Journal of Applied Biological Sciences 4 (1): 1-6, 2010
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Genetic Comparison of Fall and Spring Immigrant Forms of Endangered Caspian Salmon, *Salmo trutta caspius* (Kessler, 1870) Using Microsatellite Markers

Seyedeh Ainaz SHIRANGI[1] Mohammad Reza KALBASSI[1*] Salar DORAFSHAN[2]

[1]Faculty of Marine Science, Tarbiat Modares University, IRAN

[2]Faculty of Natural Resources, Isfahan University of Technology, Isfahan, IRAN

[*]**Corresponding Author**
e-mail: kalbassi_m@modares.ac.ir

Received : December 20, 2009
Accepted : February 15, 2010

Abstract

The Caspian salmon *S. trutta caspius* in respect to its reproductive life cycle has two immigrant forms namely fall-run and spring-run. As the Shahid Bahonar center activities on Caspian salmon stocks restoration in Klardasht of Iran has been just focused on fall-run form propagation, it could be proposed that these stocks proportions have been gradually increased. Regarding to lack of information on genetically differences between the two groups, in this study, genetic structure of these immigrant forms were analyzed by using 5 microsatellite loci. Results revealed that the most allelic frequencies were observed in fall-runs. Average of observed and expected heterozygosity in fall-runs and spring-runs were, 0.7719, 0.6108 and 0.4435, 0.5911, respectively. In both groups except Str543INRA in spring runs, all loci had deviation from Hardy-Weinberg equilibrium. Furthermore except Str543INRA in spring runs, expected heterozygosity in all loci was more than observed heterozygosity. Genetic distance between two populations and Fst, as a population distinctive index, were 0.081 and 0.025, respectively. In conclusion 5 microsatellite loci used in this study show low genetic differentiation between fall-runs and spring-runs.

Key words*: Salmo trutta caspius*, Caspian salmon, fall-run, spring-run, genetic variation, genetic differentiation, microsatellite.

INTRODUCTION

The Caspian salmon *S. trutta caspius* (Kessler, 1870) is an anadromous form and endemic subspecies fish of Caspian basin. It is distributed commonly at the western and southern coasts of the Caspian Sea. In respect of its reproductive life cycle, Caspian salmon has two immigrant forms namely fall-run and spring-run. These forms migrate to some rivers such as Terek, Kura, Sefid Rod, Nav Rod and mostly Cheshmeh-Kileh (Tonekabon) of the Caspian Sea for spawning. Fall forms migrations begin at the end of summer to the middle of fall with rather mature gonads which ready for spawning. Spring forms migrate to rivers with immature gonads and remain in rivers until next fall to reach sexual maturity for spawning. Furthermore, spring forms have more fusiform and silver body than fall forms. Therefore, there is a hypothesis that these two immigrant forms differ from each other morphologically [14].

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 [15]. *S. trutta caspius* similar to other brown trout populations is at risk of extinction and it was listed as threatened by red list (IUCN) [14]. As its propagation and stock restoration activities center in Iran (Shahid Bahonar center of Kelardasht) has been just focused on fall runs propagation and also there hasn't been achieved any successful impacts in respect to spring brood stocks propagation yet, fall run stock proportions has been increased. Besides, as a matter of fact, there is a probability that spring forms are part of fall forms affected by some interior (physiological- hormonal) and exterior (climates) parameters begin their migration in spring. Finding genetic diversity across this subspecies of *S. trutta caspius* could have great importance for aquaculture strains development, protection of small-endangered populations and biogeographical inferences [12]. Consequently, there is an urgent need to describe the current genetic diversity of fall run and spring run Caspian salmon in order to facilitate proper management based on conservation genetics. As the microsatellites has proven useful in genetic studies of brown trout populations[3, 4, 26] and no DNA based study of the current genetic structure of these two immigrant forms has been conducted, Genetic structure of the mentioned immigrant forms of *S. trutta caspius* were analyzed by using 5 microsatellite loci.

