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# Analysis of external morphology of digestive system and digestive enzyme activity of two edible native fish species; *Anabas testudineus* and *Etroplus suratensis*

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**ABSTRACT**

External morphological features of fish digestive system and digestive enzyme activity is found to be closely linked to its pattern of food and feeding. The present study was done to understand the digestive physiology of two species of edible freshwater fishes; *Anabas tesudineus* and *Etroplus suratensis*. External morphology of the digestive system and the activity of the amylase, protease and lipase in the liver, stomach and intestine of these fishes were analysed for the study. Intestine of *Anabas testudineus* had a reduced and straight pattern characteristically seen in carnivorous fishes. The significantly increased level of protease activity in the stomach and intestine of *Anabas testudineus* also indicated its inclination towards carnivory. Intestine of *Etroplus suratensis* is long and coiled as seen in herbivorous fishes. The significant increase in liver amylase activity of *Etroplus suratensis* also supported its preference for herbivorous diet. The considerable digestive enzyme activity levels along the digestive tract of these fishes suggested its omnivorous pattern of feeding. The present study thus indicated the close relation between the digestive physiology and the pattern of feeding in fishes, which could be utilised for selecting better feeding options to ensure optimal growth of these native fishes during aquaculture practices.

***Key words:*** *Native fish, Digestive system, Anabas testudineus, Etroplus suratensis*

## Introduction

Digestive system of every fish is specially related to its pattern of feeding and its unique way of adaptive evolution to its particular environment(Al-Abdulhadi, 2005). The studies on digestive physiology of fish helps to understand the feeding habits and its connection with different trophic levels, which is really advantageous in maintaining optimum fish culturing systems (Canan *et al.*, 2012). In teleost fishes, the alimentary canal starts with mouth leading to buccal cavity, pharynx, gill rakers, oesophagus, stomach, intestine, pyloric caeca, rectum and opening out through the anus (Pandey and Shukla, 2015). The digestive glands in fishes includes the liver, pancreas and gall bladder. In teleost fishes pancreas, which secretes the major digestive juices is found diffused with the liver (Biswas, 2009).

External morphological features of fish digestive system and digestive enzyme activity is found to be closely linked to its pattern of food and feeding (Al-Abdulhadi, 2005). Studies on digestive physiology of fishes could help in the understanding the mechanism of digestion in each fish type and thus in adoption of better feeding patterns for fishes and for its optimum growth and maturity during aqua culturing (Hari Sankar *et al.*, 2014). *Etroplus suratensis* is a genus of cichlid fish native to southern India and Sri

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Lanka (Pethiyagoda, 1991). It is primarily found in brackish water and is also known to tolerate fresh or sea waters for short periods (Carpenter, 2001). *Anabas testudineus* is a freshwater teleost fish found in tropical and subtropical Asia, belonging to order Anabantiformes and family Anabantidae. *Anabas testudineus* is found mostly in canals, lakes, ponds, swamps and estuaries and it have the ability to live in wide range of salinities (Menon, 1999). *Etroplus suratensis*, which is the state fish of Kerala, is a native fish species and a delicacy of Keralites (Hari Sankar *et al*., 2014; Melby *et al*., 2019). *Anabas testudineus* too is an edible indigenous fish species with high demand for aquaculture (Ahammad *et al*., 2021). Studies on the digestive physiology of *Anabas testudineus* and *Etroplus suratensis* are not done much and thus the present study was aimed to understand the relation between external morphology of digestive system and digestive enzyme activity in these two native edible fish species of Kerala, which could improve the culturing methods adopted for these native fishes.

## Materials and Methods

### Fish collection and acclimatization

Two species of fishes selected for the study, *Anabas testudineus* and *Etroplus suratensis*, were collected from Sambranikodi region of Ashtamudi Lake, Kollam with the help of local fishermen. Each fish species of approximately 40 g weight were collected for the study. The fishes were acclimatized to the laboratory conditions for one week prior to the study on digestive enzyme activity.

