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Research Article

Effects of Rotifer Feeding Frequency on Growth and Survival Rate of Early Larval Stages of Mud Crab, *Scylla olivacea*

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ABSTRACT

Crustacean larvae have the characteristics of living as carnivorous organisms. This has an impact on the length of gut evacuation time which affects the feeding pattern of the larvae. The feeding strategy of mud crab larvae is modulated by the length of gut evacuation time. Another factor that can be subject to modulation is the frequency of feeding. This research aimed to evaluate rotifer frequency feeding of early larvae stages of mud crab, *Scylla olivacea*. This research was conducted at the Mariculture Fisheries Center, Ambon with an indoor system. This research began with carrying out parental maintenance to produce larvae. Larvae are fed with rotifers with different frequencies. The treatments were the frequency of feeding rotifers, namely: Treatment 1 (F1) was the frequency of 1x a day (9:00 am); Treatment 2 (F2) was a frequency of 2x a day (9:00 am & 13:00 pm) and Treatment 3 (F3) was a frequency of 3x a day (9:00am, 13:00pm & 17:00pm). Each treatment was given 3 replications. Based on the results of this study, it showed that feeding with a frequency of three times a day (F3 treatment) was able to have a positive effect on the survival rate of zoea larvae which on the third day was 58% and the fourth day was 22% higher than the F1 and F2 treatments. The growth of larvae showed that F3 treatment was higher than F1 and F2 on the sixth day with an increase in the absolute value of growth of 0.12044 mm compared to F1 of 0.05531 mm and F2 of 0.03253 mm.

Keywords: frequency of feeding, growth, larvae, survival rate, mud crab

ABSTRAK

Larva krustase memiliki karakteristik hidup sebagai organisme karnivor. Hal ini berdampak terhadap lamanya waktu pengosongan lambung yang memengaruhi pola makan dari larva. Strategi makan dari larva kepiting bakau dimodulasi oleh lamanya waktu pengosongan lambung. Faktor lain yang menjadi subjek modulasi ini adalah frekuensi pemberian pakan. Tujuan dari penelitian adalah untuk mengevaluasi frekuensi pemberian pakan terhadap larva tahap awal kepiting bakau, *Scylla olivacea*. Penelitian ini dilaksanakan di Balai Budidaya Perikanan Ambon dengan menggunakan sistem indoor. Penelitian ini dimulai dengan melakukan pemeliharaan induk hingga menghasilkan larva. Larva kemudian diberi pakan berupa rotifer dengan frekuensi berbeda. Perlakuan pertama (F1) adalah frekuensi pemberian pakan 1x sehari (09.00); perlakuan kedua adalah frekuensi permberian pakan 2x sehari (09.00 dan 13.00) dan perlakuan ketiga adalah frekuensi pemberian pakan 3x sehari (09.00, 13.00 dan 17.00). Setiap perlakuan diulang sebanyak tiga kali. Hasil penelitian menunjukkan bahwa pemberian pakan 3x sehari (F3) berdampak positif terhadap tingkat kelangsungan hidup larva sebesar 58% pada hari ketiga dan 22% pada hari keempat dibandingkan dengan dua perlakuan lainnya (F1 dan F2). Pertumbuhan larva pada perlakuan 3 (F3) menunjukkan tren yang lebih tinggi dibandingkan F1 dan F2 pada hari kelima, dengan nilai absolut 0,12044 dibandingkan dengan perlakuan F1 sebesar 0,05531 mm dan F2 sebesar 0,03253 mm.

