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Digestive enzyme activities in mudskipper *Boleophthalmus pectinirostris* and Chinese black sleeper *Bostrichthys sinensis**

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Abstract The mudskipper *Boleophthalmus pectinirostris* and Chinese black sleeper *Bostrichthys sinensis* occupy the intertidal zone. However, both species have their own unique diet. The former is an herbivore and the latter is a carnivore. In order to reveal the relationship between digestive enzyme activities and diets in the two species, the activities of protease (P), non-specific bile salt-activated lipase (BAL) and α-amylase (A) were determined in the stomach and intestine of adult mudskipper *B. pectinirostris* and Chinese black sleeper *B. sinensis*. The results showed that the activities of protease, BAL and α-amylase in the intestine of *B. pectinirostris* were significantly (P<0.05) higher than those in the stomach. In *B. sinensis*, gastric protease activity was not different from the intestinal protease (P>0.05), while BAL and α-amylase activities of the intestine were significantly (P<0.05) higher than those of the stomach. The activity of gastric protease in *B. sinensis* was significantly (P<0.05) higher than that in *B. pectinirostris*, while the activities of intestinal protease were not different between the two fish species (P>0.05). BAL activities of the stomach and intestine in *B. sinensis* were significantly (P<0.05) higher than those in *B. pectinirostris*, while α-amylase activities of the stomach and intestine in *B. pectinirostris* were significantly (P<0.05) higher than those in *B. sinensis*. The ratios of P/BAL, A/P and A/ BAL of the digestive tract in *B. pectinirostris* were 1.5, 107.3 and 158.6, respectively; and those in *B. sinensis* were 0.2, 1.6 and 0.2, respectively. It can be concluded that food digestion in the adult *B. pectinirostris* is mainly carried out in the intestine, whereas in the adult *B. sinensis* it is initiated in the stomach and finishes in the intestine. The activities of BAL and α-amylase in *B. pectinirostris* and *B. sinensis* are well correlated with their diets. However, a clear-cut correlation between protease activity and diets is not found in these two species.

Keyword: *Boleophthalmus pectinirostris*; *Bostrichthys sinensis*; protease; non-specific bile salt-activated lipase; α-amylase; feeding habit

1 INTRODUCTION

The digestion of food into small molecules, which can be assimilated in the digestive tract of fishes, is corporately performed by the various enzymes produced from the stomach, pancreas and intestine. The profiles of three main digestive enzyme classes (proteases, lipases and carbohydrases) in fishes have been investigated for many years since it is essential for understanding the physiology of fish nutrition and solving applied nutritional problems in fish feeding (Buddington et al., 1986; Wang et al., 2002; Hakim et al., 2006; Zhu et al., 2006). For example, Uys et al. (1987) noted that, assays of the digestive enzymes in the catfish *Clarias gariepinus*, reveal that this fish is able to utilize a wide range of nutrients efficiently. Furne et al. (2005) suggest that, from the enzymatic content in the gut, a great proportion of carbohydrate could be added to the diets of carnivorous Adriatic sturgeon *Acipenser naccarii*. De Almeida et al. (2006), studying the digestive enzyme produced in response to protein and lipid, found that tambaqui *Colossoma macropomum* adapts the main digestive

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enzymes to the food intake. Furthermore, the activities of digestive enzymes in relation to diet have been examined in a variety of fishes, including herbivores, omnivores and carnivores (Sabapathy et al., 1993; Hidalgo et al., 1999; Fernandez et al., 2001). Based on these studies, the digestive patterns in different fishes in relation to their feeding habits and ecological niches have been established.

Both the mudskipper *Boleophthalmus pectinirostris* (Linnaeus, 1758) and Chinese black sleeper *Bostrichthys sinensis* (Lacépède, 1801) belong to the Gobioidei, living in the intertidal mudflats along the coasts and in estuaries. The adult *B*. *pectinirostris* is herbivorous, primarily feeding on benthic diatoms and particulate organic detritus (Zhu et al., 1993); while the adult *B*. *sinensis* is carnivorous, feeding on benthic invertebrates (shrimps, crabs, aquatic insect, etc.) and other small fishes (Chen et al., 2006). As they are considered to be excellent sea food and command a high market price in China, farming and fry production of these two fish species has been developed in mainland China since the 1980s (Hong et al., 2003). At present, these two fish species are widely cultured in the southeastern coastal waters of China in earth ponds. So far, however, artificial diets are not available for the farming of these two fish species. Thus, nutritional and digestive enzyme studies would be useful to the production of artificial diets.

