

Functional Zonation of Different Digestive Enzymes in *Eetroplus suratensis* and *Oreochromis mossambicus*

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Abstract- The major digestive enzyme activities and digestive indices were compared between *Eetroplus suratensis* and *Oreochromis mossambicus*. Pepsin-like acid proteases that acts on low pH has been identified all along the digestive tract of both the fishes. Comparatively low alpha amylase activity is shown by the *E. suratensis* and the enzyme is distributed almost equally throughout the intestinal segments in both the species. Very low alkaline protease activity is found in the stomach of both the fishes and in *O. mossambicus*, the enzyme activity diminishes extensively towards the posterior portion of the intestine whereas in *E. suratensis* the activity increases towards the posterior part. The present study showed that lipase is one of the prominent digestive enzymes in *O. mossambicus* with a remarkable specific activity throughout the digestive tract than that of *E. suratensis*. It has been noted that *O. mossambicus* has a higher values for digestive somatic index, hepato somatic index, intestinal coefficient and gut V_s standard length ratio than that of *E. suratensis* indicating its higher digestive and metabolic capabilities. The early maturity and fast growth of *O. mossambicus* can be explained by their enhanced digestive indices. The comparatively low activities of acid protease, amylase, lipase and total alkaline protease of *E. suratensis* revealed poor digestive capacity than that of *O. mossambicus*.

Index Terms- A *Eetroplus suratensis*; *Oreochromis mossambicus*; Digestion; Enzyme; Fish; Functional zonation

I. INTRODUCTION

E*etroplus suratensis*, (Pearl Spot, *Karimeen*), generally considered as the 'upper-middle class' fish has got a profile uplift as the official state fish of Kerala state in India because of its economical importance. The knowledge about digestive physiology of *E. suratensis* is very limited. Tilapias are one of the most important euryhaline finfish cultured all over the world and they represent approximately 6% of total farmed fish production [1]. *Oreochromis mossambicus* is one of the hardiest fishes in aquaculture farms. Once introduced into a habitat, they generally establish themselves very quickly. The digestive enzymes play an important role in the development and growth of fishes. The ability of the fish to utilize ingested nutrients depends on the activities of digestive enzymes present in various locations along the digestive tract.

The polyculture of different species of fish is an accepted practice to promote fish production in India. The studies on digestive proteases of several species helped in the development

of cost effective diets for their intensive farming [2, 3, 4] and the matching of an artificial diet to their nutritional needs [5]. The understanding of the functional properties and optimal conditions for hydrolysis of nutrients by digestive enzymes in fish will facilitate a more precise measurement of nutrient digestibility by a particular species. Fish can vary their feed from plankton in the summer to fish in the winter, occasionally even exploiting the bacteria and algae of the water as a source of food [6]. Digestive tissues are notoriously plastic in their responses to dietary change [7]. Albeit of the food habit, the adaptations of the digestive system of different fish species exhibit closer correlation with their diet than on their microenvironment and taxonomic category [8]. Several researchers identified variations in gut morphology in response to fasting, increases in food intake and changes in diet [7, 9]. Thus, diet is a strong predictor of both intra- and inter-specific variation in the intestinal length, indicating that fish adjust their phenotype to balance nutritional needs against energetic costs [10]. Therefore, it is necessary to analyze some morphometric parameters of the digestive tract, such as intestinal coefficient, digestive somatic index, and hepatosomatic index of the selected teleosts. The aim of the present work is to study about the acid protease, amylase, lipase and total alkaline protease digestive enzymes of *Eetroplus suratensis* and to compare with that of *Oreochromis mossambicus* in order to increase our knowledge on the digestive physiology of these cichlid species and gain information concerning its nutrition.