Kata kunci: Frekuensi pemberian pakan, pertumbuhan, larva, tingkat kelangsungan hidup, kepiting bakau

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1. Introduction

Mud crab, *Scylla sp* is one of the important economic value commodities in the Indo-Pacific region (Viswanathan and Raffi, 2015). The utilization of mud crab commodities is carried out through export for both domestic and foreign communities (Bhuiyan et al., 2021). Most of the female crabs caught were in the reproductive phase with various gonad maturity stages (Makatita, 2012; Ayhuwan, 2013). If this condition continues it will reduce the number of new individuals produced, which will have an impact on the possibility of reducing the recruitment of new individuals. So that if exploitation is carried out continuously through intensive fishing efforts by relying solely on nature, this will certainly have a negative impact on decreasing the stock of mud crab resources. To overcome this, it is necessary to carry out cultivation that is able to answer the totality of meeting needs and stock availability. Of course, the hatchery is to get larvae of good quality and able to survive through each stage of development.

Generally, mud crab larvae have the highest sensitivity so that it can lead to mass mortality in early zoea stages (Davis et al., 2004). The problem faced by crab cultivated related to the hatchery is that the survival rate is low (Maharani dan Zafran, 2005). The average survival rate of mud crab larvae in the world is 3% (Misbah, 2020). Furthermore, it is assumed that there are several factors that greatly influence the mass mortality that occurs in the larval stages of zoea and feed are one of those factors. Mass mortality usually occurs during the maintenance of zoea 2 – zoea 3 and the cause of mass death of zoea crab larvae are thought to be one of them is feeding management so that it is a limiting factor (Jantrarotai et al., 2005). Under normal conditions in nature, the diversity of live food (zooplankton) is available in sufficient quantities and can be utilized by each trophic level efficiently. However, under controlled conditions (hatchery environment), the availa-bility of feed is highly dependent on human control (Anonim, 2002). Feeding with a good and appropriate frequency will affect the survival rate of larvae, especially in the early stages which are the critical stages of larvae.

The provision of adequate nutrition will provide a high survival rate. Furthermore, feeds containing n-3 HUFA (Highly Unsaturated Fatty Acids) such as *20:5n-3* (eicosapentaenoic acid) and *22:6n-3* (docosahexaenoic acid) are essential fatty acids for marine fish larvae and crustaceans in the form of zooplankton, namely rotifers, although the nutritional content of essential fatty acids in rotifers is quite low at 0.73% (Susanto et al., 2004).

Crustacean larvae in the early stages are carnivorous in general have a longer pattern of gut evacuation time (Le Vay et al., 2001). The feeding strategy, especially in early-stage mud crab larvae, is modulated by the length of the gut evacuation time which is clearly responded to by the presence of digestible feed (Serrano, 2012). Another factor that can be subject to modulation is the frequency of feeding. Thus, this study was conducted to evaluate the frequency of feeding rotifers on the growth and survival rate of mud crab larvae, *S. olivacea*.

2. Material and methods

This research was conducted at the Mariculture Fisheries Center, Ambon with an indoor system. The tools used in the form of a plastic box 26 liters, aeration equipment, 100 mL glass beaker, thermometer, microtube (Eppendorf) 1.5 mL, pipette, pipette microproline Labasco 200-1000 mL, microscopes olympus BX51, sedgewick rafter, tissue embedding console tissue-tek, rotary microtome, waterbath, hotplate, automatic slide stainer. The materials used include the broodstock and the larvae of *S. olivacea*, rotifer, sterile seawater, and 4% formalin solution, alcohol.

Broodstock Rearing

This study began by performing maintenance on the 26 L plastic box with a base substrate of sand. The sand was replaced every four days. Female crab was maintained by feeding 6% of body weight with a larger composition namely calamari squid: fish: carrots (3: 2: 1). The feed was given once daily at 7 pm.

Larvae Rearing

The zoea larvae were reared given the feed rotifers. Rotifers were prepared the day before the larvae hatch. Rotifers were derived from the stock culture Waiheru Mariculture Center, Ambon. Rotifers were fed with phytoplankton of the type *Chlorella* sp. Larvae were successfully hatched crab female left for 15 minutes and then further sampling as many as 750 individuals to each container of treatment. The number of rotifers given to larvae was 50 ind./larvae. Water quality was measured in the morning, afternoon, and evening with a range of between 27-31°C. In this study, the parameters used as treatment were the frequency of feeding rotifers, namely: Treatment 1 (F1) was the frequency of 1x a day (9:00 am); Treatment 2 (F2) is 2x daily frequency (09.00 & 13.00) and Treatment 3 (F3) is the frequency of 3 times a day (at 09.00, 13.00 & 17.00). Each treatment was given a repeat 3 times.

