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# Digestive Enzyme Activity in Pearl Spot, *Eutroplus suratensis* from Mangalore Waters, India

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**Abstract**

The paper deals with the digestive enzyme activity in pearlspot, *Eutroplus suratensis* inhabiting the brackish waters of Mangalore area, South west coast of India. The activities of major digestive enzymes such as amylase, protease and lipase in stomach; anterior, middle and posterior portion of intestine; liver and tissue surrounding intestine were analyzed. Amylase was the most active enzyme with uniformly high levels in all seasons. High amylase activity was attributed to the nature of diet with filamentous algae as the dominate food. Protease was the second most active digestive enzyme whose seasonal variation in the activity indicated pronounced activity during pre monsoon and post monsoon seasons and subdued activity during monsoon season. Lipase activity was found to be uniformly low throughout the period of study when compared to the other two enzymes. Liver is the major source of activity in this species. Enzyme activity in the GI tract of adult *E. suratensis* showed that amylase was the most active enzyme. Intestinal mucosa was the principal site of activity followed by tissue surrounding intestine, liver and stomach. Intestinal and stomach contents, showed incidentally least activity. Protease was also recorded with considerable levels of activity, maximum being noticed in intestinal mucosa followed by tissue surrounding the intestine and liver. Stomach and intestinal contents demonstrated least protease activity. Sex-wise, however, there was significant difference in the protease, amylase and lipase activity being more active in male than in female. Lipase activity, however, was marginally more in female that in male. Total enzyme activity between juvenile and adult of *E. suratensis* of mixed sex revealed no significant difference.
**Key words : *Eutroplus suratensis*, Enzymes, Gastrointestinal tract.**

The cichlid fishes (Family : Cichlidae; Order : Perciformes) form a well known group having a wide distribution in tropical and sub tropical regions. The most popular cichlids are Tilapia which are now grouped under the genera *Tilapia, Sarotherodon* and *Oreochromis*. The pearl spot, *Eutroplus suratensis*, is an estuarine fish of commercial importance as it possesses requisite qualities for aquaculture such as good body weight, growth rate and high adaptability for food, tasty flesh and good market price (1, 2). *Eutroplus suratensis* is a widely cultured species in Indo-Pacific region (3). Being a euryhaline species, it could be acclimatized to freshwater indicating its suitability for pond aquaculture (4). Successful culture of any endemic species depends on the management of the farming system. To achieve this, information on feeding habits, digestive physiology, reproductive biology are the pre-requisites. Hence to determine the activity of digestive enzymes such as protease, amylase and lipase in the immature and mature populations, the present study was undertaken.

## Methods

Fresh samples of *Eutroplus suratensis* were caught live from Mulki estuary, Mangalore at a depths of 1.5—4.0 m and the fresh fish from fish landing centers formed the material for the present study.

The activities of major digestive enzymes such as amylase, protease and lipase in the stomach; anterior, middle and posterior portion of the intestine; liver and tissue surrounding the intestine were analyzed.

The entire alimentary canal was removed from live specimens and transferred to chilled physiological saline (0.87% NaCl). Adhering mesenteries, connective and other tissues were removed and the stomach, liver and intestine were separated at 4C. Besides, the tissue surrounding the intestine (TSI) were also

**Table 1.** Distribution of protease, amylase and lipase activity in different segments of intestine of adult *E. suratensis.* Figures are mean +/- SE of 22 determinations in duplicate made on 60 adult fish of mixed sex.