II. MATERIALS AND METHODS

Fish and preparation of crude enzyme extract

Experimental fishes of almost similar size (10-12cm) have been collected from the Fisheries station, Kerala University of Fisheries and Ocean Studies, Puthuvypu, India. The fishes were acclimated to laboratory condition for a week. A commercial diet with known proximate composition has been given *ad libitum*. The fishes were starved for approximately 12 h prior to sampling, subsequently killed by cold shock, and dissected immediately. Among bony fishes, the pancreatic tissue is usually diffused in or around the liver [11]. The exocrine pancreatic tissue of *O. niloticus* has a diffused distribution in the hepatic parenchyma and is separated from the hepatocyte cords by means of thin septa of connective tissue [12]. Thus, in the present study the whole liver consisting of diffused pancreatic tissue (hepatopancreas) and the stomach was taken as digestive organ. The stomach contents were squeezed out and rinsed with

cold distilled water to remove feed remnants. Since intestine of tilapia and pearlspot lacked visually distinct regions, they were divided into three segments of equal length and designated as anterior, middle and posterior intestine without squeezing or rinsing. Ten percent (w/v) tissues homogenate was prepared in cold Tris-HCl 50 mM buffer pH 7.2 using an electric homogenizer (KEMI Model No: KHH 1), in ice-cold condition. The homogenate was then centrifuged at 4°C at 10,000g for 10 min. The supernatant containing the enzymes was stored at -20°C until the analysis.

Methods

The tissue homogenates were purified by Trichloroacetic acid (TCA) precipitation and the precipitate of soluble protein was dissolved in 0.1M NaOH. The soluble protein content of enzyme extract was measured in comparison with BSA standards [13] by using Hitachi-2900 UV-Visible spectrophotometer.

a) Acid Protease

Pepsin like acid protease was determined by Anson's method [14] and the specific enzyme activity is expressed in Anson Unit.

b) Total alkaline protease

The total alkaline protease was estimated by using Casein as substrate [15] with a slight modification in tris buffer pH as 8. Briefly, 50µl of homogenate was mixed with 0.1M Tris buffer pH-8 having 20mM CaCl₂ and preincubated for 5 minutes at 37°C and mixed thoroughly with 1% substrate solution. The reaction was stopped by adding 12% ice cold TCA and tyrosine in the supernatant was measured at 280nm after centrifugation at 8000 rpm for 15 min. The homogenate was added to the blank tubes at the end of the incubation after adding the TCA. L-tyrosine was used as a standard, and one unit of enzyme activity (U) is defined as the amount of enzyme needed to catalyze the formation of µM of tyrosine/min/ ml of homogenate at 37°C. The specific activity is expressed as U/mg protein.

c) Lipase

Lipase was estimated spectrophotometrically by hydrolysis of p-nitrophenyl palmitate using a modified method based on that of Winkler and Stuckmann [16]. 50µl of 20mM p-nitrophenyl palmitate was mixed with 20mM Tris buffer (pH-8) containing 20mM CaCl₂, 5mM sodium cholate and 0.01% gum Arabica. 50µl of tissue homogenate was added to this reaction mixture and incubated for 10 minutes. The p-nitro phenol liberated was read at 410nm against a reagent blank.

d) Amylase

The reducing sugars liberated by the action of alpha- amylase on starch was estimated by Somogyi-Nelson method using 3,5-dinitrosalicylic acid (DNSA) [17]. One unit of activity (U) is defined as the amount of enzyme able to produce 1 mg of maltose/min/ml of homogenate at 37°C. The specific activity is expressed as U/mg protein.

Zootechnical indices

The indices are calculated as following

Digestive somatic index (DSI) = (Digestive tract weight/Body weight) X 100

Hepatosomatic index (HSI) = (Hepatopancreas weight/Body weight) X 100

Intestinal coefficient = Digestive tract length/Total fish length

Intestinal to Standard Length ratio = Total gut length/ Standard length

III. RESULTS

Pepsin like acid proteases activity has been observed all along the digestive tract of both *E. suratensis* and *O. mossambicus* (Fig. 1). Considerably high acid protease activity has been established by *O. mossambicus* than that of *E. suratensis* and the activity decreases gradually towards the posterior part of the intestine. In comparison with stomach acid protease activity in *E. suratensis* approximately two-fold increase has been observed in *O. mossambicus*. Comparatively low alpha amylase activity is shown by the *E. suratensis* and the enzyme is distributed almost equally through out the intestinal segments in both the species. However, the middle intestine showed an increased alpha amylase activity (Fig. 2). Very low alkaline protease activity is found in the stomach of both the fishes. The enzyme activity diminishes intensively at the posterior intestine in *O. mossambicus* but in *E. suratensis* the activity extends until the end of digestive tract (Fig.3) and the posterior part showed its maximum activity. The present study shows that lipase is one of the prominent digestive enzymes in *O. mossambicus* with a remarkable specific activity throughout the digestive tract (Fig. 4). Very low activity of stomach lipase is shown by *E. suratensis* (Fig. 4) compared to *O. mossambicus*. The results have been expressed as bar diagrams that represents mean ± standard deviation. Each set of bars values with different lower case letters vary significantly (p<0.05) in each tissue on the two different species (One-way ANOVA).