Survival Rate Measurement

Zoea larvae were reared and daily survival rates were calculated and at the same time, five (5) individuals were sampled from each replicate of each treatment for measurement of total body length. Histology test was used to observe the body tissue and digestive tract, if possible, from samples treated larvae feeding frequency once a day and three times a day as a comparison conducted in Laboratorium Zoology, Faculty of Science, Pattimura University. The survival rate of mud crab larvae, *S. olivacea* was calculated using the following formula (Oniam et al., 2015):

$$SR\ (\%) = \left(\frac{N_t}{N_0}\right) \times 100$$

Where, SR is the survival rate of mud crab larvae, N_t is the number of larvae of mud crab that lived until the end of the experiment, N_o = Number of larvae of mud crab at the beginning of the experiment.

Larvae Mortality Measurement

The daily larval survival rate was determined using the formula as described, wherein this data was then used to calculate the daily larval mortality rate using the following formula (Welch and Epifanio, 1995):

$$\ln N_t = \ln N_0 - Z.t$$

Where, N_t is the average value of surviving larvae in each rearing box on day t, N_0 is the number of densely stocked early larvae, Z is the mortality coefficient, t = rearing period (day)

Absolute Growth Rate Measurement

Growth parameters of larvae body length are one of the indicators were observed. This parameter will be analyzed using the absolute length growth formula as follows (Khasanah et al., 2012):

$$L = L_t - L_0$$

Where, L is the growth in absolute length (mm), L_t is the average length of larvae at the end of observation (mm), L_0 is the average length of larvae at the beginning of the observation (mm)

Measurement of total body length of larvae was used to measure the growth of *S. olivacea* larvae. The total length of which is measured by pulling the line from the leading end of the carapace (the base of the dorsal spine) to the end at the outer end of the telson.

Spesific Growth Rate Measurement

The specific growth rate is calculate based on the length of larvae (Sari et al., 2015). The formula to calculate the instantaneous growth rate of mud crab larvae as follows:

$$SGR(\%) = \frac{\ln l_t - \ln l_o}{t} \times 100$$

Where, SGR is the Spesific Growth Rate (%), L_t is the average length of larvae at the end of observation (mm), L_0 is the average length of larvae at the beginning of the observation (mm)

Histology Method

The analysis of larval histology was used the operational procedure standard of Zoology Laboratory, Faculty of Mathematics and Natural Science. It is used the paraffin method to obtain the histology sample. Larvae were fixed by 4% formalin solution for 2 x 24 hours. The fixation was continued on alcohol solution with different concentration i.a. 30%, 45%, 50%, 65%, 75%, 85%, 90%, 96% and absolute for 1.5 hours interval then it was immersed in xylol 1, xylol 2 and xylol 3 for 1.5 hours. After the fixation steps, the sample was dehydrated with paraffin 1, paraffin 2, and paraffin 3 for 1 hour.

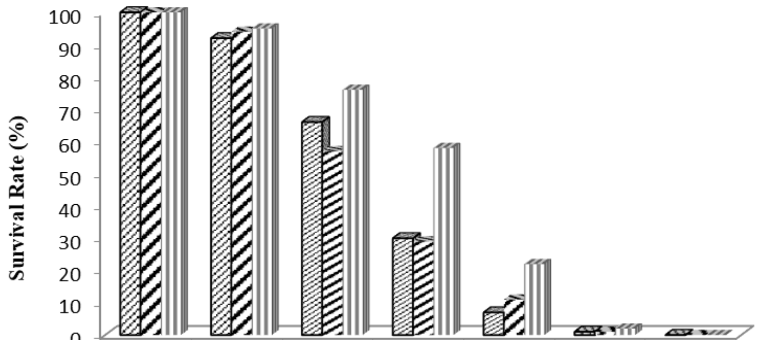
Afterward, the sample is blocked and left for 1 day. The blocked sample was cut with a rotary microtome. It was cut 3 microns thick. In the next step, the sample was stained with Hematoxylin-Eosin (HE). This process was done by slide stainer. The section was observed with Olympus Microscope BX51.