| Site of intestine | Protease | | Amylase | | Lipase | |
|---|---|---|---|---|---|---|
| | Total activity | Specific activity | Total activity | Specific activity | Total activity | Specific activity |
| Upper | 187.58 ± 2.36 | 7.07 ± 0.1 | 2297.05 ± 12.18 | 87.53 ± 12.18 | 0.88 ± 0.05 | 0.04 ± 0.00 |
| Middle | 212.68 ± 6.75 | 7.41 ± 0.24 | 2322.61 ± 5.87 | 80.79 ± 0.21 | 0.82 ± 0.02 | 0.03 ± 0.00 |
| Lower | 155.16 ± 6.09 | 5.08 ± 0.21 | 2249.00 ± 11.16 | 74.62 ± 0.36 | 1.40 ± 0.05 | 0.45 ± 0.01 |

carefully removed for enzyme assay in a search for pancreas-equivalent because a distinct pancreatic tissue is not discernible in this fish. The intestine were flushed out by cold saline and the intestine was blotted with filter paper. The intestine was later divided along its anterior-posterior axis into three segments almost in equal length which were respectively designated as anterior, middle and posterior intestine. Enzyme assays in the above tissues were carried out in the adult of both sexes during different months of the year and enzyme activity in immature fishes were also carried out for comparison with adults. The respective tissues were chopped into fine pieces and homogenized in cold saline in a potter Elvehjen-Homogenizer using a close fitting Teflon pestle and giving 6—8 strokes. The tube was chilled in crushed ice during homogenization. The crude homogenate (10% wt/vol) was filtered through folded cheese cloth, the filtrate was diluted and used fresh or stored at 0 C pending enzyme assays. Enzyme assays were carried out in the laboratory at 30 C which was the normal mean temperature of the estuary.

The protease activity was measured by the caesein digestion method. As proteolytic enzyme acts on protein, the amount of trichloro acetic acid (TCA) soluble products formed can be determined by the use of color reactions involving tyrosine. The protease activity is expressed as the amount (in μ-moles) of tyrosine liberated per min per mg tissue protein at 30 C.

Amylase activity (alpha amylase) was evaluated by reduction of 3, 5-dinitro salicylic acid by reducing groups liberated from stomach during enzymatic hydrolysis (5). The amylase activity is expressed as the amount (in μ-moles) of maltose liberated in 10 minutes at 30 C.

Beir titrimetric method was used for estimation with minor modification. The method consists of the fatty acids being liberated by the enzymes to form an emulsion of olive oil by titration with a standard alkali. The amount of standard sodium hydroxide solution used to titrate the fatty acids released is taken as an index of lipase activity of enzyme extract and is expressed as micro moles of fatty acids liberated during 18 hr of incubation at 30 C.

Specific and unit activities of enzymes were calculated as follows :

Specific activity = Total enzyme activity ÷ mg tissue protein

Unit activity = Total enzyme activity ÷ mg wet tissue.

## Results

Comparative levels of enzyme activity in stomach, intestine and liver of juvenile and adult *E. suartensis* are shown in Tables 1 to 4.

### *Protease Activity*

Protease activity : Protease registered a mean value of 141.37, 143.84 and 169.15 units of total activity in stomach, intestine and liver respectively in the juvenile where as female adults registered 75.11, 182.19

**Table 2.** Enzyme activity in intestinal mucosa of adult female and male *E. suratensis.* Values are mean +/- SE of 12 determinations in duplicate from 25 males and 35 females.

| Enzyme | Sex | Total activity (units) | Specific activity (units/mg protein) |
|---|---|---|---|
| Protease | Female | 189.19 ± 15.39 | 6.29 ± 0.54 |
| | | 188.09 ± 2.62 | 6.75 ± 0.31 |
| Amylase | Female | 2277.16 ± 19.85 | 78.98 ± 1.12 |
| | Male | 2302. ± 36.47 | 82.97 ± 4.81 |
| Lipase | Female | 0.88 ± 0.04 | 0.03 ± 0.00 |
| | Male | 0.87 ± 0.02 | 0.03 ± 0.00 |

**Table 3.** Comparative levels of enzyme activity between juveniles and adult *E. suratesis.* Values are mean + SE of 6 determinations in duplicate from 35 young ones.