The average length and weight of fishes showed very good correlation (0.99) in the case of *E. suratensis* but in the case of *O. mossambicus* it is not that good (0.73) (Table 1). *O. mossambicus* has a higher index for DSI, HSI, Intestinal coefficient and gut Vs standard length ratio than that of *E. suratensis* (Table 1).

IV. DISCUSSION

Etroplus suratensis is the prime among the cichlids indigenous to peninsular India and Srilanka. It is one of the most popular and promising species for aquaculture in India because of its high market demand and large size. With the flourishing of backwater tourism in Kerala, the demand for Pearl Spots has been on the increase. However, this species is facing serious diminution in its natural habitats owing to unrestrained exploitation and challenge by invasive species like tilapia. The general trend to exploit the most valuable species increased the threat in its maximum. It is highly nutritive, besides a good amount of meat, pearlspot include protein (16.74 %), lipids (1.15 %), carbohydrates (2.43 %), moisture (78.55%), Zn (83.04µg/g), Mn (39.44µg/g), Mg (1.15mg/g), Co (8.56µg/g), Cr (33.96µg/g), Cu (24.92µg/g), Fe (566.40µg/g), Cd (1.12µg/g) and Ni (2.64µg/g) [18]. Experimental information on the metabolic and digestive enzyme profile of Pearl Spot is inadequate for the formulation of efficient compound feeds.

Distribution and activity of intestinal digestive enzymes along the intestinal tract varies with feeding habit and intestinal

morphology [19-22]. The reports on digestive physiology of *E. suratensis* were very limited. In Chinook salmon, weight gain was found to be positively correlated with the ability of the digestive enzymes to hydrolyze diets [23]. In this study, it is found that proteases are very prominent in both stomach and intestinal segments and they are widely distributed though out the alimentary canal. Various studies on other fish digestive secretions have shown the occurrence of acid proteases that have high activity in the acidic region in the stomach and alkaline proteases acting actively in alkaline pH region in the intestine [24, 25]. Proteolytic activities at low pH have also been reported in species with prominent stomach region and a high pepsin secretion such as eel, tilapia, salmon, sea bass and trout [25, 26, 27, 28, 29]. Pepsin has been identified as the first proteolytic enzyme acting in fish digestive tract as a major acidic protease [20, 30]. Pepsin, a member of the aspartic endopeptidase family, has been identified in several species [23]. In the present examination, the acid protease such as pepsin is found to be present in all segments of the alimentary tract of both *E. suratensis* and *O. mossambicus*. It has been revealed that the peptic digestion in *Tilapia nilotica* is absent [31]. However, in the natural physiology of digestion, the acid protease will not be active in the intestinal segments due to the neutralization of acidic condition by the action of bile. For proper utilization of proteins, tilapia requires a highly acidic medium to enable biochemical digestion of protein due to thin stomach walls when compared to fishes with muscular stomach like African catfish, which relies more on mechanical breakdown of nutrients and possesses lower pepsin secretion [32].

After gastric digestion, the protein digestion is completed by the basic proteases of intestinal and pancreatic origin. A high activity of basic protease enzyme activity is observed in both species. Enzymes such as trypsin, chymotrypsin, collagenase, elastase, and carboxypeptidase have been characterized in different types of fish [33, 34]. Precursor zymogens of the alkaline proteases are secreted by pancreas [35]. High protease activity by intestinal extract at different alkaline pH range has been shown by different researchers in various species like carp [26], rainbow trout and Atlantic salmon [28], halibut and turbot [4], striped and European sea bass [33], sea bream and dentex [3], goldfish [36] and discus fish [24]. It provides evidence for the presence of minimum two major groups of alkaline proteases with different optimum pH. The *E. suratensis* has a short and less coiled intestine and the alkaline protease activity that gradually increases along the digestive tract reaches its maximum at the posterior intestine. *O. mossambicus* has a highly coiled, thin and elongated intestine with a maximal activity at the middle segment.

