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# THE EFFECTS OF WEANING TIME ON THE GROWTH AND SURVIVAL OF MUD CRAB (*Scylla olivacea*)

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*(Received 7 December 2017; Final revised 8 August 2018; Accepted 8 August 2018)*

**ABSTRACT**

Live foods such as rotifers and *Artemia* are commonly used as foods in larval rearing of mud crab (*S. olivacea*). However, the continuous availability and nutritional consistency of live foods are difficult to control. Thus, the development of artificial diets to partially or fully replaced live foods is needed to overcome the limitations of live foods. The purpose of this research was to determine the best stage at which mud crab larvae can be weaned from live foods to artificial diets. The research experiment consisted of: treatment-1, the larvae were fed with live foods from zoea-1 to megalopa stages as the control treatment; treatment-2, the larvae were fed with artificial diet from zoea-2 to megalopa stages; and treatment-3, the larvae were fed with artificial diet from zoea-3 to megalopa stages. In treatment-4, artificial diet was given from zoea-4 to megalopa stages. The growth and survival rate of larvae in treatment-1, 3, and 4 were not significantly different ($P>0.05$) but significantly different with treatment-2. Based on the present results, this study suggests that artificial diet can be given to mud crab larvae (*S. olivacea*) from the third zoea stage.

**KEYWORDS: artificial diet; larvae; live foods; *S. olivacea*; weaning period**

## INTRODUCTION

The major constraint in mud crab farming is the limited supply of seed. Currently, the seeds of mud crab were mostly collected from wild catches where the availability seasonally fluctuates (Serrano & Traifalgar, 2012). This has led to the widespread seedsstock shortage and over exploitation of the species and the farming activities have been classified as not environmentally friendly. Thus, the development of more effective hatchery techniques and reliable production of mud crab juveniles are needed to ensure the sustainability of mud crab aquaculture.

Feed is one of the factors that determine the success of an aquatic organism larval rearing in a hatchery. Currently, most hatcheries rely on live foods such as rotifers (*Brachionus plicatilis*) and *Artemia salina* nauplii. However, live foods are deemed not practical as well as economically in efficient because live foods production may account 50-75% of the total cost of aquaculture hatcheries (Dainteath & Quin, 1991). Nutritional profile of live foods is not always consistent which depends on the source, age, and cultivation techniques. Live foods also lack certain nutritional components essential for normal growth of marine larvae. Live foods can also be a vector of pathogens in larval rearing. The production of live foods in hatcheries need specialized work force and requires specific equipment and facilities (Holme, 2008). In large-scale hatchery operations, the use of live food needs to be limited periodically and replaced with artificial diets where the nutritional composition can be tailored to the needs of the larvae.

Artificial diets in the form of micro diet ensure the availability and quality of the feed. The feed can also be formulated according to the requirements of larvae. However, several studies had found that the growth performance and survival rates of mud crab larvae fed with artificial diets were not as good as those fed with live foods (Holme, 2008). The resulting poor growth was likely due to the incomplete development of the digestive organs in the early lar-

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val stage which affects the availability of digestive enzymes. The results of Jantrarotai *et al*. (2005) study on *S. olivacea* revealed that, based on histological assessment, the structure of the species' digestive organs had completely developed. Pavasovic *et al*. (2004) suggested that a successful mud crab hatchery technology requires a comprehensive understanding of the digestion process in the larvae. Such knowledge is not only very important in understanding the physiology of nutrition but also in determining the larval stadia where artificial diets can be applied.

Increased activity of digestion enzymes in larvae can be used as an indicator when artificial diets can be used. Gawlicka *et al*. (2000) suggested a similar finding that the activity of digestive enzymes is a good indicator to determine the capacity ofthe digestion system in an animal. A significant increase in enzyme activity could mean that, physiologically, larvae are ready to process feed from outside (Gawlicka *et al*., 2000). For example, the highest increase of trypsin, lipase, and amylase enzymes in *S. olivacea* larvae occurred at stadia zoea-3 (Haryati *et al*., 2014). Serrano & Traifalgar (2012) also found a similar pattern on *S. Serrata* where the increased activities of trypsin, amylase, and $\alpha$ LAP (Leucine Amino Peptidases) on *S. serrata* were observed at zoea-3 stage. The objective of this research was to determine the weaning period of *S. olivacea* larvae from live food to artificial diets.