The aim of this study was to investigate the activities of the protease, lipase and α-amylase in both *B*. *pectinirostris* and *B*. *sinensis* and to reveal the relationship between enzyme activities and feeding habits in these two fish species.

2 MATERIALS AND METHODS

2.1 Animals

Both *B*. *pectinirostris* and *B*. *sinensis* were captured in daytime from the mudflats of Funing Bay (26°53′N, 120°03′E), Fujian Province, China, in October, 2005. Seawater temperature and salinity of the sampling area were 24–25°C and 15–16. Eight *B*. *pectinirostris* and eight *B*. *sinensis* at a similar age (1^+ age) were selected in this study. The body length and body weight were 8.73–12.01 cm and 10.62–13.37 g for *B*. *pectinirostris*, and 10.87–13.36 cm and 37.13–46.26 g for *B*. *sinensis*. At this stage, debris of diatoms and organic detritus was observed in the gut of *B*. *pectinirostris*, and debris of crabs and fishes, in the gut of *B*. *sinensis*.

2.2 Treatment of the samples

The fish were transported live to the laboratory and maintained in seawater tanks for about 12 h without feeding before they were killed. The temperature, salinity and dissolved oxygen levels of the holding seawater were 24±1°C, 16 and 6.5±0.3 mg/L, and 30% of the holding water was renewed during the maintenance period. Before the experiment, no contents were found in the whole gut of either species. The stomach and intestine were removed, and identical tissues from two *B*. *pectinirostris* and two *B*. *sinensis* were each pooled as one sample and, therefore, a total of four samples (n=4) was analyzed.

The tissues were washed with iced double-distilled water, after which they were blotted dry on filter papers and then weighed. The samples were homogenized with a homogenizer (Polyton, PT-MR 2100) in iced double-distilled water at maximum speed for 3 min and centrifuged at 9 000 *g* for 30 min at 2°C. Supernatants were collected and frozen at -80°C until analysis.

2.3 Enzymatic determinations

Protease activity was assayed using 1% casein as the substrate as described by Hidalgo et al. (1999), the reaction pH was 2.5 (0.2 mol/L glycine-HCl buffer) for gastric protease (pepsin) and 7.5 (0.1 mol/L Tris-HCl buffer) for intestinal protease (alkaline protease). Non-specific bile salt-activated lipase (BAL) activity was measured in 0.25 mmol/L Tris-HCl buffer (pH 7.5) with 0.53 mmol/L p-nitrophenyl myristate as substrate (Iijima et al., 1998). α-amylase activity was determined in 0.1 mol/L phosphate-citrate buffer (pH 7.5) with 2% starch as substrate (Hidalgo et al., 1999). All the enzyme reactions were carried out at 37°C.

One unit of enzyme activity was defined as the amount of enzyme required for the formation of 1 μmol of product per minute (i.e., U). Enzyme activities were expressed as U per g of wet tissue (i.e., U g tissue^{-1}). The enzyme activity in the digestive tract of the two fishes was calculated as:

$$\frac{\text{Enzyme activity in the stomach plus the intestine (total U)}}{\text{Wet weight of the stomach plus the intestine (g tissue)}}$$

2.4 Statistical analysis

All data are presented as mean values (n=4) and standard error (±SE). The differences between sections and species for each enzyme activity were compared using Student's t-test and were considered statistically significant at the P<0.05 level.

3 RESULTS

As shown in Figs.1–3, the activities of protease, BAL and α-amylase from the intestine of *B. pectinirostris* were significantly ($P<0.05$) higher than those from the stomach, being 3.5, 9.4 and 12.3 times higher, respectively. In *B. sinensis*, gastric protease activity was not different from the intestinal ($P>0.05$) (Fig.1), while the activities of BAL and α-amylase of the intestine were significantly ($P<0.05$) higher than those of the stomach (Figs.2–3). The activities of these three intestinal enzymes were 1.2, 3.2 and 2.4 times higher, respectively, than those of the stomach in *B. sinensis*.

The activity of gastric protease in *B. sinensis* was significantly ($P<0.05$) higher than that in *B. pectinirostris*, being 2.5 times higher (Fig.1). The activity of intestinal protease in *B. pectinirostris* was 1.1 times higher than that in *B. sinensis*, however, there was no significant difference between them ($P>0.05$) (Fig.1).

