Asian Fisheries Science **18** (2005): 403-413
ISSN: 0116-6514
https://doi.org/10.33997/j.afs.2005.18.4.008
Asian Fisheries Society, Manila, Philippines

Trophic Specialization in Wild Population of *Metapenaeus monoceros* (Fabricius) during Postjuvenile Development : – Proteases and Non-specific Esterase

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Abstract

The present study is aimed at exploring the activity of proteases and non-specific esterases in the digestive tract of the wild populations of an estuarine penaeid species, *Metapenaeus monoceros*. A better understanding of the digestive capabilities of the species in their natural habitat is necessary prior to its adoption in estuarine culture processes. Penaeid proteases are of different types to handle various protein products in different pH conditions. Significant activity in both acidic (pH 2.9) and basic (pH 8.0) buffer medium indicates their existence in this species. The alkaline protease activity has been found to be equal in both sexes during their growing stages. A significant increase in alkaline protease activity in the maturing adults signifies greater protein requirement after transition from juvenile to adults. However, gradual decrease of acid protease activity from early juvenile to adult females signifies a shift of protein digestion. A sharp increase of esterase activity in juveniles is suggestive of their higher lipid utilization during maturation, with subsequent decrease in later stage. The results should therefore be invaluable for the appropriation of feeding schedule during culture or effort for conservation of this species.

Introduction

The success of rearing potentially cultivable species in brackish water depends mostly on the knowledge of their nutritional biology. In this regard, marine peneaid shrimps are reported to show species specific digestive enzyme pattern (Lemos et al. 2000). Since laboratory conditions are different from pond environment, application

of nutritional data obtained from clean-water reared shrimps is limited (Tacon 1996). Moss et al. (2001) reported significantly higher specific activities of most digestive enzymes in eutrophic pond-water reared shrimps in comparison to clean well water reared ones. Therefore, better understanding of the digestive capabilities of the animals in their natural habitat is necessary to minimize ecological degradation and improve the use of external feed inputs for appropriate growth during culture.

Post larval size-wise information regarding digestive enzymes are scanty in shrimps (Lee et al. 1984; Lee and Lawrence 1986; Hemambika and Raj 1993; Fernandez et al. 1997). Jha and Homechaudhudhuri (2004) analyzed carbohydrases from wild populations of a tropical estuarine peneaid species, *Metapenaeus monoceros* (Fabricius) which has proved to possess great potentials for culture (Chakrabarty 1981). The present study is aimed at exploring proteases and esterases from wild populations of the same species during post larval development. The characteristics of protease enzymes in different shrimp species was studied by different workers (Maugle et al. 1982a; Galgani et al. 1985). Enzyme activity in relation to larval development in shrimps has been analyzed by a number of workers (Chen and Lin 1992; Rodriguez et al. 1994.). Post juvenile protease studies also contributed significantly in understanding enzymatic variations (Lee and Lawrence 1982; Hemambika and Raj 1993). Noteworthy contributions are also available related to the nutritional effects on trypsin activity in different shrimps (Lemos and Rodriguez 1998; Lemos et al. 2000; Moss et al. 2001).

The digestion of lipids has received less attention in Crustacea. Earlier non-specific esterases have been investigated in *Litopenaeus setiferus*, *Penaeus stylirostris* and *Litopenaeus vannamei*, all by Lee and Lawrence (1982). Later Lovett and Felder (1990) studied the enzyme in *Litopenaeus setiferus* during its ontogenetic change. Moss et al.(2001) found a significant increase in enzyme activity of pond water reared *Litopenaens vannamei* than that of well-water reared shrimps.

Materials and Methods

Juvenile and adult shrimp *Metapenaeus monoceros* (Fabricius1798), were collected from high saline bheries at Port Canning of South 24 Parganas (Latitude 21.32 N to 22.20 N; Longitude 88.05 E to 89.0E), West Bengal, India, which is a part of the Hoogly-Matlah estuarine system. For the purpose of enzymatic studies shrimps of both sexes of different sizes were collected and divided into two major categories on the basis of body length, weight and maturity stages. Each major category was again differentiated into two stages, ie. the first major category immature juvenile into stages I and II, both showing no reproductive maturity. The other major category termed as maturing adult was also differentiated into stages III and IV which indicated

reproductive maturation. Detailed length-weight relationships of each category are given in table 1. Live specimens were collected during the monsoon months (July to September). Males and females were identified by the presence of appendix masculina in males and its absence in females.

