

# Genetic Diversity Between Rainbow Trout (*Oncorhynchus mykiss*, Walbaum) and Snow Trout (*Schizothorax richardsonii*, Gray) By Rapd Markers

Satesh Vasave<sup>1</sup>, Amita Saxena<sup>1</sup>, S. K. Srivastava<sup>2</sup> and A. Barat<sup>3</sup>

1. College of Fisheries, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand – 263 145.

2. Senior Scientist, Experimental Fish Farm, Champawat, Directorate of Coldwater Fisheries Research, Bhimtal.

3. Principle Scientist, Directorate of Coldwater Fisheries Research, Bhimtal.

Received: September 06, 2014 / Accepted: September 30, 2014 / Published: November 25, 2014

**Abstract:** The study of genetic diversity between Rainbow trout and Snow trout were done by using random amplified polymorphic DNA technique. The 5 RAPD primers generated a total 68 consistently scorable bands size ranging from 220-2018 bp. The percentage of polymorphic loci observed to be 17.65 % for Rainbow trout and 35.29 % for Snow trout. According to polymorphic loci the amount of genetic variation between the two species was Rainbow trout < Snow trout from low to high. The higher genetic diversity was found within the Snow trout population (0.1332), and lower genetic diversity was found for the Rainbow trout (0.0755). The Shannon index ranged from 0.1087 (Rainbow trout) to 0.1963 (Snow trout).

**Keywords:** Combretum zenkeri, benzo(a)pyrene, oxidative damage, liver function.

## 1. Introduction

RAPD procedures were first developed in 1990 (Welsh and McClelland, 1990; Williams et al., 1990) using PCR to randomly amplify anonymous segments of nuclear DNA with an identical pair of primers 8–10 bp in length. Because the primers are short and relatively low annealing temperatures (often 36–40 °C) are used, the likelihood of amplifying multiple products is great, with each product (presumably) representing a different locus. It has several advantages and has been quite widely employed in fisheries studies (Okumus Ibrahim and Çiftci Yılmaz, 2003). The method is simple, rapid and cheap, it has high polymorphism, only a small amount of DNA is required no need for molecular hybridization and most importantly, no prior knowledge of the genetic make-up of the organism in question is required (Hadrys et al., 1992).

RAPD markers allow creation of genomic markers from species of which little is known about target sequences to be amplified. This methodology has some disadvantages which include difficulty in reproducing results, subjective determination of whether a given band is present or not, and difficulty in analysis due to the large number of products. This is because RAPDs are not sensitive to any but large-scale length mutations. Therefore, variation might be underestimated (Brown and Epifanio, 2003).

In India, lack of much attempts were noticed regarding the genetic diversity between Rainbow trout and Snow trout. So this study was carried out for analyzing the genetic diversity.

---

### Corresponding author:

Amita Saxena, College of Fisheries, G. B. Pant University of Agriculture and Technology. E-mail: amitasaxena36@gmail.com.

## 2. Materials and Methods

Genomic DNA was isolated from caudal fins by the phenol–chloroform procedure (Sambrook et al., 1989). Electrophoresis was done on 0.8% agarose gel to check the quality of genomic DNA (This technique is simple, rapid to perform and capable of resolving fragments of DNA). The purified genomic DNA, dissolved in TE buffer, was taken for quantification by UV absorbance at 260 nm. The O.D. at 280 nm was also recorded for determining protein contamination.

$$\text{Conc. in } \mu\text{g}/\mu\text{l} = \frac{O.D._{260} \times 50 \times \text{Dilution factor}}{1000}$$

The ratio of O.D.<sub>260</sub>/O.D.<sub>280</sub> gives the amount of RNA or protein in the preparation. A value of 1.8 is optimum for good quantity of DNA. A value of the ratio below 1.8 indicates presence of protein in the preparation and a value above 1.8 indicates the presence RNA. Then the PCR amplification was done of the genomic DNA with primer. The RAPD products were electrophoresed in 1.2% agarose gel with ethidium bromide. 50 µg standard molecular weight marker (100bp ladder) was also loaded in one lane along with the PCR samples and run at 90 volts for 3 hour. The final gel was visualized under Gel-Doc system. Molecular weights of the bands were determined using software and computer. Gels were scored for the presence or absence of amplicon in each lane and the data was recorded as a binary matrix. If a band was present, it was recorded as “1” and if absent, as “0”. The molecular sizes of the RAPD product were estimated by their comparison with standard molecular size marker (100 bp DNA ladder), which was run parallel to the amplified products in the gel or by the help of a computer program. All calculations were carried out using the population genetic analysis software, POPGENE 1.31 (Yeh et al. 1999). The UPGMA dendrogram of population was constructed based on Nei’s (1972) genetic distances using NTSYSpc software. The UPGMA dendrogram of population was constructed based on Nei’s (1972) genetic distances using NTSYSpc software.

## 3. Results and Discussion

The population genetic studies between Rainbow trout (*Oncorhynchus mykiss*) and Snow trout (*Schizothorax richardsonii*) were carried out using RAPD marker. The RAPD marker is very cost effective and not requires any prior knowledge of the target DNA sequence.