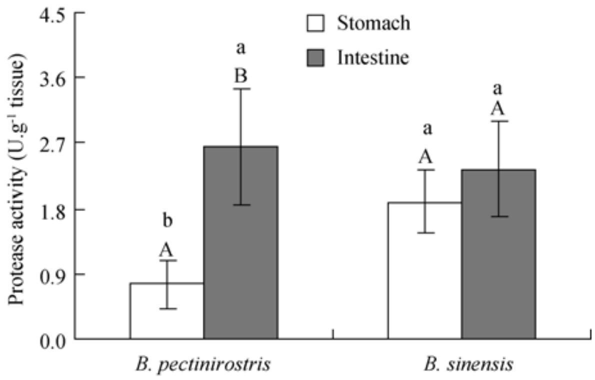

Fig.1 The activities of protease of the stomach (pepsin) and intestine (alkaline protease) in *B. pectinirostris* and *B. sinensis*

Notes: The values bearing different capital letters represent significant difference between the two organs of the same fish species ($P<0.05$), the values bearing different small letters represent significant difference between the same organs in the two fish species ($P<0.05$)

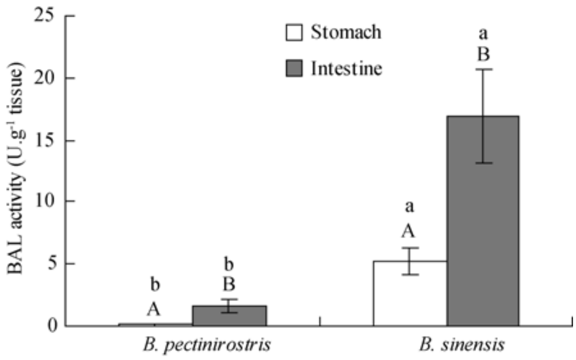

Fig.2 The activities of BAL of the stomach and intestine in *B. pectinirostris* and *B. sinensis*

Notes: The values bearing different capital letters represent significant difference between the two organs of the same fish species ($P<0.05$), the values bearing different small letters represent significant difference between the same organs in the two fish species ($P<0.05$)

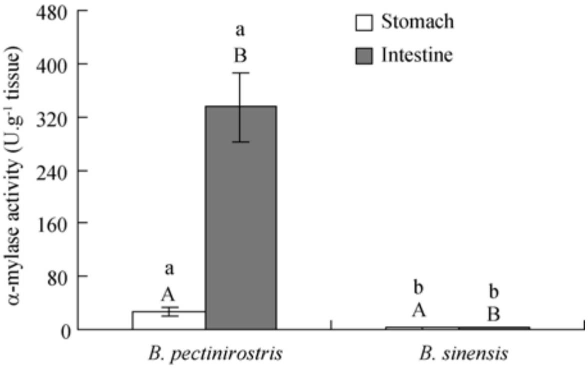

Fig.3 The activities of α-amylase of the stomach and intestine in *B. pectinirostris* and *B. sinensis*

Notes: The values bearing different capital letters represent significant difference between the two organs of the same fish species ($P<0.05$), the values bearing different small letters represent significant difference between the same organs in the two fish species ($P<0.05$)

The activities of BAL of both the stomach and intestine in *B. sinensis* were significantly ($P<0.05$) higher than those in *B. pectinirostris*, being 30.8 and 10.6 times higher, respectively (Fig.2). On the other hand, the activities of α-amylase of both the stomach and intestine in *B. pectinirostris* were significantly ($P<0.05$) higher than those in *B. sinensis*, being 14.7 and 75.8 times higher, respectively (Fig.3).

Fig.4 and Fig.5 show the ratios of P/BAL, A/P and A/BAL in the digestive tract of *B. pectinirostris* and *B. sinensis*, the values of which were 1.5, 107.3 and 158.6 in the former and 0.2, 1.6 and 0.2 in the latter, respectively.

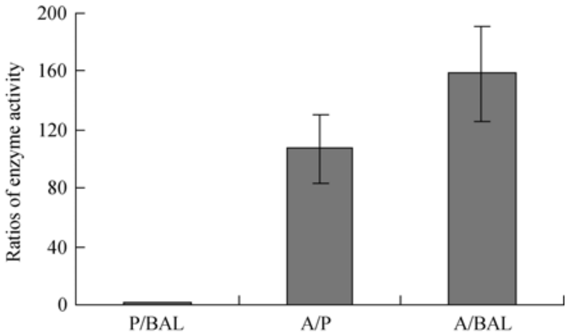

Fig.4 Ratios of protease (P), BAL and α-amylase (A) activities in the digestive tract in *B. pectinirostris*

