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DIGESTIVE ENZYME CHARACTERIZATION OF THREATENED YELLOW CATFISH *HORABAGRUS BRACHYSOMA* (GUNTHER) (TELEOSTES : SILURIFORMES: HORABAGRIDAE) AT TWO LIFE STAGES

Prasad, G. and Suneesha, I.

Laboratory of Conservation Biology, Department of Zoology,
University of Kerala, Kariavattom, Thiruvananthapuram-695 581, Kerala, India.
**E-mail: probios1@gmail.com*

Abstract: The digestive enzyme profile of *Horabagrus brachysoma,* a threatened, freshwater catfish, collected from the Periyar River of Kerala, in south India, was studied using standard methods. The results shows that in both juveniles and adults all major digestive enzymes viz. carbohydrases, proteases, and lipases were present. Among the carbohydrases, amylase, maltase and lactase were present whereas cellulase was lacking in the two life stages studied. The enzyme sucrase was present in the adults but lacking in the fingerlings. Among proteases, pepsin showed the utmost activity followed by trypsin and peptidase. The enzyme lipase was present in the intestine of adults while it was weakly present in fingerlings. Based on the findings it can be concluded that the fish has the ability to digest all major types of nutrients and possibly which will help to accept wide spectrum of feed components. The present findings will help to formulate a more economically viable and nutritionally balanced diet for the artificial rearing of this cultivable catfish.

Key words: Digestive enzyme, omnivore, ontogenic diet shift, threatened yellow catfish, *Horabagrus brachysoma,* Siluriformes, Horabagridae

INTRODUCTION

The study of digestive enzymes of fish relates its food habits to the enzymes found in the gut and is widely used in nutritional physiology as an important means of investigating the fish's digestive abilities (Fagbenro *et al.,* 2000). The ability of an organism to digest a given material is dependent on the presence of appropriate enzymes (Smith, 1980). Studies on digestive secretions in fish have helped to define the limits of dietary proteins (Twining e*t al.,* 1983) and carbohydrates (Spannhof *et al.,* 1983). Divakaran *et al.* (1983) working on the digestive enzymes in *Polydactylus sexfilis* and *Caranx melampygus,* recommended different dietary proportions of macronutrients for their best nutritive use. Hofer and Kock (1989) suggested that, from the profile of digestive enzymes, it is possible to predict the ability of species to use different nutrients. An understanding of the functioning of the digestive enzymes helps to explain nutrient digestibility (Glass *et al.,* 1989; Kolkovski, 2001). In short, studies on digestive secretions in the fish can elucidate certain aspects of the nutritive physiology and help to resolve nutritional problems, such as the matching of an artificial diet to the nutritive capabilities of fish.

A perusal of the literature revealed that information on quantitative and qualitative nature of digestive enzymes of *Horabagrus brachysoma,* a threatened yellow (Gunther, 1864), catfish is lacking even though this species has acclaimed well both in farming and ornamental. The aim of the present study was to qualitatively analyse the digestive enzymes such as carbohydrases, proteases, and lipase in the gut of fingerlings and adults of threatened yellow catfish *H. brachysoma* (Gunther, 1864) specimens obtained from Periyar River, Kerala for the first time to improve the knowledge of its nutritional physiology and digestive capabilities of this catfish. The distribution of *H. brachysoma* is presently restricted to a few river systems of Southern area of Indian subcontinent, such as Chalakudy, Periyar, Meenachil, Manimala, Pamba, Moovattupuzha, Achenkovil and Vembanadu Lake in Kerala and Aghnanashini and Kali rivers of Karnataka (Bhatt, 2001). This fish is generally present in low land areas of rivers and backwaters with mud and sand substrate, and is much relished as a food fish and has got a ready market demand in Kerala. Over exploitation, habitat destruction, pollution, sand mining and other anthropogenic stresses have drastically reduced the population of this catfish species by 60-70% in their natural habitats during the last few years (Anvar Ali *et al.,* 2007). Some biological aspects of this species such as length-weight relation ship, blood chemistry and feeding habit of this fish from lake Vembanad and some rivers are already reported elsewhere (Sreeraj *et al.,* 2006; Prasad and Anvar Ali, 2008; Anvar Ali *et al.,* 2008; Prasad and Charles, 2010) and the present study was aimed to find out the digestive enzyme profile of this fish since the nature of the digestive enzyme is highly related with the nature of the meal it can assimilate.

