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Amylase and protease activity in shrimps and prawn of Sundarbans, West Bengal, India

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Comparative assessment of amylase and protease activities in the gut and hepatopancreas extracts of *Macrobrachium rosenbergii, Penaeus monodon, P. indicus* and *Metapenaeus monoceros*, collected from Sundarban creeks along West Bengal, India, was done. α-amylase activity in gut and hepatopancreas was highest in *M. rosenbergii* followed by *P. indicus*, *M. monoceros* and *P. monodon* and the levels of α-amylase activity was found elevated in gut of all four crustacean shrimp species as compared with hepatopancrease. Increased level of protease activity in gut and hepatopancreas were observed in *P. indicus* followed by *P. monodon, M. monoceros* and *M. rosenbergii* and the protease activity level was found highest in hepatopancrease of all four crustacean species as compared to levels of gut. Increased level of α-amylase activity in *M. rosenbergii* showed that carbohydrates are the principal substrates used for energy production in *M. rosenbergii* and gut might play an important role in the digestive process of carbohydrate in shrimps and prawn.

[**Keywords**: Amylase activity, protease activity, shrimps, prawn, Sundarbans]

Introduction

The prawns can digest a wide range of foods of both plant and animal origin[1]. Although diseases pose a serious threat to the aquaculture of penaeid and non-penaeid shrimps, the production of these highly valued crustaceans continues to grow. Among the many species cultured, *Penaeus monodon* (grass shrimp, black tiger shrimp) stands out as the most important species. It accounted more than 56.8 % of coastal aquaculture production[2]. In 2013, India became the leading supplier of shrimps to the USA market, overtaking Thailand and Vietnam[3]. West Bengal is enriched with 405000 ha of estimated brackish water area for potential fish/shrimp cultivation. The Sundarbans are the largest estuarine mangrove forest in the world located in South 24 Paraganas district of West Bengal (India) and Bangladesh. Apart from several shrimp farms, Sundarbans harbours about 20 different types of shrimps and prawns species[4].

Study of digestive enzymes is an essential step towards understanding the mechanism of digestion and how the organism adapts to changes in the nutritional environment[5]. These digestive enzymes are able to hydrolyze a variety of substrates and various factors are involved in their regulation. Those factors which are involved in regulation of digestive enzymes are diet[6,7], ontogenic changes[8], body size[9], circadian rhythms[10], moulting stage[10] and even a stimulant effect from the pond water has been reported[11].

There is a need to understand the nutritional requirements of these species by analysing the digestive enzyme activities in order to formulate an effective diet to provide optimal growth. Moreover, there is lack of basic information on the digestive physiology of native shrimp and prawn species, which may cause utilization of improper feed,

resulting in poor feed digestibility and feed conversion ratio. Hence, to provide in depth insight into the enzyme activities of selected shrimps and prawns, studies were carried out on proteolytic and α-amylase activities in gut and hepatopancreas extracts of *Macrobrachium rosenbergii, Penaeus monodon, P. indicus* and *Metapenaeus monoceros*, collected from Sundarban creeks along West Bengal.

Material and Methods

The animals (n=10 for each species) as given in Table 1, were collected by using cast net and gill net from Sundarban creeks (Canning region), West Bengal and transported in icebox to the laboratory. Gut and hepatopancreas tissues from each of the species were collected aseptically, macerated and then supernatant collected from both the tissues, kept in refrigerator (-20˚C) for enzyme activity study after centrifugation (10000 rpm for 20 min) in PBS (phosphate-buffered saline) buffer. α-amylase activity was assayed using the 3, 5-dinitrosalicylic acid (DNS) method at 540 nm[12,13]. Starch was used as the substrate in the determination of α-amylase. Starch (250 µl) was incubated with the gut and hepatopancreas enzyme extract (50 µl) at 37 °C and phosphate (Na_2HPO_4 + NaH_2PO_4) buffer (pH 6.9). The reaction was terminated by adding 100 µl of 1% dinitrosalicylic acid (DNS) and boiled for 8 min. After cooling, 1000 µl of distilled water was added to the mixture. Standards were prepared using different concentrations of maltose to compare the maltose concentration in the samples.

