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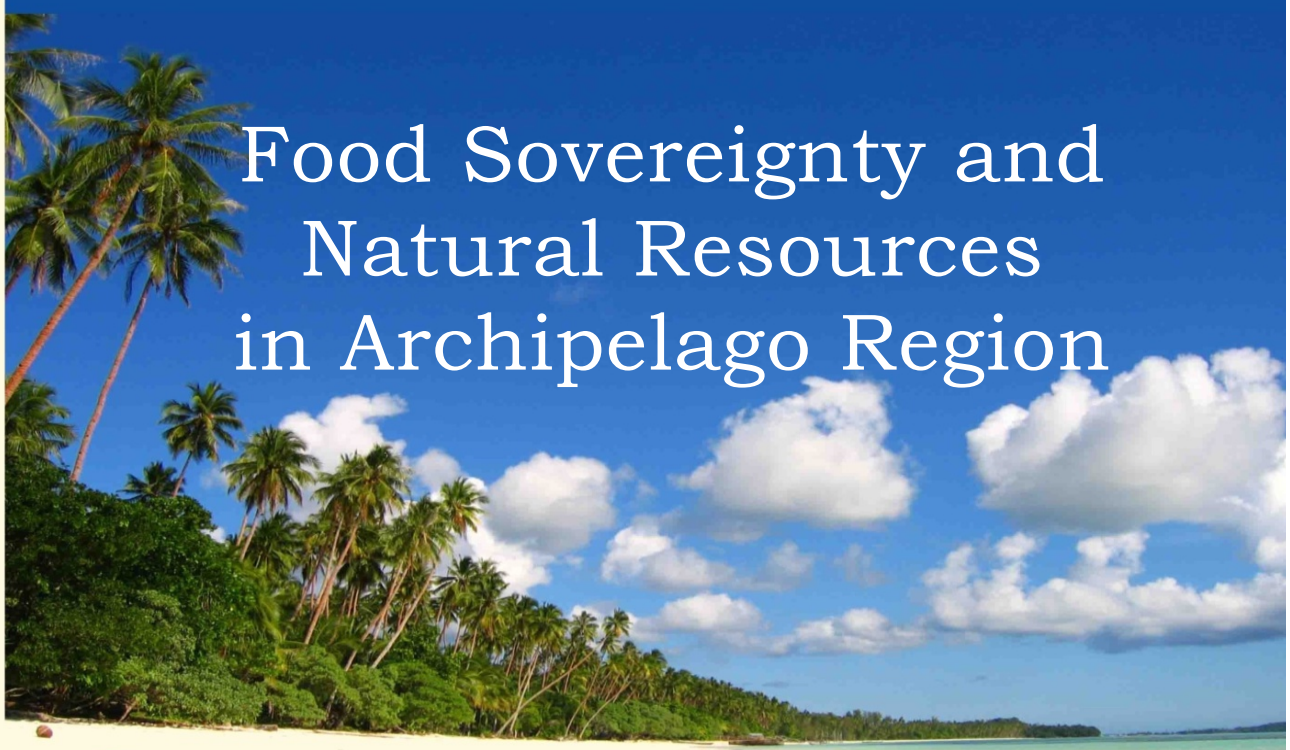

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DEVELOPMENT OF DIGESTIVE ENZYME ACTIVITY BASED ON ARTIFICIAL DIET OF FEEDING TIME ON SWIMMING CRAB LARVA (*Portunus pelagicus*)[1]

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Abstracts

One of the main problems on larvae rearing is the low survival rate when larva was being fed by artificial diet at the early larval stage. This study was conducted to examine Development of digestive enzyme activities based on feeding time of artificial diet on swimming crabs (*Portunus pelagicus)*. Five different initial feeding time were examined (i.e. A. First feeding natural food at Zoea (Z) 1; B. commercial feed at Z1; C. Z1 natural food, commercial feed at Z2; D. Z1- Z2 natural food, commercial feed at Z3 and E. Z1 –Z3 natural food, commercial feed at Z4). The parameters observed were the survival rate and enzyme activities. Results of this experiment showed that larva that was being fed by commercial diet at the Z1, Z2, Z3 failed to develop to first crab (FC) stage. On the other hand, the one that was being fed by commercial diet at the Z4 stage could develop to FC stage. The enzyme activity of protease was high in Z1 larvae and tended to decrease during larva development. Whereas the enzyme activities of amylase and lipase were lower in Z1, and tend to increase during larva development. From this research it was concluded that the first feeding time of commercial feed to larva affect the survival rate of FC, and the precise time for feeding the larvae with commercial feed was at stage Zoea 4.