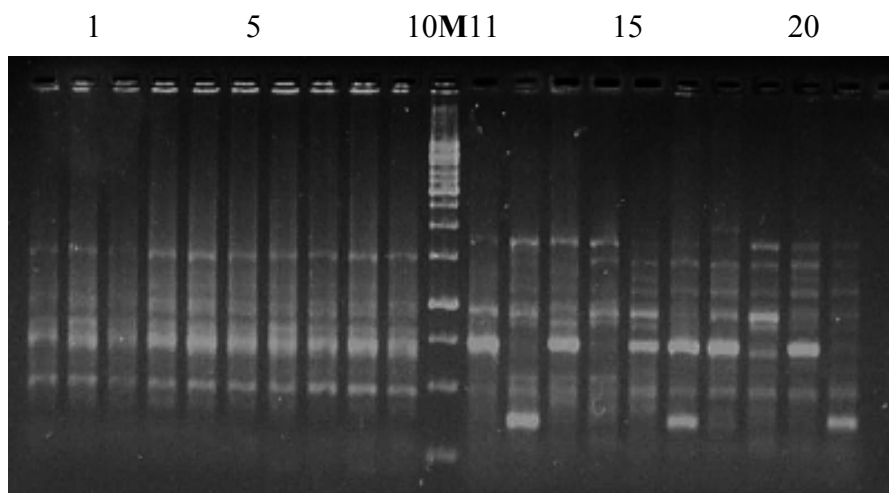
### Statistical analysis

A binary system of 0 (if the band is absent) and 1 (if the band is present) were used to score the unambiguous RAPD bands from gels. A data matrix of individual marker containing the band scoring information was transformed to allele frequencies under the assumption that each amplified band corresponds to different RAPD locus. The statistical analysis of RAPD was performed with the software POPGENE version 1.31 (Yeh et al., 1999). For all statistical tests, I have chosen a significance level  $\alpha = 0.05$  and a simulation of 1000 permutations. Genetic diversity was calculated as observed number of alleles ( $n_a$ ), effective number of alleles ( $n_e$ ) (Kimura and Crow, 1964), the number of polymorphic bands, Nei's gene diversity ' $h$ ' (Nei, 1973), Shannon's information Index (Lewontin, 1972), total genetic diversity in population ( $H_t$ ), within sample gene diversity ( $H_s$ ) for RAPD data.

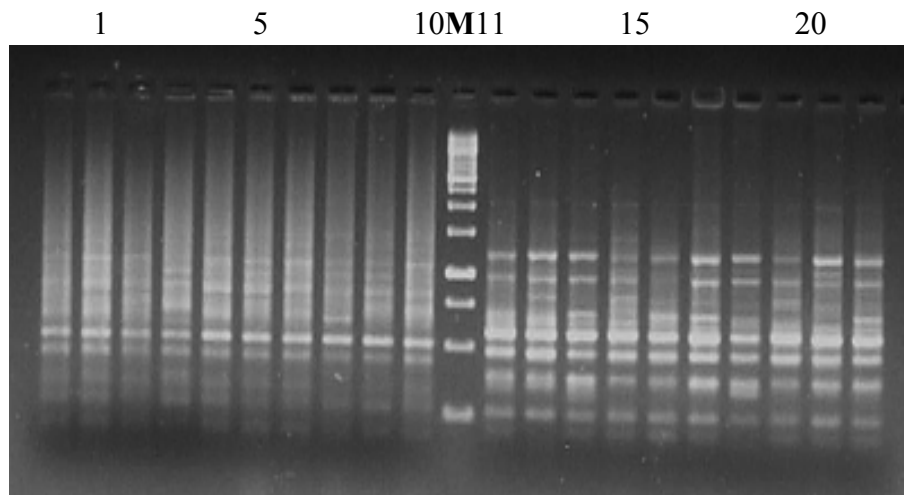
**Genetic Diversity Between Rainbow Trout (*Oncorhynchus mykiss*, Walbaum) and Snow Trout (*Schizothorax richardsonii*, Gray) By Rapd Markers**

**Table 1. Genetic variability found at 68 RAPD loci for Rainbow trout (*Oncorhynchus mykiss*) and Snow trout (*Schizothorax richardsonii*) (SD within parentheses)**

Species	Sample Nos.	na(observed no.of alleles)	ne (effective no. of alleles)	H(Nei's gene diversity)	I (Shannon's Index)	Poly. loci
Rainbow trout	10	1.1765 (0.3841)	1.1363 (0.3076)	0.0755 (0.1675)	0.1087 (0.2395)	12 (17.65%)
Snow trout	10	1.3529 (0.4814)	1.2329 (0.3581)	0.1332 (0.1959)	0.1963 (0.2818)	24 (35.29%)



**Figure 1. OPA-1 Amplified products on 1.2% agarose gel. Each lane shows different individual amplified DNA Samples of Rainbow Trout (lane 1-10) and Snow Trout (lane 11-20) and M, 1 kb molecular weight markers.**



**Figure 2. OPA-2 Amplified products on 1.2% agarose gel. Each lane shows different individual amplified DNA Samples of Rainbow Trout (lane 1-10) and Snow Trout (lane 11-20) and M, 1 kb molecular weight markers.**

