solution (formaldeid and sodium hydroxide). Band sizes were estimated using a standard AFLP DNA ladder.

Data Analysis

The bands were scored by presence (1) or absence (0) and data were transferred into Excel. Percent polymorphic loci (P), average heterozygosities (H), genetic distance were estimated using the GenAlEx v6 software. The percentages of polymorphic loci were estimated based on the percent of loci not fixed for one allele; allele frequency were analysed following Lynch and Milligan (1994). Average heterozygosity were calculated for each locus and then averaged over loci according to Lynch and Milligan (1994). Genetic distances between populations were calculated by Nei's (1978) distance and identity measures. Differentiation between and within populations of *Salmo trutta caspius* was estimated by analyzing molecular variance (AMOVA)

using GenAlEx software and dendrogram based on each individual was drawn by TFPGA v1.3 software by UPGMA analyses.

Results

The twelve AFLP primers combination generated 807 total bands in two populations and generally produced 227 polymorphic bands (Table 2, Figure 1). These twelve combinations produced range 53 to 85 total bands and 5 to 35 polymorphic bands and percentage of polymorphic bands was 28.12%.

Molecular Analysis

Allele Frequencies by Population (AFP) showed band frequency, p, q and H (heterezygosity) for each locus per population. At the end of the output, the percentage of polymorphic loci for each population was calculated. Percentage of polymorphism loci was 76.21% in fall-run population and 85.02% spring-run population and polymorphism loci mean was 80.62%.

Allelic pattern by population (APT) showed 185 and 205 bands for fall-run and spring-run, respectively and also the number of private bands of population was 21 and 41 for fall-run and spring-run, respectively (Private bands are equivalent to the number of alleles unique to a single population). Mean heterezygosity was 0.207 for fall-run and 0.227 for spring-run populations (Standard error mean heterozygosity is 0.012) (Figure 2). Nei's genetic distance of spring and fall migrating ~~Salmo trutta~~ *caspius* was 0.034 and genetic identity was 0.966.

Two-dimensional diagram of PCA (Principle Coordinates Analysis) that were drawn for the relationships between the samples (Figure 3) show that fall and spring run fishes were not in two separate groups and overlap of these two groups represented their high similarity.

Analysis of Molecular Variance (AMOVA) showed that the percentage of variation within and between populations were 94% and 6%, respectively. So difference within population was significant but was not significant between populations. Genetic differentiation of population (Fst) was 0.0603.

Table 2. Number of total and polymorphic bands of 12 combinations of *MseI\EcoRI* primers

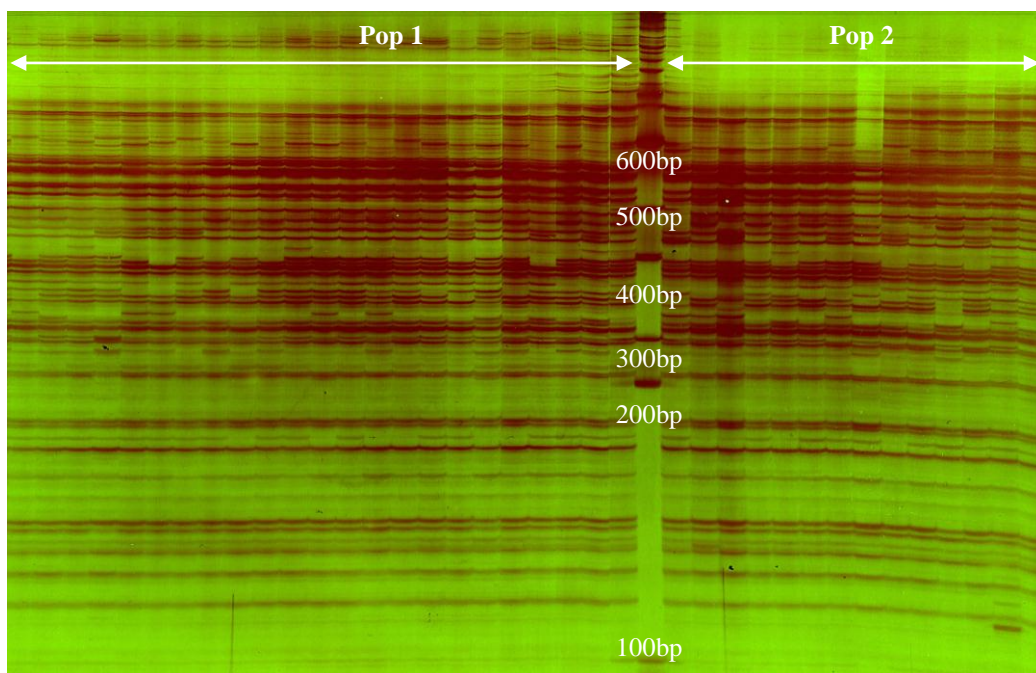| COD	Primer combination	Total bands	Polymorphic bands
A	1E/1M	82	7
B	1E/4M	63	5
C	2E/4M	63	6
D	2E/1M	80	28
F	2E/5M	56	24
G	2E/6M	58	20
H	4E/6M	58	19
I	5E/7M	85	38
J	3E/8M	64	32
K	6E/1M	61	11
L	6E/2M	84	16
M	4E/3M	53	21

