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Ontogeny and induction of digestive enzymes in *Scylla serrata* larvae fed live or artificial feeds or their combination

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Abstract. Generally, the patterns of digestive enzyme activities during ontogeny and the digestive capacities of most crab larvae are less understood. In the present study, ontogenetic patterns of enzyme activities of amylase, trypsin and leucine aminopeptidase (LAP) were investigated for the first time in developing *Scylla serrata* larvae. Moreover, the influence of diet types (live feed, artificial feed or combination of both feeds) on larval digestive enzyme activities was also examined. Results showed that amylase activity were low at early larval stages zoea 1 (Z1) to zoea 3 (Z3), exhibited enzyme activity peaks at stages zoea 4 (Z4) and zoea 5 (Z5) and declined gradually at megalopa to crab instar 1(C1) stage. Trypsin showed an elevated activity at Z2, remained low at later stages but enzyme activity rose again from megalopa until reaching the highest activity at the C1 stage. In contrast, LAP enzyme activities increased gradually from Z3, reaching a maximum at the C1 stage. Factorial ANOVA showed that enzyme activities were influenced by the developmental stage, by the type of diet, and by the interaction of these factors. Also, the use of Discriminant Analysis (DA) allowed us to show that there was a significant effect of the diet as well as development stage on digestive enzyme activities. Furthermore, DA allowed us to rank the parameters that weighted mostly in the model: trypsin>LAP>amylase in discriminating between development stages while only LAP was the predictor variable for discriminating between diets.
Key Words: *Scylla serrata*, digestive enzymes, nutritional physiology, ontogeny, live feed, artificial feed.

Introduction. The aquaculture of mud crab, *Scylla serrata* (Keenan et al 1998) in the Philippines has expanded in recent years due to increasing demands and rising market value of this commodity (Millamena & Quinitio 2000). As a consequence, rising demands of mud crab juveniles for aquaculture has led to shortage and unreliability of supply. Currently, most juveniles used in grow-out are collected from the wild and their availability has become a serious limiting factor in mud crab aquaculture.

Development of hatchery production is considered vital for the sustainable growth of mud crab aquaculture, but attempts to produce mud crab juveniles in hatchery are still unsuccessful due to problems of low and inconsistent survivals (Keenan 1999). Mortalities at all stages of development with marked peaks at early zoaeal stages (Z1-Z3) and from zoea 5 (Z5) to megalopa have been major challenges in mud crab larval rearing (Lie et al 1989). The low survival in hatchery production has been attributed to inappropriate hatchery technology due to a lack of understanding of larval developmental and digestive physiology. Pullin & Eknath (1991) have emphasized that larval digestive physiology, and probably also of larval crustacean, has been left under-researched for almost all species.

Development of a successful hatchery production technology for mud crab requires a comprehensive understanding of the digestive processes of the larvae. Although digestive capacities and enzymes involved in nutrient digestion of juvenile mud crab have been characterized (Pavasovic et al 2004), activities of these enzymes during larval development have not been fully investigated. Knowledge of the digestive processes during ontogenic development is essential in understanding the nutritional

physiology and in formulating appropriate feeds and feeding schemes necessary to satisfy the nutritional needs of the mud crab larvae.

Thus, the present study was conducted to quantify and evaluate digestive enzyme activities (Amylase, Trypsin, and LAP) during larval development. The influence of diet type (live feed, artificial feed, combination of these feeds) on the patterns of digestive enzyme activities of developing larvae was also investigated in this study.

Material and Method

Source of Larvae. Mature *Scylla serrata* broodstocks (identified according to the description of Keenan et al 1998) were obtained from the mud crab traders of Roxas City, Capiz, Philippines. Upon arrival to the laboratory, broodstocks were cleaned by brushing and disinfected by bathing in 100 mg L^{-1} formalin for 30 min. The crabs were reared individually in broodstock tanks with running water, aeration and sandy substrate. Daily, the crabs were fed *ad libitum* with squid (*Loligo* spp.) and fresh mussel meat (*Perna viridis* Linnaeus, 1758) and the animals were checked for the presence of spawned eggs. Berried females were transferred and maintained in incubation tanks (250-L capacity) with aeration. Complete tank water exchange and cleaning of tanks with faecal materials were conducted daily. Feed was not supplied to the berried females during egg incubation. During the period of egg incubation, a small sample of eggs was examined for development and health assessment. Following egg hatching, the strongly phototactic first stage zoea (Z1) larvae were collected using plankton net and transferred to experimental rearing tanks.