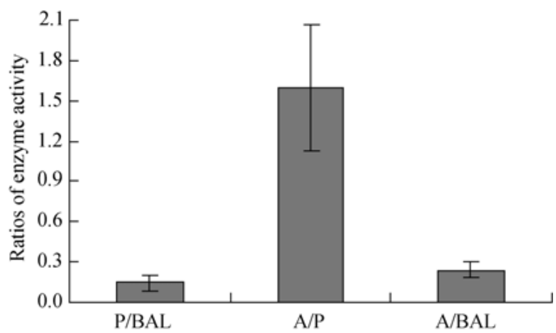

Fig.5 Ratios of protease (P), BAL and α-amylase (A) activities in the digestive tract in *B. sinensis*

4 DISCUSSION

In fishes, protein is digested initially in the stomach by pepsin and acid, and then further degraded into smaller peptides and free amino acids in the intestine by the combined actions of various alkaline proteases (e.g. trypsin, chymotrypsin and carboxypeptidases) (Hirji et al., 1982). *B. pectinirostris* is an herbivorous fish species with a poorly developed stomach (Zhu et al., 1993) and *B. sinensis* is carnivorous with a well developed stomach (Wu et al., 2007). In the present study, gastric protease (pepsin) activity in *B. sinensis* was 2.5 times higher than that in *B. pectinirostris*, while intestinal protease (alkaline protease) activity in the latter was 1.1 times higher than in the former. This suggested that pepsin is more important in protein digestion in *B. sinensis* than in *B. pectinirostris*, and alkaline protease is more necessary in protein digestion in *B. pectinirostris* than in *B. sinensis*. This is in agreement with earlier findings that in some herbivorous and omnivorous fishes that have a poorly developed stomach, such as milkfish *Chanos chanos* (Chiu et al., 1981), rabbitfish *Siganus canaliculatus* (Sabapathy et al., 1993) and *Puntius javanicus* (Chakrabarti et al., 1995), low pepsin activities are detected in their stomachs, whereas in several carnivorous fishes that have a well developed stomach, such as sea bass *Lates calcarifer* (Sabapathy et al., 1993), *Channa striatus* (Chakrabarti et al., 1995) and gilthead sea bream *Sparus aurata* (Deguara et al., 2003), high pepsin activities are found in their stomachs. Our results showed that the activities of protease, BAL and α-amylase of the intestine were 3.5, 9.4 and 12.3 times higher, respectively, than those of the stomach in *B. pectinirostris*, suggesting that food digestion is mainly carried out in the intestine of this species. This confirms the report that in *B. pectinirostris* the stomach functions firstly as a food storage organ and secondly as a digestive organ (Zhu et al., 1993). The activities of protease, BAL and α-amylase of the intestine were 1.2, 3.2 and 2.4 times higher, respectively, than those of the stomach in *B. sinensis*,. This implies that the food digestion starts in the stomach and is completed in the intestine in this species.

It has been reported that carnivorous fish species possess higher protease and lipase activities than herbivorous and omnivorous species (Kapoor et al., 1975; Sabapathy et al., 1993). However, the results from our study do not entirely agree with these reports, because the activities of intestinal protease were not significantly ($P>0.05$) different between the two fish species. Chakrabarti et al. (1995) noted that the types of diet have no bearing on the production of digestive enzymes in eleven confined-water teleost fishes. Chan et al. (2004) and German et al. (2004) investigated the digestive enzyme activities in four closely related prickleback fishes, including two herbivorous and two carnivorous species, and their results show that the activities of digestive enzymes correlate more strongly with phylogeny rather than with the fish's natural diets. Influence of the genetic strains on the activities of brush border enzymes are demonstrated in the crosses of *Oreochromis mossambicus* and *O. aureus* (Hakim et al., 2006) and in the silver perch *Bidyanus bidyanus* (Hakim et al., 2007). Furthermore, the activities of digestive enzymes are influenced by many other factors such as the ages of the fishes (Kuz'mina, 1996), temperature and season (Kuz'mina et al., 1996) and the composition of their diets (Zambonino Infante et al., 2001). Thus, the relationship between digestive enzyme activities and feeding habits in fishes is still not very clear.