Figure1. A sample of polyacrylamide gel by combination primer of 3E/ 8M. Pop 1: fall-run, and Pop2: spring-run of Caspian trout.
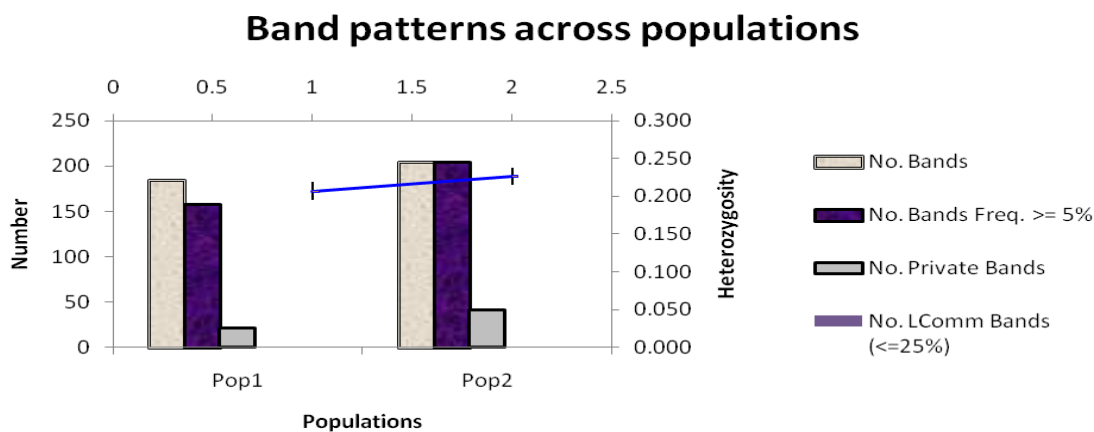

Figure 2. Allelic pattern (APT) of spring and fall migrating form of Caspian trout. Pop 1: fall-run, and Pop2: spring-run of Caspian trout.
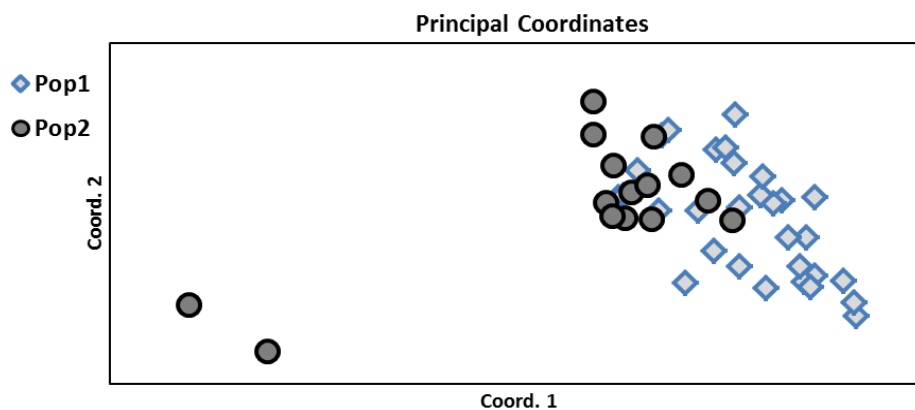

Figure 3. Two-dimensional diagram of PCA for fall and spring migrating forms of Caspian trout.
Pop 1: fall-run, and Pop2: spring-run of Caspian trout.