Data Analysis

The data was analyzed using one-way ANOVA analysis with 0.05 significance level. The data was processed using the MS-Excel office program. This analysis was used to see the effect of the frequency of feeding different rotifers on the survival rate and larval growth, especially in the early stages of *S. olivacea*.

3. Results and Discussion

Research shows that the survival rate of the *S. olivacea* larvae from the three different feeding frequency treatments rotifer ended on day 6 as presented in Figure 1. In fact, this figure showed a tendency difference in the survival rate of larvae of *S. olivacea*. Treatment frequency of feeding rotifers one and two times (F1 and F2) decreased



	0	1	2	3	4	5	6
F1	100	92	66	30	7	1	0
F2	100	94	57	29	11	1	0
F3	100	95	76	58	22	2	0

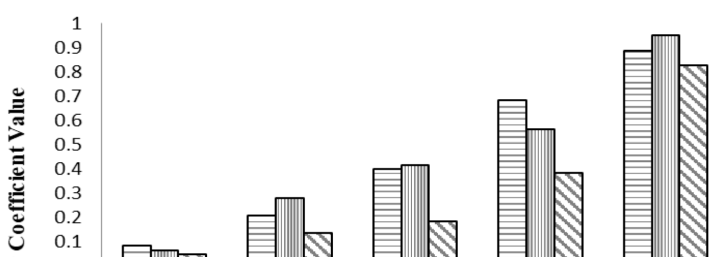
Rearing Time (days)

Figure 1. Survival Rate of Mud Crab Larvae, *Scylla olivacea* (F1: feeding frequency 1x, F2: feeding frequency 2x, F3: feeding frequency 3x)

the survival rate of larvae that are different treatment rotifer feeding three times (F3). For the F1 treatment, the percentage of larval survival on the first day of 92% decreased on the second day by 66%, then on the third and fourth days, respectively, by 30% and 7%. For the treatment of F2, the percentage survival rate of larvae on the first day by 94%, decreased on the second day by 57%, and the third and fourth days respectively by 29% and 11%. The treatment of F3 shows the difference in the survival rate of larvae is much higher especially, and on the third day of the fourth day 58% and 22%.

Based on the results of this study, it was shown that feeding with a frequency of three times a day (F3 treatment) was able to have a positive effect on the survival rate of larvae. ANOVA test results showed no difference between the three treatments (P>0.05). This means that there was no significant effect between the three treatments of different feeding frequencies in a day on the survival rate of *S. olivacea* larvae.

The survival rate of *S. olivacea* larvae is proportional to the daily mortality coefficient value obtained during the study period as shown in Figure 2. The mortality coefficient value of the mud crab larvae, S. olivacea which received different feeding frequency treatments, shows the differences that appear in the treatment. F3 with a lower mortality coefficient than the F1 and F2 treatments. This suggests that feeding with a frequency of three times a day can reduce the degree of mortality.



	1	2	3	4	5
Z-F1	0.08096	0.20776	0.39837	0.68206	0.88457
Z-F2	0.06141	0.27969	0.4139	0.56266	0.94965
Z-F3	0.04825	0.13605	0.18183	0.38108	0.82703

Rearing Time (Days)

Figure 2. Mortality Coefficient Value of *S. olivacea* Larvae (F1: feeding frequency 1x, F2: feeding frequency 2x, F3: feeding frequency 3x)

For the growth of mud crab larvae, there appears to be a difference between each treatment of the frequency of feeding on the growth of *S. olivacea* larvae during the rearing period. This shows that in fact there is a difference in larval growth, especially in the treatment of the frequency of feeding the rotifer three times (F3) which shows a tendency for the growth of the larvae to be higher when compared to the growth of the larvae in the treatment with the frequency of feeding the rotifers one and two times (F1 and F2) as presented in Figure 3.