In comparison to proteases, knowledge about carbohydrases and lipases are still lacking in many species, despite the reported importance of these enzymes [36]. Various workers have demonstrated that amylase activity is greater in omnivorous and herbivorous fish than in carnivorous fish [36, 37, 38]. Low or moderate amylase activities have been reported in other carnivorous species [39]. Comparatively a lower activity of amylase is detected in the stomach in this study and it has previously been suggested that the presence of amylase in stomach could be due to some exogenous contamination from intestinal activity [25, 32]. In the natural environment,

carbohydrates are indeed more predominant than protein. Thus, there is possibility of carbohydrate digestion beginning from the stomach [20, 39]. The neutralization of chime by bile may not be complete before it reaches the middle intestinal segment and this could be the reason for a slightly increased alpha amylase activity in that segment.

A comparative study of the activity of digestive proteolytic enzymes and amylase could reveal the capacity of different species to use protein and carbohydrates [36]. It was adumbrated that changes in digestive enzyme activity could be affected by feeding behavior and biochemical composition of food [40]. The adaptations of the digestive system of different species exhibit closer correlation with their diet rather than on their taxonomic category [8]. Antithetically, there are studies on the phylogeny which influences the pattern of amylase activity more than that by diet in prickleback fishes [41]. Earlier reports on *E. suratensis* indicated that the young ones of this species are herbivores [42, 43]. It has been observed that though it feeds on micro and macro vegetation, invertebrates such as insect larvae, bivalves, mysids and decayed organic matter are mainly consisted in its food [42, 43, 44, 45]. Carbohydrases and proteolytic activities were higher in the detritivore compared to the omnivorous and carnivorous fishes [46]. In contrary, the present study obtained comparatively higher α -amylase and both acid and alkaline protease activity in *O. mossambicus* than that of *E. suratensis*.

The carnivorous fishes have higher lipase activity compared to herbivorous and omnivorous fishes [30, 47] and it is attributed by the higher consumption of fat rich food by carnivorous fishes [35]. The lipase is not necessarily produced by the pancreas but is a property of hepatic tissue [6]. Lipolytic activity in fish is generally higher in the proximal part of the intestine and the pyloric caeca, if present. It can extend up to the lower parts of the intestine with the activity decreasing progressively. Low lipolytic activity has also been found in the stomach of several fishes but the physiological significance of gastric lipolytic activity in fish is unclear [48]. The pancreas or hepatopancreas is generally considered as the major source of digestive lipase enzymes in fish as it is in mammals [37, 49]. The lipolytic activity found in stomach is different from that of pancreatic origin, suggesting that stomach may be a source of lipases, and the intestinal flora also contributes lipolytic activities in the digestive tract of fish [48]. In the present study *O. mossambicus* possesses a high profile of lipase activity. It should be helpful in the effective digestion of lipids available in the various feeds of plant or animal origin. Comparatively a low lipase profile has been exhibited by *E. suratensis*. The anterior and middle part of both the fishes showed the most intense activity of lipase similar to the results similar to previous studies [30].

In teleosts, the gastrointestinal tract morphology generally shows specific variations with respect to diet, feeding habit phylogeny, body shape, and features that reflect functional differentiation [49, 50]. In the present study, *O. mossambicus* has a higher index for DSI, HSI, intestinal coefficient and gut Vs standard length ratio than that of *E. suratensis* indicating higher digestive and metabolic capability. The fast growth and attaining of maturity of *O. mossambicus* can be explained from the differences in digestive indices. In addition, there is a correlation between the structure of the digestive apparatus and the feeding habit of fishes [51, 52]. It is commonly emphasized that

herbivore and detritivore fish species tend to have longer, thinner and narrower intestines than carnivores [37, 51] and the intestine of omnivorous species have an intermediate length [52].