Table 1. Categorisation of collected specimens of *Metapenaeus monoceros*

Category	Stage	Sex	TL range (in cm)	Mean ± S.D.	BW range (in g)	Mean ± S.D.
Immature Juvenile	I	M	4.1-6.5	5.7161±0.5429	0.68-2.44	1.6916±0.4008
		F	4.3-6.5	5.7406±0.5876	0.72-2.53	1.7166±0.4461
	II	M	6.6-7.5	7.0352±0.3006	2.05-3.75	2.8642±0.4125
		F	6.6-7.5	6.9890±0.2987	2.25-3.80	2.9239±0.3887
Maturing Adult	III	M	7.6-9.2	8.2584±0.5089	3.25-6.45	4.5228±0.7864
		F	7.6-9.5	8.4386±0.4862	2.67-7.78	4.9997±0.8544
	IV	M	9.6-10.1	9.8133±0.1407	6.62-8.21	7.4250±0.4131
		F	9.6-11.6	10.000±0.4802	6.86-13.01	8.4006±1.4114

Abbreviations Used : M= Male; F=Female; TL=Total length; BW=Body weight

Live specimens were obtained from the early-morning hour catch (5am-7am), as diel rhythmicity in enzyme activity has been observed in shrimps (Maugle et al.1982b) and peak enzyme activities reported during this hours. They were immediately stored in ice box. In laboratory, the digestive gland (hepatopancreas) was quickly dissected out. The glands were homogenized in ice cold double distilled water (pH 7.0). The homogenized samples were then centrifuged for 30minutes in 3000rpm × 2000g at cold centrifuge. The upper most lipid layer was removed and the supernatant was collected and stored at 4˚C. The supernatant was further diluted to the required concentration with double distilled water for enzyme assay.

The protein concentration of enzyme samples were determined following Lowry et al. (1951). Both acidic and alkaline protease activity was determined following Ichishima (1970) using bovine serum albumen (Sigma,USA) as substrate. Acetate buffer (sodium acetate - glacial acetic acid) was used and adjusted to pH 2.9 and 8.0 for acidic and alkaline protease activity. The assay mixture contained 2% BSA dissolved in buffer solution (1 ml) and 1ml enzyme solution at 37.5˚C against inactivated enzyme and buffer-substrate solution as blanks. Following 1hour incubation, 5% TCA was used for haulting the reaction. Supernetant of this solution was measured in 4% Na_2CO_3 solution and double diluted folin solution at 620nm. Enzyme activity was compared from a standard curve of serial dilution of tyrosine solutions. The activity was determined following the modified method of Seligman and Kramer (1965) using α-napthyl laurate (Sigma,USA) as substrate. The reaction mixture contained 2.0 ml Tris-HCL buffer adjusted to pH 7.2, where 0.5 ml double

distlled water and 0.1% substrate solution (0.2 ml) and 0.10 ml enzyme solution was added against inactivated enzyme and other reagents. Followed by incubation period of 1hour, 1 ml of a dye Fast blue BB salt (0.4%) was added in the reaction mixtures and after waiting for 5 minutes 5% TCA was added to stop the reaction. Then 5ml of ethyl acetate was added to the mixtures and centrifuged for 5 minutes, the coloured product formed was collected from the supernatant part and measured at 530nm. Enzymatic activity was compared from a standard curve of α-napthol solutions.

Digestive enzyme activities were represented as specific activities (activity per mg of total water soluble protein in the hepatopancreas). Acidic and Alkaline Protease activity expressed as mg of tyrosine equivalent produced per mg of total water soluble protein in the hepatopancreas extract per hour of incubation. Non-specific esterase activity expressed in terms of mg of α-napthol produced per mg of total water soluble protein in the hepatopancreas extract per hour of incubation.

One way analysis of variance (ANOVA) followed by sensitive tukey test was used to distiguish between means of significant difference in enzyme activities. Treatments were taken to be significantly different if the P valules were less than 0.05.