Digestive Enzyme Activities Associated with Larval Development. Newly hatched larvae were collected and distributed in three 500 L fiberglass rearing tanks at 50 individuals L^{-1}. Tanks were provided with 35 ‰ filtered seawater (5 µm) and mild aeration to maintain oxygen and to promote homogenous dispersion of live feeds. Throughout the rearing experiment, larvae were fed a combination of rotifers (*Brachionus rotundiformes*) at a density of 100 individual mL^{-1} and *Artemia salina* at a density of 1-3 individual mL^{-1}. Larvae were randomly collected from each of the replicate tanks for the assessment of enzyme activities at every developmental stage. All sampling were conducted at 8:00 a.m.

Influence of Diet types on Digestive Enzyme Activity. In this study, the experiment was done in two parts. The first part involved feeding *S. serrata* larvae from Z1 to Z5 stages, and the second part involved feeding megalopa and juveniles; both trials aimed to assess the influence of diet types on activities of digestive enzymes. *Artemia* nauplii (A) and rotifers (R) were used as live food and commercially available microbound shrimp diet as artificial feed (AF). In the first trial, three feeding regimes were tested: Diet 1 (Live food) was composed of and *Artemia salina* at a density of 100 individual mL^{-1} of R and 1-3 individual mL^{-1} A; Diet 2 (Artificial feed) was composed of shrimp artificial feed (Argent, USA); Diet 3 (Combination) was a combination of live and artificial diets. Three replicates of 50 individuals L^{-1} were stocked in 100L fiberglass tanks. Sampling for digestive enzyme assays was done at each larval stage. Since previous works have demonstrated that feeding mud crab larvae purely with artificial feed results in total mortality at some point (Quinitio et al 1999), we reared the larvae with live food until each of the desired larval stage were produced separately then fed the dietary treatment of either pure artificial feed or combination of live and artificial feeds thereafter. Only those that were in the control group were fed continuously with live food. Feeding experiment was terminated after 48 h. For the second part, megalopa and juvenile were fed three diets: Diet 1, composed of live adult *Artemia*, given at a rate of 100% test organism biomass, Diet 2 consisted of shrimp starter diet given at a rate of 100 % biomass and diet 3 consisted of mixture of diet 1 and diet 2 in a 1:1 ratio given at a rate of 100 % biomass. Feeding experiments were done for 72 h with feeding rate in slight

excess twice daily: once in the morning and once in the afternoon. Following the feeding trial animals were randomly collected and digestive enzyme activities were measured.

Preparation of the Tissue homogenate for Enzyme Assay. Collected larval samples were homogenized in 20 volumes of cold extraction solution (50 mM citrate phosphate buffer pH 7.0) at 1:20 ratio (wet tissue weight to volume) in an Ultra Turrax homogenizer. The homogenates were centrifuged at 4000 rpm for 15 min at 4ºC and the supernatant was used as enzyme preparation. Total soluble protein was measured following the procedure of Lowry et al (1951) with bovine serum albumin as a standard. All enzyme assays were conducted within 4 h of homogenization and all samples for a single enzymatic assay were run in the same day. Blank controls, in which the reaction did not take place, were included in the routine assay.

Enzyme Assays. All assays were carried out at 25ºC and values are taken as means of triplicate estimations ± the standard error of the mean (SEM). Each assay was conducted along with appropriate controls including non-enzymatic hydrolysis.

α-Amylase activity was assayed as described by Bernfield (1955) in which the increase in reducing power of a buffered starch solution was measured with 3,5 dinitrosalicylic acid (DNS) at 546 nm. The assay mixture consisted of 0.1 mL soluble starch solution, 0.5 mL of the enzyme preparation and 0.5 mL salt solution (i. e. homogenizing solution). The reaction was stopped by adding DNS solution and the mixture was heated for 5 min in boiling water, cooled in running tap water and the absorbance read at 546 nm. Amylase activity was expressed in terms of µg maltose liberated from starch.