MATERIAL AND METHODS

Study location and fish sampling

Shahid Bahonar propagation is the only salmon propagation centers in Kelardasht of Iran, that is received

the brood stock collections of the Caspian salmon were captured from different rivers in the south Caspian basin. Fin clips of *S. trutta caspius* were collected from the center during the autumn of 2007, late winter and spring of 2008 and totally 60 fall and spring run samples were collected.

Microsatellite analysis

Genomic DNA was extracted from caudal fin, stored in 96% ethanol, using Roche DNA extraction kit (Roche, Germany). The quality and quantity of the extracted DNA were determined on 1% agarose gels. Five microsatellite loci were used in this study: strutta 12, strutta 58, OmyFgt1TUF, Str 543INRA and Str 598INRA. These loci were chosen for their ease of PCR based allele scoring and because they have been assayed in other brown trout populations [26, 3, 4, 11] and also, because of their polymorphic status based on previous studies [8, 21].Each PCR reaction was performed in a 25 µl reaction volume using 4-40 ng/mL DNA, 0.4 mM primers, 1.5 mM $MgCl_2$, concentration of Taq DNA polymerase 1-1.5U and nucleotides (dNTPs) 0.2 mM and was the same for all loci. PCR conditions were optimized for five microsatellite loci. Thermo-cycling parameters were 5 min at 94 ºC for an initial denaturation, 35 cycles of denaturation at 95ºC for 1 min, annealing temperature for 30 S and extension at 72 ºC for 1 min followed by a final extension step at 72 ºC for 10 min (Table 1).

PCR was followed by electrophoresis of products in 6% polyacrylamide gels. DNA fragments were visualized by silver staining method [25].

Data analysis

Exact tests of microsatellite deviation from Hardy-Weinberg equilibrium and calculation of allele frequency, the number of allele per locus, observed heterozygosity (H_O) and expected heterozygosity (H_E) were performed with the Gene Alex6 program [20] and GENEPOP 3.2 [22] [23]. Genetic differentiation between two immigrant forms was analyzed by calculation of F_{st} values by using Gene Alex6, furthermore AMOVA test was performed in FSTAT program [10].

RESULTS

All of five microsatellite loci for both groups showed polymorphism with numbers of alleles ranging from 7 (Strutta 543INRA, Strutta 598INRA) to 15 (Strutta12) for fall form and 2 (Strutta543INRA) to 7 (Omyfgt1) for spring form (table 2). The number of effective alleles was lower than the number of observed alleles, In all loci. Calculation of allelic richness per locus based on minimum samples for fall run and spring run were, 6.602-11.998 and 2-7 respectively (table 3). Results revealed that the most allelic frequencies were observed in fall runs. Average of observed and expected heterozygosity in fall run and spring run were, 0.7719, 0.6108 and 0.4435, 0.5911 respectively. In both groups except Str543INRA in spring form all loci showed deviation from Hardy-Weinberg equilibrium. Furthermore except Str543INRA in spring runs expected heterozigosity was more than observed heterozygosity.

Application of distance method based on the number of different alleles (Fst) between these immigrant forms was 0.025 (table 4). It showed nominal genetic differentiation between fall run and spring run. Genetic flow and genetic distance between these groups were, 9.696 and 0.081 respectively. Population assignment for two groups indicated that fall and spring runs weren't distinctive (figure 1). AMOVA test using FSTAT program showed that there is low differentiation between two immigrant forms which their intra population genetic variation was more than their inter population variation.

