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Species Specific Activity of Digestive Enzymes in Two Freshwater Prawns *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii* Juveniles

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Authors' contributions

This work was carried out in collaboration between all authors. Author AA designed, conducted the study and written the manuscript based on the advice of the author PSB who has supervised and critically reviewed the work. Authors KV and MK involved in animal collection, helped the first author in conducting the enzyme assays and literature survey. All authors read and approved the fair draft of the manuscript.

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ABSTRACT

Aim: The activity pattern of digestive enzymes (protease, amylase, lipase and cellulase) was studied in two species of freshwater prawns *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii* juveniles.
Study Design: Digestive capacity of individual species may vary. In order to see whether the activities of protease, amylase, lipase and cellulase in two species of freshwater prawns are more or less similar or not under a prescribed methodology.
Methodology: The hepatopancreas of prawns was blended and suspended in 10 mM phosphate buffer. After centrifugation at 15,000 rpm for 5 min, the supernatant was collected and used as crude enzyme source for the assays of protease, amylase, lipase and cellulase activities by adopting the standard methods.

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Results: The activity patterns of protease, amylase, lipase and cellulase were more or less similar in both the species studied. However, *M. malcolmsonii* showed little higher activities than that of *M. rosenbergii.*
Conclusion: There were no clear cut species specific activities of digestive enzymes observed between *M. rosenbergii* and *M. malcolmsonii*.

Keywords: Macrobrachium; hepatopancreas; protease; amylase; lipase; cellulase.

1. INTRODUCTION

Digestion is a key process in the metabolism of decapods crustaceans since it determines the availability of the nutrients needed for all their biological functions. The digestive enzyme activity in crustacean plays a central role in nutritional physiology and may directly or indirectly regulate growth and moult cycle, it's have been studied over the last century for applications in physiology, biochemistry and food science. Different crustacean species exhibit a particular array of digestive enzymes that reflects their different feeding habits and habitats [1]. Here Palaemonid prawns are a vital economic resource in the world's crustacean fishery industry as a major component of tropical and subtropical fisheries [2-4]. The giant freshwater prawn, *Macrobrachium rosenbergii*, is one of the most economically important cultural species worldwide. It is highly valued protein rich food and commands very good demand in both domestic and export. Next to *M. rosenbergii*, the second largest freshwater prawn, *Macrobrachium malcolmsonii* is widely distributed throughout Indian subcontinent including Bangladesh and Pakistan. These species have tremendous potentials for culture due to their fast-growing in nature [5-6]. The digestive enzymes of crustaceans have been studied over the last century for applications in physiology, biochemistry and food science [7].

In crustaceans, the digestive gland, hepatopancreas is analogous to the liver of vertebrates. Crustaceans are known to possess many of the enzymes required to breakdown key nutrients in their diet such as proteins, carbohydrates and lipids. The most common proteinases in the digestive system of decapods so far characterized are the serine proteinases, trypsin and chymotrypsin [8-9]. These enzymes have their highest activities under neutral or slightly alkaline conditions. Synthesis of non-serine proteinases, as prevailing in vertebrates, has proved contradictory in invertebrates such as crustaceans [10]. Carbohydrase activities in crustacean digestive tissues have also been extensively documented. High levels of amylase activity have been detected in crabs [11], prawns and crayfish [12-13]. Lipases have been found in many species [14], but few studies have reported on lipases in decapod crustaceans like *Litopenaeus vannamei* hepatopancreas using b-naphthyl nonanoate as substrate [15]. Endoglucanase activity has been detected in a number of commercially important crustacean species, including *Penaeus japonicas*, [16], *Euphausia superba* [17], *Cherax quadricarinatus* [18], *Scylla serrata* [19], *Macrobrachium rosenbergii* [20] and *L. vannamei* [15]. Dietary fibers, such as cellulose are associated with the delay in stomach emptying and contribute to the efficient utilization of dietary protein [21].