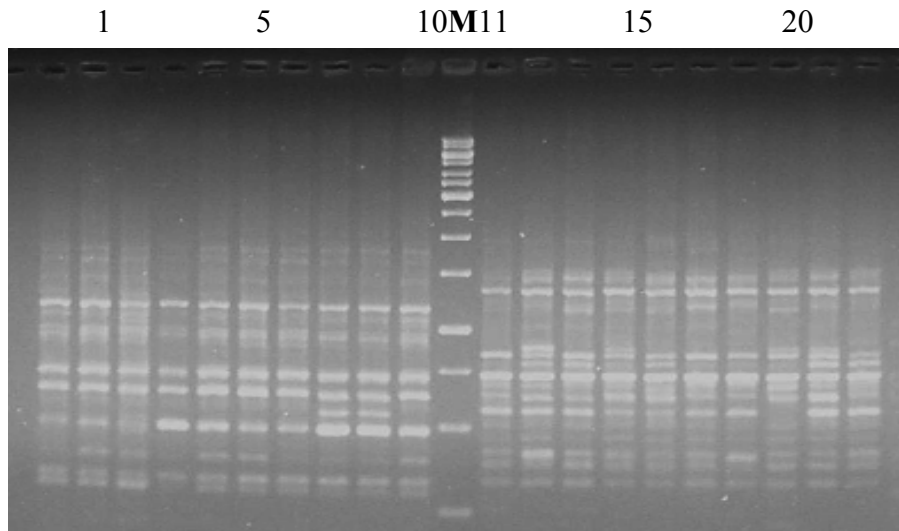


Figure 3. OPA-4 Amplified products on 1.2% agarose gel. Each lane shows different individual amplified DNA Samples of Rainbow Trout (lane 1-10) and Snow Trout (lane 12-21) and M, 1 kb molecular weight markers.

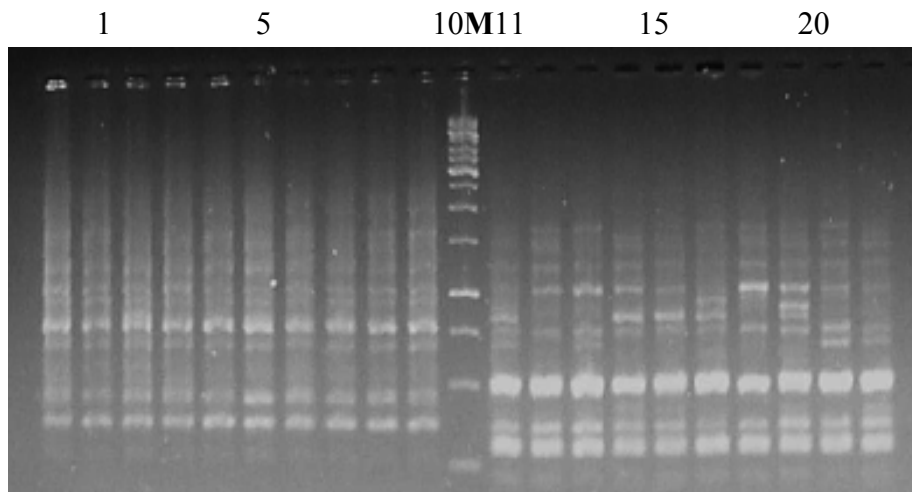


Figure 4. OPA-5 Amplified products on 1.2% agarose gel. Each lane shows different individual amplified DNA Samples of Rainbow Trout (lane 1-10) and Snow Trout (lane 11-20) and M, 1 kb molecular weight markers.

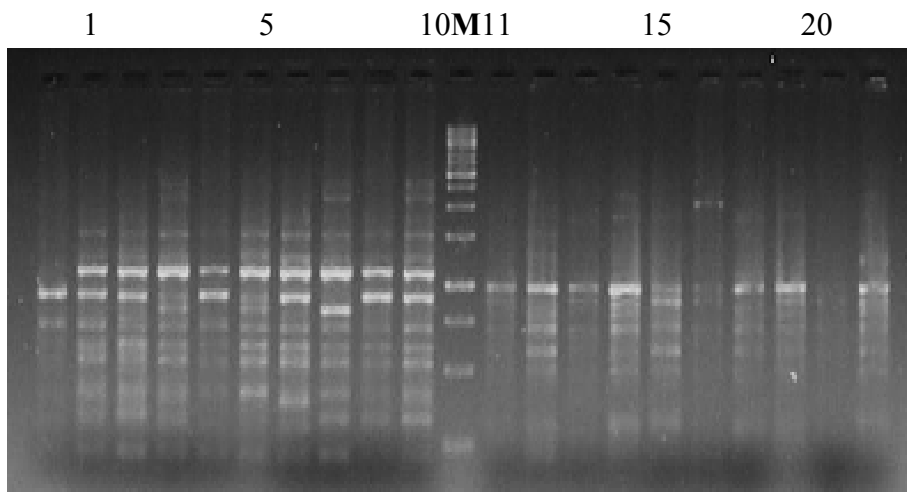


Figure 5. OPA-10 Amplified products on 1.2% agarose gel. Each lane shows different individual amplified DNA Samples of Rainbow Trout (lane 1-10) and Snow Trout (lane 11-20) and M, 1 kb molecular weight markers.

### RAPD diversity and differentiation

The 5 RAPD primers generated a total 68 consistently scorable bands size ranging from 220-2018 bp. The percentage of polymorphic loci was 17.65 % for Rainbow trout and 35.29 % for Snow trout, (Table - 1). According to polymorphic loci the amount of genetic variation between the two species was Rainbow trout<Snow trout from low to high.

According to Nei (1987), the proportion of polymorphic loci is not a good measure of genetic variation. A more appropriate measure of genetic variation is average heterozygosity or gene diversity ( $h$ ). Higher heterozygosity values indicate broader genetic diversity. In the present study, higher genetic diversity was found within the Snow trout population (0.1332), and lower genetic diversity was found for the Rainbow trout (0.0755) (Table - 1). This means that Snow trout population have a higher proportion of heterozygous genotypes than the Rainbow trout population, which was in accordance with the result of Shannon's Information index (Table - 1). The Shannon index ranged from 0.1087 (Rainbow trout) to 0.1963 (Snow trout).