## MATERIALS AND METHODS

### Source of Larvae

The source mud crab larvae used in this study came from the hatched eggs of mud crab broodstock. The broodstocks were fed daily with squid and trash fish as much as 15% of their biomass with feeding frequency twice a day at 06.00 and 18.00.

The larvae were reared in seawater with a salinity of 32-34 ppt filtered through a sand filter, then disinfected using chlorine 40 mg/L, for approximately 12 hours with strong aeration. The seawater was then neutralized using 20 mg/L thiosulfate and allowed to stand still for 1-2 hours.

Containers used for larval rearing were black plastic buckets of 25 liters in volume. Each container was filled with 20 liters of the sterilized seawater and stocked with the larvae at a density of 50 individuals $L^{-1}$.

The experiment was arranged in a completely randomized design with four treatments and three replicates (Table 1).

The live foods used in these experiments were *Brachionus* and *Artemia* nauplius, while the artificial diets was a commercial feed. From zoea-1 to 3 stages, the larvae were fed with *Brachionus* with a density of 30 individuals $mL^{-1}$. From zoea-3 to megalopa stages, the larvae were fed with *Brachionus* with an addition of *Artemia* nauplii at a density of 5 individuals $mL^{-1}$. The feeding trial was done twice daily at 07:00 and 22:00. From zoea-1 to zoea-3, the artificial diet was given as much as 5.0 mg $L^{-1}$ daily and as much as 10 mg $L^{-1}$ from zoea-3 to megalopa stages. The artificial feed was given six times daily, at 06.00, 09.00, 12.00, 21.00, 15.00, 18.00, and 21.00.

### Parameters

The experiment was terminated when all zoea-5 stage had molted to megalopa stage. The parameters measured in this study were growth, survival rate and nutritional value of the artificial diet. Mud crab larvae growth was measured by length and width of the carapace and the specific growth rate. The measurements were carried out at zoea-1 and megalopa stadia using an ocular micrometer and observed under a light microscope. The growth of length (▲L) and width carapace (▲CW) were calculated using the following formula:

$$\blacktriangle L = L_t - L_o$$

where:

▲L = growth of carapace length (mm)
$\overline{L_t}$ = length of carapace at megalopa stage (mm)
$\overline{L_o}$ = length of carapace at zoea-1 stage(mm)

$$\blacktriangle CW = \overline{CW_t} - \overline{CW_o}$$

where:
pCW = growth carapace width (mm)
$CW_t$ = carapace width at megalopae stage (mm)
$CW_o$ = carapace width at zoea-1 stage (mm)

The specific growth rate (SGR) was calculated according to the following formula:

$$SGR\ (\%) = \frac{\ln W_f - \ln W_i}{T} \times 100\%$$

where:
$W_f$ = the natural logarithm of the final weight
$W_i$ = the natural logarithm of the initial weight
T = time (days)

The survival rate (SR) was calculated when the larvae had reached the megalopae stage by using the following formula:

$$SR\ (\%) = \frac{\text{The number of larvae at megalopa stage}}{\text{The number of larvae at zoea 1 stage}} \times 100\%$$

Table 1. Arrangement of treatments based on larval development stages

| Zoea started weaning | Larva stages | | | | | |
|---|---|---|---|---|---|---|
| | Zoea-1 | Zoea-2 | Zoea-3 | Zoea-4 | Zoea-5 | Megalopae |
| Control | Live foods | | | | | |
| Zoea-2 | Overlap | Artificial diets | | | | |
| Zoea-3 | Live foods | Overlap | Artificial diets | | | |
| Zoea-4 | Live foods | | Overlap | Artificial diets | | |

Overlap: Larvae were given a combination of live food and artificial feed, each of 50% of the amount of feed (Live foods were 15 individuals $mL^{-1}$ and artificial feed was 2.5 mg $L^{-1}$)

The nutritional value of the feed was evaluated based on the results of the proximate analysis, amino acid, and fatty acid composition. Water quality parameters such as temperature, salinity, and pH were recorded daily.