The Cellulose digesting activity has been detected in *M. rosenbergii* [22]. It has been detected in a number of other crustaceans: *Carrcinus maenas* and *Cranyon cranyon* [23], *Gammarus* [24], Slipper lobster [25]. Xue et al. [26] demonstrated the presence of endogenous cellulase activity in the red claw crayfish (*Cherax quadricanatus*), and also reviewed the enzymatic requirements for cellulose digestion and absorption. The present study was aimed to determine if there any species specific activities of digestive enzymes, such as protease, amylase, lipase and cellulase in two palaemonid prawns, *M. rosenbergii* and *M. malcolmsonii*.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Prawns

The healthy juveniles of the freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879) were procured from Aqua Hatchery, Koovathur (Latitude 12.44° N; longitude 80.10° E), Kanchipuram District, Tamil Nadu, India. The healthy juveniles of the monsoon river prawn, *Macrobrachium malcolmsonii* (H. Milne-Edwards, 1844) were collected from the Lower Anaicut of the Cauvery River, south India. They were transported in oxygenated water filled polythene bags and acclimatized for two weeks to the ambient laboratory condition with ground water. The ground water satisfied the required physico-

chemical parameters (Temperature, 26±1.0℃; pH, 7.10±0.26; total dissolved solids, 0.91±0.06 g L^{-1}; dissolved oxygen, 7.10±0.30 mg L^{-1}; BOD, 35.00±1.41 mg L^{-1}; COD, 126.00±4.10 mg L^{-1}; ammonia, 0.026±0.004 mg L^{-1}). During acclimatization the prawns were fed *ad libitum* with commercially available scampi feed. In order to maintain a good and healthy water quality, more than 75% of tank water was routinely changed every day. In order to supply sufficient oxygen to the prawns and maintain an environment devoid of accumulated metabolic wastes, adequate aeration was provided. The unfed feeds, faeces, moult and dead prawns (if any) were removed by siphoning without disturbing the prawns.

2.2 Preparation of Hepatopancreas for Digestive Enzymes Assays

The digestive gland (hepatopancreas) from live prawns were removed, homogenized, suspended in 10 mM phosphate buffer (pH 7.0, 1:4 wet wt/ vol) under ice cold condition, centrifuged at 15,000 rpm for 5 min and the supernatant was collected as a crude enzyme extract. Total protein was estimated by the method of Lowry et al. [27]. Activities of digestive enzymes such as protease, amylase, lipase and cellulase from the hepatopancreas were determined by adopting the standard methods. Assays were run in triplicate.

2.2.1 Determination of protease activity

The activity of protease was estimated by the method of Furne [28]. One unit of enzyme activity represents the amount of enzyme required to liberate 1 µg of tyrosine per minute under assay conditions. The reaction mixture consisted of 0.25 ml of casein at 1% (w/v), 0.25 ml of 0.1 M glycine– NaOH buffer (pH 10.0) and 0.1 ml enzyme source. The reaction was incubated for 1 h at 37℃, then the reaction was stopped by adding 0.6 ml 8% (w/v) trichloroacetic acid solution and kept for 1 h at 2℃, then centrifuged at 1,800 g for 10 min, and the supernatant absorbance was measured at 280 nm against blank. For the blank preparation, the enzyme source was added at the end of the incubation period, just before adding trichloroacetic acid. Tyrosine solution was used as standard.

2.2.2 Determination of amylase activity

Total amylase activity was determined using the Bernfeld [29], with 2% (w/v) starch solution as substrate. The reaction consisted of 60 µL of crude extract, 375 µL of starch solution and 375 µL of 10 mM phosphate buffer (pH 7.0). After 10 min of incubation at 37℃, 100 µL of this mixture was added to 1 mL of a 3.5-dinitrosalicylic acid solution and maintained in a boiling water bath for 10 min in order to stop the reaction. Absorbance was recorded at 570 nm. Blanks for substrate and enzyme were similarly prepared, except that 10 mM phosphate buffer replaced the substrate or enzyme extract. One unit of amylase activity was expressed as mg of maltose released at 37℃ per min per mg of protein.