The total gene diversity ( $H_t$ ) in the population was 0.3296, and the genetic diversity within population ( $H_s$ ) was 0.1043. The genetic differentiation ( $G_{st}$ ) of all populations were 0.6835 with a gene flow ( $Nm$ ) = 0.2316.

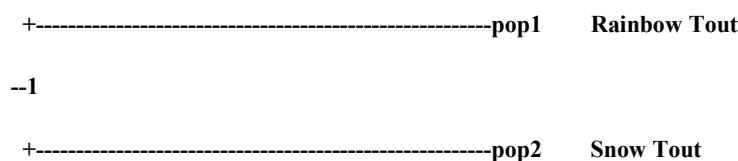
### Genetic distances and dendrogram

Using RAPD data the genetic distance between Rainbow trout and Snow trout was found to be, 0.6925. Based on these genetic distance measures, the dendrogram was made. The dendrogram showed one cluster, using RAPD markers (Figure - 6). The genetic identity between the populations from the amplified patterns of five random primers was 0.5003 (Table - 2).

**Table 2. Nei's unbiased measures of genetic identity and genetic distance (1978).**

Pop ID	Rainbow trout	Snow trout
Rainbow trout	****	0.5003
Snow trout	0.6925	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal): (POPGENE ver 1.32).



**Figure 6. Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA**

### Similarity Index and Dissimilarity Index

The similarity index between all possible pair wise comparisons of individuals from all primers was calculated and phylogenetic relationship between individuals of Rainbow trout and Snow trout samples was constructed using cluster analysis (UPGMA contained in NTSYSpc package). The results showed clustering of Rainbow trout and Snow trout clearly separated from each with two separate clusters and within that each individual were separated by separate cluster. Thus all the individuals of each species formed monophyletic species clusters.

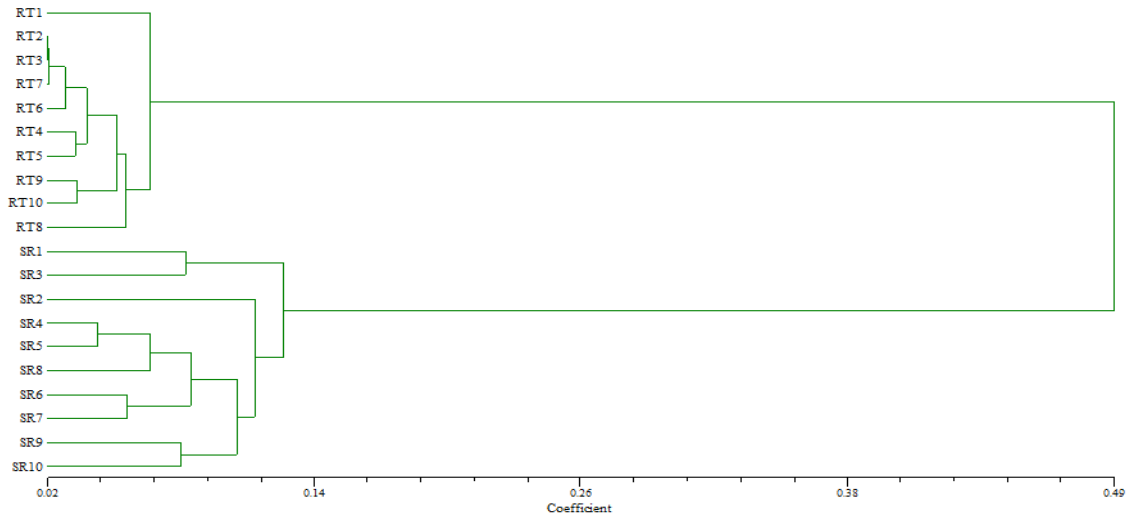


Figure 7. Phylogenetic tree produce by genetic distance between each individual samples of Rainbow Trout (RT1-RT10) and Snow Trout (ST1-ST10) by NTSYSpc 2.2