### Sampling of the fishes

The fishes of both species were anaesthetized using 0.2% Ethylene Glycol Monophenyl Ether. The fish viscera were cut opened and the alimentary canal and associated parts of the fish species were dissected and photographed to study its external morphological features. The digestive enzyme activity in the fish liver, stomach and intestine of the two fish species was done by starving the fish for 12 hr before sampling. The fishes were anesthetized using 0.2% Ethylene glycol monophenyl ether for 2 minutes and the viscera was cut opened to excise the liver, stomach, anterior intestinal segments which were then cleaned, and stored in phosphate buffer saline (1ml, pH 7.4) and kept at $^{-}80\ ^{o}C$ for doing enzyme analysis. Further the digestive enzyme activities were analysed in the homogenates of these fish tissues after homogenization and centrifugation at 10,000 rpm at $4\ ^{o}C$ for 10 minutes. The supernatant obtained was collected in pre-weighed sterile Eppendorf tubes for conducting the enzyme activity analysis.

### Measurement of digestive enzyme activity

The amylase, protease and lipase enzyme activities were analyzed in the liver, stomach and intestinal tissue homogenates of the two fish species of our study using standard protocols spectrophotometrically. The amylase enzyme activity was estimated using dinitrosalycylic acid (DNSA) reagent (Sadasivam and Manickam, 2015). The protease assay was done using Casein as the main substrate, according to the modified Sigma Aldrich method (Anson, 1938) for measuring protease enzyme activity. Lipase enzyme activity along the digestive segments of the two fish species was assayed referring Winkler and Stuckmann's method using p-nitrophenyl palmitate (pNPP) (Winkler and Stuckmann, 1979). The concentration of the enzyme was evaluated by plotting the standard graph. The activity of enzyme was measured in units of µmol/ml.

### Stastistical analysis

Independent sample t-test at 5% (0.05) level of significance was done using SPSS software version 23 for analysing the data statistically. The mean values were considered significant if $P < 0.05$ and as non-significant if $P > 0.05$. Different alphabets indicate that there is significance ($P < 0.05$), and similar alphabets shows that there is no significance ($P > 0.05$) in enzyme activity levels of similar tissues compared between two different fish species.

## Results

### External morphology of digestive system

The external morphological features of digestive system of the two fish species studied remarkably gave an indication about its feeding pattern. In *Anabas testudineus* (Fig. 1), the mouth is wider and sub-terminal in position compared to *Etroplus suratensis* (Fig 2). Short and stumpy gill rakers were seen in *A. testudineus* (Fig. 1). Oesophagus was large and distensible leading to flask shaped stomach in

*A. testudineus*. Two pyloric caeca were present in *A. testudineus*, which serve as accessory food reservoirs in fishes. *A. testudineus* had a straight and reduced intestine, characteristically seen in carnivorous fishes. Its alimentary tract ended through rectum and anus (Fig. 1). In *Etroplus suratensis* the mouth was small and terminal in position and its gill rakers were found as sieve-like, as in herbivorous fish. Oesophagus in *E. suratensis* was short and narrow which leaded to pitcher shaped stomach. Intestine is found to be elongated and coiled in *E. suratensis*; which helps to retain food for long period of time to ensure digestion, characteristically seen in herbivorous fishes. (Fig. 2).