Key Words: *larva rearing, Portunus pelagicus, commercial feed, survival*

INTRODUCTION

Development of swimming crab aquaculture is influenced by market demand so as to bring the consequences of the increasing supply for seed. Seed requirement is still obtained from wild catch so the continuity of seed availability for cultivation is very limited (Juwana, 2002). The increasing intensity of seeds catch in nature will increase the likelihood of "over fishing". In addition, natural seeds are dependent on the season which resulted in different seeds' number and size. Therefore, hatchery has been proposed as the best way to supply sufficient seeds amount with uniform size. Crab hatchery has been successfully performed on several beaches seedling. The undertaken studies has encompassed the issues of adult organism handling, mass production and seed seedling (Susanto et

al. 2003; Juwana, 2000), and food formulation (Juwana 1996; Juwana 1999; Juwana, 2002;).

Foods used in crab hatchery are generally derived from nature such as rotifers and naupliu of Artemia. However, there are some problems related to natural food such as the difficulty in preparing the food on time for the larvae, besides its expensive price (Quinitio et al. 1999); the content of n-3 HUFA (n-3 Highly Unsaturated Fatty Acid) primarily DHA (Docosahexaenoic Acids) and EPA (Eicosapentaenoic Acid) are low (Suprayudi et al. 2002), which create the crucial demand for artificial feed.

Studies in artificial feeding for swimming crab larvae are limited. Studies on mud crab larvae are generally used as the main references. The recent constraint for artificial feeding is the low larvae survival rate, consistent with Quinitio et al. (1999) study where survival rate of mangrove crab (*Scylla serrata*) larvae fed with artificial food only last until stadia zoea 2, while Syahidah dan Rusdi (2003) found the highest survival rate was only 45% for larval mud (*Scylla paramamosain*) feed with artificial food.

The low percentage of survival rate for larvae fed with artificial food were mainly caused by the lack of knowledge on the appropriate time of artificial food can be digested by larvae, as well as the availability of digestive enzymes in the larval stadia development. In order to improve the survival rate of crab larvae, a study on the effects of feeding time on the survival rate of crab larvae in relation to the development of digestive enzyme activity has been completed for small crab larvae (*Portunus pelagicus*).

RESEARCH METHOD

Time and Place

The study was conducted in 3 months at the Research and Development Centre of Marine Fish (BPPILAPU) Pangandaran, Kudat District, and enzyme analysis was carried out in the Laboratory of Microbiology and Biochemistry, Biological Resources and Biotechnology Research Center, Bogor Institute of Agriculture (IPB).

Artificial Food

Artificial food used was Lanzy ZM-Shrimp with compositions of 48% protein, 13% lipid, 2.5% crude fiber and 8% moisture content.

Brachionus sp

Brachionus sp obtained from bulk cultures, using 1 ton concrete tank. Food used for *Brachionus* sp was *Nannochloropsis* sp of mass culture, produced from 14 tons concrete tank. For the purpose of enrichment, *Brachionus sp* initially introduced into 10 liters sea water in the enrichment tank with salinity 33-34 ppt, density 1000 ind / ml and aerated water. Fish

oil and corn oil were respectively place with the proportion of 15% and 85% as much as 1 ml were added with 0.1 g egg yolk. This mixture will be set in 200 ml water and emulsified for 2 min (Suprayudi et al. 2002). Once emulsified, the media is inserted into the enriched *Brachionus* sp container for 8 hours (Karim, 1998).

Artemia

Artemia sp used was Inve no. 2, and to produce nauplius of *Artemia* sp the cysts were incubated in a hatching container filled with seawater with the salinity of 30-33 ppt. Hatched density of Cysts were 5 grams / liter of sea water. Containers used for hatching cysts made from conical based fiber glass with capacity 40 liters and equipped with aeration. Shell of hatched cysts into nauplius was separated by unwavering water for 10 minutes, then harvested by opening valve at the container bottom then placed in a mesh size no. 120 sieve. Next, nauplius was enriched with fish and corn oil and were put into 10 liters sea water, with the container salinity 33-34 ppt, density 200 ind / l (Karim, 1998) and aerated. Fish oil and corn oil were respectively place with the proportion of 15% and 85% as much as 1 ml were added with 0.1 g egg yolk. This mixture will be set in 200 ml water and emulsified for 2 min (Suprayudi et al. 2002). Once emulsified, the media is inserted into the enriched *Brachionus* sp container for 8 hours (Karim, 1998).