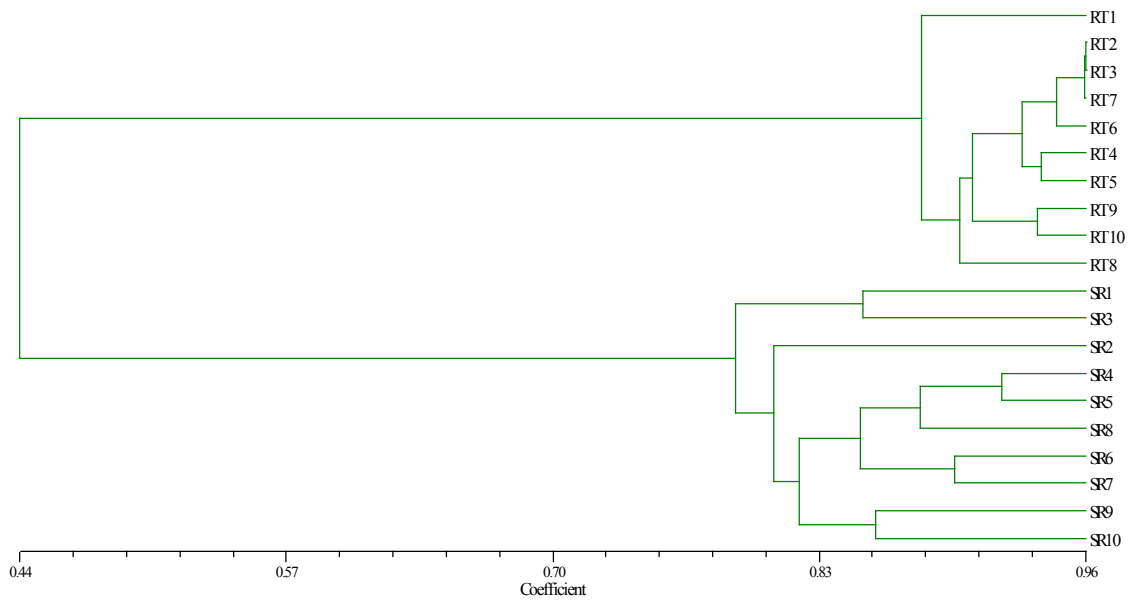


Figure 8. Dendrogram obtained by the Jaccard similarity index and method of UPGMA for Rainbow Trout (RT1-RT10) and Snow Trout (ST1-ST10).

"Science Stays True Here"

Biological and Chemical Research, Volume 2014, 40-51 | Science Signpost Publishing

**Table 3. Matrix obtained by the Jaccard Similarity Coefficient for Rainbow Trout (RT1-RT10) and Snow Trout (ST1-ST10) by using NTSYSpc 2.2.**

	RT1	RT2	RT3	RT4	RT5	RT6	RT7	RT8	RT9	RT10	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST 10
<b>RT1</b>	1																			
<b>RT2</b>	0.918	1																		
<b>RT3</b>	0.878	0.959	1																	
<b>RT4</b>	0.878	0.959	0.918	1																
<b>RT5</b>	0.896	0.939	0.938	0.938	1															
<b>RT6</b>	0.878	0.959	0.918	0.918	0.898	1														
<b>RT7</b>	0.878	0.959	0.958	0.918	0.938	0.958	1													
<b>RT8</b>	0.820	0.900	0.898	0.898	0.917	0.898	0.898	1												
<b>RT9</b>	0.875	0.880	0.840	0.878	0.896	0.917	0.878	0.896	1											
<b>RT10</b>	0.896	0.939	0.898	0.938	0.917	0.938	0.938	0.878	0.936	1										
<b>ST1</b>	0.433	0.406	0.375	0.397	0.403	0.375	0.375	0.381	0.410	0.403	1									
<b>ST2</b>	0.446	0.463	0.433	0.477	0.439	0.433	0.433	0.439	0.446	0.462	0.698	1								
<b>ST3</b>	0.492	0.462	0.431	0.453	0.460	0.431	0.431	0.438	0.468	0.460	0.851	0.759	1							
<b>ST4</b>	0.468	0.439	0.409	0.431	0.438	0.431	0.409	0.438	0.468	0.438	0.776	0.827	0.804	1						
<b>ST5</b>	0.476	0.448	0.418	0.439	0.446	0.439	0.418	0.446	0.476	0.446	0.780	0.830	0.843	0.918	1					
<b>ST6</b>	0.484	0.455	0.424	0.446	0.453	0.424	0.424	0.431	0.460	0.453	0.796	0.811	0.755	0.860	0.827	1				
<b>ST7</b>	0.483	0.453	0.422	0.468	0.452	0.422	0.422	0.429	0.459	0.452	0.809	0.860	0.800	0.875	0.840	0.896	1			
<b>ST8</b>	0.517	0.484	0.453	0.476	0.484	0.476	0.453	0.460	0.492	0.484	0.776	0.759	0.804	0.878	0.880	0.860	0.837	1		
<b>ST9</b>	0.452	0.424	0.394	0.415	0.422	0.415	0.394	0.422	0.452	0.422	0.755	0.774	0.857	0.820	0.824	0.769	0.780	0.784	1	
<b>ST10</b>	0.468	0.439	0.409	0.431	0.438	0.431	0.409	0.438	0.468	0.438	0.776	0.792	0.840	0.840	0.843	0.860	0.837	0.840	0.857	1

Genetic markers have many other potential uses in managing farmed and wild stocks, for example identifying origin of escaped farm stock in Atlantic salmon, rainbow trout and Atlantic cod (Glover 2010) and defining potential introgression of genes from farm escapes into wild populations of salmon (Skaala et al. 2006).

In the present study 5 primers generated a total number of 68 fragments, with the approximate size ranging from 220 to 2018 bp. This wide range of band sizes is comparable to the results of studies carried out by other authors. For example, Hatanka and Galetti, (2003); Dergam *et al.*, (2002); Arnishi and Okimoto, (2004) and Grapputo *et al.* (2006) also employed 3 to 5 random primers in different fish species from different locations and found the total numbers of amplified fragments varied from 31 to 74 with a size ranging from 300bp – 1500bp. Callejas and Ochando (2002) and Das *et al.* (2005) used a maximum of 5 and 15 random primers, respectively and observed more numbers of amplified fragments (270 and 449) in fish species. However, Yoon and Kim (2001) employed only 5 random primers and observed a maximum of 1344 numbers of amplicons. The presence of more numbers of fragments might be due to the presence of more priming site at the template DNA with the particular series of Operon primers employed in their study.