2.2.3 Determination of lipase activity

The lipase activity was assayed by Furne et al. [28]. A reaction mixture containing PVA solution-emulsified substrate (1 ml), McIlvaine buffer at pH 7.0 (0.5 ml), and supernatant from the homogenates (0.5 ml) was incubated for 4 h at 37ºC. Afterwards, 3 ml of 1:1 ethanol-acetone solution was added to stop the reaction and break the emulsion. Phenolphthalein in ethanol 1% (w/v) was added to the reaction mixture and titrated with 0.01 M NaOH. One unit of lipase was defined as the amount of enzyme required to hydrolyse 1.0 micro equivalent of fatty acids from triacylglycerols in 1 h at pH 8 and 37ºC.

2.2.4 Determination of cellulase activity

Cellulase activity was determined by the method of Gonzalez-Pena et al. [30]. The gastric fluid from individual prawns was transferred to 1.5 ml centrifuge tubes, homogenized in 1 volume of 10 mM ice cold sodium citrate at pH 7.0 with a motorized homogenizer at maximum speed for 1 min and centrifuged at 4℃ at 10,000 g for 3 min. The supernatant was then transferred to a new tube and stored at -20℃ until analysis. Each prawn's hepatopancreas was homogenized in 1 volume of 10 mM sodium citrate at pH 7.0 with a motorized homogenizer at maximum speed for 1 min. After this, the homogenate was centrifuged at 10,000 g for 5 min at 4℃. The supernatant was then treated as for gastric fluid. Take 1 ml of 1% microcrystalline cellulose, 1 ml of 0.1 M phosphate buffer and 1ml of enzyme extract solution in a test tube. Incubate the test tubes for 1 h at 37℃. After 1 h, stop the reaction by the addition of 0.5 ml of dinitrosalicylic acid reagent. Note the absorbance at 540 nm. Deduce the value from the standard curve prepared using glucose. One unit of cellulase is defined as the amount of enzyme per ml which releases one µg of glucose per minute.

3. RESULTS AND DISCUSSION

Table 1 depicts the activity values of *digestive enzymes*, protease, amylase, lipase and cellulase in the hepatopancreas of *M. rosenbergii* and *M. malcolmsonii* juveniles. The activities of these enzymes were found to be significantly higher in *M. malcolmsonii* than that of *M. rosenbergii.* The difference was maximum in lipase, followed by amylase, cellulose and protease. The quantum of activity was in the order of protease > amylase > lipase > cellulase (Table 1; Fig. 1).

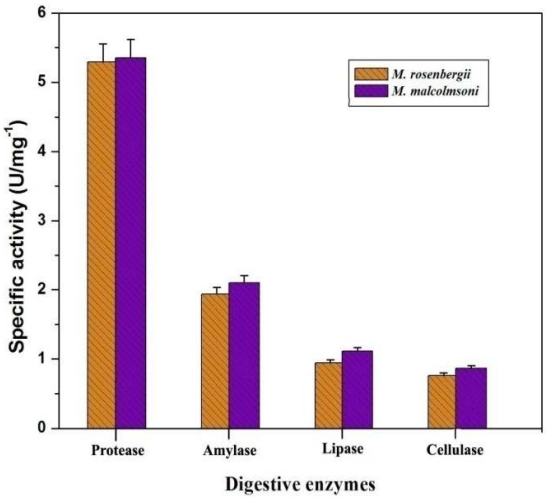

Fig. 1. Species specific activities of protease, amylase, lipase, and celulase (U mg −1) in the hepatopancreas (HP) of freshwater prawn *M. rosenbergii* and *M. malcolmsonii*

Data are reported as means±SD of three replicate analyses

Studies on digestive physiology and enzyme activity provide important basic knowledge for the assessment of nutritional status in farmed shrimp [31]. Most digestive proteases from decapods are reported to be serine proteases (more recently called serine endopeptidases), including trypsin and chymotrypsin, which seem to be the most important crustacean digestive enzymes [32-33]. The present investigation signifies that the hepatopancreas of *M. rosenbergii* and *M. malcolmsonii* contains enzyme such as protease, amylase, lipase and cellulase. It has been reported that the presence of proteinases and peptidases is an important adaptive advantage.