Larvae Supply

Adult Maintenance

Adult stock as many as 10 individuals (9 females and 1 male) were placed in a maintenance concrete pool with dimension 3.2 x 1.8 x 1.2 m and filled with sea water salinity 33 - 34 ppt, and temperatures 30 - 31^{o}C. The pool is equipped with circulatory system and aerated. The adult crabs fed with squids 2 times a day. Fifty percent water replacement was conducted once, every 2 weeks. Every 2 or 3 days, depending on the left out food, the pool was being cleaned from food residue. Mature adults were observed by the presence of eggs with yellow color. The adults with egg were left for 3 more days in the pool then moved to 150 l aquarium with sand bottom, circulation system was "double bottom" and aerated. Eggs observations were conducted intensively every morning to see discoloration. If the egg color had changed from yellow to brown, entirely black, and microscopically the outskirts had been orange then the adults were immediately transferred into a hatching container in the afternoon.

Egg Hatching

Egg hatching container made of rounded 500 l fiber glass volume was filled with ± 450 l sterilized seawater with salinity 30 ppt. The water was added with formalin 2 ppm and strongly aerated. The next day the adults

crab would hatch egg in the container. After hatching, adults were moved back to maintenance pool and maintained as before. Hatched larvae in the container were being cleaned up from impurities using sieves. Larvae sampling for the research only for healthy larvae (active and swim on the water surface) performed by first turning off aeration in approximately 5 minutes.

Larvae Rearing and Data Collection

Containers and Media

54 pieces 2 liters round plastic jars filled with 1 liter sterilized seawater with salinity 30 ppt were used for the study. The sea water was initially filtered with cotton and chemically treated with chlorine at a dose of 15 ppm. The water then stored in 1 ton tank and flowed with circulation pump through 30 Watt UV. Sterilized water was used as medium in plastic jars. All plastic jars were placed in the "water bath", controlled by thermostat at a temperature 30^{o}C, in order to maintain the stability of media temperature. Each jar was weakly aerated using a hose connected to a Pasteur pipette to maintain oxygen solubility. Aeration source came from "root blower". Dissolved oxygen and temperature of media respectively 5.86 to 6.01 mg / l and 29.5 - 30^{o}C, whereas water pH ranged from 7.72 to 7.84 and salinity was 30 ppt.

Experimentation

Prior to experimentation, plastic jars, as research container, were placed randomly in a "water bath", filled with 1 liter seawater and weakly aerated. Thirty individuals then will be place into containers and being feed based on the research treatment.

Table 1 Amount of Natural and Artificial Food Given Based on Stadia

Stadia	*Brachionus* sp (ind/ml)*	Nauplius *Artemia* (ind/ml)*	Artificial feed (mg/l/day)**
Z1	40 *	TD	0.5
Z2	40 *	TD	2.0
Z3	40 *	0.5*	4.0
Z4	40 *	1.0*	6.0
Megalopa	TD	4.0*	8.0

Remaks : * Suprayudi *et al.* (2002) ; **Susanto *et al.* (2003); TD= no feeding

Frequencies for artificial feeding were 4 times a day, at 8 am, 11 am, 2 pm and 5 pm. The research media was changed every morning to avoid food residue and to separate the larval stadia.

Enzyme sampling

Samples for digestive enzymes analysis (protease, amylase and lipase) on crab larvae was taken separately from larval rearing of feed test. A 6 tons rectangular concrete tank filled with 6 tons water and aerated was used to put 50 ind / l zoea 1 larval stadia. Larvae were fed only with natural food (enriched *Brachionus* sp and nauplius of Artemia) during stadia zoea 1 to megalopa. Sampling was conducted on each stadium and prior to enzyme sampling, the larvae was left fasted for 4 hours in a 2 liter jar. Larvae taken for each stadia were 0.5 – 1.0 grams using strainer and rinsed with fresh water and then will be placed in plastic bags and stored in Freezer (-20^{o}C).