Evaluation of the genetic diversity between Rainbow Trout and Snow trout was carried out by using 10-mer random primers and found a total of 77.94% polymorphism. The *Schizothorax richardsonii* has shown more polymorphism (35.29 %) than the *Onchorhynchus mykiss* (17.65%). It is because of the as Snow Trout collected from the natural waters and this permit wide range of individuals present other than siblings and less probability of mating within same parental stock. Whereas Rainbow trout was collected from the cultured stock in a pond and there are more chances of inbreeding. Whereas, Das *et al* (2005) observed the varied range of 42.6%, 31.7%, 30%, 19.2%, 16.8% and 14.3% polymorphic loci in different carp species. Liu and Chu-Wu (2006) also calculated very high (86.00 – 92.11%) polymorphic loci ratio in five species of snappers using the RAPD technique. Hence, in the present study polymorphisms of alleles found to be less in comparison to other reports.

RAPD markers show dominance property and so, the gene frequency estimates calculated from RAPD data is less accurate than those obtained from co-dominant markers (Lynch and Milligan, 1994).

In this study, Nei's genetic diversity was found to be higher in Snow Trout than Rainbow Trout. The genetic distance between Rainbow Trout and Snow trout was found to be, 0.6925. They show significant genetic differentiation due to low level of gene flow (0.2316). Difference in population gene diversity ( $H_s$ ) and total gene diversity ( $H_t$ ) indicate much genetic differentiation among the population. The overall genetic diversity,  $H_t = 0.3296 \pm 0.0394$ , is largely varied to the average subpopulation genetic diversity,  $H_s = 0.1043 \pm 0.0159$ , which revealed a more genetic differentiation among the Rainbow Trout and Snow trout populations studied ( $G_{st} = 0.6835$ ). Lopes *et al.* (2007) observed moderate genetic variability index ( $P > 42.0\%$ ) in *S. brasiliensis*, when compared to other migratory fish species.

The RAPD technique has been used for phylogenetic relationship in different fish species (Bardakci and Skibinski, 1994; Callejas and Ochando, 2002; Barman *et al.*, 2003; Bardakci *et al.*, 2004; Das *et al.* (2005); Aranishi and Okimoto, 2004; Li and Chu Wu, 2006). Phylogenetic analysis by RAPD showed two different clusters between two populations (Rainbow Trout and Snow trout). Again Jaccard coefficient values gave different cluster for each of the individual samples used for the study. The similarity index between all possible pair wise comparisons of individuals were calculated (Nei 1978). The similarity index value within the individuals of Rainbow trout obtained was 0.820 - 0.959 while in Snow trout individual's ranges from 0.698 - 0.918 and between the Rainbow and Snow trout it ranges from 0.375 – 0.517. This indicates that Snow trout individuals have wide range of similarity index value.



## Acknowledgement

Authors are thankful to Dean, CFSc, Dean PGS, GBPUAT, Pantnagar, India and Director of Directorate of Coldwater Fisheries Research, Bhimtal, India for providing research facilities. One of the authors Mr. Satish Vasave (JRF) is thankful to ICAR for the financial grant to pursue this research.

## References

- [1] Ahmed, M.M.M.; Ali, B.A. and El-Zaeem, S. (2004). Application of RAPD markers in fish: part I- Some genera (Tilapia, Sarotherodon and Oreochromis) and species (Oreochromis aureus and Oreochromis niloticus) of tilapia. *Int. J. Biotechnol.*, 6:86–93.
- [2] Aranishi, F. and Okimoto, T. 2004. Genetic relationship between cultured populations of Pacific oyster revealed by RAPD analysis. *Journal applied Genetics*, 45(4):435-443.
- [3] Avise, J.C. 1994. *Molecular Markers, Natural History, and Evolution*. Chapman and Hall, New York, NY. 511 pp.
- [4] Bahy A. Ali, Tian-Hua Huang, Da-Nian Qin & Xiao-Mei Wang, 2004. A review of random amplified polymorphic DNA (RAPD) markers in fish Research. *Reviews in Fish Biology and Fisheries*, 14: 443–453.
- [5] Bang, X., Kai-Jian, W., Gui-Rong, Z., 2006. Genetic diversity of five freshwater mussels in genus *Anodonta* (Mollusca: Bivalvia) revealed by RAPD analysis. *Acta Hydrobiologica Sinica* 30, 684-691.
- [6] Baradakci, F. and Skibinski, D.O.F. 1994. Application of the RAPD technique in Tilapia fish: species and subspecies identification. *Heredity*, 73: 117-123.
- [7] Bardakci, F.; Tatar, N. and Hrbek, T. 2004. Genetic relationships between Anatolian species and sub- species of *Aphanis Narda*, 1827 (Pisces, Cyprinodontiformes) based on RAPD markers. *Biologia*, Bratislava, 595:559–566.
- [8] Barman, H. K., Barat, A., Yadav, B. M., Banerjee, S., Meher, P. K., Jana, R. K., Reddy, P. V. G., 2003. Genetic variation between four species of Indian major carps as revealed by random amplified Polymorphic DNA assay. *Aquaculture* 217, 115-123.
- [9] Bartfai, R., Egedi, S., Yue, G. H., Kovacs, B., Urbanyl, B., Tamas, G., Horvath, L., Orbath, L., 2003. Genetic analysis of two common carp brood stocks by RAPD and microsatellite markers. *Aquaculture* 219, 157-167.
- [10] Begg, G.A., C.P. Keenan and M.J. Sellin, 1998. Genetic variation and stock structure of school mackerel and spotted mackerel in northern Australian waters. *J. Fish. Biol.*, 53: 543-559.
- [11] Brown, B. and Epifanio, J. 2003. Nuclear DNA. E. M. Hallerman (Ed.), *Population genetics: principles and applications for fisheries scientists*. American Fisheries Society, Bethesda, Maryland: 101-123.
- [12] Caccone, A.; Allegrucci, G.; Fortunato, C. and Sbordoni, V. 1997. Genetic differentiation within the European sea Bass (*D. labrax*) as Revealed by RAPD-PCR assay. *J. Heredity*, 88: 316-324.
- [13] Callejas, C.; Luskova, V. and Ochando, M.D. 2004. A contribution to the genetic characterization of some species of the genus *Gobio* (Cyprinidae). *Folia Zool.*, 53(4): 433 – 436.
- [14] Callejas, C. and Ochando, M.D. 2002. Phylogenetic relationships among Spanish *Barbus* Species (Pisces, Cyprinidae) shown by RAPD markers. *Heredity*, 89: 36–43
- [15] Cortinhas, D. S.; Cristina, M.; Chirlei, G.; Jose, P.A.; Bueno, N. R.; Matoso, D.A. and Margarete, C.M. 2010. A prime inference on genetic diversity (RAPDs) in the marine fish *Atherinella brasiliensis* (Teleostei, Atherinopsidae) from Southern Brazil. *Acta Zoologica*, 91: 242-248.
- [16] Das, P.; Prasad, H.; Meher, P.K.; Barat, A.K. and Jana, R.K. 2005. Evaluation of genetic relationship among six *Labeo* species using Random amplified polymorphic DNA (RAPD). *Aquaculture Research*, 36:564-569.

