SUMMARY

The Indian minor carp *Labeo dero* (Family *Cyprinidae*) contributes to major share in capture among the minor carps. It has less bones, and soft flesh when young. Moreover, it has high fecundity and it forms a commercially important food fish (Chondar, 1999). These properties make it a commercially attractive species for culture.

*Labeo dero* (Hamilton, 1822) is basically a Gangetic carp having a natural distribution in the Indus river system; the Ganga river system, the Brahmaputra and the Mahanadi River. The fish inhabits freshwater rivers, reservoirs, lakes, “bheels”, “jheels” and ponds.

For the significance attached to the species, effective conservation and propagation assisted rehabilitation strategies, need to be urgently planned. There is no recorded information on any class of genetic markers and stock structure in *L. dero*. The genetic variation information can provide data that can be used to provide scientific basis for evolving measures of conservation and management of natural populations by using molecular genetic methods.

The study was done under three broad segments: i) Identification of polymorphic microsatellite and allozyme markers, ii)
Quantification of genetic variability and distribution patterns within and between the sub-populations and iii) Estimation of gene flow and population structure of *L. dero* natural population for identification of distinct genetic stocks.

In *L. dero*, for identification of allozyme loci, a total of 18 different allozymes were tried on 15 different individuals collected from distinct geographical locations. The standardisations of conditions were done for the tissue samples (liver / muscle), volume of sample extract to be loaded and the running time for every enzyme system. The standardized conditions were then used on the 18 enzyme systems; which gave twenty eight loci, out of which 8 i.e. *EST-1*, *EST-2*, *PGDH*, *GPDH*, *GPI*, *PGM-2*, *XDH* and *MDH-2* were polymorphic. A total number of alleles ranged from 2 to 5.

Through cross species amplification of 62 microsatellite primers from related species, a total number of 19 microsatellite loci were identified in *L. dero*. Polymorphism was detected at eight microsatellite loci, the number of alleles ranged from 3 to 9. The 8 polymorphic loci used for population genetic studies in *Labeo dero* were *MFW-1*, -15, -17, -24, -26, *Ca-12*, *R-12* and *R-3* and size range of alleles observed at these loci 169-189 bp, 136-198 bp, 186-214 bp,
159-165 bp, 106-118 bp, 152-192 bp, 115-134 bp and 92-128bp, respectively.

In allozyme analysis, the mean number of alleles per locus ranged from 1.28 (Beas and Satluj) to 1.43 (Tons) and mean observed heterozygosity from 0.029 (Beas) and 0.071 (Tons). At three allozyme loci \textit{EST-1*}, \textit{EST-2*} and \textit{XDH*}, genotype frequencies significantly deviated from the Hardy-Weinberg expectation. For testing for Hardy-Weinberg equilibrium, out of total 72 tests performed, significant deviations were detected for 17 tests, after sequential Bonferroni corrections to probability levels (P<0.0014). The results exhibited significant deficiency of heterozygotes (+ve F\textsubscript{is}) at different allozyme loci and localities.

In microsatellite analysis, the mean number of alleles ranged from 3.75 (Beas) to 5.38 (Jiabharali) and the observed heterozygosity value over all loci ranged from 0.0295 (Satluj) to 0.4395 (Gerua). Significant deviation was detected, after sequential Bonferroni procedure to probability levels (P<<0.00091). The probability values showed significant deviation in all population at seven loci. At those loci, where HW disequilibrium was observed, heterozygosity deficiency was evident from the positive F\textsubscript{is} value.
At allozyme loci, one locus pair (EST-1* and PGDH*) showed linkage disequilibrium in *L. dero*. While at microsatellite loci did not found linkage disequilibrium.

For bottleneck analysis, the allozyme loci were analyzed under Infinite Allele model (IAM) of mutation and microsatellite loci under Infinite Allele model (IAM) and two Phase modal (IAM and TPM) of mutation to detect if the population have undergone any genetic bottlenecks in the recent past. In allozyme analysis, significant probabilities for excess of heterozygosity were found in *Labeo dero* samples from samples of river Tons (Wilcoxon P = 0.04688) however, for microsatellite analysis, from two localities, Brahmaputra (Wilcoxon P = 0.01953) and Mahanadi (Wilcoxon P = 0.3906) under IAM and from Ganga (Wilcoxon P = 0.03906), Kosi (Wilcoxon P = 0.01953) and Gerua (Sign Test P = 0.00845 and Wilcoxon P = 0.01172) localities under TPM. Mode-shift was observed at microsatellite loci in samples of river Mahanadi.

Pairwise comparison to assess allelic homogeneity and pairwise $F_{st}$ and probability values were analysed for allozyme and microsatellite markers in *L. dero* from nine different collections, to assess the genetic divergence in *L. dero*. Allozyme analysis revealed that
25 out of possible 36 tests had significant allelic heterogeneity, after Sequential Bonferroni correction to the probability level. Out of a total of 36 pair wise comparisons, thirty two pairs indicated significant (p<0.05) divergence between samples from different drainages. The mean $F_{st}$ of all the loci across all collections was 0.059. The pairwise $F_{st}$ values ranged from 0.01496 (Satluj and Beas) to 0.24492 (Brahmaputra and Tons).

In microsatellite analysis, total of 36 tests for allelic homogeneity over all loci were performed and all the population pairs that were significant (P<0.05) except between Satluj and Beas and, Yamuna and Ganga, and Gerua and Ganga. The mean $F_{st}$ value over all collections and all loci was 0.019, revealing 1.9% of the total genetic variation was due to variation among samples, and there exists genetic substructure in *L. dero* population. The pairwise $F_{st}$ values ranged from 0.0008 (Satluj and Beas) to 0.14 (Mahanadi and Tons).

The present investigation revealed that genetic differentiation was found in *L. dero* natural population through both the allozyme and microsatellite markers. High significant divergence of Mahanadi samples from all the samples is supported by both the markers. In Indus system, Satluj and Beas were not genetically different. *L. dero* from Indus and Ganga river system were most divergent from that of Mahanadi. It is
worth mentioning here that *L. dero* from Mahanadi need further scientific investigation.

Evidence for presence of more than one genetic stocks of *L. dero* is drawn from partitioning of variability (F-statistics). On the basis of the distribution of genetic variation indicated by allozymes and microsatellites five different genetic stocks of *L. dero* from the four river basins can be identified i.e. (i) from Indus river system (Satluj and Beas), (ii) Ganges river system, including Ganga, Yamuna, Kosi, Gerua, (iii) Tons, (iv) Bhramaputra (Jiabharali) and (v) Mahanadi. The results provided the evidence that *Laboe dero* in different rivers in India has distinct populations structure. In broad range, comparing across the riverine basins *L. dero* in Indus, Ganges, and Mahanadi are genetically different and conservation and management strategies need to be planned accordingly.