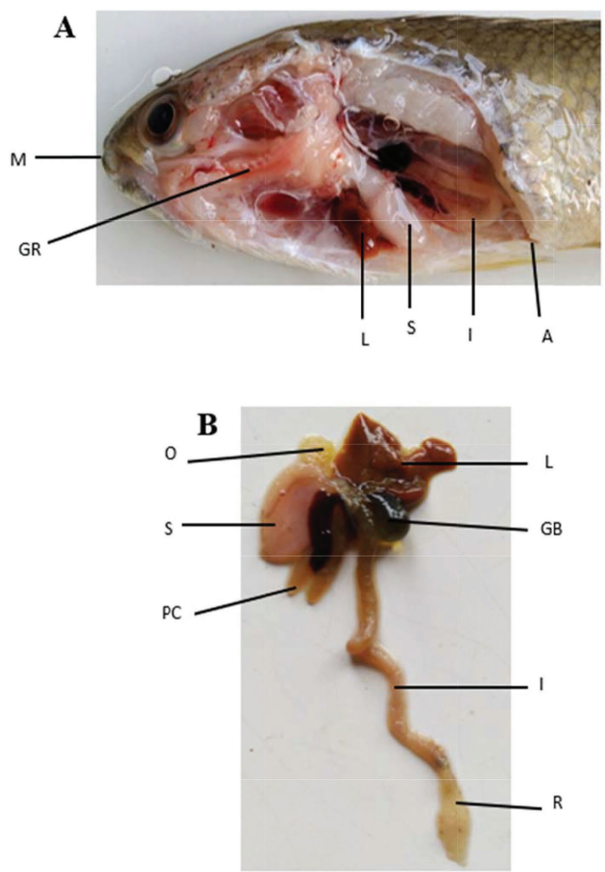


**Fig. 1.** Photographs showing the external morphology of digestive system of *Anabas testudineus*; Viscera cut opened view(**A**), Alimentary canal dissected and displayed view(**B**): showing Mouth (M), Gill rakers (GR), Oesophagus (O), (Flask shaped) Stomach (S), Pyloric caeca (PC), Liver (L), Gall bladder (GB),(Straight) Intestine (I), Rectum(R), Anus (A).

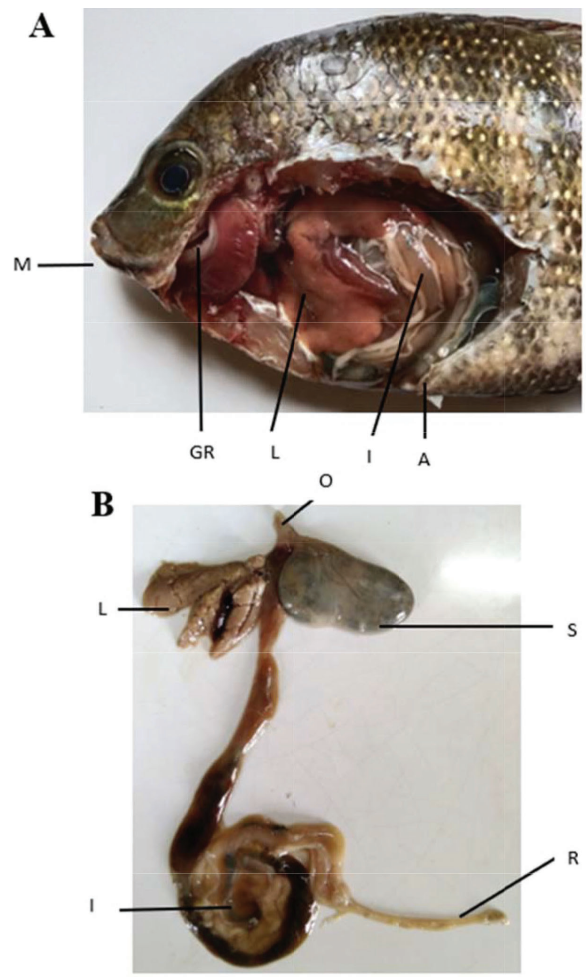


**Fig. 2.** Photographs showing the external morphology of digestive system of *Etroplus suratensis*; Viscera cut opened view (**A**), Alimentary canal dissected and displayed view (**B**): showing Mouth (M), Gill rakers (GR), Oesophagus (O), (Pitcher shaped) Stomach (S), Liver (L), (Coiled) Intestine (I), Rectum(R), Anus (A).

### Digestive enzyme activity in liver, stomach and intestinal tissues

The study on digestive enzyme activities in *Anabas testudineus* and *Etroplus suratensis* denoted remarkable relation to its feeding habits. The liver amylase activity (Fig. 3) in *E. suratensis* was significantly ($P < 0.05$) higher compared to the liver amylase enzyme activity in *Anabas testudineus*. The stomach and intestinal amylase activity in *Etroplus suratensis* showed significant ($P < 0.05$) decrease compared to the stomach and intestinal amylase enzyme activity in *Anabas testudineus*. Considerable liver amylase activities in *Etroplus suratensis*, indicated its ability to digest carbohydrate rich plant based food materials. The stomach and intestinal protease activity (Fig. 4) showed significant (P< 0.05) increase in *A. testudineus* compared to stomach and intestinal protease activity in *Etroplus suratensis*, which suggested their preference towards more animal-based protein rich food materials. The liver protease activity showed significant (P< 0.05) increase in *E. Suratensis* compared to the protease activity in liver of A. testudineus. The liver lipase activity (Fig. 5) in *E.suratensis* showed a significant (P< 0.05) increased level and the stomach lipase activity showed a significant (P<0.05) decrease compared to that of in *Anabas testudineus*. Thus the digestive enzyme activity levels showed significant (P < 0.05) regional level differences along the digestive segments compared between the two fish species studied.