V. CONCLUSION

The comparative study on digestive enzymes such as acid and alkaline protease, amylase and lipase activity in *E. suratensis* and *O. mossambicus*, shows that *O. mossambicus* would digest the dietary proteins and fats better than *E. suratensis* and its amylase pool would enable this fish to digest carbohydrates at herbivorous levels. When compared, *O. mossambicus* exhibit the omnivorous gut characteristics like long and highly coiled intestine, but *E. suratensis* has a short intestine. The knowledge about the digestive enzymes and digestive capabilities would be advantageous for diet manufacturing, as carbohydrates could be added at a greater proportion than protein and thereby save on feed manufacturing costs. In turn, the higher proportion of protease enzymes and amylase with respect to lipase in pearlspot may help to reduce the fat content in feed, thereby increase in shelf life of artificial feed for this species. This knowledge will be helpful to understand the competitive feeding strategies of native and alien species.

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Table 1: Length-Weight relationships and digestive indices of *Etroplus suratensis* and *Oreochromis mossambicus*

	TL	SL	TW	COR	TGL	TGW	HW	SW	DSI	HIS	IC	Gut Vs SL Ratio
<i>E. suratensis</i>	11.950±1.31	9.333±1.0	42.167±14.89	0.99	41.167±5.74	0.37±0.06	0.613±0.42	0.165±0.03	0.956±0.307 ^a	1.332±0.475 ^a	3.445±0.303 ^a	4.411±0.391 ^a
<i>O. mossambicus</i>	11.283±0.64	8.833±0.41	21.19±2.58	0.73	65.50±21.64	0.977±0.24	0.305±0.14	0.133±0.05	4.584±0.874 ^b	1.475±0.701 ^a	5.755±1.61 ^b	7.353±2.11 ^b

TL- Total Length, SL- Standard Length, TW- Total Weight, COR-Correlation between Total length and Total weight, TGL- Total Gut Length, TGW- Total Gut Weight, HW- Hepatopancreas Weight, SW- Stomach Weight, IC- Intestinal coefficient. Length was measured in centimeter and weight in grams and they were reported as mean ± Standard deviation (SD). Digestive somatic index (DSI), Hepato-somatic index (HIS), Intestinal coefficient and Gut Vs Standard Length Ratio were reported as mean ± SD. On each columns values with different lower case letters vary significantly (P<0.05) in each tissue on the two different species (One-way ANOVA)