Trypsin-like activity was determined according to Geiger & Fritz (1988) using the specific substrate BAPNA (Benzoyl-arginine-*p*-nitroanilide). The assay mixture consisted of 1.25 mL of the substrate solution, 0.1 mL of purified trypsin solution and buffer in a final volume of 2.25 mL. The reaction was started by adding BAPNA solution for 5 min and was stopped by adding 0.25 mL of 30% acetic acid. The absorbance of the supernatant was read at 405 nm and the enzyme activity was expressed as micromoles of product formed min^{-1} mL^{-2} of enzyme preparation. The activity of purified enzyme preparation was subtracted from the total trypsin-like activity.

Leucine Aminopeptidase (LAP) is a proteolytic enzyme which hydrolyzes the peptide bond adjacent to a free amino acid group. It is called aminopeptidase because it rapidly catalyzes the hydrolysis of leucine-containing peptides. However, it also catalyzes the hydrolytic release of other amino acids located at the *N*-terminal end of various polypeptides and proteins. The assay system for LAP consisted of 1.0 mL of 60 mM Tris-HCl buffer pH 8.5, 1.4 mL of 0.001 M of L-leucine-*p*-nitroanilide and 0.3 mL of enzyme preparation. The reaction was stopped by the addition of 30% acetic acid. Enzyme activity was expressed as micromole of *p*-nitroaniline formed at 405 nm.

Statistical Analysis. Statistical analysis of the data was performed using a graph-statistical software package (Statistica, Stat Soft., Inc., USA and Sigma plot 11, Systat, USA)). Homogeneity of variances and normality were tested (using Levene's test and Shapiro–Wilk's test, respectively) before analyzing the data with an ANOVA. Post hoc analysis among groups after finding significant differences were performed by Tukey tests, depending on the data, with the level of significance preset at $P<0.05$. Data were reported as mean ± standard error (or residual error in the case of multivariate or two-way ANOVA).

To explore the question of whether mud crab larvae and juveniles had distinctive digestive enzyme status profiles when fed live food, artificial feed, or combined live and artificial feed, Discriminant Function Analysis (DFA) was used. The canonical discriminant analysis (CDA) was used for classifying a set of observations into predefined classes (e.g. mud crab stages of development: Z1, Z2, Z3, Z4, Z5, M, CI). The purpose was to determine the class of an observation based on a set of variables known as predictors or input variables (e.g. enzyme activity). Prior to running the CDA, data were checked for multivariate normality, homogeneity of variances and covariances, and correlations

between means and variances. Computationally, the test performs a canonical correlation analysis that determines the successive functions and canonical roots (the term root refers to the eigenvalues that are associated with the respective canonical function). Eigenvalues can be interpreted as the proportion of variance accounted for by the correlation between the respective canonical variates. The maximum number of functions will be equal to the number of groups minus one, or the number of variables in the analysis, whichever is smaller. The first function maximizes the differences between the values of the dependent variable, while the second function is orthogonal to it (uncorrelated with it) and maximizes the differences between values of the dependent variable, controlling for the first factor (Stat Soft 2007). Differences among developmental stages were measured directly by means of the Wilk's λ-criterion. The χ2-test for Wilk's λ was used to test the significance of the overall difference between the centroids in feeding regime. The relative importance of the original variables in separating the developmental stages of the groups was gauged by standardized values.

Results. Amylase activity were relatively low at the early larval stages (Z1-Z3) but increased significantly in stages (Z4 - Z5) until the megalopa stage and declined to midrange level upon reaching the C1 stage (Figure 1). Trypsin-like activity exhibited low activity levels at Z1 stage, showed a slight increase in activity at Z2 then enzyme activity decreased to a low level from Z3 to Z5. Upon reaching the megalopa stage trypsin-like activity increased sharply reaching the highest activity at the C1 stage (Figure 2). The exopeptidase LAP, on the other hand, showed slightly higher activities at initial larval stage (Z1) and the enzyme activity incrementally increased until Z4 stage. An abrupt and significant increase in LAP activity (highest enzyme activity) was observed at Z5 stage but LAP activity gradually decreased from the megalopa to the C1 stage (Figure 3).