| Site of GI tract/its associated organ | Juvenile | | Adult | | | |
|---|---|---|---|---|---|---|
| | | | Female | | Male | |
| | Total activity | Unit activity | Total activity | Unit activity | Total activity | Unit activity |
| | | | **Protease** | | | |
| Stomach | 141.37 ± 5.87 | 433.66 ± 18.00 | 75.11 ± 1.18 | 75.11 ± 1.18 | 94.66 ± 1.68 | 67.61 ± 1.20 |
| Intestine | 143.84 ± 3.40 | 345.78 ± 8.17 | 182.19 ± 15.39 | 196.80 ± 14.84 | 188.09 ± 2.62 | 302.13 ± 29.58 |
| Liver | 169.15 ± 3.04 | 604.12 ± 10.87 | 164.42 ± 5.22 | 164.42 ± 5.22 | 141.55 ± 2.15 | 141.58 ± 2.15 |
| | | | **Amylase** | | | |
| Stomach | 1857 ± 34.34 | 5698.09 ± 105.31 | 174.63 ± 12.18 | 1874.63 ± 12.18 | 2012.20 ± 5.58 | 1437.28 ± 3.99 |
| Intestine | 2012.13 ± 43.32 | 4837.00 ± 103.99 | 2277.16±19.85 | 2390 ± 64.30 | 2302.32 ± 36.47 | 3660.63 ± 31.91 |
| Liver | 2159.49 ± 23.22 | 7712.45 ± 82.94 | 2349.39 ± 12.17 | 2349.39±12.17 | 2208.18 ± 9.74 | 2208.18 ± 9.74 |
| | | | **Lipase** | | | |
| Stomach | 0.50 ± 0.01 | 1.54 ± 0.02 | 0.89 ± 0.20 | 0.89 ± 0.20 | 0.83 ± 0.03 | 0.59 ± 0.02 |
| Intestine | 0.68 ± 0.01 | 1.64 ± 0.02 | 0.88 ± 0.04 | 0.96 ± 0.05 | 0.87 ± 0.02 | 1.38 ± 0.12 |
| Liver | 1.29 ± 0.01 | 4.64 ± 0.04 | 1.60 ± 0.20 | 1.60 ± 0.20 | 1.19 ± 0.01 | 1.19 ± 0.01 |

and 164.42 units of total activity in these parts of GI tract respectively. Male, on the other hand, showed 94.66, 185.09 and 141.55 units in the above organs respectively. However, values of protease unit activity of juvenile were higher compared to either sexes ; stomach, intestine and liver of juveniles thus showing a mean value of 433.66, 345.78 and 604.12 units compared to 75.11, 196.80 and 164.42 units in female and 94.66, 188.09 and 141.55 units in male in the above mentioned tissues/organs respectively.

### *Amylase Activity*

Amylase total activity levels in stomach, intestine and liver of juvenile showed a mean value of 1857.6, 2012.13 and 2159.49 units respectively as compared to 1874.63, 2277.16 and 2439.39 units in female and 2012.20, 2302 and 2208.18 in male respectively in the above organs/tissues. Unit activity, however, was marked with 5698.09, 4837.00 and 7712.45 units in the above organs of juveniles where as in female they were 1874.63, 2390.92 and 2349.39 units and in male 1437.28, 3660.63 and 2208.18 units respectively in the above organs/tissues.

### *Lipase Activities*

The comparative levels of lipase total activity observed between juveniles and adults revealed that, mean values of total activity of juveniles were 0.50, 0.68 and 1.29 units in stomach, intestine and liver respectively, whereas among adults female registered 0.89, 0.88 and 1.60 units and male 0.83, 0.87 and 1.19 units of total activity in the above tissues/organs respectively.

Unit activity recorded were 1.54, 1.64 and 4.60 in juvenile and 0.89, 0.96 and 1.60 units in adult female and 0.59, 1.38 and 1.19 units in the adult male in the stomach, intestine and liver respectively.

The data indicate that liver is the main source of the maximum lipase activity compared to stomach and intestine in both juvenile and adult. Protease and amylase activities were generally high in all the three tissues/organs of GI tract in both sexes (Table 1). Comparison of total activity and unit activity between juvenile and adult *E. suratensis* however, revealed no significant difference ($\chi^2 = 4.6$, $P>0.05$, Friedman test).

