CHAPTER - 6

SUMMARY AND CONCLUSION

6.1 Summary

The aim of present study was to evaluate phenotypic (morphometric and meristic indices) and genotypic (RAPD fingerprinting) characters in the population of an endangered fish, *Tor putitora* (Hamilton) in the lentic and lotic waters of Himachal Pradesh. The findings of the study are summarized below:

6.1.1 Quantitative phenotypic analysis

6.1.1.1 Comparative morphometric characterization

During the study twenty-one morpho-meristic traits are investigated between stocks of *Tor putitora* population originating from two distinct drainages in Himachal Pradesh, viz., Indus drainage system (Seer stream & manmade Pong reservoir) and Gangetic drainage system (Giri River). Mean values of morphometric measurements are compared and no significant differences are observed among the mean indices values except in eye diameter (ED)/head length (HL), which differs at 5 percent level of significance among all the three stocks. The highest mean values of most of the morphometric characters viz., standard length (SL)/total length (TL), pre dorsal distance (PreDD)/TL, post dorsal distance (PostDD)/TL, length of dorsal fin (LDF)/TL, depth of dorsal fin (DDF)/TL, length of anal fin (LAF)/TL, length of pectoral fin (LPF)/TL, length of pelvic fin (LpF)/TL, minimum body width (MinBW)/TL, maximum body width (MaxBW)/TL, distance between pectoral and pelvic fin (DistPec&Pel)/TL, distance between pelvic and anal fin (DistPel&Anal)/TL, length of caudal fin (LCF)/TL, length of caudal peduncle (LCP)/TL, head depth (HD)/HL, pre orbital distance (PreOD)/HL and inter orbital distance (IOD)/HL are observed within the Pong reservoir stock and are not significantly different from those of freely flowing waters of Seer stream and Giri river. Mean values of various morphometric characters show relatedness among the stocks from natural water bodies viz., Seer stream and River Giri than the stock from impounded water.
body i.e. Pong reservoir at 5% level of significance. This may be attributed to the fact that population in Pong reservoir has developed as self-sustaining population.

Various body measurements in relation to total length of *Tor putitora* are calculated for correlation coefficient (r) and regression coefficients (values of a and b in regression equations) and regression equation between an independent character (X) versus various dependent characters (Y) are extrapolated. All the body and head measurements during the present study are found to be under the high correlation coefficient range (0.90-1.00) among the three stocks of *T. putitora*. High degree of positive correlation (r> 0.91) with less than one percent level of significance (p< 0.05) between independent (total length and head length) and respective dependent morphometric characters indicate that morphometric characters increase in direct proportion to independent characters.

The ‘b’ values of different variable characters (Y) in proportion to total length (X) in *Tor putitora* from Seer stream indicate that the rate of growth in standard length (SL) is the highest (0.752) and the lowest (0.130) for minimum body width (MinBW). While in relation to head length, ‘b’ value is the highest (1.091) for inter-ocular distance (IOD) and the lowest (0.057) for head depth (HD).

The ‘b’ values of different variable characters (Y) in relation to total length (X) of *Tor putitora* from Giri River show that the rate of growth in standard length (SL) is maximum (1.054) and minimum (0.069) for minimum body width (MinBW). On the other hand in relation to head length, ‘b’ is maximum (0.769) for head depth (HD) and minimum (0.243) for eye diameter.

The ‘b’ values of different variable characters (Y) in relation to total length (X) of *Tor putitora* from Pong reservoir indicate that the rate of growth in standard length (SL) is the highest (0.853) and the lowest (0.034) for length of pelvic fin (LpF). On the other hand, ‘b’ is the highest (1.119) for interorbital distance (IOD) and the lowest (0.042) for head depth (HD) in relation to head length.
Various morphometric characters in three stocks of *T. putitora* population are grouped following Vladykov’s categories. Out of twenty-one characters studied, eighteen characters in seer stream, fifteen characters in each of Giri river and Pong reservoir are genetically controlled. While three characters in each of three stocks are environmentally controlled and three characters in each of Giri river and Pong reservoir lies under intermediate category. Majority of morphometric characters in *Tor putitora* are genetically controlled as they exhibit narrow range differences with 86% genetically controlled characters in Seer stream stock and 72% in Giri River and Pong reservoir stock each, *Tor putitora* is placed under Vladykov’s category of restricted distribution.

6.1.1.2 Comparative Meristic analysis of *Tor putitora* stocks

The meristic characters observed among all the three stocks of *Tor putitora* show no significant differences except lateral line scale counts which differ significantly among the three stocks of *Tor putitora* (t-test; p<0.05). This may lead to conclude that meristic counts are independent of body size and there is no change in meristic counts with increase in body length observed.