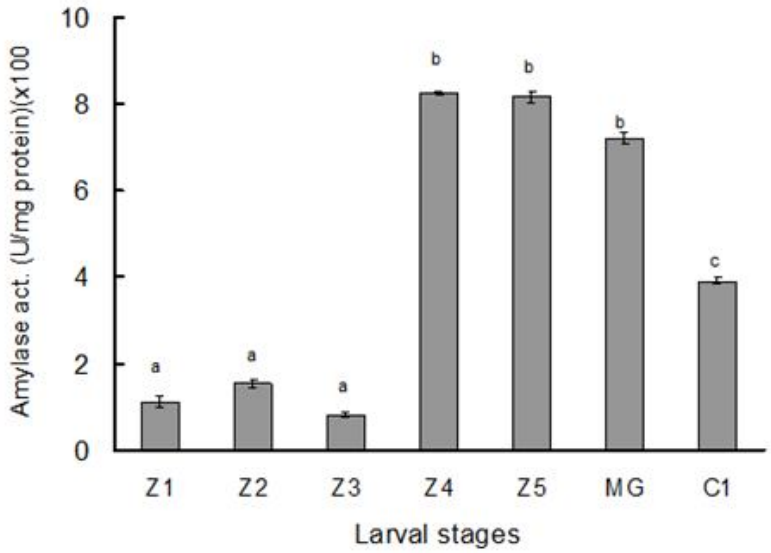

Figure 1. Changes in specific amylase activity in *Scylla serrata* during larval development from Zoea I to the first crab stage (error bars indicate standard error of means). Z, zoeal stages I-V; MG, megalopa stage; C1, first crab stage.

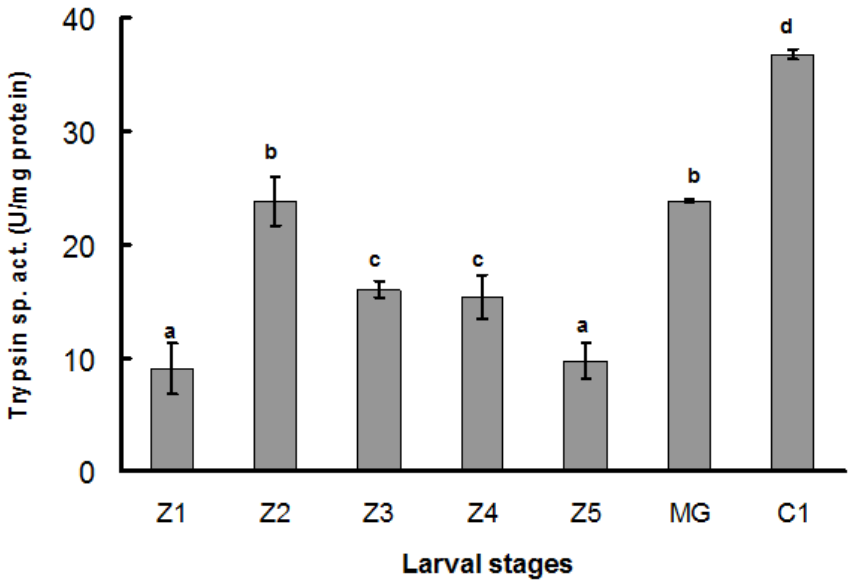

Figure 2. Changes in specific trypsin-like activity in *Scylla serrata* during larval development from Zoea I to the first crab stage (error bars indicate standard error of means). Z, zoeal stages I-V; MG, megalopa stage; C1, first crab stage.

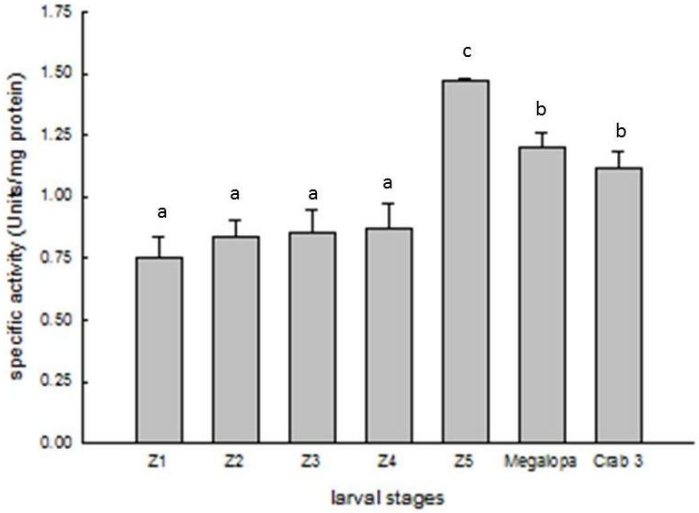

Figure 3. Changes in specific leucine aminopeptidase activity in *Scylla serrata* during larval development from Zoea I to the first crab stage (error bars indicate standard error of means). Z, zoeal stages I-V; MG, megalopa stage; C1, first crab stage.