The absolute growth value of F1 treatment on the first day is 0.01332 mm until the fourth day is 0.02518 mm. There is an increasing trend on the chart that the absolute value is 0.047266 mm on the fifth day and 0.05531 mm on the sixth day (Figure 3). It is seen that there is increasing however it is not much different. The specific growth rate of F1 treatment on the first day is 2.73% and the percentage increase in the daily growth value of larvae on the next days is not significant and it is 1% respectively (Figure 4). Otherwise, the absolute growth value of F2 treatment on the first day to the fourth day is not much different even on the fourth day to the sixth day is a small relative (0.03133 mm to 0.03253 mm). The percentage increase in the daily growth value of larvae is not significant. The instantaneous growth rate of larvae is in 1% range.

The absolute growth of the F3 treatment is a slightly different trend from the F1 dan F2 treatment. The absolute value on the first day is

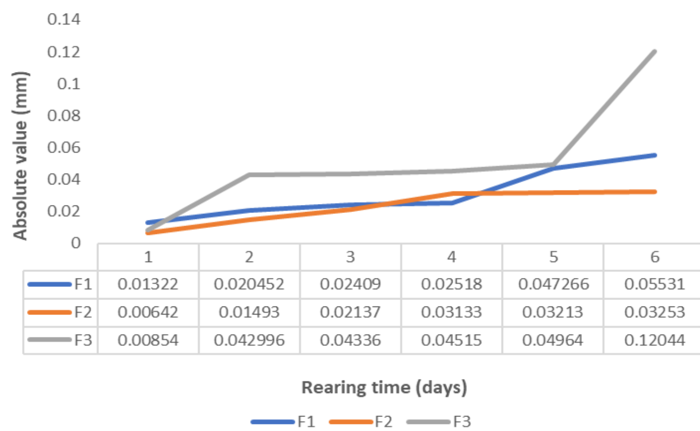


Figure 3. Absolute Growth of Mud Crab Larvae, *Scylla olivacea* (F1: feeding frequency 1x, F2: feeding frequency 2x, F3: feeding frequency 3x)

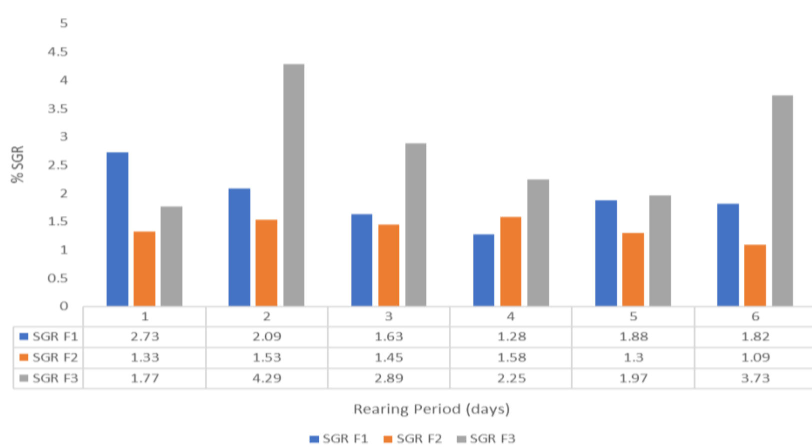


Figure 4. The Specific Growth Rate of Mud Crab Larvae (F1: feeding frequency 1x, F2: feeding frequency 2x, F3: feeding frequency 3x)

0.00854. The top of an upward trend is seen on the second day of 0.042996 mm and the sixth day of 0.12044 mm. The percentage of larval daily growth is increased on the second day by 4.29% and the sixth day by 3.73% (Figure 4) however, the results of the ANOVA test showed that there was no difference (P>0.05) from the frequency of feeding treatment on the growth of *S. olivacea* larvae.