Table 1 Species and their size of samples collected from Sundarban creeks

Species	Body weight (g)
Macrobrachium rosenbergii	78.10±9.04
Penaeus monodon	61.24±6.18
Metapenaeus monoceros	40.31±5.41
Penaeus indicus	46.18±8.24

The amount of soluble protein in the gut and hepatopancreas enzyme extracts was determined by Lowry's method[14] using bovine serum albumin as a standard protein. Then 50 µl of gut and hepatopancreas extract sample or the blank was added to freshly mix complex-forming reagent. The mixture was then incubated at 37°C for 30 minutes in boiling water bath. After 30 minutes, 250 µl of Folin reagent was added kept for incubation at 37°C for 10 min and the absorbance was taken at 660 nm. Activity of protease was determined by using Anson method[15]. Enzyme extract (gut and hepatopancreas) 200 µl was mixed with substrate (0.65% of casein in 25 mM Tris-HCl buffer) at 37°C for 60 min and after incubation Trichloroacetic acid TCA (5%) was added to attenuate the reaction. This mixture was centrifuged at 10000 rpm for 10 min and the released amino acids were measured as tyrosine by the method of Folin and Ciocalteu[16].

The data were statistically analyzed by statistical package SPSS version 16 and the mean values were compared by one way ANOVA followed by Duncan's multiple range test (DMRT) to determine the significant differences between the means. Comparisons were made at the 5% ($p < 0.05$) probability level.

Results

The species and size of shrimp and prawn used for the study are presented in Table 1. α-amylase activity level in gut was significantly ($p < 0.05$) high in *M. rosenbergii* (4.083 U mg^{-1} protein) followed by *P. indicus* (2.896 U mg^{-1} protein), *M. monoceros* (1.336 U mg^{-1} protein) and *P. monodon* (0.493 U mg^{-1} protein) (Fig 1) and in the hepatopancreas significantly ($p < 0.05$) increased levels of amylase activity was found in *M. rosenbergii* (2.27 U mg^{-1} protein), followed by *P. indicus* (1.993 U mg^{-1} protein), *M. monoceros* (1.233 U mg^{-1} protein) and *P. monodon* (0.132 U mg^{-1} protein) (Fig 2).

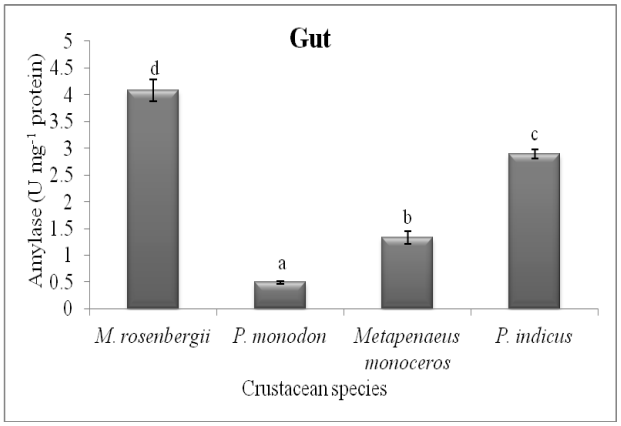

Fig. 1 - α-amylase activity in the gut of different shrimp and prawn species collected from Sundarban creeks (Values with different superscript are significantly different ($p < 0.05$) and express as mean with the standard error bar).

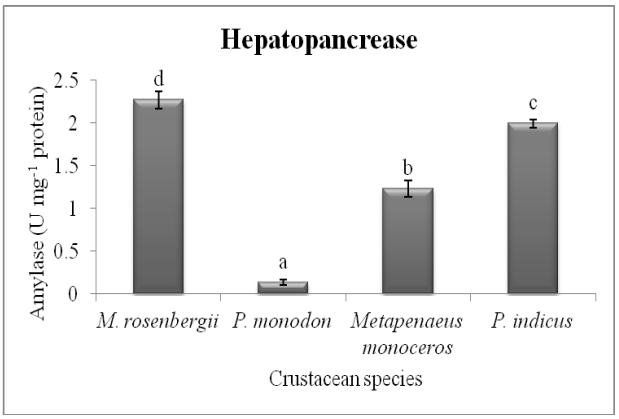

Fig. 2 - α-amylase activity in the hepatopancraese of different shrimp and prawn species collected from Sundarban creeks (Values with different superscript are significantly different ($p < 0.05$) and express as mean with the standard error bar).

The α-amylase activity level was found significantly ($p < 0.05$) elevated in gut of all four-crustacean species (*M. rosenbergii* (4.083 U mg^{-1} protein), *P. indicus* (2.896 U mg^{-1} protein), *M. monoceros* (1.336 U mg^{-1} protein) and *P. monodon* (0.493 U mg^{-1} protein)) as compared with hepatopancrease (Fig 3-6). Significantly ($p < 0.05$) increased level of protease activity in gut were observed in *P. indicus* (0.1141 U mg^{-1} protein) followed by *P. monodon* (0.0615 U mg^{-1} protein), *M. monoceros* (0.045 U mg^{-1} protein) and *M. rosenbergii* (0.0015 U mg^{-1} protein) (Fig 7).