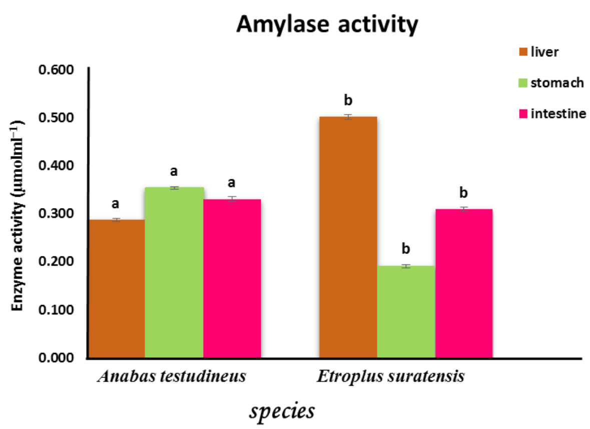


**Fig. 3.** Graph showing variation in amylase activity along the digestive segments of *Anabas testudineus* and *Etroplus suratensis*. Each bar is the mean value and error bar shows the SD. Different alphabets in the same tissue indicates significant (P < 0.05) difference in enzyme activities among the species as per independent sample t-test at 5% level of significance, and similar alphabets indicates that there is no significance (P > 0.05).

## Discussion

The external morphological features of digestive system of fish is specific to the functioning levels of its digestive enzymes, which is closely related to the feed type selected by the fish (Al-Abdulhadi, 2005). Carnivorous fishes are found to have a straight and reduced intestine and the protease enzyme activity along its digestive segments is found to be comparatively higher than in herbivorous fishes. The her-

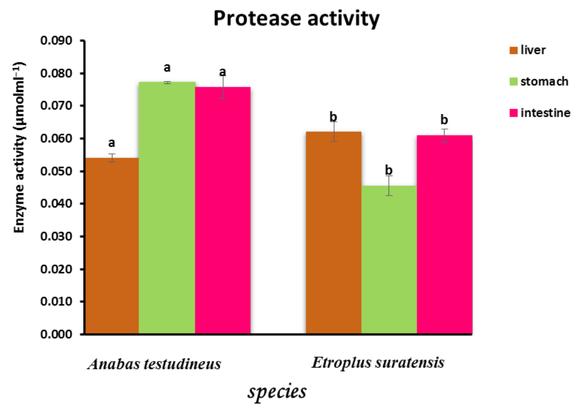


**Fig. 4.** Graph showing variation in protease activity along the digestive segments of *Anabas testudineus* and *Etroplus suratensis*. Each bar is the mean value and error bar shows the SD. Different alphabets in the same tissue indicates significant (P < 0.05) difference in enzyme activities among the species as per independent sample t-test at 5% level of significance, and similar alphabets indicates that there is no significance (P > 0.05).

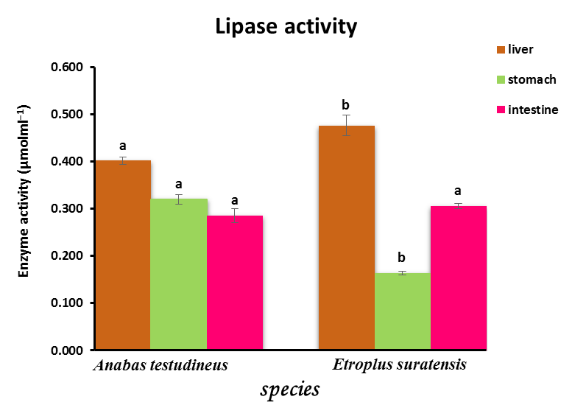


**Fig. 5.** Graph showing variation in lipase activity along the digestive segments of *Anabas testudineus* and *Etroplus suratensis*. Each bar is the mean value and error bar shows the SD. Different alphabets in the same tissue indicates significant (P < 0.05) difference in enzyme activities among the species as per independent sample t-test at 5% level of significance, and similar alphabets indicates that there is no significance (P > 0.05).