**Statistical Analysis**

To evaluate the effect of the treatments on growth and survival rate, the data were analyzed using analysis of variance (ANOVA). If there were significant differences among the treatments, Turkey's W-test (Steel & Torrie, 1991) was used to determine the treatments that produced the best response. The quality of the feed was evaluated descriptively by comparing the nutritional value of the artificial diet with the larvae requirements.

## RESULTS AND DISCUSSION

Results of the analysis of variance showed that the differences in the weaning of live foods with artificial diet were significantly affect ($P < 0.05$) on width and length carapace growth as well as the individual specific growth rate of mud crab larvae from zoea-1 to megalopae stages. The larvae fed with artificial diet started from zoea-3 to zoea-4 had insignificantly different ($P > 0.05$) of growth rates compared with the larvae fed only with live foods started from zoea-1 to megalopae. However, the former had higher growth rates which were significantly different ($P < 0.05$) compared with the larvae fed with artificial diets started at zoea-2.

The average survival rate of mud crab larvae from zoea-1 to megalopae stages is presented in Table 3.

Analysis of variance showed that the difference in the starting stage of the weaning of live foods to artificial diet significantly affects ($P < 0.05$) the survival rate of larvae from zoea-1 to megalopae. The larvae fed with artificial diet from zoea-3 to zoea-4 had no significant difference ($P > 0.05$) of survival rates compared with the larvae fed only with live foods started from zoea-1 to megalopae. Similar to the

Table 2. Average growth in length and width carapace (mm) and the specific growth rate (%) of *S. olivacea* from zoea-1 to megalopae stages

| Replacement of live foods with artificial diets | Growth of carapace (mm) | | Individual specific growth rate (%) |
|---|---|---|---|
| | Length | Width | |
| Control | 3.13 ± 0.482[a] | 1.18 ± 0.146[a] | 0.0246 ± 0.0021[a] |
| Zoea-2 | 2.83 ± 0.108[b] | 1.04 ± 0.040[b] | 0.0209 ± 0.0013[b] |
| Zoea-3 | 3.03 ± 0.107[a] | 1.19 ± 0.020[a] | 0.0249 ± 0.0021[a] |
| Zoea-4 | 3.15 ± 0.072[a] | 1.20 ± 0.021[a] | 0.0264 ± 0.0015[a] |

Description: control: larvae fed with live foods from zoea 1 to megalopa stages; the same letter within the same column indicates no significant difference in the level of 5%

Table 3. The average survival rate (%) of mud crab (*S. olivacea*) larvae from zoea-1 to megalopae stages

| Replacement of live foods with artificial diets | Survival rate (%) |
|---|---|
| Control | 35.57 ± 7.01[a] |
| Zoea-2 | 7.77 ± 1.37[b] |
| Zoea-3 | 37.80 ± 4.91[a] |
| Zoea-4 | 47.10 ± 1.84[a] |

Description: control: larvae fed with live foods from zoea-1 to megalopae; the same letter indicates no significant difference in the level 5%

growth rates, the survival rates of the former were significantly different ($P<0.05$) than that of the larvae fed with artificial diets started at zoea-2. The average survival rate of mud crab larvae fed with the artificial diet started at zoea-2 stage was only 7.77%. The larvae fed with the artificial diet started from zoea-3 and zoea-4 and only fed with live foods had much higher survival rates of 37.80%, 47.10%, and 35.57%, respectively.