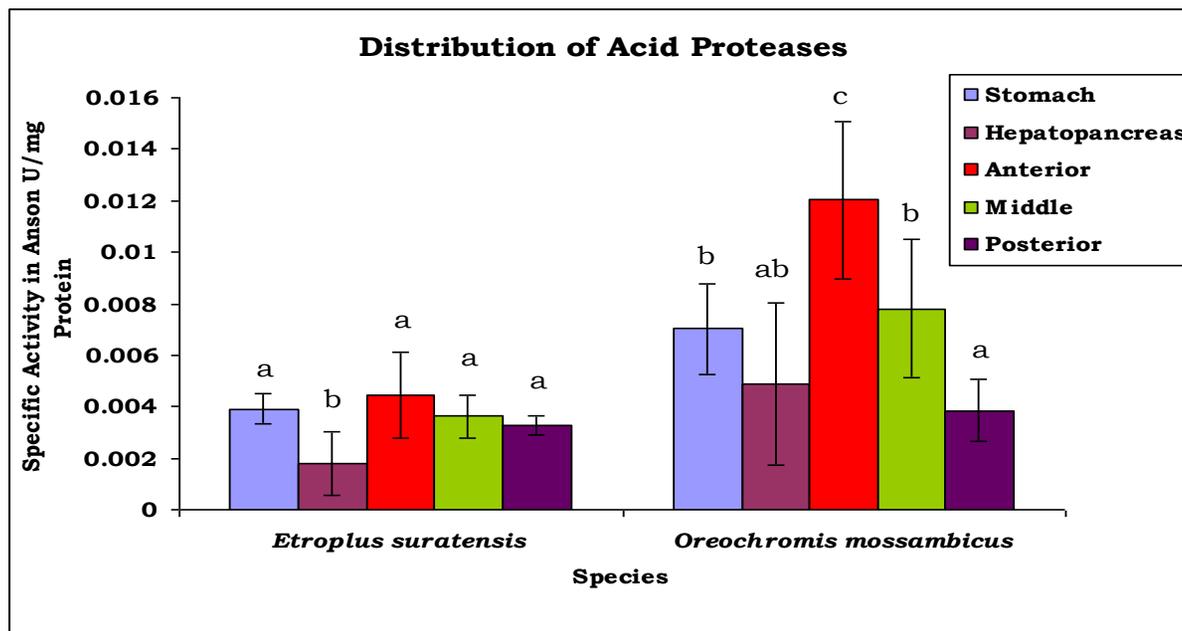


Figure 1: Distribution of acid protease along the digestive segments of *E. suratensis* and *O. mossambicus*

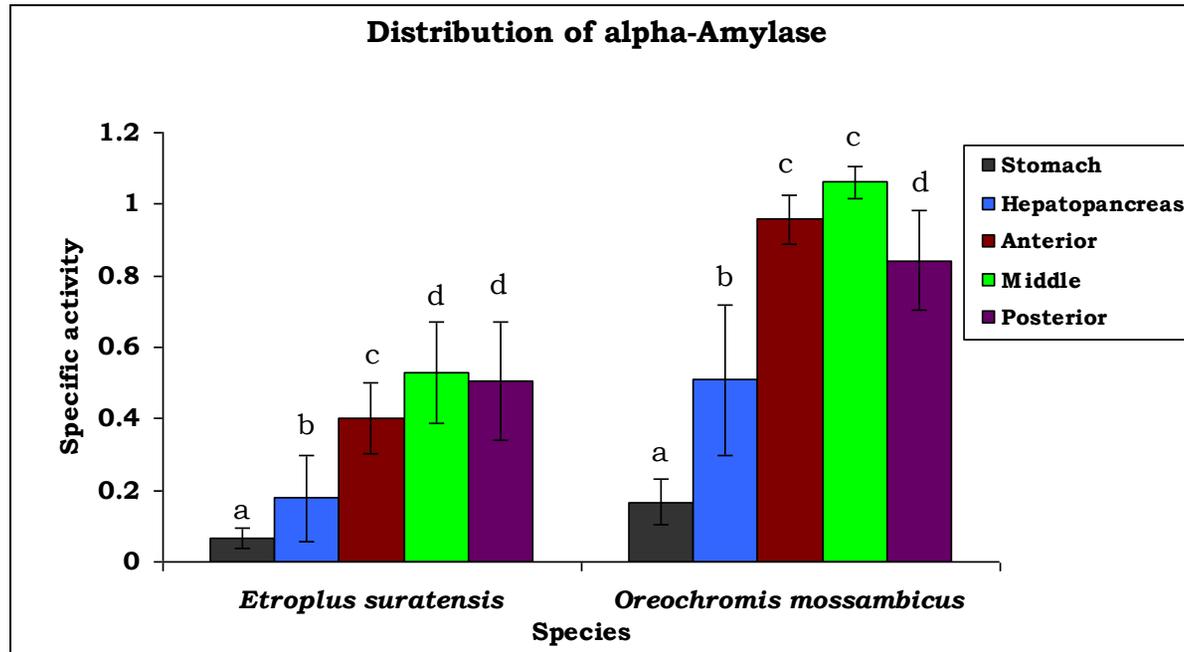


Figure 2: Distribution of alpha-amylase along the digestive segments of *E. suratensis* and *O. mossambicus*