Results

Table 2. Enzyme activities in different categories of *Metapenaeus monoceros*

Category	Stage	Sex	Acid Protease (Mean± S.D.)	Alkaline Protease (Mean± S.D.)	Esterase (Mean± S.D.)
Immature Juvenile	I	M	0.0174 ± 0.0019	0.0265 ± 0.0010	4.7840 ± 0.2143
		F	0.0299 ± 0.0026 ♦	0.0272 ± 0.0015	7.0957 ± 0.1858 ♦
	II	M	0.0155 ± 0.0014	0.0274 ± 0.0016	13.5792 ± 1.0147 *
		F	0.0208 ± 0.0008 # ♦	0.0264 ± 0.0015	14.00452 ± 0.5004 #
Maturing Adult	III	M	0.0163 ± 0.0003	0.0297 ± 0.0011	10.31027 ± 0.3880 *
		F	0.0208± 0.0009 ♦	0.0278 ± 0.0018	12.27648 ± 0.4185 # ♦
	IV	M	0.0181 ± 0.0009	0.0383 ± 0.0021 * ♦	8.48753 ± 0.5104 *
		F	0.0139 ± 0.0008 # ♦	0.0245 ± 0.0002 #	7.61271 ± 0.1935 #

Abbreviations Used : M= Male; F=Female

* denotes significant difference in enzyme activity (P < 0.05) between males of different stages.
\# denotes significant difference in enzyme activity (P < 0.05) between females of different stages.
♦ denotes significant difference in enzyme activity (P < 0.05) between males and females of any stage

Acid protease activity expressed as mg (mean ± S.D.) of tyrosine equivalent produced per mg of total water soluble protein in the extract per hour of incubation.
Alkaline protease activity expressed as mg (mean ± S.D.) of tyrosine equivalent produced per mg of total water soluble protein in the extract per hour of incubation.
Esterase activity expressed as mg (mean ± S.D.) α-napthol produced per mg of total water soluble protein in the extract per hour of incubation.

Acid Protease (Figure 1)

Variations in relation to growth stages

No significant difference in enzyme activity was found between males of different stages ($P>0.05$). Females showed significant decrease in enzyme activity from stage I to II and also from III to IV ($P<0.05$). Enzyme activity of stages II and III of growth was almost equal.

Sex-wise variations

Mean difference in enzyme activity was found to be significant in between all stages of males and females ($P<0.05$). A higher titer of enzyme activity was found in females than males in stages I to III of growth, but mean enzyme activity of males in the stage IV was higher in comparison to females.

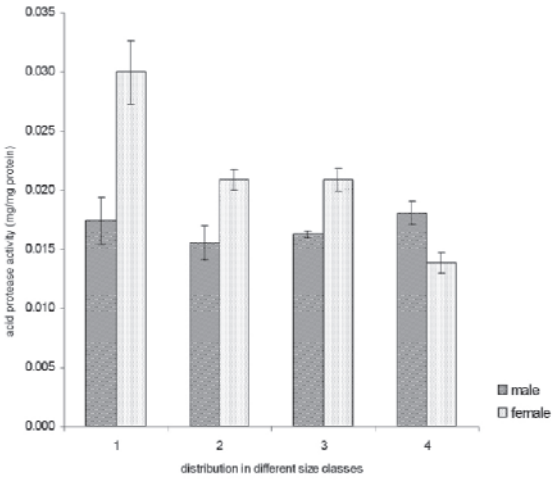

Fig. 1. Acid protease activity expressed as mg (mean ± S.D.) of tyrosine equivalent produced per mg of total water soluble protein in the extract per hour of incubation.

* denotes significant difference in enzyme activity ($P < 0.05$) between males and females of any stage

Alkaline Protease (Figure 2)

Variations in relation to growth stages

The results indicate that the increase in enzyme activity in males from stages III to IV was only statistically significant($P<0.05$).However,adecreasing trend in such enzyme activity in females from stages III to IV was noticeable and statistically significant ($P<0.05$).

Sex-wise variations

The males of only the growing stage IV showed significantly increased value ($P<0.05$) when compared with females of the same stage.

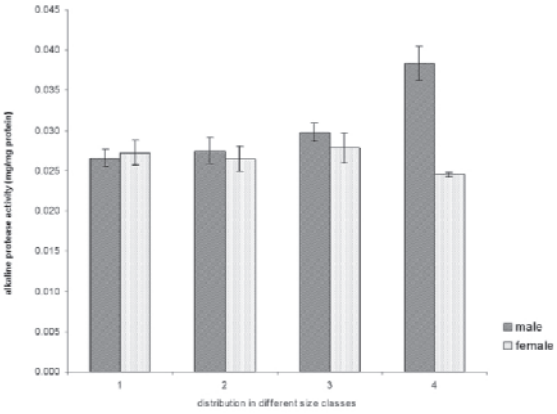

Fig. 2. Alkaline protease activity expressed as mg (mean ± S.D.) of tyrosine equivalent produced per mg of total water soluble protein in the extract per hour of incubation.