The UPGMA dendrogram was constructed on the basis of the inter population genetic similarity. Cluster analysis results produced by UPGMA method in TFPGA software revealed that high similarity exist in both groups of immigrant fishes (Figure 4).

Discussion

AFLP is a multilocus PCR-based DNA fingerprinting technique (Liu, 2007); AFLP's most important point is its capacity for inspecting an entire genome for polymorphism and its reproducibility (Blears *et al.*, 1998). As compared to other markers such as RFLP and RAPD, AFLP provides a much greater level of polymorphism and covers large genomes well. However, AFLPs has been rarely applied in animal ecological and evolutionary studies (Sonstebo *et al.*, 2007).

Due to the importance of accurate identification and separation of spring and fall migrating Caspian trout (*Salmo trutta caspius*) and their status of being endangered species, AFLP technique was used for assessing the genetic variation.

Concerning ~~Salmo~~ *trutta*, genetic diversity among different populations was studied by using various methods (Apostolidis *et al.*, 2008; Ostergren, 2006; Charles *et al.*, 2005; Elo *et al.*, 1997), but the application of AFLP in ~~Salmo~~ *trutta* L. was only carried out by Sonstebo *et al.* (2007). The number of primer combinations that was applied for AFLPs usually varied from three to nine, while the number of polymorphisms varied from 30 to 400 (Sonstebo *et*
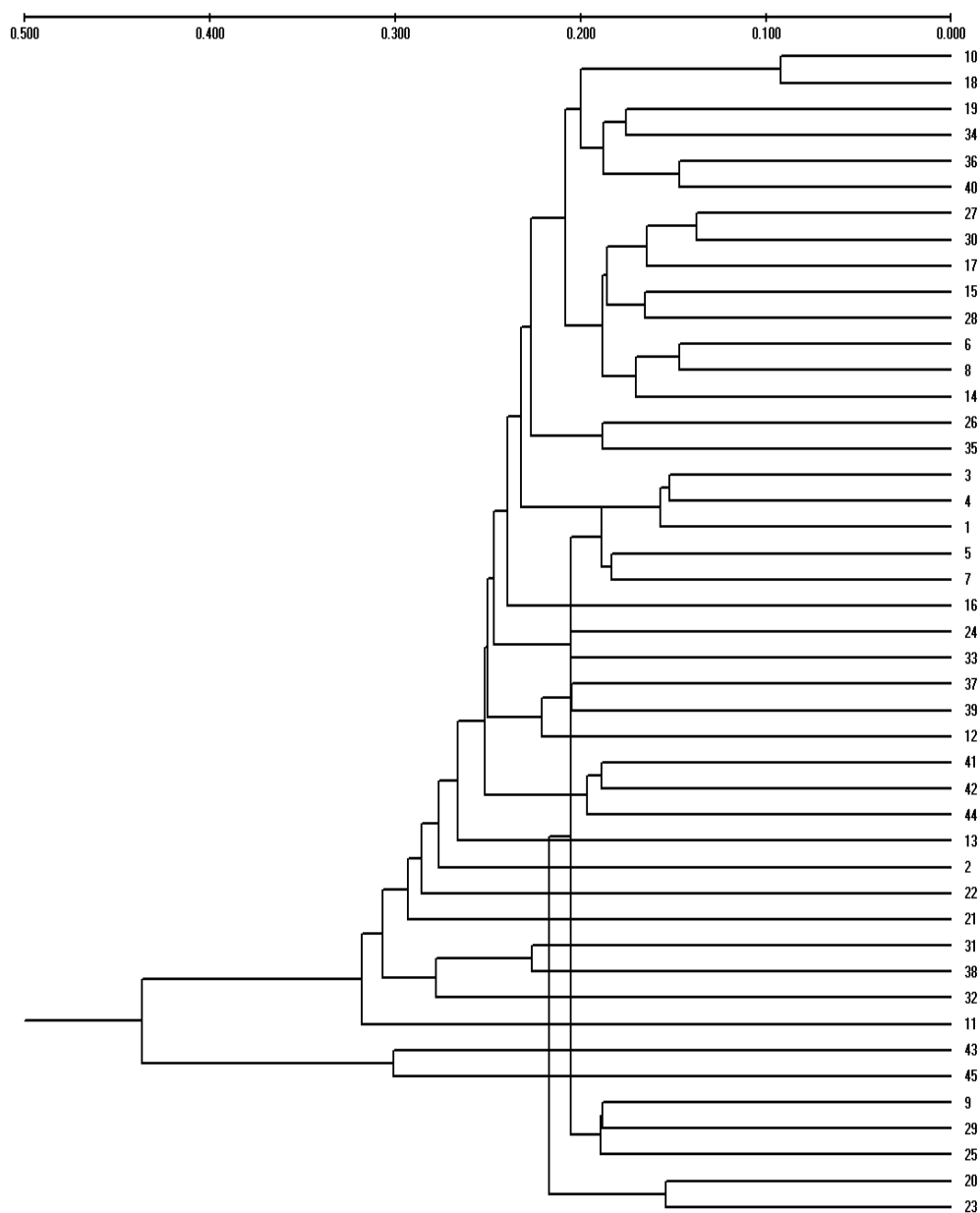