It has been reported that the higher levels of cellulase activity observed from crayfish and prawn [34], given that freshwater crustaceans tend to consume a greater proportion of plant material in their diets compared with marine species [20,35]. Regarding protease, a sharp decrease in activity was observed as the temperature increased, presumably as a result of thermal inactivation. At high temperature, unfolding of enzyme molecule occurred, leading to the loss in activity. As per literature, enzymatic extracts of the two species are species-specific and may be used for identification of species as done using different proteins from different species [36] or, by identification of isoenzymes for population studies as done for *P. vannamei* [8]. The high levels of proteolytic activity have been detected in the hepatopancrease of several prawns [37]. Much lower levels of total protease activity have been reported in some crustaceans, such as the red claw crayfish, that prefer diets based on detritus or plant material [12].

Amylase in the midgut gland of *F. subtilis*, *L. schmitti*, and *L. vannamei* exhibited maximal activity between 40℃ and 50℃, as previously recorded for other crustaceans and fish [19]. The ability of prawns to use a particular carbohydrate source may have both a digestive and metabolic origin. Prawns appear to be able to utilize

Table 1. Species specific activities of protease, amylase, lipase, and celulase in the hepatopancreas (HP) of freshwater prawn *M. rosenbergii* and *M. malcolmsonii*

Parameters	Species specific activities of digestive enzymes (Unit mg^{-1} protein; *(×10^2 Unit mg^{-1} protein)		*t*-value/ *P*<
	M. rosenbergii	***M. malcolmsonii***	
Protease	5.34±0.08	5.42±0.07	-22.00/ 0.002
Amylase	1.91±0.01	2.13±0.02	-38.10/ 0.001
Lipase*	0.95±0.05	1.12±0.02	-9.82/0.010
Celulase	0.72±0.03	0.83±0.02	-7.20/0.019

Each value is mean ± SD of 3 individual observations; Values are significant at P< 0.05

complex carbohydrates better than simple ones such as glucose [38-39]. Dietary monosaccharide are rapidly absorbed, but are poorly utilized. Soluble starch appears to be the most suitable carbohydrate source for the giant freshwater prawn *M. rosenbergii* [21].

Lipid reserves available in crustaceans for energy is small, with only 8% of body lipids stored as triacylglycerides in the mid gut gland [40]. This could explain the lowest values of lipase activity in different animals, including shrimp, such as *P. setiferus* [41]. In crustaceans, neutral lipids are preferentially catabolized during fasting, while polar lipids are conserved to fulfill structural roles [42]. Catabolism of triacylglycerides achieved by lipases is scarcely studied in crustaceans; however, there is a variation of digestive enzymes, such as proteinases (trypsin and chymotrypsin) during starvation conditions in shrimp [43]. Penaeid shrimps are ideal crustacean models to study sequential changes of digestive enzymes during development because all larval stages are free swimming rather than embryonated and metamorphosis to adult morphology and habits takes several weeks [44]. In crustaceans, lipases have been detected in crab *Carcinus mediterraneus* [45] and shrimps such as, *P. setiferus* [41] in *P. monodan* [46].

In the present study, the cellulase activity speckled widely among the hepatopancreas of *M. malcolmsonii* and *M. rosenbergii* species sampled in this study, but usually emerged to be associated with dietary preference. Particularly the cellulase activity was higher in *M. malcolmsonii* followed by the *M. rosenbergii.* In earlier study the higher levels of cellulase enzyme activity observed for crayfish and prawn [47], given that freshwater crustaceans tend to consume a greater proportion of plant material in their diets compared with marine species [48]. A reduction in dry matter and crude protein digestibility with increasing levels of cellulose on diets has been observed in other species of *P. monodon* [49]. However, it appears that much of this digested cellulose is not absorbed and so remains in the gastro intestinal tract and contributes to dry matter in the faces [30].