The relatively low growth and survival rates of *S. olivacea* larvae fed with the artificial diet starting at zoea-2 stage was probably caused by the inability of the larvae to digest artificial diets at this stage. This means that less energy and material are available to support the growth and survival of the larvae. Haryati *et al*. (2015) study showed that the activity of trypsin, á amylase, and lipase enzymes in larvae fed with the artificial diet from zoea-2 which were lower and significantly different compared to the larvae fed only with live foods from zoea-1 to megalopae and larvae fed artificial diets from zoea-3 and 4 stages. The study findings of Serrano & Traifalgar (2012) on *S. serrata* also showed a similar pattern where the increased activity of trypsin, amylase, and $\alpha$ LAP (Leucine Amino Peptidases) were high in zoea-3 larvae. Holme *et al*. (2006) also found that zoea-3 larvae when fed a diet composed of 50% MBD and 50% live *Artemia* have higher survival and better growth development to reach the next stage (zoea-4) compared to those fed 100% live *Artemia*. They argued that the MBD contained nutrient (s) that were beneficial for zoea survival and development, which might be either lacking or available at a limited level in live *Artemia*. Other studies on fish and penaeid shrimp species also found similar results where formulated diet particles fed in conjunction with live food organisms (termed 'co-feeding') to early larval stages generally supported better survival and growth (Holme, 2008). This can probably be explained by the reduced digestive capabilities in the early larval stages when digestion is heavily reliant on enzymes obtained from live foods.

The results of the proximate analysis are presented in Table 4. The protein, carbohydrates (NFE), and lipid ranged from 44.53% to 58.58%, 22.43% to 29.65%, and 6.15% to 11.86%, respectively.

The essential amino acid composition was presented in Table 5. The levels of eight amino acids were higher in *Brachionus* than those in nauplius *Artemia* and artificial diet. Only one essential amino acid methionine, in *Brachionus* was lower than that in the nauplius *Artemia* or in artificial diet. However, the deficiency of this amino acid can be replaced by amino acid cysteine which is a non-essential amino acid. Amino acids threonine, lysine, and histidine in *Artemia* nauplius were lower than in *Brachionus* and artificial diets. Arginine amino acid level was higher in *Artemia* than in *Brachionus* and artificial diets.

The differences in the growth and survival rate of larvae in this study were suspected to be caused by the nutritional value of feed and the ability of larvae to digest feed. Feed protein content ranged from 44.53% to 58.58%. According to Genodepa *et al*. (2004), protein requirement of *S. serrata* at megalopae stage was 79.4%, whereas according to Catacutan (2002), protein requirement of *S.serrata* juveniles ranged from 34.2% to 51.8%. Based on the protein content, the feed used have a good quality.

The carbohydrate content (NFE) ranged from 22.43% to 29.65%. The dietary requirement and utilization of carbohydrates by mud crabs are largely unknown. However, one study by Catacutan *et al*. (2003) found that *S. serrata* larvae were able to digest a wide range of carbohydrates. The fat content ranged from 6.15% to 11.86%.

Table 4. Value of proximate analysis of feed (% dry weight)

| Composition | *Brachionus* | *Artemia* nauplii | Artificial | |
|---|---|---|---|---|
| | | | Diets-1 | Diets-2 |
| Protein | 54.23 | 58.58 | 45.69 | 44.53 |
| Lipid | 11.86 | 6.15 | 9.01 | 9.37 |
| Fiber | 6.64 | 7.72 | 2.13 | 1.43 |
| NFE | 26.17 | 22.43 | 29.16 | 29.65 |
| Ash | 1.01 | 5.12 | 13.99 | 14.80 |

Amino acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are essential in the crustacean diet. Tyrosine and cystine might serve as semi-essential amino acids as their presence in the diet reduces the requirement of phenylalanine and methionine, respectively (Guillaume, 1997). Based on the amino acids composition, threonine, lysine, and histidine in *Artemia* nauplius were lower than that of *Brachionus* and artificial diets. The level of the amino acid methionine in *Brachionus* was lower than that of nauplius *Artemia* and artificial diets and its role can be replaced by amino acid cystine. Amino acid requirements are qualitatively varied among life stages and very little research has been done to determine amino acid requirements in the development of crustacean larvae (Bengston, 1993).

The fatty acid composition in *Brachionus*, *Artemia* nauplius, and artificial diet is presented in Table 6.