Figure 4. UPGMA dendrogram showing the phylogenetic relationship among all individual of fall and spring run ~~Salmo~~ *trutta caspius*. Number(s) 1-30: fall run, 31-45: spring run.

al., 2007). In this study, 12 primer combinations were used which produced 227 polymorphism bands. Sonstebo *et al.* (2007) applied 6 primer pairs to identify genetic difference among 11 lake populations of brown trout (*Salmo trutta* L.) which produced 178 polymorphism bands. Percentage of polymorphism loci in spring-run (85.02%) was higher than fall-run (76.21%). This difference could relate to the sample size. Simmons *et al.* (2006) in a study on channel catfish recognized the effects of sample size on the allele frequency; as the sample sizes increase, the frequency of alleles can change. In this regard, the allele frequency differences could be potentially over-estimated or under-estimated in some populations because of the small sample sizes. There were no significant differences as to the levels of heterozygosity between fall-run and spring-run. Mean heterozygosity in fall-run (0.207) was lower than spring-run (0.227). It indicates higher genetics diversity in spring-run un comparison with fall-run. Sonstebo *et al.* (2007) found that the mean of gene diversity in the *Salmo trutta* L. was 0.18 which shows low genetic diversity in brown trout populations. Genetic distance based on Nei's genetic distance was 0.034 which indicated high similarity (0.966) between fall-run and spring-run population, while Nei's Genetic distance calculated by Sonstebo *at al.*, (2007) in *Salmo trutta* L. at population level was 0.878. Analysis of Molecular Variance (AMOVA) showed the difference within populations (0.94%) was significant, while they were not between populations which is due to the genetic difference existed between individuals that is similar to the obtained results by Sonstebo *et al.* (2007). They showed there are high variations within populations while these differences were few among populations of ~~Salmo~~ *trutta* L. and concluded the incident could be due to the migrations occurred between lake populations. Likely great part of variation observed between populations is due to environmental differences and it has been often assumed that at least a part of this variation is determined genetically (Allendorf *et al.*, 1987). Genetic variation between fall run and spring run populations, revealed no significant genetic differentiation between them ($F_{st}= 0.060$).

The present study was performed by considering the high resolution feature of AFLP in genetic population, 12 applied primer combinations showed that genetic variation was not significant between fall and spring migrating Caspian trout. The two migrating forms reproduce synchronously in the autumn; this way of reproduction is similar to those described in brown trout populations consisted of a mixture of anadromous and resident individuals where no genetic differences are described among both life strategies (Hansen *et al.*, 2000, Hansen, 2002, Charles *et al.*, 2005). Morphological, demographical and ecological characteristics differ in these two forms (Frost and Brown 1967; Campbell, 1977; Baglinière *et al.*, 2000). No differentiation between the coexisting anadromous and resident forms was reported. Charles *et al.* (2005) concluded that resident and anadromous trout have a naturally reproducing unit in this river system. Also, Klemesten *et al.* (2003) explained that the body size and acclimatization vary in resident and migratory froms in the same populations and also between fishes of small streams and large rivers in the same area. The observed variations appear to be rather the result of phenotypic plasticity and partly as an effect of genetic selection. Considering that no genetic differences were found between two forms, it seems that spring type is probably a part of fall run population.