4. CONCLUSION

In conclusion, there were no clear cut species specific activities of digestive enzymes observed between *M. rosenbergii* and *M. malcolmsonii*, high amylase and protease activity and low cellulase activity in both species can be linked to their carnivorous feeding habits. Total protease, amylase, lipase and cellulase enzyme activity was similarly higher in *M. malcolmsonii* than in *M. rosenbergii*. Several factors affect the activity of enzymes may be temperature and pH. Therefore, further investigations on digestive enzymes are required to improve knowledge existing on their interaction with different factors intrinsic to prawn nutrition (such as dietary composition or growth stage); all these features can offer interesting perspectives for further studies, with exciting and promising applicative purposes for aquaculture development.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ceccaldi HJ. Anatomy and physiology of the digestive system, in *Crustacean* nutrition, D'Abramo LR, Conklin DE, DM Akiyama. Eds. The World Aquaculture Society. 1997;261-291.
2. Holthuis LB. Shrimp and prawns of the world. An annotated catalogue of species of interest to fisheries. FAO Species Catalogue, Fish Synopsis. 1980;125(1):1–27.
3. Mantelatto FLM, Barbosa LR. Population structure and relative growth of freshwater prawn *Macrobrachium brasiliense* (Decapoda, Palaemonidae) from Sao Paulo State, Brazil. Acta Limnologica Brasiliensia. 2005;17(3):245-255.
4. Hossain MY, Ohtom J, Jaman A, Saleha J, Robert LVJ. Life history traits of the Monsoon River prawn *Macrobrachium malcolmsonii* (Milne-Edwards, 1844) (Palaemonidae) in the Ganges (Padma) River, Northwestern Bangladesh. J. Freshw. Ecol. 2012;27:131-142.
5. Kanaujia DR. Indian River prawn *Macrobrachium malcolmsonii* and minor species of commercial importance. In:

Purushan KS, Kumar MB, Dinesh K, Editors. Souvenir, International Symposium of Freshwater Prawns. August 20–23, College of Fisheries, Kerala Agricultural University, Kochi. 2003;51-56.