MATERIALS AND METHODS

Specimens of *Horabagrus brachysoma* required for the present study was caught live using gill net (25 mm, 40mm) from the Periyar River. For the digestive enzyme analysis, live fingerlings and adults of *H. brachysoma* obtained were transported to the laboratory and kept in separate glass aquaria supplied with clean water. The size of the fingerlings ranged from 73 to 84 mm (mean 77 mm) and weight between 3.6 and 5.4 gm (mean 4.37 gm) whereas the adult sizes ranged from 222 to 234 mm (mean 229 mm) and weight between 89.7 and 112.95 gm (mean 102.88 gm). The two sets of fish, 25 numbers each were maintained in the wet lab and starved for 48 hours to clear the alimentary canal. They were anaesthetized with benzocaine and dissected to remove the entire guts, later separated into stomach and intestine. The different gut regions were pooled and homogenized using cold neutralized potassium hydroxide and the homogenates then centrifuged for 15 minutes at 4800 rpm for 30 min at 4^{o}C. The supernatants were collected, frozen in sample vials and stored at -20^{o}C until assayed for the digestive enzymes.

Benedict's qualitative reagents were used for the qualitative assay of carbohydrates following the methods of Olatunde *et al.* (1988). For the qualitative assays of proteases followed the method of Balogun and Fisher (1970). The method of Ogunbiyi and Okon (1976) was used to determine lipase activity qualitatively.

RESULTS AND DISCUSSION

The summary of qualitative distribution of enzymes in the fingerlings and adults of *H. brachysoma* is presented in Table 1. Among carbohydrases, amylase showed widest distribution in regions of the alimentary canals of the fingerlings and adults. However, in adults' intestine showed higher amylase activity than that in fingerlings. This was followed by other carbohydrases like maltase, lactase and sucrase. The enzyme maltase and lactase also showed highest activity in the intestine of adults. Sucrase was absent in fingerlings, while it was weakly present in the stomach and intestine of adults. The enzyme cellulase was totally absent in the alimentary canal of both fingerlings and adults. Among proteases pepsin showed highest activity only in the stomach of both fingerlings and adults. The alkaline proteases such as trypsin and peptidase were present in the intestine of fingerlings and adults. The enzyme lipase activity was predominant in the intestine of adults. But in fingerlings it is weakly present in the intestine only.

Table 1. Qualitative distribution of digestive enzymes in fingerlings and adults of *Horabagrus. brachysoma*

	Fingerlings		Adults	
Enzyme	stomach	Intestine	Stomach	Intestine
CARBOHYDRASES				
Amylase	+	++	+	+++
Sucrase	-	-	+	+
Maltase	+	++	+	+++
Lactase	+	++	+	+++
Cellulase	-	-	-	-
PROTEASES				
Pepsin	+++	-	++	-
Trypsin	-	+++	-	++
Peptidase	-	+	-	+
LIPASE	-	+	+	++

+= weak, ++= moderate, +++= strong, -= nil.