There is a great plasticity in the migratory behaviour of stocks of a wide variety of salmonids, influenced by genetic, environmental, biological and morphological factors (Jonsson, 1982; Palm and Ryman, 1999; Nordeng, 1983; Northcote, 1997; Klemetsen *et al.*, 2003). Two of these factors are body size and age. Caspian trout fry is usually released at a size of 5-15 gr in rivers. In anadromous trout, there are differences in body size and age among migrants. Larger and older individuals descend to sea earlier in the year than smaller and younger ones (Klemetsen *et al.*, 2003). The mentioned behavior has been detected in smolts (Rasmussen, 1986; Bohlin *et al.*, 1993, 1996), sub-adults and adults (Jonsson and Jonsson 2001). There are several possible reasons for the size and age segregation including osmoregulatory capacity and vulnerability to predation. On the other hand, Forseth *et al.* (1999) studied the time of migration in relation to growth and metabolic rate in migrating forms of juvenile brown trout. Also, river size can affect the time of freshwater brown trout ascent (klemetsen *et al.*, 2003). Sea trout, spawning in large rivers, mounts fresh water about 6 months before arriving at the spawning grounds (Campbell, 1977), whereas trout spawning in small brooks often mount just prior to the time of spawning (Klemetsen *et al.*, 2003). It may be concluded that the larger individuals migrate first from rivers to the Caspian Sea and also return first (in spring) to the rivers for spawning. These parameters could be indicative for the presence of the two forms in the Caspian Sea.

It seems that these groups have been originated from one population. Therefore, more attention should be paid to protect this valuable endemic species through monitoring management plans of rearing complexes including the modification of sex ratios in mating and the prevention of breeding among different river populations so that to preclude further decrease in the population of this economically and genetically valuable species.

Acknowledgments

References

Abdoli, A. 2000. The Inland Water Fishes of Iran, 1st ed. Museum of Nature and Wild Life of Iran, Tehran, 378 pp.

Allendorf, F.W. and Ryman, N. 1987. Genetic management of hatchery stocks. In: N. Ryman and F. Utter (Eds.), Population Genetics and Fishery Management. University of Washington Press, Seattle: 141–159.

Apostolidis, A.P., Madeira, M.J., Hansen, M.M. and Machordom, A. 2008. Genetic structure and demographic history of brown trout (*Salmo trutta*) populations from the southern Balkans. Freshwater Biology, 53: 1555-1566. doi: 10.1111/j.1365-2427.2008.01988.x

Baglinière, J.L., Ombredane, D. and Marchand, F. 2000. Critères morphologiques pour l'identification des deux formes (rivière et mer) de truite (*Salmo trutta* L.) présentes sur un même bassin. Bulletin Francias de la Pêche et de la Pisciculture, 357/358: 375-386.

Barannik, V., Borysova, O. and Stolberg, F. 2004. The Caspian Sea region: environmental change. Ambio, 33: 45-51. doi: 10.1579/0044-7447-33.1.45

Blears, M.J., de Grandis, S.A., Lee, H. and Trevors, J.T. 1998. Amplified fragment length polymorphism (AFLP): A review of the procedure and its applications. Jouranl of Industrial Microbiology and Biotechnology, 21: 99-114. doi: 10.1038/sj.jim.2900537

Bohlin, T., Dellefors, C. and Faremo, U. 1993. Optimal time and size for smolt migration in wild sea trout (*Salmo trutta*). Canadian Journal of Fisheries and Aquatic Sciences, 50: 224-232. doi: 10.1139/f93-025

