CHAPTER – 1

INTRODUCTION

Fishes comprise nearly half of global vertebrate species. The group is represented by 27,977 valid species including 11,952 primary freshwater, 12,457 secondary freshwater and 3,568 exclusively marine species (Nelson, 2006), however the total number of fishes has increased to 32,700 in the year 2013 (FishBase, 2013). The proportion of fish diversity in the Indian subcontinent is about 8.9% of the world ichthyofauna, consisting of approximately 2,500 species - 930 primary and secondary freshwater species, and 1,570 marine species (Nelson, 2006). The primary freshwater fish diversity in India consists of 667 species, represented by 62% cyprinoids, 26% siluroids and 12% other groups (Jayaram, 1999, 2006; Menon, 1999; Ponniah and Gopalakrishnan, 2000; Ponniah and Sarkar, 2000; Nelson, 2006; Vishwanath et al., 2007; Devi and Indra, 2009).

The cyprinoids commonly called as carps with about 3969 species, form the largest group of fishes contributing greatly towards inland fisheries in Southeast Asia, including India (Eschmeyer and Fong, 2013). Among the carps, the mahseer fishes of the genus Tor are regarded as the best sport and most sought after food fish in the region (Thomas, 1897; McDonald, 1948).

The genus Tor includes ten valid species viz. T. putitora (Hamilton, 1822), T. tor (Hamilton, 1822), T. khudree (Sykes, 1839), T. progenius (Gray, 1833), T. kulkarni (Menon, 1992), T. musullah (Sykes, 1839), T. mosal (Hamilton, 1822), T. malabaricus (Jerdon, 1849), T. neilli (Day, 1869) and Tor barakae (Arunkumar and Basudha, 2003) in different parts of India (Menon, 1992; Devi and Indra. 2009; Eschmeyer and Fong, 2013).

Among the mahseer fishes, T. putitora is a large sized fish attaining a maximum length of 274 cm (Rahman, 1989) and maximum weight of 54 kg (Banarescu and Coad, 1991). It is of immense commercial fishery importance in the Himalayan foothills (Thapa,
1994). It is commonly known as ‘The Golden Mahseer’ because of the characteristic golden colouration of its fins; sometimes also referred to as ‘yellow-finned mahseer’, ‘grey hound mahseer’ and ‘thick-lipped mahseer’ (Jha and Rayamajhi, 2012). The golden mahseer is widely distributed in the Hindkush Mountains and Himalayas (Day, 1878; Jhingran, 1983), Northeast Hills (Petr, 2002), Bangladesh (Talwar and Jhingran, 1991), Bhutan (Rajbanshi and Csavas, 1982), Myanmar (Oo, 2002) and Thailand hill ranges. In India, it is widespread in Jammu & Kashmir, Himachal Pradesh, Uttrakhand, Uttar Pradesh, Bihar, Darjeeling, Sikkim, Assam, Meghalaya, Mizoram, Manipur and Nagaland (Rainboth, 1990, Jha and Rayamajhi, 2012). In Himachal Pradesh, the golden mahseer occurs in all major rivers, lakes and reservoirs. It is a highly precious and most sought after fish of the Pong reservoir.

*T. putitora* is column feeder, being omnivorous during the adult stage but feeds on periphytic algae and diatoms in juvenile stage (Sehgal et al., 1971; Pathani and Das, 1979; Badola, 1979; Nautiyal and Lal, 1984, 1985; Shreshtha, 1990). It prefers water with pH ranging from 7.4 - 7.9 and temperature varying from 13°C to 30°C (Shreshtha, 1999). It avoids very cold waters, and congregates by the hundreds in the lower reaches of the rivers during the winter (Day, 1889). According to an FAO report (1997), *Tor putitora* needs water temperature above 15°C to breed and can withstand temperature above 30-35°C, it should not be called a cold water fish but rather a warm water fish.

In response to various physical, chemical and biological stimuli, *T. putitora*, like other mahseer fishes migrates to small tributaries during the breeding season which is from June to August (Beaven, 1877; Desai, 2003; Nautiyal, 1994; 2002). First flooding caused by snowmelt is a signal for the adults to migrate upstream towards the glacier fed tributaries during prespawning period from March to April to lay their gametes (sperm and ova). Coinciding with the onset of winter in October-November, juveniles and the spent adults move downstream to their feeding areas in larger rivers where they grow older and larger
(Malik and Negi, 2007). The complete mahseer stock does not migrate, as many of the potential brooders have been found to be present in major streams of its habitat.