Various enzymes derived from different digestive organs of the gastrointestinal tract appear concurrently and cooperate tightly to maximize food utilization. The fingerlings and adults had widely spread out carbohydrases in their stomach and intestine, however highest carbohydrase activity was recorded in the intestine of adults. Generally, a low level of carbohydrase activity during the fingerlings. Stage and a high level during the adult stage suggest a minor metabolic role during the early stages and a more important role during the adult stages, such as search of food, migration and reproduction (Kori-Siakpere 1999). Among carbohydrases, amylase is widely distributed in the stomach and intestine of both fingerlings and adults. Amylases are found in most fishes, omnivores, herbivores as well as carnivores (Kapoor *et al.,* 1975). The high level of amylase activity in *H. brachysoma* indicates that starch is readily hydrolysed and probably plays an important role in energy metabolism. The activity of maltase in the stomach and intestine of fingerlings and adults ensures that the starch converted into maltose by the amylase in the alimentary canal finally gets digested into glucose. According to Barrington (1957) maltose is said to be the main product of the action of amylase and in view of this it is expected that maltase will closely associate with it. Maltase enzyme has been reported from many omnivores such as *Cyprinus carpio L.* (Kawai and Ikeda, 1971), rabbit fish, *Siganus canaliculatus,* sea bass, *Lates calcarifer* (Sabapathy and Teo, 1993) and *Clarias anguillaris* (Kori-Siakpere, 1999) African bony tongue fish, *Heterotis niloticus* and the intestine of African electric catfish, *Malapterurus electricus* (Fagbenro *et al.,* 2001), Olantunde *et al.* (1991) stated the importance of lactase in the gut of fish, and attributed its presence to the possibility of plant materials similar to lactose being present in diatoms, desmids and other algae normally eaten by fish. Here the presence of a variety of carbohydrases in *H. brachysoma* indicates the ability of this species to digest a wide range of vegetable matters in their food components such as leaf litter, shoots of aquatic plants, seeds, plant fibres, rice etc. This information is in agreement with that of the feeding habit of this fish, the adults feed more on plant mater than diet of animal origin. Among carbohydrases, cellulase was not detected in any region of the alimentary canal of both the fingerlings and the adults. Most studies conclude that fishes lack indigenous cellulose (Fish, 1960; Ugwumba, 1989). The fish that show cellulase activity may have gut of microflora capable of cellulose digestion as reported by Stickney and Shunway (1974).

In this study, activity of proteases was found to be higher in the fingerlings than in the adults. Similar observations are reported from some other omnivorous fishes such as S*arotherodon galileaus* (Akintude, 1984). This higher activity of proteases in fingerlings than in adults could be attributed to the high protein content of the fingerling food. In *H. brachysoma,* pepsin is found only in the stomach region, while trypsin and peptidase activities were higher in the intestinal region. Proteolytic enzymes take part in the utilization of protein and consequently, affect growth, which is greater in fingerlings than in adults. The gut content analysis of this fish also shows that animal matter is the second major food, rich in protein. Pepsin thus may play a major role in protein digestion. Pepsin is believed to be responsible for the initial and partial hydrolysis of proteins in the stomach, subsequently completed by the combined action of trypsin and peptidase in the intestine. The enzyme pepsin has been detected in the stomach of many teleosts (Kapoor *et al.,* 1975). The end products of pepsin digestion are large polypeptides, oligopeptides and some amino acids. The enzyme peptidase present in the intestine takes part in the final phase of protein digestion and this could be the reason for the presence of peptones in the same region.

The present results show reduction in protease enzyme activity as the fish increase in size. Dabrowski and Goglowski (1977) found the highest protease-like enzymes in carp fry and predominantly pepsin-like enzymes in trout, while white fish larvae are characterized by trypsin-like enzymes although they possess a stomach. They also exhibited a constancy and reduction of proteolytic activity in relation to body weight. The study of digestive enzymes in African electric cat fish *Malapterurus electricus* made by Fagbenro *et al.* (2001) reported that among proteases, pepsin shows highest activity in the stomach, and the fish also showed a predatory dietary habit. Sabapathy and Teo (1993) while studying digestive enzymes in the gut of the omnivorous fish *Siganus canaliculatus* and the carnivorous *Lates calcarifer,* reported that pepsin activity is higher in the carnivorous species and it relies mainly on pepsin for the digestion of protein, whereas trypsin and chymotrypsin activities were higher in the omnivorous fish. In the present study also, the fingerlings showed high pepsin activity in the stomach than in adults and the feeding habits of fingerlings are more towards a carnivorous nature than that of adults and there is marked shift in the feeding pattern of the species from a carnivorous feeding habit to a more pronounced omnivorous feeding habit.

Peptic activity in fish occurs in acid conditions (pH 2-4) and alkaline protease activity is more in intestine (Smith 1980). This indicates that digestion with alkaline protease takes place after acid protease digestion and these results points to the potentiality of the fish for digesting protein irrespective of the food habit as reported by Chakrabarti *et al.* (1995).