On the basis of present observations the meristic formula is summarized as:

D = 12 (4/8), P=15 (1/14), p=9 (1/8), A=8 (3/5), C=17, Ll= 23-28, Ltr=7

6.1.2 Quantitative genotypic analysis

6.1.2.1 RAPD analysis

Genomic DNA samples extracted from fresh blood preserved in 95% ethanol from Seer stream, Giri river and Pong reservoir stocks of *Tor putitora*, are amplified with 18 decamer random primers. Of which 10 decamer primers (OPAA-01, OPAC-09, OPAC-11, OPAC-12, OPAH-06, OPAH-10, OPB-05, OPB-08, OPB-10 and OPY-02) generated polymorphic patterns, which are clear and easy to interpret are selected for further analysis. Primer OPB-05 and OPB08 are most reproducible primers among the 10 RAPD primers used in this study, since the numbers of bands produced are 11 each. While the minimum number of bands 4 are produced by OPAC-09 primer. On the other hand, the primer OPB-05 produces
the most shared markers among the 3 stocks of *Tor putitora* population which account 8 bands.

The total number of RAPD bands produced in Seer stream, Giri River and Pong reservoir stocks of *Tor putitora* are 88 bands, of which 42 bands are polymorphic among three stocks and account of 47.72% proportion of polymorphic loci among 3 stock of *Tor putitora*. The percentage of polymorphic loci is higher in Seer stream and Giri river population of *Tor putitora* than in Pong reservoir population is indicative of relatively high level of genetic variation among Seer stream and Giri river than Pong reservoir population. The pair-wise inter-population genetic dissimilarity observed among Seer and Giri (0.272), among Seer and Pong (0.211) and among Giri and Pong (0.258) (Table 5.14). The genetic similarity value of 3 stocks is found to be highest among Seer and Pong (0.788) than in Giri and Pong (0.762) and lowest in Seer and Giri (0.727) (Table 5.14). This may be attributed to the mixing of Seer and Pong reservoir stock as of same Indus river system. However, all the three stocks of *T. putitora* show almost similar level of intrapopulation similarity indices viz. 76% in Seer stream, 80.76% in Giri River and 81.16% in Pong Reservoir implying that individuals within each population are genetically close to each other.

### 6.1.2.2 UPGMA dendrogram

UPGMA dendrogram based on Jaccard’s similarity matrix (Table 5.19) and genetic distance is constructed (Fig. 5.67). This measurement indicated the segregation of Seer stream, Giri River and Pong Reservoir population of *T. putitora* into two distinct clusters: the Pong reservoir and Seer stream produces one cluster whereas Giri River population belongs to another cluster. Two major clusters attributed to their variable River systems as Pong reservoir (Beas basin) and Seer Stream (Sutlej Basin) are belonging to Indus River System while Giri River (Yamuna basin) belongs to Ganga River System. UPGMA dendrogram shows 66% similarity among the three stocks of *T. putitora* and approximately
70% similarity among the Seer stream and Pong reservoir stocks forming second major cluster.

Minor clustering within the Pong reservoir is lesser in number than within the Seer stream and Giri stream stocks of *T. putitora*, indicates that inbreeding of the Pong reservoir population have taken place sharing the similar ancestral gene pool with less genetic diversity within the Pong reservoir population, while number of minor clusters are more in Seer stream and Giri river populations in *T. putitora* indicates that sharing and mixing of gene pool is least among these populations attributed to their naturally uninterrupted migrating routes allow them to migrate freely and reproduce.

**6.1.2.3 Principle Component Analysis (PCA)**

Analysis covariance matrix of RAPD profiling of *T. putitora* in Seer Stream, Giri River and Pong reservoir populations indicated that the first five Principle Components (PCs) explained about 66.71% of variance of the RAPD profiling (Table 5.20). Principal Components Analysis does not require any prior information about the groups in RAPD analysis. In the present study 88 observations are made on 36 variables from Seer stream, Giri river and Pong reservoir stocks of *T. putitora*. Among the resultant 36 principal components, first (PC-1), second (PC-2), third (PC-3), fourth (PC-4) and firth (PC-5) principal components accounted for 66.71% of total cumulative variation.

PC-1 explains 28.19% of the variation, PC-2 explains 17.22% of variation, PC-3 explains 12.91% of variation, PC-4 explains 4.745% and PC-5 explains 3.64% of additional variation. XY scatter diagram obtained by plotting these five (PC-1, PC-2, PC-3, PC-4 and PC-5) principal components show variable clusters indicating Seer stream and Giri river stocks are closer than Pong reservoir stocks of *T. putitora* population.