Bohlin, T., Dellefors, C. and Faremo, U. 1996. Date of smolt migration depends on body-size but not age in wild searun brown trout. Journal of Fish Biology, 49: 157-164. doi: 10.1111/j.1095-8649.1996.tb00012.x

Campbell, J.S. 1977. Spawning characteristics of brown trout and sea trout *Salmo trutta* L. in Kirk Burn, River Tweed, Scotland. Journal of Fish Biology, 11: 217-229. doi: 10.1111/j.1095-8649.1977.tb04115.x

Charles, K., Guyomard, R., Hoyheim, B., Ombredane, D. and Bagliniere, J.L. 2005. Lack of genetic differentiation between anadromous and resident sympatric brown trout (*Salmo trutta*) in a Normandy population. Aquatic Living Resources, 18: 65-69. doi: 10.1051/alr:2005006

Coad, B.W. 2000. Criteria for assessing the conservation status of taxa (as applied to Iranian freshwater fishes). Biologia, 55: 537-555.

Elo, K., Ivanoff, S., Vuorinen, J.A. and Piironen, J. 1997. Inheritance of RAPD markers and detection of interspecific hybridization with brown trout and Atlantic salmon. Aquaculture, 152: 55-65. doi: 10.1016/S0044-8486(96)01529-3

Englbrecht, C.C., Freyhof, J., Nolte, A., Rassmann, K., Schliewen, U. and Tautz, D. 2000. Phylogeography of the bullhead *Cottus gobio* (Pisces: Teleostei: Cottidae) suggests a pre-Pleistocene origin of the major central European populations. Molecular Ecology, 9: 709-722. doi: 10.1046/j.1365-294x.2000.00912.x

Forseth, T., Næsje, T.F., Jonsson, B. and Harsaker, K. 1999. Juvenile migration in brown trout: a consequence of energetic state. Journal of Animal Ecology, 68: 783-793. doi: 10.1046/j.1365-2656.1999.00329.x

Frost, W.E. and Brown, M.E. 1967. The Trout, Collins. New Naturalist, London, 286 pp.

Hansen, M.M., Ruzzante, D.E., Nielsen, E.E. and Mensberg, K.L.D. 2000. Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). Molecular Ecology, 9: 583-594. doi: 10.1046/j.1365-294x.2000.00898.x

Hansen, M.M. 2002. Estimating the long-term effects of stocking domesticated trout into wild brown trout (*Salmo trutta*) populations: an approach using microsatellite DNA analysis of historical and contemporary samples. Molecular Ecology, 11: 1003-1015. doi: 10.1046/j.1365-294X.2002.01495.x

Jonsson, B. 1982. Diadromous and resident trout *Salmo trutta* L.: Is their difference due to genetics? Oikos, 38: 297-300. doi: 10.2307/3544668

Jonsson, B. and Jonsson, N. 2001. Polymorphism and speciation in Arctic charr. Journal of Fish Biology, 58: 605-638. doi: 10.1111/j.1095-8649.2001.tb00518.x

Kakehi, Y., Nakayama, K., Watanabe, K. and Nishida, M. 2005. Inheritance of amplified fragment length polymorphism markers and their utility in population genetic analysis of *Plecoglossus altivelis*. Journal of Fish Biology, 66: 1529-1544. doi: 10.1111/j.0022-1112.2005.00683.x

Kassam, D., Seki, S., Rusuwa, B., Ambali, A.J.D. and Yamaoka, K. 2005. Genetic diversity within the genus Cynotilapia and its phylogenetic position among Lake Malawi's mbuna cichlids. African Journal of Biotechnology, 4: 1195-120.

Kiabi, B.H., Abdoli, A. and Naderi, M. 1999. Status of the fish fauna in the South Caspian basin of Iran. Zoology in the Middle East, 18: 57-65. doi: 10.1080/09397140.1999.10637782

Klemetsen, A., Amundsen, P.A., Dempson, J.B., Jonsson, B., Jonsson, N., O'Connell, M.F. and Mortensen, E. 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecology of Freshwater Fish, 12: 1-59. doi: 10.1034/j.1600-0633.2003.00010.x

Liu, Z.J. (ed) 2007. Aquaculture Genome Technologies. Blackwell Publishing, Australia, 551 pp. doi: 10.1002/9780470277560

Liu, Z.J. and Cordes, J.F. 2004. DNA Marker Technologies and their Application in Aquaculture Genetics. Aquaculture, 238: 1-37.
doi: 10.1016/j.aquaculture.2004.05.027

Liu, Z.J., Karsi, A., Li, P., Cao, D., Ju, Z. and Dunham, R. 2003. An AFLP-based genetic linkage map of channel catfish (*Ictalurus punctatus*) constructed by using an interspecific hybrid resource family. Genetics, 165: 687-694.