Statistical Analysis

The design of experiments

The experiment used a Completely Randomized Design (CRD) with 5 treatments and 3 replications (Steel and Torrie, 1991). The used treatments were as follow:

Treatment A

Stadia	Z1	Z2	Z3	Z4	Megalopa
Natural food	■	■	■	■	■
Artificial Food					

Treatment B

Stadia	Z1	Z2	Z3	Z4	Megalopa
Natural food					
Artificial Food	■	■	■	■	■

Treatment C

Stadia	Z1	Z2	Z3	Z4	Megalopa
Natural food	■				
Artificial Food		■	■	■	■

Treatment D

Stadia	Z1	Z2	Z3	Z4	Megalopa
Natural food	■	■			
Artificial Food			■	■	■

Treatment E

Stadia	Z1	Z2	Z3	Z4	Megalopa
Natural food	■	■	■		
Artificial Food				■	■

Remarks : ■ = Larvae Feeding

Data Analysis

Data analysis applied was ANOVA and if there were differences between treatments then the analysis will be followed by Duncan's Multiple Range Test (Steel and Torrie, 1991). Analysis for enzyme activity protease, amylase and lipase data were plotted in charts and graphs of enzyme development for each stadium and will be descriptively discussed.

Variables

Survival rate

Crab larvae survival rate was calculated using formula:

$$SR = Nt / No \times 100\%$$

Where: SR = larvae survival rate

No = number of larvae at the beginning of experiment

Nt = number of larvae at the end of experiment

Intermolt Period

Intermolt larval period was obtained using formula developed by Suprayudi et al (2004):

$$Dt = \Sigma Nt / N \Sigma$$

Where: N = Number of larvae within a stadia at any given time

t = Time

Chemical Analysis

Larvae Digestive Enzymes

Observation of digestive enzymes was conducted for each stadia: zoea 1, zoea 2, zoea 3, zoea and megalopa 4. Observed enzymes included protease, amylase, and lipase.

RESULTS AND DISCUSSION

Observation results of crab larvae (*Portunus pelagicus*) survival rate intra stadia and carapace width are shown in Table 2.

Table 2 Survival rates (%) between larval stadia and carapace width (mm) of swimming crab

	Stadia							wide Carapax (X±SD)	n
	Z1-Z2	Z2-Z3	Z3-Z4	Z4-M	M-FC	Z1-Z4	Z1-FC		
A	47.78[a]	74.82[a]	80.84[a]	68.38[a]	88.89[a]	26.67[a]	15.55[a]	1.50±0.14[a]	2
B	14.45[b]	*	-	-	-	-	-	-	-
C	35.56[a]	**	-	-	-	-	-	-	-
D	40.00[a]	65.00[a]	64.02[a]	***	-	16.67[a]	-	-	-
E	41.11[a]	68.49[a]	80.37[a]	31.19[b]	50.00[b]	22.22[a]	3.33[b]	1.25±0.07[a]	2

Remarks : *Larvae deceased in day 7, **Larvae deceased in day 11, ***Larvae deceased in day 16 Same notation in the same column, no significance difference (P<0.05)

The survival rate intra stadia Z1-Z2 (Table 2) shows that the natural food early on stadia Z1 (treatment A, C, D and E) has a higher survival rate (P <0.05), compared to artificial food in early stadia Z1 (treatment B). There are no differences among survival rates intra Z2-Z3 and Z3-Z4 (P> 0.05), while the survival rates of the Z4-M and M-FC shows that natural food on early stadia Z1 is better than artificial food in early Z4 stadia. The survival rate of larval swimming crab of stadia Z1-Z4 suggesting that there are no differences in natural feeding early stadia Z1 (P> 0.05) with artificial food in early stadia Z3 and Z4. However the survival rate Z1- FC with natural food treatment since the early stadia Z1 is higher (P <0.05) than the initial treatment of artificial food on stadia Z4. Carapace width FC did not differ (P> 0.05) for natural food treatment in early stadia of Z1 compared to artificial food treatments on stadia Z4.

Intermolt period (time between molting) larvae at each stadia during the study are shown in Table 3.