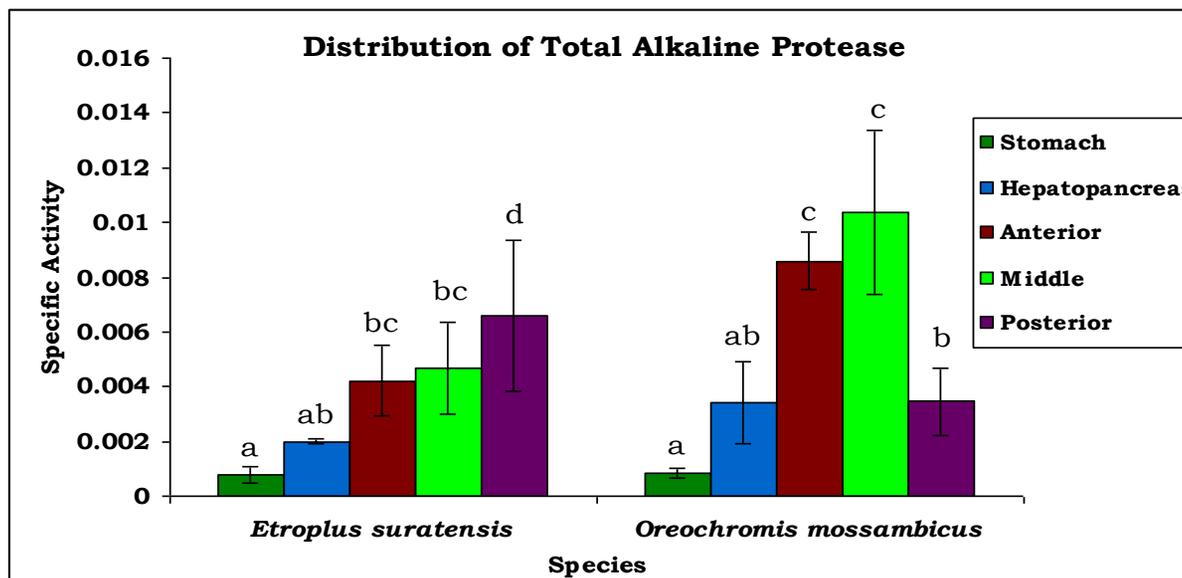


Figure 3: Distribution of total alkaline protease along the digestive segments of *E. suratensis* and *O. mossambicus*

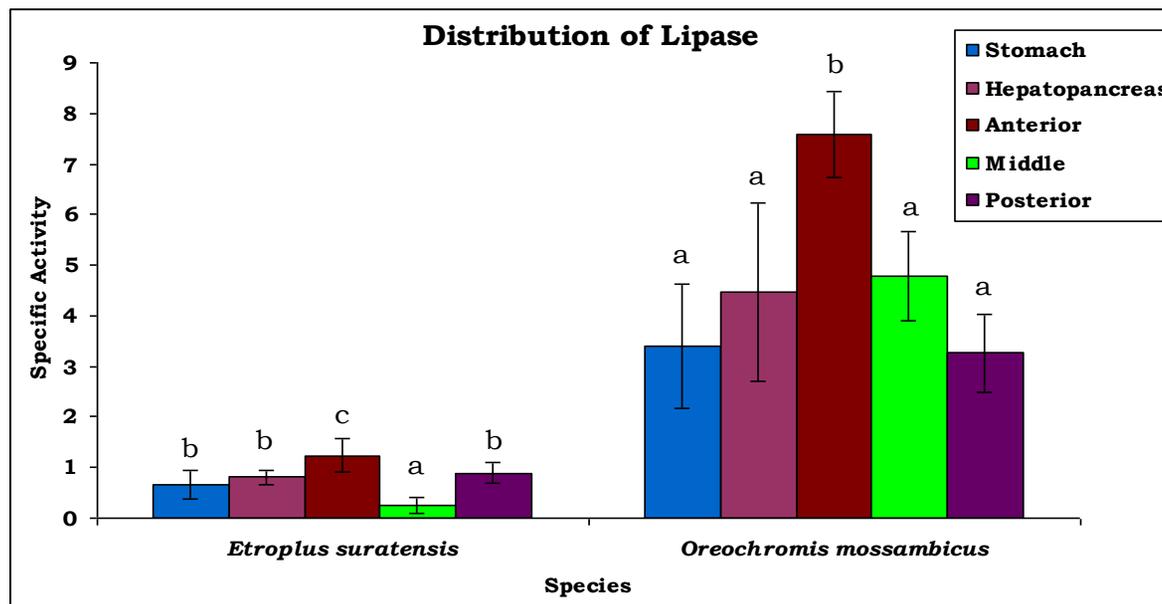


Figure 4: Distribution of lipase along the digestive segments of *E. suratensis* and *O. mossambicus*