Lynch, M. and Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers. Molecular Ecology, 3: 91-99. doi: 10.1111/j.1365-294X.1994.tb00109.x

Money, T., Reader, S., Qu, L.J., Dunford, R.P. and Moore, G. 1996. AFLP based mRNA fingerprinting. Nucleic Acids Research, 24: 2616-2617. doi: 10.1093/nar/24.13.2616

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

Niksirat, H. and Abdoli, A. 2009. On the status of the

critically endangered Caspian brown trout, *Salmo trutta caspius*, during recent decades in the southern Caspian Sea basin. Zoology in the Middle East, 46: 55-60. doi: 10.1080/09397140.2009.10638328

Nordeng, H. 1983. Solution to the "Char problem" based on Arctic Char (*Salvelinus alpinus*) in Norway. Canadian Journal of Fisheries and Aquatic Sciences, 40: 1372-1387. doi: 10.1139/f83-159

Northcote, T.G. 1997. Potamodromy in Salmonidae: living and moving in the fast lane. North American Journal of Fisheries Management, 17: 1029-1045.

Okumuş, I. and Çiftci, Y. 2003. Fish population genetics and molecular markers: II-molecular markers and their applications in fisheries and aquaculture. Turkish Journal of Fisheries and Aquatic Sciences, 3: 51-79.

Ostergren, J. 2006. Migration and genetic structure of *Salmo salar* and *Salmo trutta* in Northern Swedish rivers. PhD thesis, Uppsala: Swedish University of Agricultural Sciences, 32 pp.

Palm, S. and Ryman, N. 1999. Genetic basis of phenotypic differences between transplanted stocks of brown trout. Ecology of Freshwater Fish, 8: 169-180. doi: 10.1111/j.1600-0633.1999.tb00068.x

Rasmussen G. 1986. The population dynamics of brown trout (*Salmo trutta* L.) in relation to year-class size. Polskie Archiwum Hydrobiologii, 33: 489-508.

Sedgwick, S.D. 1995. Trout farming handbook. Alden Press, Oxford.

Simmons, M., Mickett, K., Kucuktas, H., Li, P., Dunham, R. and Liu, Z. 2006. Comparison of domestic and wild channel catfish (*Ictalurus punctatus*) populations provides no evidence for genetic impact. Aquaculture, 252: 133-146. doi: 10.1016/j.aquaculture.2005.11.006

Sivasundar, A. and Hey, J. 2003. Population genetics of Caenorhabditis elegans: the paradox of low polymorphism in a widespread species. Genetics, 163: 147-157.

Sonstebo, J.H., Borgstrom, R. and Heun, M. 2007. A comparison of AFLPs and microsatellites to identify the population structure of brown trout (*Salmo trutta* L.) populations from Hardangervidda, Norway. Molecular Ecology, 16: 1427-1438. doi: 10.1111/j.1365-294X.2007.03256.x

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, V., Hornes, T., Frijters, M., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23: 4407-4414. doi: 10.1093/nar/23.21.4407

Wang, Z.Y., Tsoi, K.H. and Chu, K.H. 2004. Applications of AFLP technology in genetic and phylogenetic analysis of penaeid shrimp. Biochemical Systematics and Ecology, 32: 399-407. doi: 10.1016/j.bse.2003.10.006

Whitehead, A., Anderson, S.L., Kuivila, K.M., Roach, J.L. and May, B. 2003. Genetic variation among interconnected populations of *Catostomus occidentalis*: implications for distinguishing impacts of contaminants from biogeographical structuring. Molecular Ecology, 12: 2817-2833. doi: 10.1046/j.1365-294X.2003.01933.x