Table 3 Intermolt period (day) for each crab larvae stadia

Perlakuan	Stadia					
	Z1	Z2	Z3	Z4	M	FC
A	1.5[a]	3.9[a]	7.2[a]	10.9[a]	14.1[a]	16.6[a]
B	1.8[b]	4.5[b]	*	-	-	-
C	1.8[b]	5.8[c]	**	-	-	-
D	1.6[a]	4.4[ab]	7.9[b]	13.3[b]	***	-
E	1.6[a]	4.4[ab]	7.5[c]	11.9[c]	16.4[b]	17.0[a]

Remarks : Z = Zoea, M = Megalopa, FC (first crab); *Larvae deceased in day 7, **Larvae deceased in day 11, ***Larvae deceased in the day 16 same notation in the same column, no significance difference (P<0.05)

Z1 stadia development time also does not show any differences (P> 0.05) between treatments A, D and E, compared to treatments B and C. Length of time for development of C Z2 indicated that treatment C required longer development time (P <0.05), compared with other treatments. The A treatment does not show any different development length (P> 0.05) compared to treatments D and E. Time length for development of B treatment in stadia Z2 does not differ (P> 0.05) from treatments D and E. Time length taken by larvae to reach the Z3 and Z4 were different (P> 0.05) between natural food treatment in early stadia of Z1 (treatment A) with artificial food treatment in early stadia of Z3 (treatment D) and Z4 (treatment E). The time length to achieve megalopa larvae was different (P> 0.05), but did not differ (P> 0.05) for larval development time to achieve FC between natural food treatment in early stadia of Z1 (treatment A) compared to initial treatment of natural food in stadia Z4 (treatment E).

Activities of digestive enzymes protease, amylase and lipase at each crab larvae stadia, rotifers and Artemia nauplius are presented in Table 4.

Tabel 4 Activities of digestive enzymes protease, amylase and lipase at each crab larvae stadia, rotifers and Artemia (unit/minute/gram)

Digestive enzymes	Z1	Z2	Z3	Z4	Mega-lopa	Rotifer	Nauplius Artemia
Protease	0.2290	0.1590	0.1540	0.1260	0.0630	0.006	0.080
Amilase	0.0011	**	0.0015	0.0014	0.0020	0.0011	0.0008
Lipase	0.5166	0.3783	1.1431	1.5449	3.4011	0.4974	3.3957

Remarks : *Analysis result at Microbiology and biochemistry laboratories, Living Resources and Biotechnology Research Center IPB , ** value outliers

Protease enzyme activity shows a decrease in each stadium; from Z1 to megalopa, while protease enzyme in rotifer (0,006 units / min / g) and Artemia nauplius (0.080 units / min / g) are below the value of protease enzyme activity in stadia Z1 and Z3 . Amylase, as the opposite of protease enzyme activity, tended to increase at each stadia crab larvae. Amylase enzyme activity in rotifers same with amylase enzyme activity in stadia Z1 (0.0011 units / min / g) but activity of the enzyme amylase on Artemia nauplius (0.0080 units / min / g) is lower than the activity of the enzyme amylase on stadia Z3. Furthermore, lipase activity tended to increase at each stadia where in rotifers is 0.4974 units / min / g almost equal to the activity of lipase in larval stadia Z1, whereas lipase activity in Artemia nauplius (3.3957 units / min / g) is higher than the activity of lipase in larval stadia Z3 .

Survival rate of larval swimming crab at the end of study intra stadia between was different from the early treatment with natural food in stadia Z1 and early treatment with artificial food in stadia Z4. Both treatments could produce first crab (FC) at the end of the study, whereas other treatments did not result in FC. Treatment of artificial food in early stadia Z1 only survived until day 7, whereas treatment of early artificial food on stadia Z2 and Z3 only survived between day 11 until day 16. Differences between natural food treatments on early stadia of Z1 and artificial food in early stadia of Z4 were affected by the early artificial food treatments.

Treatment of artificial food in early stadia of Z1 showed the lowest (14.45%) larvae survival rate between Z1-Z2 stadia compared to other treatments. The condition was influenced by the incapability of larvae to hydrolyze artificial food and only utilize a food reserve backup since hatched. In addition, development of digestive system had not been perfect or reached definitive form yet. Due to the incomplete system, digestive enzyme production which reflected by enzyme activity was still low, and the larvae was not able to digest artificial food having no enzymes, and only able to survive until day 7. This condition was similar to the early artificial food treatment of stadia Z2, where larvae could only survive up to day 11. Gawlicka et al. (2000) stated that the activity of digestive enzymes was a good indicator to determine the capacity of digestion; high activity may indicate that larvae were physiologically ready to process external food.