The golden mahseer is endangered and included in the IUCN Red Data Book, 2012 (Sharma, 2009; Jha and Rayamajhi, 2011). The main threats are from overfishing, and loss and deterioration of habitat including the breeding grounds (Stone, 2007). Besides, the construction of a number of dams across rivers has disrupted the migration of *T. putitora*, thus affecting its feeding and breeding. Decline in its population has been observed in most of the Indo-Gangetic drainages over the west (Sehgal et al., 1971; Joshi, 1988; Sunder and Joshi, 1977; Sehgal, 1994) and central Himalaya (Shrestha, 1997). The stress on the mahseer population is not only due to their overexploitation for its table value but also due to the rise in developmental activities, especially the growing number of hydroelectric-cum-irrigation projects which have fragmented and deteriorated its natural habitat (Nautiyal and Singh, 1989).

Consequently, the golden mahseer is no more available in Kashmir, and some areas in Himachal Pradesh and Uttrakhand (Kumar, 1999; Khan and Sinha, 2000). Its migration into the Kangra valley has been completely stopped due to the construction of Pong Dam across the river Beas (Petr, 2002). However, it has established self-reproducing stocks in the fragmented populations (Petr, 2002).

It is estimated that its population has declined by more than 50% and it may reach even up to 80% with the construction of more dams (Jha and Rayamajhi, 2012). Golden mahseer serves as a primary food source as well as a source of economic security, and because it is central to the biodiversity of freshwater eco-system, the subject of this fish must be promoted to the level of a key policy issue to be addressed by all facets of the national community. It is, therefore, very important to conserve this fish not only for the livelihood of people but for sustainability and economy of the Himalaya.
The major theme in conservation biology is the preservation of diversity such as genetic, morphological, behavioral and other aspects of variation on different organization levels from whole ecosystems to species and lower levels (Ford, 2004). Deoxy-ribo nucleic acid (DNA) techniques increase insight into genetic variation at different functional levels, whereas morphology and morphological variation has been a continuous base for studies even long before Darwin (1859). The morphological variation in a species is an important component, which repeatedly has been shown to correlate with factors such as diet, habitat and predation risk in fishes (Helland et al., 2009; Schluter et al., 2010). In India, recent significant efforts have resulted in developing successful cell cultures and cell lines from *Tor putitora*, *Lates calcarifer* and *Labeo rohita* (Lakra et al., 2005; 2006 a, b).

Variation within and between populations and stock discrimination within exploited species are important issues not only in fisheries management but also for conservation programmes and to recognition of groups within a species which are largely reproductively isolated from each other. Many nongenetic methods of stock discrimination are available for achieve varying degrees of success in distinguishing breeding stocks (Okumus and Ciftci, 2003). Morphological and meristic characters have both heritable (genetic) and nonheritable (environmentally influenced) components. However, natural selection and evolutionary history can shape morphological characters but differences among populations, subspecies or species may also be influenced or determined by the environment (Okumus and Ciftci, 2003).

Morphometrics is the quantitative analysis of organism’s shape and integral component in evolutionary ecology and developmental studies in biology, while taxonomists and systematists use morphological information to describe and diagnose species (Shearer, 1994). Morphometric includes external measurements of an organism, while meristic counts deal with serial counts of body elements (Talwar and Jhingran, 1992). Morphological characters including meristic counts and body proportions often vary along a geographic gradient (Lindsey, 1988). Morphometric analysis also helps to understand the relation between body parts (Carpenter, 1996). The morphometric relationship between length and weight can be used to assess, the well being of individuals
and to determine possible differences between separate unit stocks of same species (King, 2007). The assessment of intra and inter population variation, could also determine how and to what extent differences among individuals are shaped into the differences that separate races and species. In India, intra-specific level variation has been assessed in finfishes and shellfishes within and between populations that provided information on stock structure of the prioritized endangered and commercial fish species. (Punia et al., 2005; Lal et al., 2004 a, b; Mohindra et al., 2001; 2004, 2005; Gopalakrishnan et al., 2006, 2004; Singh et al., 2004). The comparative study on phenotypic characterization of Tor putitora from the Himachal Pradesh has not been reported yet, which would be very useful for its and Himalayan sustainability.