The results of observations during the study showed that the frequency of feeding rotifers had an effect on the percentage of survival and the average absolute growth of mud crab larvae, *S. olivacea,* although statistically there was no difference. Survival rates and growth are two things that are interrelated with one another. Through the results of this study, the results showed that larvae were given the frequency of feeding rotifers three times a day (F3) had a higher percentage of larval survival or survival rates than the other two treatments, namely F1 and F2, and corresponded to the mortality coefficient value. The mortality coefficient value of the F3 treatment was lower than the other two treatments. This means that through feeding three times, the survival rate of the larvae increases so that the mortality of the larvae is reduced. This is thought to occur because the rotifer is consumed three times every four-hour interval, and is supported by the high prey ability of the rotifer so that the available energy obtained can be used to meet the needs of larvae in the early stages. The availability of a sufficient amount of feed helps the larvae to survive

The factor that influences the level of survival of larvae is the availability of feedstocks (Castine et al., 2008; Ikhwanuddin et al., 2012). In addition, the increased survival of mud crab larvae is also due to an increase in the rate of larval predation on prey (Widodo et al., 2010). The rate of predation itself is influenced by the presence of food for prey, as Welch and Epifanio (1995) stated that the decapod crustacean larvae itself are a type of encounter feeder that is highly dependent on encounters with prey.

In addition, the preying ability of crab larvae is also supported by the suitability of the feed with the size of the larva's mouth opening. The size of the mouth opening of the larvae is estimated to be 0.1 mm (Widodo et al., 2010), while the size of the rotifers is in the range of 150-180 µm (Susanto et al., 2004). In this study, the size of the rotifer used as feed for larvae through the sieve was 100 µm.

Factually, feeding with a frequency of three times can meet the supply of energy needed by larvae, especially in the early stage which is a critical stage, when compared to the frequency of one and two times a day, considering that crab larvae have lecithotrophic properties (Millamenia and Bangcaya, 2001) and planktotrophic (Al-Aidaroos, 2013). Therefore, sufficient energy is needed, as soon as the larvae hatch to maintain the stability of their bodies in order to be able to defend themselves and to prevent gaps from occurring as a result of the process of shifting the use of food from egg yolks (endogenous feeding) to the process of taking food from outside (exogenous feeding) (Widodo et al., 2010). The energy needs for the maintenance of the body must be met first and if it is in excess it will be used for growth (Purba, 2012). If this gap occurs in the rearing system, it will interfere with the larvae themselves physiologically and can result in high mortality as in larvae fed with a frequency of only one and two times a day.

In connection with that, the aspect of providing energy also guarantees the need for *S. olivacea* mud crab larvae, so that it has a relationship with the length of digestion time which zoea larvae can digest feed with an optimal ratio for one hour (Nghia et al., 2007). The length of time for taking food in the stomach of *Scylla serrata* larvae was 80 minutes or 1 hour 20 minutes (Serrano, 2012).

Through radiotracer studies, early-stage *Scylla serrata* larvae have a shorter duration of food present in the intestine, the same is true for trout larvae (Genodepa et al., 2006). This means that the early-stage larvae have a faster gut evacuation time. The evacuation of feed in the intestines of catfish larvae (*Clarias gariepinus)* took place more quickly during continuous feeding than discontinuous feeding (Garcia-Ortega et al., 2010). In this regard, the results of this study can be presumed that feeding with a frequency of three times every four hours can approach the length of time required to take feed naturally. This indicates that the uninterrupted feeding activity of crab larvae during the early zoea phase will make a good contribution to the physiological larvae to be able to maintain their survival, development, and growth.