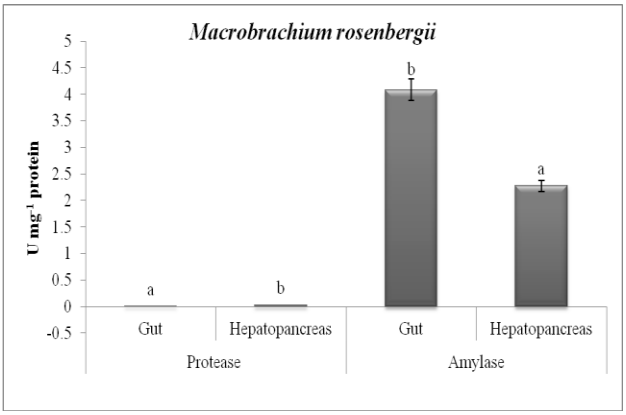

Fig. 3 - α-amylase and protease activity in the gut and hepatopancreas of *M. rosenbergii* collected from Sundarban creeks (Values with different superscript are significantly different ($p < 0.05$) and express as mean with the standard error bar).

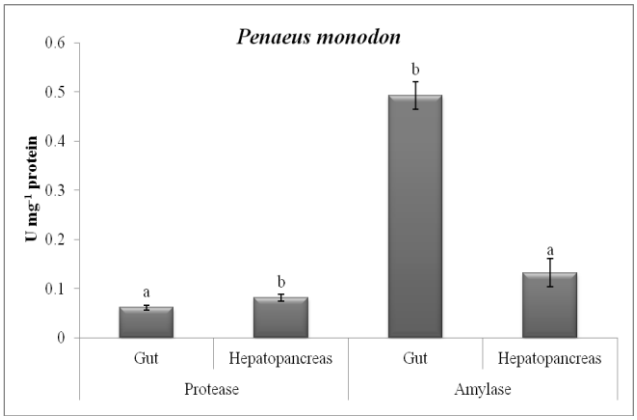

Fig. 4 - α-amylase and protease activity in the gut and hepatopancreas of *P. monodon* collected from Sundarban creeks (Values with different superscript are significantly different ($p < 0.05$) and express as mean with the standard error bar).

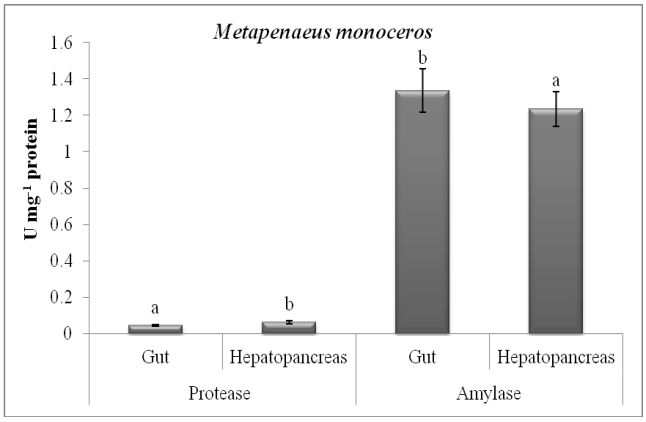

Fig. 5 - α-amylase and protease activity in the gut and hepatopancreas of *M. monoceros* collected from Sundarban creeks (Values with different superscript are significantly different ($p < 0.05$) and express as mean with the standard error bar).

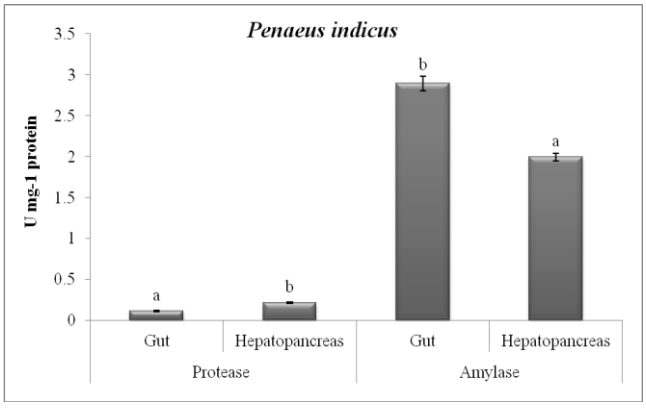

Fig. 6 - α-amylase and protease activity in the gut and hepatopancreas of *P. indicus* collected from Sundarban creeks (Values with different superscript are significantly different ($p < 0.05$) and express as mean with the standard error bar).