6. New MB, Valenti WC, Tidwell JH. Freshwater prawns: Biology and farming, Eds. New MB, Valenti WC, Tidwell JH, Abramo LRD, Kutty MN. John Willey and Sons, UK. 2010;544.
7. Jiang ST, Moody MW, Chen HC. Purification and characterization of proteases from digestive tract of grass shrimp (*Penaeus monodon*). J. Food Sci. 1991;56:322-326.
8. Sainz JC, García-Carreno F, Cordova-Murueta J, Cruz-Hernández P. Whiteleg shrimp (*Litopenaeus vannamei*, Boone, 1931) isotrypsins: Their genotype and modulation. J. Exp. Mar. Biol. Ecol. 2005; 326:105-113.
9. Tsai IH, Lu PJ, Chuang JL. The midgud chymotrypsins of shrimps (*Penaeus monodon, Penaeus japonicus* and *Penaeus penicillatus*). Biochim. Biophys. Acta. 1991;1080:59-67.
10. Gildberg A. Aspartic proteinases in fishes and aquatic invert ebrates. Comp. Biochem. Physiol. 1988;91B:425-435.
11. Brethes JC, Parent B, Pellerin J. Enzymatic activity as an index of trophic resource utilisation by the snow crab *Chionoecetes opilio* (O. Fabricius). Journal of Crustacean Biology. 1994;14:220-225.
12. Figueiredo MSRB, Krickker JA, Anderson AJ. Digestive enzyme activities in the alimentary tract of redclaw crayfish, *Cherax guadricarinatus* (Decapoda; Parastacidae). Journal of Crustacean Biology. 2001;21:334-344.
13. Omondi JG, Stark JR. Studies on digestive proteases from midgut glands of a shrimp, *Penaeus indicus*, and a lobster, *Nephrops norvegicus*: Part 1 proteolytic activity. Applied Biochemistry and Biotechnology-Part A Enzyme Engineering and Biotechnology. 2001;90(2):137-153.
14. Berner LD, Hammond GE. Phylogeny of lipase specificity. Lipids. 1970;6:558-562.
15. Moss SM, Divakaran S, Kim BG. Stimulating effects of pond water on digestive enzyme activity in the pacific white shrimp *Litopenaeus vannamei* (Boone). Aquaculture Research. 2001;32: 125-131.
16. Yokoe Y, Yasumasu I. The distribution of cellulose in invertibrates. Comparative Biochemistry and Physiology. 1964;13: 323-338.
17. Chen CS, Chen HY. Purification and properties of carboxymethyl cellulase from antarctic krill *Euphausia superba*. Journal of the Chinese Biochemical Society. 1983; 12:61-70.
18. Xue XM, Anderson AJ, Richardson NA, Xue GP, Mather PB. Characterisation of cellulase activity in the digestive system of the redclaw crayfish (*Cherax quadricarinatus*). Aquaculture. 1999;180: 373-386.
19. Pavosovic M, Richardson NA, Anderson AJ, Mann D, Mather PB. Effect of temperature and diet on digestive enzyme profiles in the mudcrab, *Scylla serrata*. Aquaculture. 2004;242:641-654.
20. Gonzalez –Pena M, del C, Anderson AJ, Smith DM, Moreira GS. Effect of dietary cellulose on digestion in the prawn *Macrobrachium rosenbergii*. Aquaculture. 2002;211:291-303.
21. Gomez Díaz G, Nakagawa H. Effects of dietary carbohydrates on growth and body components of the giant freshwater prawn, *Macrobrachium rosenbergii*. Aquat. Living Resour. 1990;3:99-105.
22. D'Abramo LR, Moncreiff C, Holcomb F, Montanez J, Buddington RA. Ascorbic acid requirement of juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquaculture. 1994;128:269-275.
23. Kristensen JH. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. Mar. Biol. 1972;14: 130-142.
24. Chamier A. Cellulose digestion and metabolism in the freshwater amphipod *Gammarus pseudolimnaeus* Bousfield. Freshwater Biology. 1991;25:23-40.
25. Johnston DJ, Yellowlees D. Relationship between dietary preferences and digestive enzyme complment of the slipper lobster *Thenus orientalis* (Decapoda: Scyllaridae). J. Crustacean Biol. 1998;18:656-665.
26. Xue XM, Anderson AJ, Richardson NA, Anderson AJ, Xue GP, Mather PB. Characterization of cellulose activity in the digestive system of the red claw crayfish (*Cherax quadricanatus)*. Aquaculture. 1999;180:373-386.