Figure 5a shows the histological slice larval body mud crab *S. olivacea*. The digestive system of the mud crab *S. olivacea* at the zoea stage (zoea1 – zoea5) can be divided into three parts foregut, midgut, and hindgut. The foregut consists of the mouth, esophagus, and stomach. The midgut consists of the intestines, the anterior midgut caeca, the posterior midgut caeca, and the midgut or hepatopancreas gland. Both the anterior and posterior midgut caeca were not found in the early-stage larvae of *S. olivacea* zoea

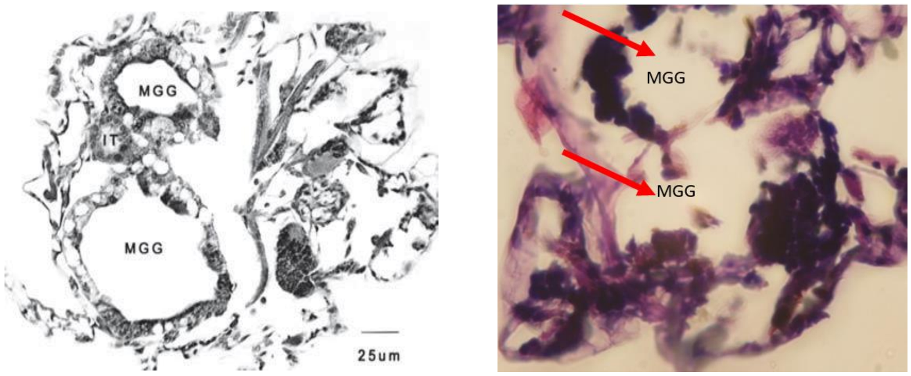


Figure 5. Comparison of histological cross-section photo zoea first larval stage mud crab *Scylla olivacea*. MGG: Midgut gland (Source: a. Jantrarotai et al., (2005); b. research documentation using 100x magnification)

(zoea 1 and 2), but from the third to the fifth zoea these structures developed. Midgut glands can be found throughout the developmental zoea (zoea 1-zoea 5) (Jantrarotai et al., 2005). The same thing was seen in this study, namely the histological results of stage I zoea larvae of S. olivacea, which showed that the midgut structure was still simple (Figure 5b).

From the study of the digestive histology of *S. olivacea* larvae, it was found that the entire digestive system of the third zoea larvae was fully developed (Jantrarotai et al., 2005). Therefore, high energy is required for larval development. As a result, the energy remaining for molting may be insufficient causing high mortality during the development of zoea larvae from the second stage to the third stage of zoea. Therefore, the live feed is absolutely necessary during the early stages of the zoea period because it contains complete nutrients including free amino acids. In addition, the live feed can undergo autolysis in the rudimentary digestive tract. However, in a later stage (zoea 3- zoea 5), more suitable artificial feed (artificial plankton) can be used to complement or replace live feed because the digestive system of *S. olivacea* larvae is fully developed. Furthermore, it was also explained that for larvae that were given natural food and artificial feed, there was a difference in the process of evacuating food in the stomach, namely that larvae given artificial food took longer to digest and take in the intestines compared to larvae given natural food (Serrano, 2012).

Generally, the size of the larvae is usually represented by the length of the body of the larva itself (Chen et al., 2011). The length of nauplius can be a promising approach because the increase in length will correspond to its development and can be an indicator of the development of each sub-stage of nauplii in particular (Racotta et al., 2003). This is in accordance with the graph shown in Figure 4, which shows that there is a tendency to increase, especially for the frequency of feeding rotifers three times (F3). There were two peaks of increase in larval growth, especially on the second and sixth days. This is thought to occur due to a developmental process, namely a change from the stage of zoea 1-zoea 2 and then zoea 2-zoea 3. As is known, the growth of crustaceans is known as discontinuous growth which is characterized by changes in body mass that increase after a process known as molting occurs. the transition from one stage to the next life stage through the replacement of the old carapace (exsuvi). Usually, the development process from one stage to the next is also followed by morphological changes.