* denotes significant difference in enzyme activity ($P < 0.05$) between males and females of any stage

Esterase (Figure 3)

Variations in relation to growth stages

Mean differences were significant in all stages of males ($P<0.05$). Males showed an increase in activity from stages I to II, while decrease in activity was found between stages II and III as well as stages III and IV of growth. Females also showed significant mean differences in all stages ($P<0.05$). From stages I to II of growth there was an increase in enzyme activity. When compared between stages II and III and stages III and IV of growth, the activity decreased. The general trend of enzyme activity showing an increase from stages I to II followed by a decrease from stage II to III and III to IV in both sexes was similar.

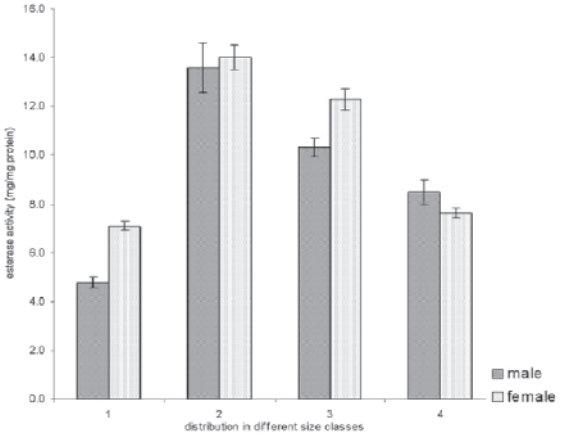

Fig. 3. Non-Specific Esterase activity expressed as mg (mean ± S.D.) α-napthol produced per mg of total water soluble protein in the extract per hour of incubation.

* denotes significant difference in enzyme activity ($P < 0.05$) between males and females of any stage

Sex-wise variations

Significant mean difference was only found between the males and females of growth stages I and III ($P<0.05$). In these stages females had higher activity than males.

Discussion

Proteins are indispensable nutrients for the structure and function of all living organisms including shrimps. Since proteins are continually being used by the animal for growth and repair of tissues, a continuous supply of proteins or its component amino acids is needed. Due to its importance protein has been the most studied nutrient. Dietary studies have recommended a protein level of 30%-57% for various species of shrimps (Shiau et al. 1991). D'Abramo and Sheen (1994) emphasized on a careful examination of the reported estimate of protein requirement from studies in different species as because it was dependent on quality (essential amino acid profile) of dietary protein, age or physiological state and even environment of the shrimps.

Several proteases have been examined. Trypsin like protease is one of them. Galgani et al. (1985) purified and analysed the properties of shrimp trypsin from *Marsupenaeus japonicus*. The optimum pH of its activity was found between 8.0-

8.3. Honjo et al. (1990) characterised trypsin from *Fenneropenaeus indicus*, the optimum pH ranging from 6.5-11.0. Lu et al. (1990) purified two trypsins from *P. monodon* with very low optimum pH around 2.4. Besides, chymotrypsin, carboxy peptidase A and B and leucine-aminopeptidase activity have been reported from shrimps (Lovett and Felder 1990). However, pepsin is not identified in shrimps. As Crustacean trypsin is able to act on native protein (Lu et al. 1990), the shrimps do not seem to 'require' pepsin (Dall 1992). Lan and Pan (1993) observed protease activity from hepatopancreas of *Penaeus monodon* to occur in two pH optima 7.5 and 4.0. The pH optima of protease activity from digestive system of *Farfantepenaeus californiensis* was found to be between 6.0 and 10.0, maximum activity occurring at around 8.0 (Vega-Villasente et al. 1995). All these signify that penaeid proteases are of different types or capacity to handle protein products in different pH conditions. In the present study, positive result of protease activity in both acidic (pH 2.9) and basic (pH 8.0) buffer medium indicates their existence and activity in *Metapenaeus monoceros*. Presence of polychaetes, other Crustaceans, Insect larvae part, Molluscs, fish scales as identified from the foregut of experimental shrimps gave evidence of protein components in their daily feeding routine.