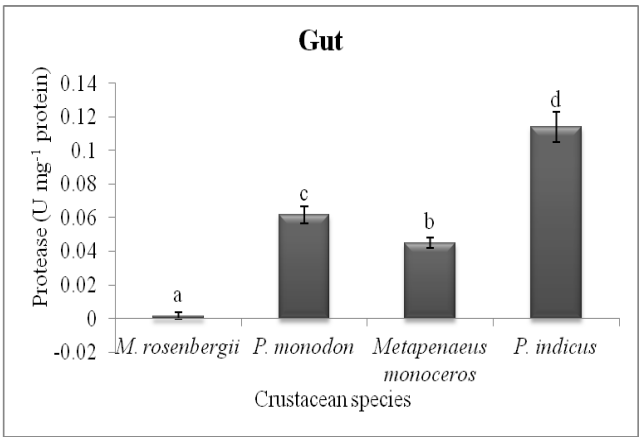

Fig. 7 - Protease activity in the gut of different shrimp and prawn species collected from Sundarban creeks (Values with different superscript are significantly different ($p < 0.05$) and express as mean with the standard error bar).

Similar trend in protease activity levels was observed in hepatopancrease with significantly ($p < 0.05$) high value were observed in *P. indicus* (0.212 U mg^{-1} protein) followed by *P. monodon* (0.081 U mg^{-1} protein), *M. monoceros* (0.0642 U mg^{-1} protein) and *M. rosenbergii* (0.031 U mg^{-1} protein) (Fig 8). The protease activity level was found significantly ($p < 0.05$) increased in hepatopancrease of all four-crustacean species (*P. indicus* (0.212 U mg^{-1} protein), *P. monodon* (0.081 U mg^{-1} protein), *M. monoceros* (0.0642 U mg^{-1} protein) and *M. rosenbergii* (0.031 U mg^{-1} protein)) as compared to levels of gut (Fig 3-6).

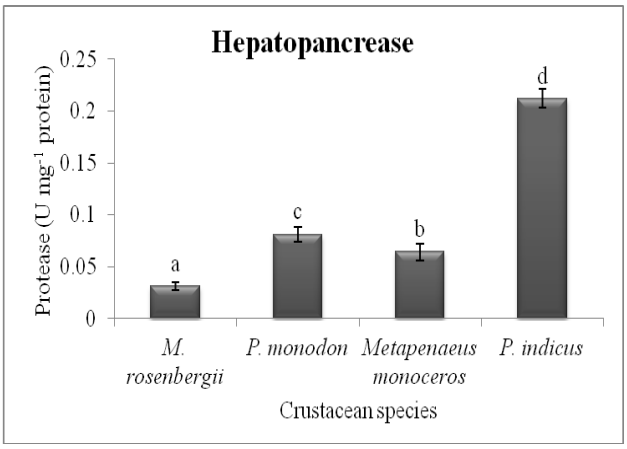

Fig. 8 - Protease activity in the hepatopancreas of different shrimp and prawn species collected from Sundarban creeks (Values with different superscript are significantly different ($p < 0.05$) and express as mean with the standard error bar).

Discussion

Knowledge of digestive enzymes of aquatic organisms of economic importance is important, as the enzyme profile of a given species is closely related to its feeding habits and its capability to digest food items[17]. Hepatopancreas or midgut gland accounts for 3-4% (w/w) of whole body weight of shrimp and prawn species and is the most important digestive organ, followed by stomach (0.6%) and intestine (0.5%). Specific activities of digestive enzymes are higher in hepatopancreas than in stomach or intestine[18]. In addition to the proteases, α-amylase, cellulase, and chitinase were found in various digestive organs of shrimp and prawn[18,19]. The nutritional state and growth of shrimp/prawn do not necessarily correlate well with the activities of protease, lipase, or amylase[20,21]. However, examples of positive correlation were found between digestive enzyme activities and growth[22,23]. The digestive enzyme activities of various shrimp and prawn species showed a good correlation between digestive enzyme activity and food habits. The protein levels for optimal shrimp growth were suggested to be positively correlated to the proteolytic enzyme activity and negatively to α-amylase activity[18]. A comparative study of the activity of digestive proteolytic enzymes and α-amylase could reveal the capacity of different species to use protein and carbohydrates[24]. Therefore, the analysis of the digestive enzyme (proteases and amylase) activities in the hepatopancreas and gut can be correlated with the nutritional state of the species[25]. The present study shown the proteolytic and α-amylase activities in gut and hepatopancreas extracts of *M. rosenbergii*, *P. monodon*, *P. indicus* and *M. monoceros* and more emphasis has been given to study the enzyme activity in different organs (i.e. hepatopancrease and gut), and difference in the enzyme activity in different shrimp and prawn species.