bivorous fishes are found to have a highly coiled intestinal pattern with higher amylase activity for enabling the digestion of plant materials (Hidalgo *et al*., 1999; Fange and Grove, 1979). Omnivorous fishes on the other hand have an intermediate gut length that is seen in carnivorous and herbivorous fish (Dutta and Hossain, 1993).

The presence of subterminal and wider mouth is a feature specific to predatory bottom feeding fishes. Small and terminal mouth is a specification seen in filter feeders that feeds on minute planktons (Biswas, 2009). *Anabas testudineus* have sub-terminal and wider mouth, short and stumpy gill rakers as found in omnivorous fishes. *Etroplus suratensis* have a terminal short mouth, gill rakers form sieve-like structure as seen in herbivores across the gill slits for for filtering water to retain microscopic plankton in the buccal cavity. Oesophagus is distensible in carnivorous fish which suits for its predacious nature, where as it is short and narrow in herbivorous and omnivorous fishes. Oesophagus is a large and distensible tube, leading to a flask shaped stomach in Anabas. Oesophagus is short and narrow in *E. Suratensis* leading to pitcher shaped stomach. Stomach shape varied in different species of fish, it showed flask shape in *A. testudineus* and pitcher shaped in *E. suratensis* (Shafi, 2000). Intestine is found to be elongated and coiled, in herbivorous fish for enabling the digestion of plant materials. Anterior part of the intestine gave rise to two pyloric caeca in *A. testudineus*, which helps to increase the absorption surface of intestine (Shafi, 2000). Straight and reduced intestine present in *A. testudineus*, is usually seen in carnivorous fishes. Intestine in Etroplus is found to be long and coiled type characteristically seen in herbivorous fishes.

The herbivorous fishes showed higher amylase activity for ensuring the digestion of plant materials (Hidalgo *et al*., 1999; Fange and Grove, 1979). *E. suratensis* is found to select much plant resources along with the fat and protein content in their feed and thus is mostly herbivorous in nature (Melby *et al*., 2019). Significantly higher level of amylase activity in liver of *E. suratensis* compared with that of *A. testudineus* showed its incline towards herbivorous pattern of feeding. Proteolytic activity is found to be lower in herbivorous fish species, than in omnivorous and carnivorous species (Hofer and Schiemer, 1981). Proteolytic activity is found to decrease with the increase in gut length of fishes and it is found to increase with decrease in gut length of fishes. Anabas is found to be described as an omnivorous fish which opts for much carnivorous diet (Mustakim *et al*., 2020). *A. testudineus* had a significantly higher protease activity in the stomach and intestinal tissues, compared to that of *E. suratensis* which indicated its preference towards carnivorous pattern of feeding. Higher liver lipase activity in *E. suratensis*, suggested its ability to handle fatty food sources. *E. suratensis* showed a significant decreased level of stomach lipase activity as shown in previous studies (Hari Sankar *et al*., 2014)

Enzyme assay study comparing the activity of enzymes amylases, proteases and lipases in similar digestive tissues between the two fish species showed significant variations in most of the digestive tissues, suggesting its omnivorous feeding nature with more preference for carnivory in *A. testudineus* and for herbivory in *E. suratensis*. Thus, through the present study the external morphological features of the digestive system and the pattern of digestive enzyme action clearly indicated its relation towards the type of food and feeding habits of two species of fishes our study.

## Conclusion

The study on digestive enzymes in fishes helps to understand the nutritional status and feeding adaptations of fishes, which is essential to maintain and design appropriate feeding patterns for developing new feed formulations for aquaculture practices. The present study indicated the close relation between the digestive physiology and the pattern of feeding in the edible native fish species selected for the study, which could be used for further research works to ensure its optimal aqua culturing possibilities in the future.

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### Conflict of interest

The authors do not have any conflict of interest.

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