Eicosapentaenoic fatty acid (EPA) in *Brachionus*, *Artemia* nauplius, and artificial diets were relatively high. Fatty acid DHA was detected in rotifers but its level was diminutive compared to artificial diets. Fatty acid DHA was not detected in *Artemia* nauplius. Takeuchi (2000) suggested that n-3 HUFA especially EPA and DHA are the essential ingredients for the larvae to survive and support the growth of the carapace. Studies have shown that *S. serrata* needed the enzyme for *de novo* synthesis of long chained unsaturated fatty acids (HUFA) (Sheen & Wu, 1999). The deficiency of n-3 HUFA eicosapentaenoic acid (EPA) and doxosahexaenoic acid (DHA) has been identified as cause of low survival, longer intermolt period, and narrower carapace width (Suprayudi *et al*., 2004a). The results of a research by Suprayudi *et al*. (2004b) showed that rotifers enriched with *Nannochloropsis* had EPA levels ranged from 0.94 to 1.46 % and very low DHA. In this study, EPA level in Artemia ranged from 0.27 to 0.39 % and DHA was untraceable. Based on the content of EPA and DHA, artificial diets quality were relatively the same with in both *Brachionus* and *Artemia.*

The water temperature measured during the experiment ranged between 24°C-30°C. Mardjono *et al*.

Table 5. Amino acid composition of *Brachionus*, *Artemia* nauplii, and artificial diets (% crude protein)

| Amino acid | *Brachionus** | *Artemia* nauplii* | Artificial | |
|---|---|---|---|---|
| | | | Diets-1** | Diets-2** |
| Phenyllanine | 2.67 | 1.61 | 1.82 | 1.64 |
| Leusine | 4.18 | 2.83 | 2.91 | 2.63 |
| Isoleusine | 2.33 | 1.79 | 1.65 | 1.47 |
| Metionine | 0.54 | 0.82 | 0.78 | 0.73 |
| Valine | 2.88 | 1.91 | 1.98 | 1.77 |
| Threonine | 2.19 | 0.85 | 1.76 | 1.67 |
| Arginine | 3.15 | 4.45 | 2.81 | 2.53 |
| Histidine | 1.10 | 0.36 | 0.90 | 0.78 |
| Lisine | 4.67 | 1.24 | 2.19 | 1.85 |
| Triptofan | Nd | Nd | Nd | Nd |

Description: nd = not detected; * = Haryati (2002); ** = analysis result in the Integrated Laboratory Bogor Agriculture Institute

Table 6. Fatty acid composition in *Brachionus*, *Artemia* nauplius, and artificial diet (% lipid)

| Fatty acid | Carbon | *Brachionus** | *Artemia* nauplii* | Artificial | |
|---|---|---|---|---|---|
| | | | | Diets 1** | Diets 2** |
| Miristic | C14:0 | 2.26 | 1.70 | 3.34 | 3.04 |
| Pentadeconic | C15:0 | 0.12 | - | 0.33 | 0.28 |
| Palmitic | C16:0 | 9.38 | 12.20 | 14.57 | 12.40 |
| Stearic | C18:0 | 1.96 | 2.60 | 3.23 | 2.81 |
| Oleic | C18:1 | 2.69 | 30.7 | 14.80 | 12.53 |
| Linoleic | C18:2n6 | 1.52 | 9.30 | 8.24 | 8.71 |
| Linolenic | C18:3n3 | 0.40 | 3.30 | 0.05 | 0.07 |
| Arachidonic (ARA) | C20:4n6 | 1.40 | 4.60 | 0.241 | 0.39 |
| Eicosapentaenoic (EPA) | C20:5n3 | 4.39 | 6.50 | 3.76 | 4.08 |
| Docosahexaenoic (DHA) | C22:6n3 | 0.89 | - | 1.72 | 4.81 |

Description: *= Haryati (2002); **= analysis results in Integrated Laboratory Bogor Agriculture Institute

(1994 ) suggested that the optimum temperature in larval rearing of mud crab ranges from 24°C to 31°C. The salinity of the media during the study was 30 ppt which was well within the optimum salinity range suggested by Mardjono *et al*. (1994) of 30-35 ppt. The pH level for all treatments during the study was maintained at 7.5-8.0. According to Shelley *et al*. (2011) mud crabs should be maintained in media with a pH range of 7.5-8.5

## CONCLUSION

The present study suggested that weaning in larval rearing of *S. olivacea* can be done after zoea stage-3.

## ACKNOWLEDGEMENTS

The authors wish to thank the Hasanuddin University that has funded this research through the Research Fund BOPTN 2014.

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