Most protease enzymes in penaeids are found to act in optimum alkaline pH. In the present study alkaline protease (pH 8.0) shows almost equal activity in males and females (except stage IV) of tested *Metapenaeus monoceros*. The activity is also almost same in both males and females during their growing stages, a pointer towards their almost equal protein requirement. Protease activity was found to be influenced by the type of protein in the gut of *Marsupenaeus japonicus* (Maugle et al. 1982b). Lee et al. (1984) found that in *Litopenaeus vannamei*, the level and source of protein and the size of the animal, had an impact on enzyme activity. Dietary studies in *Litopenaeus schmitti* showed highest protease (trypsin) activity, when the dietary protein was increased up to 25%-35% (Galindo et al. 1992). Thus, significant increase in enzyme activity in already maturing adult males from stage III to IV may be possible due to higher protein demand in order to provide more energy to prepare themselves for reproduction. A significantly higher enzyme activity in females of stage III in comparison to stage IV females has been seen. It signifies greater protein requirement immediately after transformation from juveniles to adults.

Acid protease (pH 2.9) in females of different stages showed marked variations, suggesting its importance in protein food management. Highest value was obtained during early juvenile stages (stage I). Gradual decreasing trend in activity probably signifies a shift of protein digestion from acid to alkaline medium. The activity of acid protease particularly high in female juveniles probably indicates the strategy of the young developing juveniles to obtain and utilize proteinaceous food at the maximum. Therefore, the capacity of protein digestion at different pH is an added advantage. The enzyme activity in males however is similar in all stages. Both acid

and alkaline protease activities are greater in males of stage IV possibly in response to their higher protein demand. Significant differences in enzyme activities between males and females of all the stages gives an idea about the variations in handling the protein products in natural food in a variety of different ways.

Lipids are required in the diets of shrimps for their energy value, and as sources of essential fatty acids, fat-soluble vitamins, sterols and phospholipids. Even digestive gland lipid serves as an energy store during starvation and more normally in preparation for moulting (Barclay et al. 1983). Fat splitting enzymes are mostly found to be esterases rather than lipases. In the present study detection of non-specific esterase activity from all developmental stages of shrimps of either sex supports the view. A dietary lipid level of 6%-10% is found to be optimum for different species of shrimps (Lim 1998). High levels (>10%) are usually associated with significant retardation of weight gain (Shiau 1998). Biochemical analysis of prey animals of *Penaeus esculentus* from nature is a clear evidence of this fact (Dall et al. 1991). However, the optimal level of dietary lipid is based upon the amount of dietary energy. Chuntapa et al. (1999) showed that the diet containing lipid : carbohydrate ratio (% wt / wt) of ~1:4.6 gave the highest growth rate in comparison to other diets in *Penaeus monodon*. Even growth rates of shrimps (*Penaeus monodon*) are found to be significantly affected by lipid source.

In the present observation a sharp increase in esterase activity (two fold or more) from stage I to II in both sexes is suggestive of their higher lipid utilization as the break down products of lipid are considered as potential energy source and stored for future life. Nutrient reserves, derived primarily from the hepatopancreas are required from the on set of maturation to support gonadogenesis (the synthesis of ovaries and testes) and gametogenesis (the development of oocytes or sperms). Once gonadal tissue and oocytes have formed, vitellogenesis (the production and accumulation of egg yolk) is the dominant metabolic activity. The yolk is composed of different nutrients including lipids (phospholipids, triacylglycerides and cholesterol).

Hepatopancreas is the major organ of absorption, processing and storage of dietary lipids. Higher activity of esterase at the transition of immature juvenile to maturing adult (stage II) form might be necessary for utilizing and depositing fatty substances in increased amount. During maturation hepatopancreatic neutral lipid and phospholipids are processed and transported to the ovaries (Khayat et al. 1994). As the gonads are in their developing form, the stored lipid from hepatopancreas being used, further storage in the hepatopancreas seems unnecessary. Therefore, gradual decrease in enzyme activity (from stages II to III) is observed. Significant decrease in enzyme activity from stages III to IV can also be explained in this way. As lipid storage in hepatopancreas is more essential in females for its accumulation in egg yolk and formation of lipid globules in eggs, the demand of lipid material is higher in females than in males. Significantly higher activity of esterase in stage I and

III females in comparison to males is confirmatory of the view.

Acknowledgment

The authors are indebted to Head, Department of Zoology, University of Calcutta and Faculty, Department of Zoology, Presidency College for the encouragement and administrative support. The financial support of Labonya Prova Bose Trust, Calcutta is gratefully acknowledged.

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