The influence of diet type and larval developmental stages on digestive enzyme activities is presented in Table 1. Diet type, developmental stage and their interaction were observed to affect the levels of amylase activity. Regardless of diet type, amylase activities tended to increase as the developmental stage progress from Z1 to Z4 or Z5. At the megalopa stage amylase activity declined and increased again at C1 stage. On the other hand, larvae fed with live feeds exhibited a significantly lower amylase activity at stages Z1 to Z3 than larvae fed with artificial feed and combination of these feeds. No apparent effects of diet type could be observed in the pattern of amylase activity at later developmental stages.

Table 1

Two-way analysis of variance (ANOVA) of digestive enzyme activities (mean ± standard error) at various development stages of *S.serrata* larvae and juveniles fed live food, artificial feed or combination of the two foods. Means with different letters are significantly different (P<0.05). Asterisk at P values denotes significant differences

Factors		Digestive enzymes		
Diet	Stages	Amylase	Trypsin	LAP
Live food	Z1	0.222±0.008^{c}	0.0092±0.0025^{c}	0.0069±0.0014^{d}
	Z2	0.278±0.045^{c}	0.0083±0.0015^{c}	0.0047±0.0007^{d}
	Z3	0.310±0.014^{c}	0.0954±0.0007^{a}	0.0043±0.0005^{d}
	Z4	3.155±0.303^{a}	0.0494±0.0075^{b}	0.0230±0.0029^{c}
	Z5	1.728±0.376^{b}	0.0471±0.0053^{b}	0.0242±0.0018^{bc}
	M	1.869±0.145^{b}	0.0032±0.0017^{c}	0.0275±0.0008^{b}
	CI	3.373±0.481^{a}	0.1021±0.0015^{a}	0.0387±0.0004^{a}
Artificial feed	Z1	0.249±0.009^{d}	0.0051±0.002^{d}	0.0021±0.0001^{d}
	Z2	0.340±0.123^{d}	0.0053±0.0002^{d}	0.0050±0.0008^{cd}
	Z3	0.330±0.055^{d}	0.0958±0.0004^{b}	0.0031±0.0004^{d}
	Z4	1.840±0.316^{b}	0.0200±0.0039^{c}	0.0119±0.0007^{abc}
	Z5	3.895±0.171^{a}	0.0272±0.0032^{c}	0.0101±0.0013^{abc}
	M	1.237±0.335^{c}	0.0076±0.0045^{d}	0.01917±0.0089^{ab}
	CI	2.297±0.068^{b}	0.1096±0.0003^{a}	0.0211±0.0091^{a}
Combination	Z1	0.247±0.029^{d}	0.0037±0.0011^{c}	0.0041±0.0017^{e}
	Z2	1.216±0.143b^{c}	0.0038±0.0002^{c}	0.0044±0.0008^{de}
	Z3	0.344±0.010^{d}	0.0899±0.0022^{b}	0.0023±0.0002^{e}
	Z4	1.173±0.029^{bc}	0.0082±0.0030^{c}	0.0084±0.0023^{cd}
	Z5	2.905±0.159^{a}	0.0097±0.0062^{c}	0.0089±0.0019^{c}
	M	0.881±0.158^{cd}	0.0031±0.0008^{c}	0.0284±0.0003^{b}
	CI	1.870±0.860^{b}	0.1097±0.0021^{a}	0.0367±0.0003^{a}
ANOVA	Overall	*P*-value		
Diet (A)	0.0001*	0.044*	0.0001*	0.0001*
Dev. Stage (B)	0.0001*	0.0001*	0.0001*	0.0001*
Interaction (A x B)	0.0001*	0.0001*	0.0001*	0.0151*

Diet type, larval developmental stage and their interaction significantly affect the larval trypsin-like enzyme activity (Table 1). At every developmental stage trypsin-like enzyme exhibited a similar pattern of activities regardless of the diet type. However, influence of diet type on larval trypsin-like enzyme activity was apparent at stages Z3 to Z5, wherein larvae receiving live feeds had significantly higher enzyme activities than the larvae fed the two other diet types. Similarly, larval LAP enzyme activity was also influenced by the developmental stage, the type of diet and the interaction of these factors. The pattern of larval LAP enzyme activities tended to be similar in all developmental stages at any of the dietary treatments. However, significantly higher LAP enzyme activities could be observed at larval stages Z4 and Z5 of larvae fed with live diets than those fed the other diets at the same larval stages.