The difference between zoea 1 and zoea 2 is eye development, where zoea 1 has attached eyes and zoea 2 has stalked eyes (Pattirane and Pattiasina, 2020). Furthermore, the difference between zoea 2 and zoea 3 is the "seta" in the six maxilliped parts (Madhu and Madhu, 2008). These organs are vital parts that function as supports in the early stages of larvae to help obtain food. The decapod crustacean larvae itself is a type of encounter feeder that is very dependent on encounters with prey (prey) so that with eye conditions that are attached and not working properly, the help of other vital organs is needed, namely the antenna and maxilliped (Welch and Epifanio, 1995).

Antenna organs function to detect environmental chemical conditions (chemoreceptors) to determine the presence of prey and then it will be captured and destroyed using a maxilliped (Hui, 2006). On the other hand, when the eye is on the stem, it is assumed that the eye function has been running well so that the ability to prey is better and this has a good impact on the energy supply and growth of *S. olivacea* larvae.

The graph of the growth of *S. olivacea* larvae given F3 treatment turned out to have a better tendency than F1 and F2 treatments. This is presumably not only because of the feeding characteristics and characteristics of the larvae but also because of the role of nutrients and enzymes in rotifer feed which can support the larvae to obtain energy in accordance with the morphological conditions of the early-stage larvae. The feed given to the larvae of *S. olivacea* was rotifer from BPBL culture which was fed with baker's yeast, then given *Chlorella* sp. Rotifer feed given yeast has a low nutritional value with a HUFA (Highly Unsaturated Fatty Acids) content of 1.3% (Melianawati et al., 2006). But on the other hand, indirectly, *Chlorella* sp with a HUFA (Highly Unsaturated Fatty Acids) content of 29% can boost the nutritional value of rotifers so that it can contribute to larval growth, especially in the early stages (Chilmawati and Suminto, 2010).

Thus, presumably through feeding with a frequency of three times a day every four-hour interval to help keep early larval stage can get the nutrients in order to accumulate all the energy needed for growth. In addition to the nutritional content possessed by rotifers to support larval life, it is suspected that there is a role for endogenous and exogenous enzyme content for the larvae themselves in conformity with the simple morphological structure of the stomach, especially for early-stage larvae.

The ability of crustacean larvae to produce digestive enzymes is closely related to the development of the digestive tract (Serrano, 2012). The digestive process itself occurs in the foregut with enzymes produced by the digestive glands (tubular appendage) and formed by the digestive tubules (Hui, 2006). Some of the food is digested and then passes through the foregut to the midgut which is the center of enzyme activity and nutrient absorption and the end of the digestive process.

The type of feed can affect the pattern of digestive enzyme activity of mud crab larvae (Serrano and Traifalgar, 2012). This is because the development of the early stages of larvae is highly dependent on the presence of live feed as a contribution to the presence of exogenous enzymes to aid digestion which will be released into the larval digestive tract through zymogen autolysis to activate endogenous enzymes in the larval stomach (Serrano, 2012).

Mud crab larvae, *Scylla serrata* fed live feed showed high endopeptidase and exopeptidase enzyme activity (Serrano & Traifalgar, 2012). Endopeptidase (trypsin) and exopeptidase (LAP) enzymes are a group in the protease enzyme group that is responsible for digesting protein so that it is easily absorbed in the form of amino acids (Fujaya, 2004). High enzyme activity and supported by the fast rate of digestion and transport of feed in the stomach of the larvae are indicators that are assumed to increase the growth of mud crab larvae. So it is suspected that this can also be experienced by the mud crab *S. olivacea* in this study.

4. Conclusions

It can be concluded that a higher survival rate of mud crab larvae, *S. olivacea* in the early stages was obtained by feeding rotifers with a frequency of three times (F3) every four hours. The larval growth of the early stages of mud crab *S. olivacea* through feeding rotifers with a frequency of three times also increased. It is hoped that there will be further research on feeding with a frequency of more than three times to see how far the survival rate and growth of mud crab larvae are to pass the critical stage and enter the next zoea stage.

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