Both *B. pectinirostris* and *B. sinensis* inhabit the intertidal zone, but their food niches are isolated and they belong to different families. It can be inferred that they have developed two different digestive strategies due to their phylogeny and the diet differences between them. Intestinal protease activity in *B. pectinirostris* was slightly higher than that in *B. sinensis*, and this could be a digestive strategy adopted by the herbivorous *B. pectinirostris* to maximally utilize the low protein content in its natural diet. Hidalgo et al. (1999) pointed out that no differences exist in proteolytic activities to classify fishes as either omnivorous or carnivorous. It has been suggested that to make up for the lower amount of protein available in their diet, herbivorous fishes appear to increase consumption rate and enzyme production (Hofer, 1982). Moreover, as the vegetal proteins are more difficult to digest than animal proteins (Hidalgo et al., 1999), the same amount of protein consumed requires a 10 times higher proteolytic activity in fish feeding on grass than in fish feeding on meal worms (Hofer, 1982). Thus, the high protease activity presented in the herbivorous or omnivorous fishes should be acceptable. Our results indicated that the herbivorous *B. pectinirostris* possesses a high potential for protein digestion. Hence, it is suggested that it is necessary to add animal protein to the conventional diet of this species. On the other hand, the low activity of intestinal protease in *B. sinensis* could have resulted from gut plasticity and the repressing of digestive enzyme production when its biosynthetic and other costs (of maintenance) exceed the benefits provided (Karasov, 1992; Caviedes-Vidal et al., 2000).

Dietary lipids of marine fishes are mainly composed of wax esters and triacylglycerols that contain a high proportion of polyunsaturated fatty acids, which are more resistant to hydrolysis by pancreatic lipase (Chen et al., 1990). Therefore, the most important digestive lipase in marine fish appears to be BAL not pancreatic lipase (Izquierdo et al., 2000). In this study, BAL activities of both the stomach and intestine in the carnivorous *B. sinensis* were significantly (P<0.05) higher than those in the herbivorous *B. pectinirostris*. This is consistent with previous reports that fishes feeding on a high lipid diet exhibit higher lipase activities than those consuming a low lipid diet (Izquierdo et al., 2000; Tengjaroenkul et al., 2000). On the contrary, German et al. (2004) found that the two herbivorous fishes *Cebidichthys violaceus* and *Xiphister mucosus* show higher BAL activity than two carnivorous fishes *X. atropurpureus* and *Anoplarchus purpurescens*, and they suggest that the high lipase activity in the herbivorous fishes is able to extract all the lipids available from their algal diet and whatever animal materials that the two herbivorous fishes ingest. Our results showed a much higher A/BAL ratio (158.6) in the herbivorous *B. pectinirostris* than that (0.2) in the carnivorous *B. sinensis*. This agrees with the point that dietary lipids play an important role as a source of energy for carnivorous fishes where the availability of carbohydrate for energy is very low (Watanabe, 1982). On the other hand, the P/BAL ratio (0.2) in *B. sinensis* was lower than that (1.5) in *B. pectinirostris*, and this P/BAL value is also lower than the protease/lipase ratio of the digestive tract in the carnivorous Adriatic sturgeon *Acipenser naccarii* and the rainbow trout *Oncorhynchus mykiss* (Furne et al., 2005). This suggests that lipids are more important than proteins as a source of energy to the carnivorous *B. sinensis*.

Most reports on α-amylase in fishes conclude that herbivorous or omnivorous fishes have higher α-amylase activities than carnivorous fishes (Kapoor et al., 1975; Sabapathy et al., 1993; Hidalgo et al., 1999; Fernandez et al., 2001; Chan et al., 2004). Our results agreed with this conclusion. In the present study, significantly lower α-amylase activity was detected in the digestive tract in *B. sinensis* than in *B. pectinirostris*, indicating that *B. sinensis* has a low ability to utilize carbohydrate. Munilla-Morán et al. (1996) noted that digestion of carbohydrates is at low rates in three carnivorous fish species, and α-amylase is not considered fundamental in their digestive processes. Low amylase activity is detected in the carnivorous *Acipenser transmontanus*, although the diet of the fish contains a high level of carbohydrate (Buddington et al., 1986). Some researchers suggest that a ratio of α-amylase activity to protease activity could be used as a criterion to define an animal's feeding habits, i.e. a high A/P ratio being for herbivores and omnivores and low A/P ratio for carnivores (Biesiot et al., 1990). This case has been found in a number of fish species (Hidalgo et al., 1999). The A/P ratio (107.3) of the digestive tract in the herbivorous *B. pectinirostris* was considerably higher than that (1.6) in the carnivorous *B. sinensis*, which coincides with their feeding habits.

The present study showed that protease, BAL and α-amylase exist in both *B. pectinirostris* and *B. sinensis*. As a high potential for protein digestion is presented in the herbivorous *B. pectinirostris*, it is necessary to introduce animal protein into its conventional diet. On the other hand, a low α-amylase activity and a high BAL activity were found in *B. sinensis*, and so low level carbohydrate diets would be more appropriate for this species. Future studies are needed to investigate the appropriate percentages of proteins in *B. pectinirostris* diets and the contribution of lipids to the dietary energy budget in *B. sinensis*.

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