To further clarify the interrelationship of diet types, larval developmental stages and digestive enzyme activities, data were subjected to a Discriminant Function Analysis (DFA) to find the linear combination of the variables (enzyme activities) that best described the developmental stage and the diets of mud crab larvae. Discrimination by developmental stage produced two discriminant functions (Table 2) while only one discriminant function was generated when discrimination was by diet types. Standardized discriminant function coefficients (Table 3) indicated that the stage of development in mud crab larvae was strongly discriminated by the first function contributing 79.6% of the variance, and this function weighted most heavily by the trypsin-like activity, followed by LAP activity and amylase activities in this order. The

scatterplot of canonical scores (Fig. 4) when the data was further subjected to canonical determinant analysis (CDA) confirmed that function 1, largely by the trypsin-like activity, could discriminate clearly stages Z1 and Z3 which were plotted much further to the right and the megalopa stage to the left. The second function provided some discrimination between the other five stages but was not nearly as clear as that provided by the first canonical function. On the other hand, diet did not discriminate developmental stages significantly and the scatterplot of canonical scores (Fig. 5) showed almost random distribution of canonical scores.

Table 2

Summary of the results of the canonical discriminant analysis (only significant discriminant function are shown) of the developmental stages in the mud crab *Scylla serrata* using specific enzyme activities (amylase, trypsin and leucine aminopeptidase (LAP) as predictor variables

	Function	Eigenvalue	Canonical correlation	Wilks Lambda	Chi-square	d.f.	*P*
Dev. stage	1	4.04	0.974	0.120	67.91	10	0.0000*
	2	0.70	0.895	0.589	14.41	4	0.0000*
Diet	1	0.02	0.140	0.	1.22	2	0.5437^{NS}

* = significant; NS = not significant

Table 3

Standardized discriminant function coefficients for each of the significant discriminant functions by development stage and by diet

Development stage			Diet	
	1	2		1
TRYPSIN	1.119	-0.056	LAP	-1.164
LAP	-0.435	0.752		
AMYLASE	-0.128	0.695		
Eigenvalue	18.494	4.039		0.093
Cum.prop.	0.796	0.970		0.818

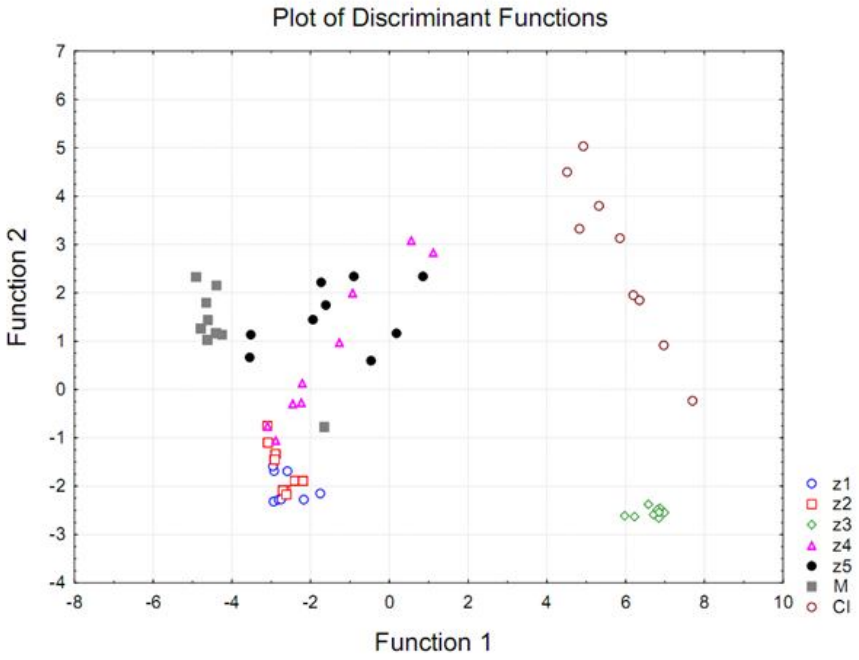

Figure 4. Plots of the first two axes for the canonical discriminant analysis of the developmental stages in the mud crab *Scylla serrata* using specific enzyme activities (amylase, trypsin and leucine aminopeptidase (LAP) as predictor variables.