## Discussion

The moderately acidic pH in the anterior part of the alimentary canal suggests digestion in stomach. In fish, the digestive enzyme activity strongly relates to pH of gut (6). For instance, an acidic environment characteristic of stomach digestion and pepsin action has been expressed in carnivorous rainbow trout.

**Table 4.** Mean pH values in the different parts of the alimentary canal of *E. suratensis.* Mean values are based on 6—10 determinations from each part.

| Parts of the alimentary canal | Mean ± SE | Range |
|---|---|---|
| Buccal cavity | 6.45 ± 0.21 | 6.00 – 7.50 |
| Oesophagus | 5.83 ± 0.23 | 4.50 – 6.70 |
| Stomach | 5.02 ± 0.18 | 4.50 – 6.30 |
| Upper intestine | 5.55 ± 0.31 | 4.50 – 7.50 |
| Middle intestine | 7.02 ± 0.15 | 6.30 – 8.00 |
| Lower intestine | 7.30 ± 0.15 | 6.20 – 8.00 |
| Rectum | 8.00 ± 0.12 | 7.20 – 8.50 |
| Liver | 6.33 ± 0.05 | 6.00 – 6.50 |

However, pearl spot being a herbivore, amylolytic activity is predominant in intestinal mucosa. Accordingly the distal part of the alimentary canal showed slightly alkaline pH (Table 4). Bittertech (7) determined tryptic and amylolytic activities on different intestinal segments of stomachless silver and big head carps and found out optimum pH at 8.3 and 7.0 respectively in those fishes. Though foregut lumen of silver carp had a low pH of 6.4 the fish compensated the deficiency by having higher enzyme concentration. The liver of *E. suratensis* with a pH of 6.33 was the major source of lipase activity.

Although predominantly herbivorous fish, the intestine of pearl spot appears to be capable of digesting dietary proteins. However, total amylase activity levels were observed to be uniformly higher compared to protease and lipase throughout the period of observation indicating that amylase is a key digestive enzyme which plays an important role in the digestion process of *E. suratensis.*

The relative activity of enzymes correlates with feeding habits of *E. suratensis*. Herbivorous fish generally possess higher amylase activity as compared to carnivorous fish (8). Amylase do occur in most omnivorous fish such as cyprinids (9, 10) and herbivorous fish such as Tilapia (11, 12) and milk fish (13).

In the present study amylase activity was found to be more pronounced during pre-and post-monsoon months. During these periods, fish were found to feed actively on filamentous algae. This period of active feeding coincided with the post spawning phase of the fish which seem to suggest that feeding behavior is dependent on energy requirement of the body. The energy requirement of a fish is influenced by age, breeding period, general activity and temperature of water (14). The development due to loss of energy in the deposition of egg.

Activities of protease, amylase and lipase in gastro intestinal (GI) tract of *E. suratensis* indicated the following major features : Amylase found to be most active enzyme with uniformly high levels in all seasons. High amylase activity is attributed to the nature diet with filamentous algae as the dominate food. Protease was the second most active digestive enzyme whose seasonal variation in the activity indicated pronounced activity during pre monsoon and post monsoon seasons and subbed activity during monsoon season. Lipase activity was found to be uniformly low through the period of study when compared to the other two enzymes. Liver is the major source of activity in this species. Survey of enzyme activity (total activity – units) in the GI tract of adult *E. suratensis* showed that amylase was the most active enzyme. Intestinal mucosa was the principal site of activity followed by tissue surrounding intestine, liver and stomach. Intestinal and stomach contents showed incidentally least activity. Protease was also recorded with considerable levels of activity, maximum being noticed in intestinal mucosa followed by tissue surrounding intestine and liver. Stomach and intestinal contents demonstrated least protease activity. Sex wise, however, there was significant difference in the protease, amylase and lipase activity being more active in males than I females. Lipase activity, however, was marginally more in females that in males. Total enzyme activity between juvenile and adult of *E. suratensis* of mixed sex revealed no significant difference ($\chi^2 = 4.6$, $P > 0.05$, Friedman test).

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