- [17] Davisson M., T., J. E. Wriggitt and L. M. Atherton, 1973. Linkage of LDH loci in the teleost genus *Salvelinus*. *Genetics* 73: 645-658.
- [18] Dergam, J.A.; Paiva, S.R. and Schaeffer, C.E. 2002. Phylogeography and RAPD-PCR variation in *Hoplias malabaricus* (Blanch, 1794) (Pisces, teleostei) in southeaster Brazil. *Genetics and Mol. Biology*, 25, (4): 379-387.
- [19] El-Alfy, S. H.; Abdelmordy, M. and Salama, M. S. 2009. Genetic Variation among Nile Tilapiine Fishes (Perciformes: Cichlidae) Assessed by Random Amplified Polymorphic DNA (RAPD) Analysis. *Research Journal of Cell and Molecular Biology*, 3(1): 63-70.
- [20] Elo, K., Ivanoff, S., Jukka, A., Vuorinnen, J. A., Piironen J., 1997. Inheritance of RAPD markers and detection of interspecific hybridization with brown trout and Atlantic salmon. *Aquaculture* 152, 55-56.
- [21] El-Zaeem, S.Y. and Ahmed, M.M.M. 2006. Genetic differentiation between sex reversal and normal of Full- Sib Nile Tilapia, *Oreochromis niloticus* based on DNA fingerprinting. *Research Journal of fisheries and hydrobiology*, 1(1): 1-5.
- [22] Ferguson, A.; Taggart, J.B.; Prodohl, P.A.; McMeel, O.; Thompson, C.; Stone, C.; McGinnity, P. and Hynes, R.A. 1995. The application of molecular markers to the study and conservation of fish populations, with special reference to salmon. *Journal of Fish Biology*, 47:103-126.
- [23] Foo, C.L., Dinesh, K.R., Lim, T. M. Chan, W.K., Phang, V.P.E. 1995. Inheritance of RAPD markers in the guppy fish, *Poecilia reticulata*, *Zoological Science*, 12: 535.
- [24] Grapputo, A.; Bisazza A. and Pilastro, A. 2006. Invasion success despite reduction of genetic diversity in the European population of eastern mosquito fish (*Gambusia holbrooki*), *Italian Journal of Zoology*, 73(1): 67-73.
- [25] Hadrys, H.; Balick, M. and Schierwater, B. 1992. Application of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.*, 1:55-63.
- [26] Hatanaka, T. and Galetti Jr., P.M. 2003. RAPD markers indicate the occurrence of structured population in a migratory fresh water fish species. *Genetic Molecular biology*, 26:1415–4757.
- [27] Hatanaka, T. & P. M., Galetti Jr., 2004. Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae) *Genetica*, 3: 239-244.
- [28] Kimura, M. and Crow, J.F. 1964. The number of alleles that can be maintained in finite populations. *Genetics*, 49:725-738.
- [29] Kirpichnikov, V. S. 1981. Genetic bases of fish selection. Translated by G. G. Gause. Springer, Berlin.
- [30] Li and Chu-Wu 2006. Genetic diversity and Molecular markers of five Snapper species. *Journal of Agricultural Biotechnology*, 14(3): 349-355.
- [31] Liu, Z. J., Li, P., Argue, B. J., Dunham, R. A., 1998. Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their F1, F2 and backcross hybrids. *Animal Genetics* 29, 58-62.
- [32] Liu, Z.J; Li, P.; Argue, B.J. and Dunham, R.A. 1999. Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. *Aquaculture*, 174(1-2):59-68.
- [33] Lopes, C. M., Almeida Simoes, D. A. F., Orsi, M. L., Britto, S. G. D. C., Sirol, R. N. and Sodre, L. M. K. 2007. Fish passage ladders from Canoas complex – Paranapanema river: evaluation of genetic structure maintenance of *Salminus brasiliensis* (Teleostei: Characiformes). *Neotrop. Inichthyol.*, vol.5.
- [34] Lu, R. and Rank, G.H. 1996. Use of RAPD analysis to estimate population genetic parameters in the Alfalfa leaf cutting bee, *Megachile rotundata*. *Genome*, 39: 655-663.
- [35] Lynch, M. and Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers. *Molecular ecology*, 3:91-99.
- [36] Marle-Koster, E.V. and Nel, L.H., 2003. Genetic markers and their application in livestock breeding in South Africa: a review. *S. Afr. J. Anim. Sci.* 33, 1–10.