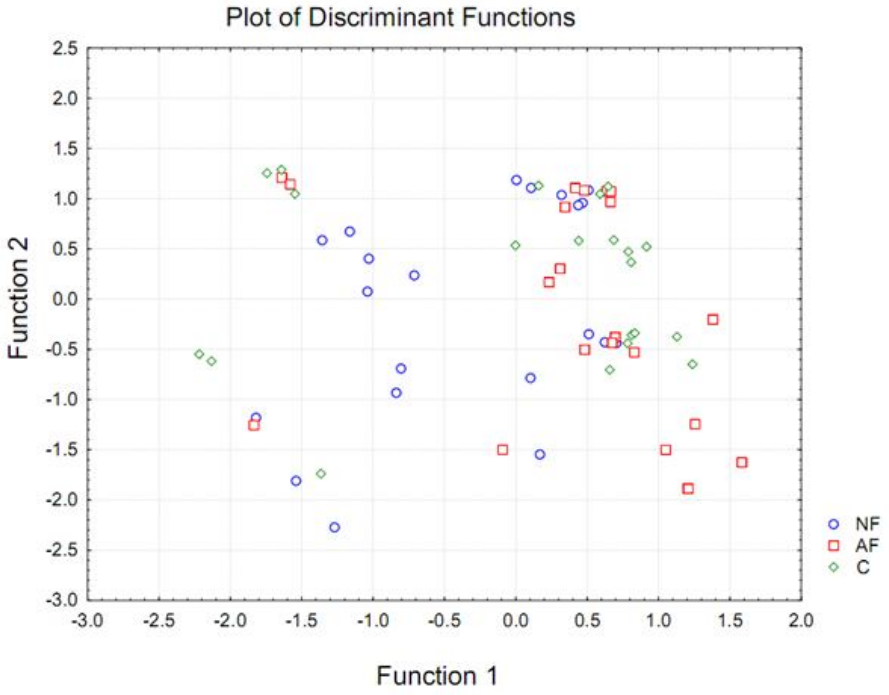

Figure 5. Plots of the first two axes for the canonical discriminant analysis of the different dietary treatments (NF=natural (live) food; AF=artificial feed; C=combination of the two foods NF+AF) in the mud crab *Scylla serrata* using specific enzyme activities (amylase, trypsin and leucine aminopeptidase (LAP) as predictor variables.

Discussion. The ability of crustacean larvae to produce digestive enzymes is closely related to the development of the digestive tract, partly genetically modulated which may be stage-specific and partly diet-induced. High levels of enzyme activity have been detected in penaeid larvae right after hatch (Jones et al 1991) and in *S. paramamosain* larvae, specific activities of protease, amylase, cellulose and lipase was found to vary among different developmental stages (Hong et al 1995). In agreement to these findings, our results showed that low levels of amylase on the early larval stage indicated the carnivorous nature of the early stage of this decapod crustacean. Total amylase activity sharply increased at around Z4 to megalopa stage and declined upon metamorphosis to crab. This considerable increase in activity could suggest a shift from carnivory to omnivory feeding nature. The slight decline in the activity at the crab stage could be indicative of the omnivorous feeding nature typical of the juveniles. Similar pattern have been observed in the larvae of freshwater prawn, where it is demonstrated that amylase activity is extremely low at early stages but is highly active at stage VII- XI (Kamarudin et al 1994). Furthermore, the same pattern has also been observed in *P.serratus larvae* (Van Wormhoudt 1980).

Like any other decapod crustacean larvae, trypsin-like activity was detected as early as the first larval stage. A slight peak was observed in Z2 stage, declined thereafter and stabilized in stages Z3 to Z5 (Fig. 2). Later stages showed a pattern of increased activity reaching a maximum at the crab stage. In freshwater prawn, tryptic-like activity decreased upon the first feeding and tended to decline in subsequent stages. However upon reaching the sixth stage a dramatic increase in activity was noted (Kamarudin et al 1994). Similar patterns have been reported in other decapod species, carridean shrimp, *H. americanus* and *P. elegans* (Van Wormhoudt 1980; Beisiot 1986). The increase in tryptic-like activity up to the crab stage augurs well with the predatory nature of the larva at this stage. Moreover, it is assumed that this sharp increase in trypsin-like activity was due to the completion of the development of the digestive apparatus with concomitant increase in size of the midgut gland (hepatopancreas).