The larvae survival rate between stadia Z2-Z3 did not show any significant differences, as well as the survival rates between stadia Z3-Z4. These suggested that the effect of artificial food in early stadia Z3 and Z4 were no different from the natural food on early stadia of Z1. The indifference of larvae survival rate was influenced by the ability of larvae Z3 stadia to utilize artificial food which reflected in the increasing activities of enzyme amylase and lipase. Increased amylase and lipase enzyme activities due to digestive tract of larvae began to flourish as gastric mill, filter and hepatopancreas gland. According to Li (1990), Li and Li (1995) in Li et al. (1997), improvement of digestive system of mangrove crab larvae was indicated by an increase in development of gastric mill, filter and hepatopancreas gland. Basis form of gastric mill starts to appear on stadia Z3 and approaching perfection in stadia Z5. Ceccaldi (1989) stated that the development of gastric mill in crustaceans occurred during the process of metamorphosis, and one functions of hepatopancreas in crustaceans was to synthesize and to secrete digestive enzymes.

Larvae survival rate was also supported by contribution of external digestive enzymes. Before larvae were given artificial food, they had been feed with naturally food (rotifers and Artemia). Munilla-Moran et al. (1990) in Kolkovski (2001) stated that there was a contribution of digestive enzymes by rotifer and Artemia to turbot (*Scophtalmus maximus*) fish by 43-60% for protease, esterase 89-94% and amylase 15-27%. Although there were no differences between early treatments with artificial food in stadia Z3 and Z4; with natural food on early stadia of Z1, but the length of development time of stadia Z2, Z3 and Z4 are significantly different.

The difference in development time indicated that the use of artificial food by larvae was slow and could be expected to affect energy produced which further influenced larval development. In addition, observations during the study showed that most larvae die during molting (change stadia). The shortage of n-3HUFA in larvae food was the main problem, which was able to accelerate the development of inter-stadia molting. Takeuchi et al. (1999) in Suprayudi et al. (2002) stated that n-3HUFA was effective in accelerating the development of inter-stadia and helped to maintain a high rate of survival in larvae of "swimming crab" (*Portunus trituberculatus)*.

Survival rates of Z4-M and M-FC in natural food treatment in early stadia of Z1 provided the best survival rates (68.38% and 88.89%) compared to artificial food treatment in early stadia of Z4 which respectively only 31.19% and 50.00%. Furthermore, time length development for Z4 and megalopa were faster on natural food treatment in early stadia of Z1, compared to artificial food treatment in early stadia of Z3 and Z4.

The difference indicated that stadia Z4 and megalopa larval development of the digestive tract had been perfect and reached definitive form so they were able to digest and absorb artificial food. Perfection on larval digestive system will increase the production of digestive enzymes which were reflected by higher activity of amylase and lipase enzyme with the increase of larva stadia. The availability of digestive enzymes in larvae was very helpful in digesting artificial food.

The survival rate intra stadia of Z1-Z4 had no significant effect among natural food treatments in early stadia of Z1 and early artificial food treatments on stadia Z3 and Z4, but there were significant differences in survival rates between Z1-FC stadia between natural food treatment in early stadia Z1 and early artificial food treatment on stadia Z4.

The difference was mainly due to high mortality rate of larvae during molting which caused by deficiency of artificial feed in n-3HUFA especially DHA and EPA, which decreased number of larvae of the next stadia, and amplified by cannibalism especially in stadia megalopa. Observations in the study indicated that larval mortality rate was high and cannibalism occurred for crab larvae mainly during megalopa stadia.

Takeuchi et al. (1999) in Suprayudi et al. (2002) reported that DHA and EPA had different roles in the development of crab larvae. Suprayudi (2004)[a] suggested Artemia feeding with EPA 0.71 to 0.87% and DHA from 0.49 to 0.72% to maintain high survival rate, larval development time and carapace width.

CONCLUSION AND RECOMMENDATION

Based on the study results, it can be concluded that:

1. Protease enzyme activities tend to decrease at each stadia while amylase and lipase activities tend to increase.
2. The appropriate time for artificial food on larval swimming crab (*Portunus pelagicus*) is at the zoea 4 (Z4) stadia.

Based on the above conclusion, this study recommends the implementation of artificial food feeding on larval swimming crab (*Portunus pelagicus*) rearing at zoea 4 stadia.