The 95% variance threshold method provided satisfactory results. The choice at 95% of the total variance is relatively high and this approach appears promising because there is low risk that many of the components that are retained will not summarize noise or non-trivial components not included.
6.2 Conclusion

It is observed in this study on *Tor putitora* that either the phenotype analysis based on a large number of morphometric character indices and meristic counts, or genotype analysis based on RAPD fingerprinting can be used to discriminate fish populations with the same results up to the intra specific level, or both the phenotype and genotype analyses can be used to assess the degree of phenotypic plasticity shown by the populations. Nonsignificant differences in phenotypic characters indicate population homogeneity among Seer stream, Giri river and Pong reservoir stocks. A more definite conclusion, however, may be reached with longer number of samples including other rivers (if possible to collect) with TRUSS network analysis for morphometric characters and faster evolving molecular markers such as microsatellite loci. The level of phenotypic and genotypic variation provides the raw material for the selective improvement of a stock for the selective improvement of a stock for sustainable aquaculture production.

By this study, we have revealed, for the first time, the intra-population and inter population genetic variability between the three populations of *T. putitora* in Himachal Pradesh, India. The results of the present study can be used as a baseline for further study involving this threatened species in the country.

6.2.1 Implications for conservations

Due to high levels of endemism and human pressure, fresh water fish faunas around the world are under serious threats (Magurrun, 2009). Threats of freshwater fish species require special attention because historical influences on distribution and diversity patterns may be more evident in freshwater fishes than in other taxonomic groups (Rosenfeld, 2002). Seer stream, Giri river and Pong reservoir population of *Tor putitora* show narrow and restricted range distribution, as results reveals that most of the morphological characters lies under the genetically controlled category with less than 10% level of range difference.
Narrow endemic or restricted range species are conservation targets due to intrinsic biological features (IUCN 2001 and Gaston 1996). They indicate sites for which there are few spatial options for conservations (Eken et al., 2004 and Lawler et al., 2003). Most important, sympatric occurrence of restricted range species is a valuable indicator of areas of endemism (Rosen, 1978; Rosenfeld, 2002 and Hausdorf, 2002). Alarmingly, restricted range species tend to be the most poorly respondents biodiversity targets in site selection analyses (Lawler et al., 2003 and Araujo et al., 2001).

Present results highlight the importance of refining the morphometric characterization in the various stocks of *T. putitora* population available in India for its careful revision and complication of detail restricted narrow range distribution. Results of the present study should be considered as a crucial step in threat assessments or priority setting exercises in regions that combine low intra population diversity and high rates of habitat loss. To avoid under estimation in the number of restricted-range population of *Tor putitora* detected, other distributional range should also be evaluated.

Pong reservoir population of *Tor putitora* is now considered as selfrestrained population with high endemism and low genetic variability due to Pong dam generating intercepted migratory routes of this migratory fish, needs attention of conservation measure. The location of narrow endemic species, along with complimentary conservation targets such as maintenance of migratory routes of large commercially important fish species (Barthem et al., 1991; Petrere, 1996 and Agostinho et al., 2004) should be decisive in locating hydropower projects. However, hydropower plants are still causing harm to entire natural fresh water communities around the globe (Dudgeon et al., 2006, Anderson et al., 2006 and Fearnside, 2006). *Tor putitora* being is a critical component of conservation strategies that should be integrated to other conservation targets in conservation planning initiatives. Characterization of genetic diversity is a necessary requirement for the improvement, used and conservation of genetic resources (Chandra et al., 2010). Maintaining genetic diversity has become a major issue in conservation biology as it is generally thought to be important for the overall species viability and the
potential for evolutionary responses to environmental change (Meffe and Carrol, 1997). Loss of genetic diversity could lead to a decline in a species ability to cope with changing environment and demographic fluctuations both in the short and long term (Milligan et al., 1994). Genotypic characterization of *Tor putitora* stocks and populations is an underexplored area as little is known about the genetic variability and similarity within and between the population of *T. putitora*, research is needed to developed and standardize techniques for their genetic studies. Also, the reproductive biology of *T. putitora* is underexplored and techniques for its induced breeding and artificial propagation can be used to conserve the species through captive breeding programs and also to generate new employment opportunities.

High level of genetic similarity and low genetic differences between the populations of *Tor putitora* in present study suggested lower differentiation rate between populations and could be result of genetic drift, as also observed in to populations of Neotropical fish *Brycon lundii* (Wasko and Galetti Jr., 2002).

Different strategies for *Tor putitora* conservation such as in situ (Protection sties), ex-situ (live gene banks, cryopreservation of fish gametes and embryos), general awareness among fishing communities about the importance of environment and fish resources conservation, can contribute to conversation, optimum utilization and recovery to *Tor putitora* population.

Approaches employing several DNA marker systems *viz.* microsatellite and D-loop methods may increase the accuracy of genetic studies of the various populations of *Tor putitora*. Thus, further genetic analysis, including other localities and at different times along the year, using micro satellite and mitochondrial DNA markers will further enhance the genetic knowledge of *Tor putitora* in greater detail to be used in reproductive and restocking programs.