1. INTRODUCTION

Global production of fish from aquaculture has grown substantially in the past decade, reaching 52.5 million tonnes in 2008, compared with 32.4 million tonnes in 2000. Aquaculture continues to be the fastest-growing animal food producing sector and currently accounts for nearly half (45.6 percent) of the world's food fish consumption, compared with 33.8 percent in 2000. The Asia–Pacific region continues to dominate the aquaculture sector, accounting for 89.1 percent of global production, with China alone contributing 62.3 percent of global production. The second largest producer of carps is India (15.7%) followed by another 10.2 % of all carps is produced by Bangladesh, Myanmar, Viet Nam, Indonesia and Pakistan, collectively.

The world total aquaculture production of Indian major carps viz., *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* is 2.42 mmt, 1.22 mmt and 0.474 mmt respectively, sharing 4.34%, 2.19% and 0.85% of the total world aquaculture production (55.68 mmt) in 2009 (FAO, 2011). Similar to global trends, Indian fisheries has made great strides during the last five decades with the production levels increasing from 0.75 mmt in 1950's to over 7.845 mmt in 2009 (FAO, 2011). Of the total, about 48.33% (3.79 mmt) comes from aquaculture that has placed the country as the second largest world aquaculture producer. Though, the country possesses a large number of potential cultivable carp species, it is only three Indian major carps viz., catla, rohu and mrigal, together contribute a lion's share of 86.63% of total freshwater aquaculture production. The freshwater aquaculture resources of the country are huge in terms of 2.36 million hectares of ponds and tanks and 1.07 million hectares of beels, jheels and derelict waters. In addition to 0.12 million km of canals and 2.05 million hectares of reservoirs that could be put to different fish...
culture practices or even culture based capture in case of large water sheets India is one of the richest in terms of freshwater resource (FAO, 2008).

The “Fish for all” Program initiated by the world fish centre under the chairmanship of Dr. M.S. Swami Nathan is steadily adding further impetuous to fisheries. Fish originating from both culture and capture fisheries sources can make significant contributions to improve and diversify dietary intakes and promote nutritional wellbeing among most population groups. Fish have a highly desirable nutrient profile and provide an excellent source of high-quality animal protein that is easily digestible and of high biological value. Fatty fish, in particular, are an extremely rich source of essential fatty acids, including omega-3 polyunsaturated fatty acids that are crucial for normal growth and mental development, especially during pregnancy and early childhood. Fish are also rich in vitamins (fat-soluble vitamins A, D and E, and water-soluble vitamins, B complex) and minerals (especially calcium, phosphorus, iron, selenium and iodine in marine products). Therefore, fish can provide an important source of nutrients, particularly for those whose diets are lacking in other animal-source foods.

Feed accounts for the major part of production cost in aquaculture. Development of economical feed mixture is an important prerequisite for fish culture in which growth of fish is influenced by dietary protein quality and quantity provided other physiological requirements are fulfilled. Aquaculture of cyprinids is mainly under semi incentive pond culture conditions with only a small proportion (2 to 3%) cultivated intensively using relatively low cost artificial diet. Over the past decades significant progress has been made to reduce the potential effect of feed on the environment and inadequate formulation and poor utilization of dietary nutrients (Kaushik, 1995). However only a little attention has been paid to the optimization of
production, which involves supply of adequate amount of essential nutrients from using well-identified sources. Nutritional research with carp under controlled condition was mainly concerned with common carp (*Cyprinus carpio*), with limited work under taken with other Cyprinids such as grass carp (*Ctenopharyngodon idella*) Indian major carps, rohu (*L. rohita*), mrigal (*C. mrigala*) and catla (*C. catla*).

In culture operations, the efficiency of a feed in promoting maximum growth in animal depends not only on its nutritive profile, but also on the animal's inherent ability to consume, digest, absorb and metabolize the nutrients, which the feed contains. The available data show that there is a remarkable homogeneity in the data on requirements of different species of teleosts (NRC, 1993). Since adoptive changes in the intestinal morphology depending on natural feeding habit seem to exist, possible differences in nutrient utilization might be expected. Digestive processes in fish are less known than in animals, though the data obtained in fish so far show that the digestive enzyme studied are qualitatively similar to those observed in other invertebrates.