Molecular data have proven useful for clarifying the taxonomic relationships, species identification and assessment of stock structure and defining species boundaries in morphologically conservative or highly variable groups of freshwater fish (Ward, 2000). Genetic data have become increasingly important in assessing the gene flow between populations, which is crucially important for the maintenance of genetic diversity. Loss of genetic variability due to population fragmentation has been reported in some neo tropical migratory fishes (Agostinho et al., 2004). Sapna (1999) and Vijayakumar (1992) have reported genetic homogeneity in the stocks of grey mullet (Mugil cephalus) from east and west coasts of India. Similarly, Menezes (1993; 1994a,b), Verma et al., (1994, 1996) and Jayasankar et al., (2004) using allozymes, RAPD and morphometrics data have recognized populations of Indian mackerel (Rastrelliger kanagurta) and oil sardine (Sardinella longiceps) that belong to the same unit stock. Preliminary analysis on seahorses (Hippocampus kuda) using sequence information of cytochrome b gene of mtDNA, sampled from Arabian Sea and Bay of Bengal revealed that these two populations are genetically close to each other (Thangaraj et al., 2003). The development of molecular technique for genetic analysis has led to a great increase in our knowledge of fish genetics and or understanding the phylogeny and genetic relations in fishes.
Measuring genetic diversity in wild fish population, or aquaculture stocks is essential for interpretation, understanding and effective management of these populations or stocks. Genetic variation at molecular level has been used in population genetic studies by employing a variety of technical approaches and a wide range of new molecular techniques (Lehmann et al., 2000; Jayasankar, 2004; Jin et al., 2006; Harris et al., 1991; Mjolnerod et al., 1997; Coughlan et al., 1998; Norris et al., 1999; Wasko and Galetti, 2002; Barman et al., 2003; Hassani et al., 1999; Mataso et al., 2004; Jayasankar, 2004; Jin et al., 2006), species identification (Palumbi and Cipriano, 1998; Prioli et al., 2002), monitoring fisheries (Menezes et al., 2006), aquaculture (Liu et al., 1998; Barriga-Sosa et al., 2004) and enhancement operations (Marzano et al., 2003). Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and microsatellite have been applied to address various fisheries issues such as stock identification, mixed stock analysis (Hanfling et al., 2005), conservation, domestication and taxonomy (Callejas and Ochando, 1998; Prioli et al., 2002). Genetic markers are also used to understand the pattern of migration of fish stocks, nature of breeding populations and in conservation and taxonomy and systematics with varying degrees of success (Melamed et al., 2002).

RAPD technique was first introduced in 1990. It is a technique based on the PCR amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence (Welsch and McClelland, 1990; Williams et al., 1990). RAPD has been used in population studies in fisheries, and can be used efficiently for geographic analysis of populations with differential degrees of geographic isolation (Brahmane et al., 2006), differentiating sex-chromosomes (Iturra et al., 1998), genetic inheritance (Elo et al., 1997), gene-mapping (Liu et al., 1999), fish conservation (Fritzch and Rieseberg, 1996; Dioh et al., 1997), stock identification and population analysis in fish (Partis and Wells, 1996; Dong and Zhou, 1998; Bartfai et al., 2003; Mohd. Ahmed et al., 2004; El-Zaeem et al., 2006; El-Zaeem et al., 2012; El-Zaeem and Ahmed, 2006; Faddagh et al., 2012). The RAPD technique has been used as a source of genetic markers to quantify genetic variations of the sub populations in the migratory fishes.
1.1 AIM AND OBJECTIVES

The aim of the present study was to evaluate phenotypic (morphometric and meristic indices) and genotypic (RAPD finger printing) characters in the population of an endangered fish, *Tor putitora*, in lentic and lotic waters of Himachal Pradesh. To achieve the aim, following objectives were set:

(i) Identification of stream site for the collection of golden mahseer, *Tor putitora* specimen from Himachal Pradesh, India.

(ii) Identification of phenotypic characteristics from the freshly captured and preserved specimens of golden mahseer, *Tor putitora* from Himachal Pradesh, India.

(iii) Detection of RAPD pattern for determination of genetic variation among the different populations of golden mahseer, *Tor putitora* inhabiting in different streams and rivers Himachal Pradesh, India.

(iv) To analyze the polymorphism among the different populations of golden mahseer, *Tor putitora* from Himachal Pradesh, India.