REFERENCES

Ceccaldi HJ. 1989. Anatomy and physiology of digetistive tract of crustaceans decapods reared in aquaculture; *In* Advances in tropical aquaculture, workshop at Tahity, Franch Polynesia, Feb. 20 March 4. *Ifremer*. *Actes de collogue* 9 : 253-256

Juwana S. 1996. Studi pendahuluan tentang peranan pakan buatan dalam budidaya rajungan *portunus pelagicus,* (Portunidae, decapoda). *Oceonologi dan Limnologi di Indonesia*. Pusat Penelitian dan Pengembangan Oceanologi dan Pusat Penelitian dan Pengembangan Limnologi-LIPI. 29:1-13

Juwana S. 1999. Studi untuk membuat diet optimal bagi burayak rajungan; bentuk dan lama penyimpanan bahan baku. *Oceonologi dan Limnologi di Indonesia*. Pusat Penelitian dan Pengembangan Oceanologi dan Pusat Penelitian dan Pengembangan Limnologi-LIPI. 31:25-39

Juwana S. 2000. Produksi masal benih rajungan (*portunus pelagicus) di* Puslitbang Oceanologi-LIPI. Jakarta. Kepadatan optimal burayak dan pasca burayak. *Oceonologi dan Limnologi di Indonesia*. Pusat Penelitian dan Pengembangan Oceanologi dan Pusat Penelitian dan Pengembangan Limnologi-LIPI. 32:13-25

Juwana S. 2002. Crab culture technique at RDCO-LIPI. Jakarta. Indonesia, 1994 to 2001. *Proceedings workshop on marinevulture in Indonesia. Mataram, Lombok Island.* Research center for oceanography-LIPI, Institute of marine science Norwegian Bergen-Norway. P. 144

Karim MY. 1998. Aplikasi pakan alami (*Brachionus plicatilis* dan nauplius artemia) yang diperkaya dengan asam lemak omega-3 dalam pemeliharaan larva kepiting bakau (*Scylla serrata* forskal). (Thesis) : Bogor. PPs Institut Pertanian Bogor.

Kolkovski S. 2001. Digestive enzymes in fish larvae and juveniles-implications to formulated diets. *Aquaculture*, 200: 181-201

Li S, C Zeng, H Tang, G Wang, Q Lin. 1997. Investigations into the reproductive and larval culture biology of the mud crab, *Scylla paramamosain:* A Research overview. *In* Keenan CP and A Blackshaw (Eds.). Mud crab aquaculture and biology. *Proceedings of an international scientific forum held in* Darwin, Australia, 21-24 April 1997. ACIAR *Proceedings* Pp :121-139

Quinitio ET, F Parado-Estepa, V Alava. 1999. Development of hatchery techniques for mud crab *Scylla serrata* (Forskal). Comparison of feeding schemes. *In* Keenan, C.P. and Blackshaw (Eds.). Mud crab Aquaculture and Biology. *Proceedings of an International Scientific Forum held in Darwin,* Australia, 21-24 April 1997. ACIAR Proceedings, Pp : 125-130.

Steel AM, JH Torrie. 1991. *Prinsip dan prosedur statistika. Cetakan kedua.* Jakarta. PT. Gramedia, Jakarta. 491 p

Suprayudi MA, T Takeuchi, K Hamasaki. 2004[a]. Effect of *Artemia* enriched with eicosapentaenoic and docosahexanoic acid on survival and occurance of molting failure in megalop larvae of the mud crab *Scylla serrata. Fisheries sci.* 70:650-658

Suprayudi MA, T Takeuchi, K Hamasaki, J Hirokawa. 2002. The effect of N-3HUFA content in rotifers on the development and survival of mud crab, *Scylla serrata,* larvae. Suisanzoshoku, *Japan Aquaculture Society.* 50 (2): 205-212.

Susanto B, M Marzuqi, I Setyadi, D Syahidah, N Permana, I Rusdi, Haryanti. 2003. Produksi masal benih rajungan (*Portunus sp*) melalui perbaikan manajemen pakan dan kontrol biologi. Laporan Teknisi Balai Besar Riset Perikanan Budidaya Laut, Gondol, Bali. 21 p.

Syahidah D, I Rusdi. 2003. The effect of different initial feeding time of micro diets as artificial feed to survival rate of mud crabs larvae, *Scylla paramamosain*. *In International Seminar on Marine and Fisheries* 15-16 December 2003, Jakarta Convention Center. ISBN 979-98165-1-3.