Table 1 Flanking primers, annealing temperature, observed Size range and Genbank Accession number of 5 microsatellite loci

Locus	Primer	Size (bp)	Temp. (°C)	Genbank Accession No.
Strutta58	5'-AACAATGACTTTCTCTGAC-3 5'-AAGGACTTGAAGGACGAC-3'	102-190	57	U60223.1
Strutta 12	5'-AATCTCAAATCGATCAGAAG-3' 5'-AGCTATTTCAGACATCACC-3'	124-216	57	U60220.1
OmyFgt1TUF	5'-AGATTTACCCAGCCAGGTAG-3' 5'-CATAGTCTGAACAGGGACAG-3'	187-263	59	BX9272291.8
Str543INRA	5'-ATTCTTCGGCTTTCTCTTGC-3' 5'-ATCTGGTCAGTTTCTTTATG-3'	119-169	60	AB001062.1
Str591INRA	5'-CTGGTGGCAGGATTTGA-3' 5'-CACTGTCTTTCGTTCTT-3'	146-198	59	AB001066.1

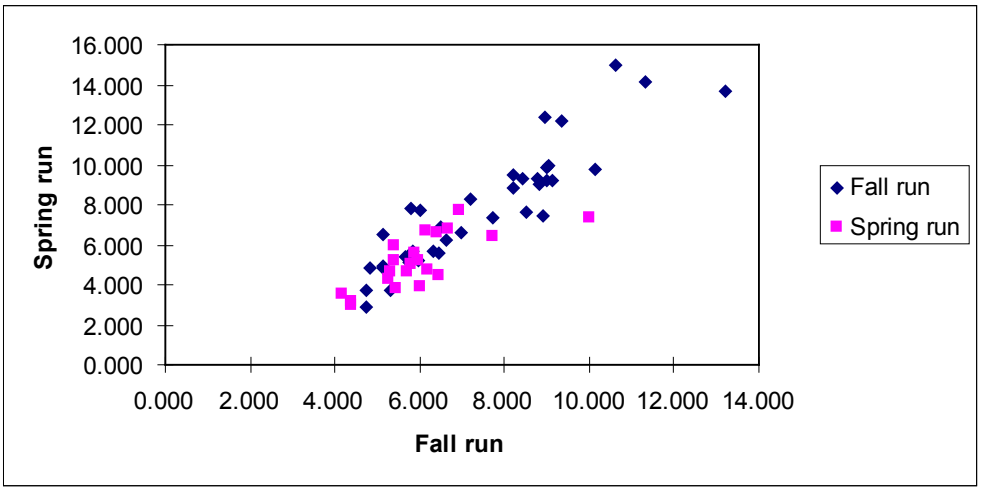

Figure 1 population assignment for fall and spring runs of the Caspian salmon

DISCUSSION

In this study no alleles were found that identify strictly differentiation of fall run and spring run. Highest allelic frequency was related to fall runs. If all alleles have the same frequencies and they are not affected by rare or private alleles (P<0.01), the number of effective alleles per population will equal to the actual number of alleles [17]. In order to lower number of effective alleles compare with the number of observed alleles in all loci, It is assumed that all alleles of this study have been affected by rare alleles. As average of observed and expected heterozygosity in fall run were higher than spring run (table 2), genetic variation in fall run was more than spring run. As the previous studies on the Caspian salmon populations revealed that there was the genetic variation between all populations [16, 21]. Furthermore, the mean heterozygosity in this study was significant (0.7143) and all 5 loci were polymorphic, there was genetic diversity between fall run and spring run. The number of alleles in this study compare with some other studies on brown trout was low [4, 26] but it was in agreement with the previous studies of the Caspian salmon populations. In spite of genetic variation between fall run and spring run populations, no significant genetic differentiation was found between them (F_{st}= 0.025). In the similar study on *S.trutta*, it was proven that no differentiation between the anadromous and resident forms coexisting in the Oir basin was found but there was a large amount of variation among them [4]. As well, investigating of genetic variation and population structure of *S. trutta* populations in two Swedish rivers, using microsatellites showed that genetic variation among these population was high while genetic differentiation of them was low [19]. In other study, Banks *et al*. (2000) in order to assess genetic diversity within and among the four runs (winter, spring, fall and late fall) of Chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley using 10 microsatellite loci reported that in spite of low genetic differentiation among subpopulations, substantial genetic divergence was found among runs [2]. In this study, AMOVA test using Fstat program showed that there is differentiation between two immigrant forms which intra population genetic variation was more than inter population. On the basis of assignment test results (figure1), only Str543INRA in spring run was in accordance with Hardy-Weinberg equibrilium. Observed deviation from Hardy-Weinberg in other loci could be derived from reduction of heterozygosity [21, 26]. As all fall run loci showed deviation from Hardy-Weinberg disequilibrium, It is illustrated that fall run fishes have several distinctive populations. Owing to expected heterozygosity in all loci except Str543INRA in spring run was more than observed heterozygosity, it could be confirmed that inbreeding was performed among them.