An amylase is a digestive enzyme that catalyses the hydrolysis of carbohydrate into simple sugars. Amylase has been poorly studied in crustaceans[26]. In this study, α-amylase activity in gut was highest in *M. rosenbergii* followed by *P. indicus*, *M. monoceros* and *P. monodon* and in the hepatopancreas increased levels of α-amylase activity was found in *M. rosenbergii*, followed by *P. indicus*, *M. monoceros* and *P. monodon*. The α-amylase activity level was found elevated in gut of all four-

crustacean species as compared with hepatopancrease which showed that gut might play an important role in the digestive process of carbohydrate in shrimps and prawn. The present study is supported by various researchers who demonstrated that alpha-amylase and cellulase activities in the midgut gland, intestine, and stomach of *M. rosenbergii* are much higher than those found in the marine species, *P. monodon*, *P. japonicus*, and *P. penicillatus*[18]. Diaz-Herrera *et* al.[27] found that carbohydrates are the principal substrates used for energy production in larval and postlarval *M. rosenbergii*. Hidalgo *et* al.[24] reported that α-amylase activity is greater in omnivorous and herbivorous fish than in carnivorous fish. Chan *et* al.[28] mentioned that the activity of α-amylase follows a pattern influenced more by phylogeny than by diet. On contrary, Fernandez *et* al.[17] pointed out that the adaptations of the digestive system of different species exhibit closer correlation with their diet rather than on their taxonomic category. This has also confirmed by the observation made by Kuzmina[29], which indicated that changes in digestive enzyme activity is affected by feeding behaviour and biochemical composition of food. Enzyme assay in this present study revealed high amylase activity in *M. rosenbergii* which indicates that carbohydrates may be important energy sources in prawn diets as compared to shrimp diets.

Protease (also called peptidase or proteinase) is an enzyme that facilitates the breakdown of proteins and several major classes of proteases (i.e., trypsin, chymotrypsin, carboxypeptidases, aminopeptidases and astacin) have recently been identified in crustacean's digestive systems. Trypsin is a major proteolytic enzyme which normally exhibits high activity in crustaceans. Interestingly, unlike vertebrate trypsin, crustacean trypsin can hydrolyze native proteins[30]. Proteases are the most assessed digestive enzymes in crustaceans and play a key role in the overall assimilation of nutrients[31]. These enzymes are also very important for the metabolism and growth of penaeids due to their fundamental role in providing essential amino acids. Hence, the release of essential amino acids may be dependent on the effective hydrolysis of proteins by digestive proteases[32]. In the present study increased level of protease activity in gut were observed in *P. indicus* followed by *P. monodon, M. monoceros* and *M. rosenbergii*. Similar trend in protease activity levels was observed in hepatopancrease with highest value were observed in *P. indicus* followed by *P. monodon, M. monoceros* and *M. rosenbergii*. The protease activity level was found highest in hepatopancrease of all four-crustacean species as compared to levels of gut, which indicates that hepatopancrease might be playing an important role in the protein hydrolysis. The study is supported by D'Abramo and Sheen[33] who demonstrated that like other penaeid shrimp, the major digestive proteases secreted by the hepatopancreas of *P. monodon* are trypsin[34], chymotrypsin, and carboxylpeptidase[35].

Interestingly, the increased levels of α-amylase activity were observed in *M. rosenbergii* followed by *P. indicus*, *M. monoceros* and *P. monodon*, indicates that *M. rosenbergii* more efficiently utilizes carbohydrates as a source of energy as compared to other three species of shrimp. Moreover, gut of all four-crustacean species showed higher levels of α-amylase activity which indicate that gut might be playing an important role in digestive process of carbohydrate. The protease activity level was found highest in *P. indicus* followed by *P. monodon, M. monoceros* and *M. rosenbergii* showed that shrimp species more efficiently utilized protein diets as compared to *M. rosenbergii*. Increased levels of protease activity were observed in hepatopancrease of all four-crustacean species as compared to levels of gut, which indicates that hepatopancrease might be playing an important role in the protein hydrolysis. Our results build an understanding about the digestive physiology of the shrimp and prawn species collected from Sundarbans and provide a basic experimental model to study the activity of different digestive enzymes in crustaceans. However, further work is needed to determine the function of these digestive enzymes in response to different diets having varying levels of protein and carbohydrate in order to develop knowledge of the nutritional requirements of crustacean species and optimize a cost effective and environment-friendly feed formulation for farmed prawn and shrimp species.

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