The study of digestive enzymes in three tropical cat fishes *Physailia pellucida, Eutropius niloticus* and *Schilbe mystus* made by Olatunde and Ogunbiyi (1977) suggest that relatively high combined proteases and low amylase activities can be correlated with the proportion of protein and carbohydrates in their diets. The diet of these fishes mainly consisted of insects, fishes and crustaceans, although plant material was also found in their stomachs in a small quantity. Cockson and Bourne (1990) studied the digestive enzymes of omnivorous fish *Clarias mossambicus* and herbivorous fish *Tilapia shiranachilwae* and found that protease activity was distributed throughout the entire gastrointestinal tract of *C. mossambicus* whereas in *T. shirana chilwae,* protease activity was limited to the anterior intestine. In the present work also, the digestive enzyme profile can be correlated well with the feeding habit.

Fishes, insects and poultry wastes were probably the most important source of dietary lipids which implies the utilization of fatty acids as the energy source. In the present observation, a high lipase activity was detected in the intestine of adults, while in fingerlings it was weakly present. High lipase activity owing to high dietary lipid reveals the important role of lipids as the energy source (Divakaran *et al.,* 1999). The wide distribution of lipase in the alimentary canal of adults agrees with the findings of Borlongan (1990) in the milkfish *Chanos chanos.* It was also detected in the alimentary canals of the adults of *Clarias anguillaris* (Fagbenro *et al.,* 2001), African bony tongue fish, *Heterotis niloticus* (Fagbenro *et al.,* 2000) and the intestine of African electric catfish, *Malapterurus electricus* (Fagbenro *et al.,* 2001). But little data exist to date on the relation of the fat content of the food to lipase production. The indication from the previous studies is that there is no lipase adaptation.

Chesley (1934) noted that there was no correlation between the fat content in the food of a fish and lipase activity. He also reported that Brazoria species, a marine teleost consuming much fatty food, had a concentration of lipase not higher than those of other fish which fed on a diet containing less fat. Agarwal *et al.* (1975) stated that lipase activity did not seem to be correlated to the fat content of the diet. In contrast to this a positive correlation between lipase activity and lipid digestibility in mahseer *(Tor khudree)* (Bazar and Keshavanath, 1993), rohu *(Labeo rohita)* (Gangadhar *et al.,* 1997) and the European sea bass *(Dicentrarchus labrax)* (Peres and Oliva-Teles, 1999) has been reported. Similarly a straight correlation between lipase and dietary lipids is observed in the pyloric caecum and anterior intestine of tambaqui *(Colossoma macropomum)* (De Almeida *et al.,* 2006). Prasad and Anvar Ali (2008) while reporting the feeding habit of *H. brachysoma* at two life stages from the same river revealed that the fish is an omnivore and the adults preferring plant matter diets over animal tissues and exhibiting an ontogenic diet shift from a more pronounced carnivorous feeding habit to a general omnivorous habit. The present results supports the earlier observations that the fish is an omnivore (Prasad and Anvar Ali, 2008) and it can also be postulated that *H. brachysoma* has the capacity to digest lipid containing food effectively since enzyme responsible for the digestion of this component is present in the early as well as later stages of its life history.

CONCLUSION

The digestive enzyme profile of *H. brachysoma* reveals that the fish has an omnivorous feeding habit, with the adults preferring plant matter diets than animal tissues. In the fingerlings the protease enzymes are more pronounced and in adults carbohydrases are more intense, this shows an ontogenic diet shift in this catfish. Since the fingerling stage is the stage of maximum growth and hence of high protein requirement and high utilization, which could be the reason for the high protease activity in this period. Another important finding is that the ability of this catfish to feed on various diets with all the most of the nutrients and this adaptability to the varying ecological profiles suggests that it is an opportunistic feeder. The wide spectrum feeding habit will be an added advantage for the survival of this nocturnal catfish in different riverine habitats where food derived from allochthonous will be a major share and the results will help to formulate more economically viable diet for the profitable rearing of this species.

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