27. Lowry OH, Rosebrough NJ, Farr AL, Randall RS. Protein measurement with the folin phenol reagent. J. Biol. Chem. 1951;193:265-275.
28. Furne M, Hidalgo MC, Lopez A, Garcia-Gallego M, Morales AE, Domenzain A, Domezain J, Sanz A. Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. Aquaculture. 2005;250:391-398.
29. Bernfeld P. Amylases. In: Colowick SP, Kaplan NO, (Eds.). Methods in Enzymology. Academic Press, New York. 1955;149-158.
30. Gonzalez-Pena, M.del C, Alex JA, Smith DM, Moreira GS. Effect of dietary cellulose on digestion in the prawn *Macrobrachium rosenbergii*. Aquaculture. 2002;211:291-303.
31. Jones DA, Kumlu M, Le Vay L, Fletcher DJ. The digestive physiology of herbivorous, omnivorous and carnivorous crustacean larvae: A review. Aquaculture. 1997;155:285-295.
32. Garca- Carreno FL, Hernandez – Cortez MP, Haard NF. Enzymes with peptidase and proteinases activity from the digestive systems of a freshwater and a marine decapod. Journal of Agricultural and Food Chemistry. 1994;42:1456-1461.
33. Fernandez L, Oliva M, Carrillo O, Van Wormhoudt A. Digestive enzymes of *Penaeus notialis* during reproduction and moulting cycle. Comparative Biochemistry and Physiology Part B. 1997;118:1267-1271.
34. Crawford AC, Richardson NR, Mather PB. A comparative study of cellulase and xylanase activity in freshwater crayfish and marine prawns. Aquaculture Research. 2005;36:586-592.
35. Tacon AGJ, Akiyama DM. Feed ingredients. In: Crustacean Nutrition. Advances in World Aquaculture. (ed. by D'Abramo LR, Conklin DE, Akiyama DM), World Aquaculture Society, Baton Rouge, L A, USA. 1997;6:411-472.
36. Hasnain A, Hasan Arif S, Ahmad R, Pandey RB. Identification of species marker bands in native and SDS-PAGE patterns of soluble muscle proteins of four species of genus Channa (Channidae: Channiformes) with evidence of some intraspecies eterogeneity. Asian Fisheries Sci. 2005;18:49-58.
37. Le Moullac G, Klein B, Sellos D, Van Wormhoudt A. Adaptation of trypsin, chymotrypsin and aamylase to casein level and protein source in *Panaeus vannamei* (Crustacea, Decapoda). Journal of Experimental Marine Biology and Ecology. 1996;208:107-125.
38. New MB. A review of dietary studies with shrimp and prawns. Aquaculture. 1976; 9:101-144.
39. New MB. Freshwater prawn culture: A review. Aquaculture. 1990;88:99-143.
40. Chandumpai A, Dall W, Smith DM. Lipid-class composition of organs and tissues of the tiger prawn *Penaeus esculentus* during the molting cycle and during starvation. Mar. Biol. 1991;108:235-246.
41. Lovett DL, Felder DL. Ontogenic change in digestive enzyme activity of larval and postlarval white shrimp *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). Biology Bulletin. 1990;178:144-159.
42. Stuck KC, Watts SA, Wang SY. Biochemical responses during starvation and subsequent recovery in postlarval pacific white shrimp, *Penaeus vannamei*. Mar. Biol. 1996;125:33-45.
43. Muhlia-Almazan A, Garcia Carreno FL. Influence of molting and starvation on the synthesis of proteolytic enzymes in the midgut gland of the white shrimp *Penaeus vannamei*. Comp. Biochem. Physiol. 2002; 133:383-394.
44. Ying D, Ting-Ting W, Yu-Feng W, Xue-Jie G, Cai-Feng Y. Activities of digestive enzymes during embryonic development in the crayfish *Procamabrus clarkii* (Decapoda). Aquac. Res. 2009;40/41:1-6.
45. Slim C, Ahmed F, Nabil M, Heykel T, Hafedh M, Youssef G. Crab digestive lipase acting at high temperature: Purification and biochemical characterization. Biochimie. 2007;89: 1012-1018.
46. Deering DMJ, Hewitt DR, Brock I. Triacylglycerol digestion by the leader prawn *Penaeus monodon*. J. World Aquacult. Soc. 1996;27:359-361.
47. Crawford AC, Richardson NR, Mather PB. A comparative study of cellulase and xylanase activity in freshwater crayfish and marine prawns. Aquaculture Research. 2005;36:586-592.

48. Tacon AGJ, Akiyama DM. Feed ingredients. In: Crustacean Nutrition. Advances in World Aquaculture, (ed. by D'Abramo LR, Conklin DE, Akiyama DM). World Aquaculture Society. 1997;6:411-472.

49. Koshio S, Teshima S, Kanazawa A, Watase T. The effect of dietary protein content on growth, digestion efficiency and nitrogen excretion of juvenile kuruma prawns, *Penaeus japonicus*. Aquaculture. 1993;113:101-114.

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