Genetic differences between subpopulations will evolve in the course of time if there is little or no gene flow between them [6] Gene flow rates of 10% or less may justify treatment as separate stocks [5]. Consequently, calculation of gene flow indicated that the amount of gene flow between two immigrant forms were high (N_m= 9.696). With high levels of migration and gene flow between populations, the similarity of populations increases [18] and there isn't considerable genetic differentiation among them.

If amount of Fst, as a population distinction index is 0 - 0.05, genetic differentiation will be little [28]. In this study, Fst indicated weak genetic differentiation between two groups. On the other hand, genetic distance between runs was 0.081. It revealed nominal genetic differentiation, too. As F_{IS} parameter was more than zero (0.188), indicating inbreeding and intermixing [28] of fall run and spring run Caspian salmon fishes. The loss of genetic variation, due to prolonged selection, loss of heterozygosity due to (random) inbreeding or isolation may result in a decrease of the potential adaptability of a population [9]. The dangers inherent in subdivision of fish populations are that inbreeding and genetic drift will

lead to fixation of genes, loss of fitness (vigour, viability, fecundity, resistance to disease) and ultimately extinction of local populations [9]. Allelic richness was positively correlated with effective population size at founding. The results indicate that considerations concerning effective population size in hatcheries must be taken seriously to promote high levels of genetic variation among individuals and minimize loss of genetic diversity [1]. Bottlenecks due to small number of breeders are common in hatchery reared commercial stocks [27] Consequently, the hatchery produced brood stocks face the risk of reduction of the genetically effective population size (N_e), resulting in excessive inbreeding and loss of genetic variation. Ryman and Laikre (1991), showed how supportive breeding may reduce effective population size and therefore accelerate the loss of genetic diversity within wild populations [24]. This loss may reduce the viability of individuals, for example through reduced heterozygosity, and it may also impact the potential evolution of new adaptations by populations over the long term.

CONCLUSION

This study indicates high levels of Polymorphism in *S. trutta caspius* detectable with microsatellite primers developed from brown trout and rainbow trout. Results of the present study demonstrate that, we may assume the samples are from same population. It means one population exists in the southern part of the Caspian Sea. This is just a preliminary study and it is necessary to continue the study in order to increase the certainty of the assumption of having one population in the southern part of the Caspian Sea. 5 sets of microsatellite primers produced replicable amplification in *S. trutta caspius*. However, they show low genetic differentiation between fall and spring runs.

Results suggest that there is evolutionary conservation of the flanking regions for these loci among related taxa. The cross amplification between salmon species is consistent with earlier findings that primers developed in one species often work in other related species. It seems that spring form is probably a part of fall run population and the deference between them could be attributed to physiology or environmental factors. Artificial breeding practices may inadvertently decrease the genetic variation of the Caspian salmon population by breeding related individuals or by the use of small numbers of parents as brood stocks and these two immigrant forms may have affected bottleneck. Consequently, inbreeding and genetic drift are increased with reduction of effective population size.