- [37] Moore, S.S., Whan, V., Davis, G.P., Byrne, K., Hetzel, D.J.S. and Preston, N. 1999. The development and application of genetic markers for the Kuruma prawn *Penaeus japonicus*. *Aquaculture*, 173: 19-32.
- [38] Mulcahy, D.L., Cresti, M., Sansavini, S., Douglas, G.C., Linskens, H.F., Bergamini, G., Vignani, R. and Pancaldi, M., 1993. The use of random amplified polymorphic DNAs to fingerprint apple genotypes. *Sci. Hort.* 54, 89–96.
- [39] Naish, K.A.; Warren, M.; Baradaka, F.; Skibinski, D.O.F.; Carvalho, G.R. and Mair, G.C. 1995. Multilocus DNA fingerprinting and RAPD reveal similar genetic relationships between strains of *Oreochromis niloticus* (Pisces: Cichlidae). *Mol. Ecol.*, 4: 271-274.
- [40] Nei, M. 1972. Genetic distance between populations. *American Naturalist*, 106:283-292.
- [41] Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci., USA*, 70: 3321-3323.
- [42] Nei, N., Li, W. H., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA*. 76, 5269-5273.
- [43] Nei, M. 1987. *Molecular evolutionary genetics*. Columbia Univ. Press, New York, 512 pp.
- [44] Nilsson, J. and Schmitz, M. 1995. Random amplified polymorphic DNA in Arctic char. *Nordic J. Freshwater research*, 71:372-377.
- [45] Okumus Ibrahim and Yılmaz Çiftçi, 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.
- [46] Phang, V.P.E.; Foo, C.L.; Dinesh, K.R.; Lim, T.M. and Chan, W.K. 1995. Inheritance of RAPD markers in the Guppy fish (*Poecilia reticulata*). *Zoological science*, 12: 535-541.
- [47] Postlethwait, J.H., Johnson, S.L., Midson, C.N., Talbot, W.S., Gates, M., Ballinger, E.W., Africa, D., Andrews, R., Carl, T. and Eisen, J.S., 1994. A genetic linkage map for the zebrafish. *Science* 264, 699–703.
- [48] Saiki, R.K.; Gelfand, D.H.; Stoffel, S.J.; Higuchi, R.; Horn, G.T.; Mullis, K.B. and Erlich, H.A. 1988. Primer directed enzymatic amplification of DNA with thermostable DNA polymerase. *Science*, 239: 487-491.
- [49] Sambrook, J.; Fritsch, E.F. and Maniatis, T. 1989. *Molecular cloning: a laboratory manual*, 2 edn. Cold spring Harbor Laboratory press, New York.
- [50] Sands, C. J. E.; Jarman, S. N. and Jackson, G.D. 2003. Genetic differentiation in the squid *Moroteuthis ingens* inferred from RAPD analysis. *Polar Biol.*, 26:166–170.
- [51] Schlotterer, C., 2004. The evolution of molecular markers – just a matter of fashion? *Nat. Rev. Genet.* 5, 63–69.
- [52] Thomas, S., 2001. Genetic characterization of *Clarius batrachus* (Linnaeus, 1758) based on Isozyme polymorphism and DNA markers. Ph.D thesis. Central Institute of Fisheries Education, Mumbai. pp 1-71.
- [53] Turan, C., 1997. Population Structure of Atlantic herring, *Clupea harengus* L., in the Northeast Atlantic using Phenotypic and Molecular Approaches. Ph.D. Theses. The University of Hull. Hull. U.K.
- [54] Wang, L., Song, L. S., Lei, L. H., Wei, X., 2003b. Comparison of genetic diversity between populations of *Chlamys farreri* revealed by AFLP and RAPD analysis. *Journal of Zoology* 4, 33- 38.
- [55] Wang, C., Li, S., 2004a. Phylogenetic relationships of Ornamental (Koi) carp, Oujiang colour carp and Long-fin carp as revealed by mitochondrial DNA COII gene sequences and RAPD analysis. *Aquaculture* 231, 83-91.
- [56] Welsh, J., McClelland. M., 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* 18, 7213-7218.
- [57] Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey, S. V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18, 6531-6535.
- [58] Williams, R. N., Evans, R.P. and Shiozawa, D.K. 1998. Genetic analysis of indigenous cutthroat trout populations from northern Nevada. Clear Creek Genetics Lab Report 98-1 to Nevada Department of Wildlife, Reno, Nevada, 30 pp.

- [59] Yeh, F.C.; Yang, R.C. and Boyle, T. 1999. POPGENE version 1.31, The Microsoft Window based freeware for Population Genetic Analysis, Edmanton, Alberta: University of Alberta, available in the internet at <http://www.ualberta.ca/~fyeh/fyeh>.
- [60] Z.J. Liu, J.F. Cordes, 2004. DNA marker technologies and their applications in aquaculture genetics. *Aquaculture* 238: 1 –37
- [61] Zinning, M., Zhimeng, Z., Xianshi, J., Qisheng, T. and Yongquan, Su. 2004. Analysis of RAPD and mitochondrial 16s rRNA gene sequence from *Trichiurus lepturus* and *Eupleuragrammus muticus* in the yellow sea. *In progress in Natural science*, 14 (2): 125-131.