It has been observed that in fish, proteins together with lipids are the major sources of energy. Although poorly utilized by fish, carbohydrates do have a wide range of digesting enzymes (Phillips, 1969). The nature of relative activity of digestive enzymes in fish correlates with the nature of fish normal diet. Practical diets for carps are often formulated with little concern for the biological and environmental consequences of poor digestive or metabolic utilization of dietary ingredients. Considerable variability in the optimal protein levels (25-50%) in the diets of common carp has been reported (Kaushik, 1995). The difference in the digestibility values of the same proteins between species can arise because of the difference in terms of their enzyme complement concentration and proteolytic ability (kinetics) and the
studies pertaining to the kinetics (Km and Vmax) are very few in carps. Thus, an understanding of the digestive enzyme complement is extremely important in diet formulation. Understanding the optimized conditions for digestive enzymes activities will also enable better comprehension of nutrient digestibility in fish (Glass et al., 1989; Kolkavski, 2001). Study on protease activities has also helped in the development of more rapid and accurate in vitro digestibility assays (Ezquerra et al., 1998). Characterization of protease activities in several fish species in order to facilitate formulation of feed, prediction of inhibition by anti nutritional factors in plant based ingredients and development of suitable feeding regimes based on internal rhythm of protease secretion has been carried out (Alarcon et al., 1999; Outzen et al., 1996; Rivera, 2003). In comparison to proteases, studies on carbohydrases especially amylases are still lacking in Indian major carps cultured species despite reports on important role of these enzymes in other fishes (Hidalgo et al., 1999; Fernandez et al., 2001).

The purification and characterization of the digestive enzymes may provide an insight into the environment in which the enzymes operate with efficiency during utilization of nutrients by fishes. Such a study, also gains significance in the era of custom made artificial diets, since knowledge of natural activators and inhibitors of these enzymes can help in deciding on the appropriate sources and levels of carbohydrates in feeds (Roychan and Chaudhari 2001).

The development of digestive functions can influence survival in early life history. At the time of hatching, the alimentary canals of the teleosts is undifferentiated and generally remains stomach less throughout the larval phase. The conclusion of differentiation, including formation of a functional gastric region, usually occurs during metamorphosis of the larvae to juveniles. Concurrently the
adult diet is assumed (Tanaka et al., 1973). Thus among aquatic animals, greater emphasis has been placed in larval nutrition studies in which enzymes profiles have been used to formulate appropriate diets. Information describing changes in digestive secretion and enzyme activity are necessary for understanding larval digestive mechanism and can assist studies on feeding habits and nutrition requirements. This knowledge can also be applied towards improvement of larval rearing techniques for fish culture. For example, heavy mortalities have been reported for the larvae of *L. rohita* (Jhingran, 1991) under nursery rearing in ponds. There is a minimum period of total or partial dependence by the larvae on live food organisms before they switch over to artificial feeds (Mookerji and Rao., 1999). No study is made correlating the development of the digestive enzymes in the larvae and fry of fish with their growth, more so specifically in the larvae of the Indian Major Carp *Labeo rohita*.

Rohu (*Labeo rohita*) is the most important among the three Indian major carp species used in carp polyculture systems. This Indo-Gangetic riverine species is the natural inhabitant of the riverine system of northern and central India, and the rivers of Pakistan, Bangladesh and Myanmar. In India, it has been transplanted into almost all riverine systems including the freshwaters of Andaman, where its population has successfully established. The species has also been introduced in many other countries, including Sri Lanka, the former USSR, Japan, China, Philippines, Malaysia, Nepal and some countries of Africa. The traditional culture of this carp goes back hundreds of years in the small ponds of the eastern Indian states (FAO, 2006 - 2012).
The present program of work encompasses the following objectives.

- To partially purify and characterize the amylase and trypsin from the adult fish of *L. rohita*.
- To estimate the rate of hydrolysis of nutrients by the partially purified digestive enzymes in bioreactor studies in terms of protein and carbohydrate breakdown.
- To evaluate the expression of different digestive enzymes in the larvae of *L. rohita*.
- To determine and correlate the activities of amylase and proteases (trypsin, chymotrypsin and total proteases) in the digestive tract of *L. rohita* of different size groups with biometric parameters.
- Evaluation of digestive enzymes in nutritional studies involving protein and carbohydrates from different sources.