Acknowledgment

The authors wish to thank the Tarbiat Modares University for the financial support and its laboratory of genetics and biotechnology of aquatics. They also thank Shahid bahonar propagation center for collecting the samples. This work would have not been possible without the kindly help of Mrs Shirin Jamshidi

Table 2 Summary of microsatellite data: number of observed and effective alleles per locus, tests for deviation from Hardy-Weinberg equilibrium, expected (H_E) and observed (H_O) heterozygosity in fall and spring runs of the Caspian salmon

Forms	Tests	Strutta58	Strutta 12	OmyFgt1TUF	Str543INRA	Str591INRA
Fall	No. of observed alleles	11	15	10	7	7
	No. of effective alleles	5.776	5.358	4.511	2.771	3.991
	H-W. test	** *	** *	** *	** *	** *
	H_E	0.8382	0.8245	0.7890	0.6479	0.7597
	H_O	0.5405	0.6486	0.6486	0.5135	0.7027
Spring	No. of observed alleles	5	5	7	2	6
	No. of effective alleles	2.911	2.617	3.706	1.446	3.459
	H-W. test	*	**	*	Ns	**
	H_E	0.6367	0.5961	0.7150	0.2937	0.7140
	H_O	0.4783	0.3478	0.6087	0.3487	0.4348

*Significant at the 0.05 level; ** Significant at the 0.01; ns. not Significant

Table 3 Calculation of Allelic Richness in fall and spring runs of the Caspian salmon (Gene Alex6)

Form / Locus	Fall	Spring	Both
Strutta58	9.113	5	8.463
Strutta12	11.998	5	10.338
Omyfgt1TUF	8.873	7	8.287
Str543INRA	6.602	2	5.856
Str598INRA	6.741	6	6.949

Table 4 F Statistics and Gene flow (N_m) of the Caspian salmons

Locus / Test	Strutta58	Strutta12	Omyfgt1TUF	Str543INRA	Str598INRA	Mean
F_{IS}	0.282	0.281	0.128	0.056	0.193	0.188
F_{IT}	0.303	0.296	0.141	0.093	0.209	0.209
F_{ST}	0.029	0.021	0.015	0.040	0.020	0.025
N_m	8.299	11.610	16.090	6.059	11.990	9.696

$N_m = 0.25(1 - Fst)/Fst$

REFERENCES

[1] Aho T, Ronn J, Piironen J, Bjorklund M. 2006. Impacts of effective population size on genetic diversity in hatchery reared brown trout (*Salmo trutta L.*) populations, Aquaculture. 56: 244-248.

[2] Banks MA, Rashbrook VK, Calavetta MJ, Dean CA, Hedgecock D. 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of Chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley, Can. J. Fish. Aquat. Sci. 57: 915- 927.

[3] Cagigas ME, azquez EV, Blanco G,. Sanchez JA. 1999. Combined assessment of genetic variability in populations of brown trout (*Salmo trutta L.*) based on Allozymes, microsatellites, and RAPD markers, Marine biotechnology. 1: 286-296.

[4] Carlsson J, Nilsson J. 2001. Effects of Geomorphlogical Structures on Genetic Differentiation among Brown Trout (*Salmo trutta*) Populations in a Northern Boreal River Drainage: Transactions of the American Fisheries Society, 130: 36- 45.

[5] Carvalho GR, Hauser L. 1995. Molecular genetics and the stock concept in fisheries. Molecular Genetics in Fisheries. London, Chapman and Hall. 55-80.

[6] Chakraborty R, Leimar O. 1987. Genetic variation population. N. Ryman and F.M. Utter (Eds.), Population Genetics and Fishery Management. Washington: University of Washington. 89-120.

[7] Charles K, Guyomard R, Hoyheim B, Ombredane D, Baglinière JL. 2005. Lack of genetic differentiation between anadromous and resident sympatric brown trout (*Salmo trutta*) in a Normandy population, Aquat Living Resour, 18: 65- 69.