In the present study, LAP enzyme activity exhibited a prominent peak at stage Z5, gradually decline upon metamorphosis to megalopa until reaching the C1 stage. The increase of LAP enzyme activities in the present study seemed to coincide, as reported by Ong (1964), with the physiological changes that happen at stage Z5 in *S. serrata* larvae such as the development of the gastric mill, and increase in the size of the hepatopancreas. In the southern king crab, *Lithodes santolla*, a lecithotrophic species, activities of alanine aminopeptidase (AAP), an exopeptidase like LAP, exhibit gradual increases in activity as the developmental stages progress until reaching a peak at stage CI. This pattern of LAP activity is in agreement with the findings of the present study, except that LAP peak activity was observed at stage Z5.

In the developing larvae, enzymatic response to diet is partly predetermined by genetic control, gut morphology and trophic level, but there are evidences demonstrating that diet could influence digestive enzyme activities (Jones et al 1997). To date, among decapods larvae, only the penaeids and late stage carideans can utilize artificial diets successfully. It was hypothesized that penaeids possesses a well developed anterior midgut diverticulae (AMD) and hepatopancreas while the other crustaceans do not, making them able to efficiently digest and utilize artificial formulated diets. The present findings indicate that in *Scylla serrata larvae,* developmental stage, type of diet and their interactions significantly influenced the pattern of digestive enzyme activities. Larvae fed live feed exhibited a significantly lower amylase activity at stages Z1 to Z3 than larvae fed with artificial feed and combination of these feeds. Moreover, larvae fed the live diet also exhibited significantly higher digestive protease activities (trypsin-like and LAP enzyme activities) at stages Z3 to Z5 than did larvae fed artificial diet or combination of these diets. These findings indicate that the type of diets could influence the pattern of digestive enzyme activities of mud crab larvae. It has been hypothesized that early developing larvae rely on the enzymes present in live food organisms to assist digestion (Kumlu 1999). Enzymes in live prey are released to the digestive tract of crustacean larvae by autolysis of zymogens, which activate the endogenous enzymes within the larval gut (Kumlu & Jones 1995) and the present findings are in agreement with these

earlier works on other species. Research work on total or partial replacement of live food by artificial feed to Z1 larvae of *S. serrata* have shown that larvae fed with combination of live and artificial diets have a lower growth performance and exhibited a higher incidence of deformities than larvae fed the control diet of live food only (Quinitio et al 1999). Also, Z1 larvae do not survive to stage Z3 when fed a formulated prawn diet alone (Quinitio et al 1999). The same investigators have been successful in causing the molting of the mud crab larvae to Z3 under co-feeding with live and artificial diets but still the best overall survival is recorded for larvae fed a full ration of live food. In the present study, live feeds enhanced proteases activity of mud crab larvae and this could be the explanation for the better performance of larvae when fed with live diets.

It is quite complicated to determine which enzyme activities were significantly affected by the diet or developmental stage (as probably genetically controlled variable) as main effects but many trends were clearly apparent from visual inspection of the data although not significantly by statistical criteria. Accordingly, we used multivariate statistical methods to combine individual enzyme activities into enzyme function profiles. With the enzyme activity profile established by Discriminant Analysis (DA), we were able to show that there was a significant effect of the diet as well as development stage upon enzyme activities. Furthermore, DA allowed us to rank the parameters that weighted mostly in the model: trypsin-like>LAP>amylase in discriminating between development stages and only LAP was the predictor variable for discriminating between diets.

Conclusions. Collectively, the findings of this study show that Amylase, Trypsin-like and LAP were already active, at the early developmental stage (Z1-Z3) of *Scylla serrata.* However, amylase activities at the early larval stages were found uniformly low in contrast to those of the proteases (LAP and trypsin-like enzyme), indicating the carnivorous nature and the inability of the larvae to digest carbohydrates. Furthermore, the interactive effects of larval developmental stage and type of diets significantly influenced enzyme activities. Overall, feeding *S. serrata* larvae live diet promoted significantly higher digestive enzyme activities, suggesting the importance of live food as sources of exogenous enzymes for the larvae. The use of Discriminant Analysis (DA) allowed us to show that there was a significant effect of the diet as well as development stage on digestive enzyme activities. It also allowed us to rank the parameters that weighted mostly in the model: trypsin>LAP>amylase in discriminating between development stages while only LAP was the predictor variable for discriminating between diets.

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