[8] Dorafshan S. 2007. Chromosomal handlings of the Caspian salmon (*Salmo trutta caspius*) and Rainbow trout (*Oncorhynchus mykiss*) and comparison of the growth in F1. Partial Full fillment for Degree of fisheriy Phd. College of Marine Science, Tarbiat Modares University, 46-52.

[9] Ferguson A, Taggart JB,. Prodohl PA, McMeel O, Thompson C, Stone C, McGinnity P, Hynes RA. 1995. The application of molecular markers to the study and conservation of fish populations, with special reference to *Salmo*. Journal of Fish Biology. 47103-126.

[10] Goudet J. 2001. FSTAT, a program to estimateand test gene diversities and fixationindices (version(2) (9)3).Availablefrom http://www.unil.ch/izea/softwares/fstat.html.

[11] Hansen MM, Ruzzante DE, Nielsen EE, Mensberg KLD. 2000. Microsatellite and Mitochondrial DNA Polymorphism Reveals Life-History Dependent Iinterbreeding between Hatchery and Wild Brown Trout (*Salmo trutta L.*). Molecular Ecology. 9: 583-594.

[12] Hassanien HA, Elnady M, Obeida A, Itriby H. 2004. Genetic diversity of Nile Tilapia revealed by

randomly amplified polymorphic DNA (RAPD). *Aquaculture Research*. 35:587-593.

[13] Jug T, Berrebi P, Snoj A. 2005. Distribution of non-Native Trout in Slovenia and their Introgression with Native Ttrout Population as Observed through Microsatellite. Biological Conservation, 123:381-388.

[14] Kiabi BH, Abdoli A, Naderi M. 1999. Status of the fish fauna in the South Caspian basin of Iran. Journal of Zoology. 18:57-65.

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

[16] Navidi Moghadam Foumani R. 2005. Investiagation of Genetic variation in Caspian salmon (*Salmo trutta caspius*) populations of Tonekabon by using microsatellite markers, MSc thesis, science faculty, The university of Guilan.

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

[18] Neigel JE. 1997. A comparison of alternative strategies for estimating gene flow from genetic markers. Annual Review of Ecology and Systematics. 28:105-128.

[19] Ostergren J. 2006. Migration and genetic structure of *Salmo salar* and *Salmo trutta* in Northern Swedish rivers. PHD thesis, Faculty of forest science, Department of aquaculture science, Umea, Swedish university.

[20] Peakall R, Smouse PE. 2006. GENALEX6: genetic analysis in Excel. Population genetic software for teaching and research. Nolecular Ecology Notes. 6:288-295.

[21] Rafiee A. 2006. Investiagation of Caspian salmon (*Salmo trutta caspius*) Genetic variation, MSc thesis, science faculty, The university of Guilan.

[22] Raymond M, Rousset F. 1995. An exact test for population differentiation. Evolution. 49:1280-1283.

[23] Raymond M, Rousset F. 2004. GENEPOP. http://www.wbio-med.curtin.edu.au/genepop.

[24] Ryman N, Laikre L. 1991. Effects of supportive breeding on the genetically effective population size. Conserv Biol. 5:325–329.

[25] Sambrook J, Fritsch EF, Maniatis T. 1989. Electrophoresis of RNA through Gels Containing Formaldehyde: Molecular Cloning, 2nd edn. Cold Spring Harbor, NY:CSH Laboratory Press. 743–745.

[26] Sonstebo JH, Borgstrom R, Heun M. 2007. Genetic Structure of Brown Trout (*Salmo trutta*) from the Hardangervidda Mountain Plateau (Norway) Analyzed by Microsatellite DNA: A Basis for Conservation Guidelines. Conserv Genet. 8:33- 44.

[27] Verspoor E, Jordan WC. 1989. Genetic variation at the Me-2 locus in the Atlantic salmon within and between rivers: evidence for its selective maintenance. Fish Biology. 35:205-213.

[28] Wright BS. 1951. The genetical structure of populations Annual Eugenics. 15:323-354.