Crablet of Mud Crab *Scylla Olivacea* Production from the Different Stages of Larvae fed *Artemia* Nauplii Enriched Using *Nannochloropsis* sp

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Abstract: Improvement of feed quality for mangrove crab larvae rearing is one of the important factors to increase of crablet production. The aim of the research was to know the influenzing of enriched *Artemia* nauplii using *Nannochloropsis* sp fed to the different stages of larvae *Scylla olivacea* on crablet production. Twelve units of tanks volume 250 L filled with saline water salinity 30 ppt, aerated, then stocked with new hatched mud crab larvae zoea-1 at the density 100 ind./L. The larvae zoea-1 were fed rotifer, *Brachionus* sp. and after zoea-3, beside fed by rotifer, the larvae were also fed by *Artemia* naupli. Four treatments were tested, namely: A). *Artemia* nauplii enriched using *Nannochloropsis* sp. was given to the larvae zoea-3 until develop to megalop stage. B). *Artemia* nauplii enriched using *Nannochloropsis* sp was given to the larvae zoea-4 until develop to megalop stage. C). *Artemia* nauplii enriched using *Nannochloropsis* sp was given to the larvae zoea-5 stage until develop to megalop stage. D). *Artemia* nauplii without enriched using *Nannochloropsis* sp. was given to the larvae zoea-3 until develop to megalop stage. Result of the research showed that the highest of Larvae Development Indexes and Megalop Occurence Indexes was obtained in treatment A and resulted the highest of crablet production, then followed by treatment C and B and those were significantly different ($P<0.05$) with the crablet production in treatment D. The use of Artemia nauplii enriched by *Nannochloropsis* sp. to feed larvae, *S. olivacea* stage zoea-3 to zoea-5 until develop to the megalop stage is one of the key factor to enhance the crablet production. By this finding, the crablet production in hatchery will be increased and the mud crab culture in brackishwater pond able to developed.

Keywords: *Scylla olivacea;crablet; Nannochloropsis sp: Artemia; enriched*

1. Introduction

Mud crab commonly also called mangrove crab is an important source of income for small scale coastal rural people in some part area of Indonesia. There are recognized any four species of mangrove crab namely; *Scylla serrata, S. transquebarica, S. olivacea* and *S. paramamosain* (Keenan *et al.,* 1998). *S. olivacea* is dominant species of mangrove crab in South Sulawesi waters, Indonesia (Herlinah and Septiningsih, 2015). Due to high economic value of the mud crab, it has been impacted to the high suppresing of that mangrove crab in the wild and the previous study was indicated an overexploitation of mangrove crab, *S. olivacea* in the mouth river of Cenranae, Bone Regency, South Sulawesi, Indonesia (Gunarto *et al.*, 1999). To minimize an overexploitation, the mangrove crab culture in the brackishwater pond where the seed from hatchery could be developed. Earlier research on mangrove crab seed production was reported in some countries such as Taiwan (Chen and Cheng, 1985); Phillipphina (Quinitio *et al*, 2001); Japan (Hamasaki *et al*, 2002), Malaysia (Anuar *et al*, 2011) and Indonesia (Yunus *et al*, 1997). However, crablet production in hatchery is still low. Karim (2006) and Suprayudi *et al*. (2012) reported that the lower of mangrove crab production was caused by the low of feed quality. An improving of feed quality is very important and crucial to be conducted to enhancing mangrove crab larvae viability. Bioencapsulated of rotifer as well as *Artemia* nauplii using fatty acid such High Unsaturated Fatty Acid (HUFA) was recomended before they were fed to the mangrove crab larvae. It was due to EPA (*Eicosa Pentaenoic Acid*) and DHA (*Docosa Hexaenoic Acid*) content in rotifer, *Brachionus plicatilis* and *Artemia* nauplii relatively low (Yousefian and Najafpour, 2011; Adloo *et al.*, 2012).

To improve EPA and DHA content in rotifer and *Artemia* naupli, beside enriched using HUFA, they can be also enriched using algae, such *Chlorella* spp (Truong *et al*., 2007b). Shigeki and Hamasaki (2012) reported that rotifer enriched using HUFA and fed to the *S. serrata* larvae is able to more enhanced larvae development. However, mortality syndrome often occured in molted larvae (Hamasali *et al*., 2002a). Ferreira *et al*. (2009) stated that rotifer fed sufficient of *Nannochloropsis gaditana* would twofold increase of protein, lipid and carbohydrate content. The DHA/EPA ratio also increase from 0.063 in rotifer without enriched with *Nannochloropsis* sp to 0.147 in rotifer enriched with *Nannochloropsis* sp (Gunarto and Herlinah, 2013). Whereas Gunarto and Herlinah (2015) reported that rotifer was enriched using HUFA the DHA/EPA ratio was increased 69.23%, while *Artemia* nauplii enriched with HUFA the DHA/EPA ratio was increased only 28.72%. Enhancing of DHA/EPA ratio in rotifer and artemia is very important to increasing the succeed of larvae zoea stage develop to the next stage (Truong *et al.*, 2007a).

Nannochloropsis sp is one of the best phytoplankton as a feed for rotifer, *Brachionus* sp. Jean and Hur (2011), stated that the content of fatty acid 22: 6n-3, DHA and 20 5n-3, EPA in *Nannochloropsis* sp was 0,29% and 34,88%, respectively. *Nannochloropsis* sp concentrated or alive was used to enriched rotifer and *Artemia* nauplii (Matthew *et al*., 2006) and fed to fish/crustacean larvae would improve larvae vitality, it was because of EPA dan DHA content in rotifer as well as in *Artemia* nauplii were increased (Okauchi, 2004).

The mangrove crab stages consist of five level of zoea stage, namely zoea-1, zoea-2, zoea-3, zoea-4 and zoea-5, then megalop stage and lasted is crablet stage. In what level of zoea stage the most effective to the larvae were started be given fed *Artemia* nauplii enriched by *Nannochloropsis* sp in order to enhancing crablet production. The aim of the research was to find out the effect of enriched *Artemia* nauplii using *Nannochloropsis* sp fed to the different stage of larvae *S. olivacea* on crablet production.

2. Materials and Method

Research was conducted during January to February 2016 in wet laboratory at Station of Research Institute for Coastal Aquaculture at Marana, Maros - Indonesia. Twelve units of conical fiberglass tanks volume 250 L was filled with 200 L of sterile sea water salinity 30 ppt with aeration system. The healthy new hatched of mangrove crab larvae were stocked in those tanks at the density of 100 ind./L. Water temperature and dissolved oxygen in the tanks was 29-30^{o}C and 7-8.5 mg/L respectively.

Rotifer were given to the larvae zoea-1 to zoea-3 as feed at the density of 20 ind./mL. After larvae develop to the zoea-3, the treatment of feeding using *Artemia* nauplii enriched with *Nannochloropsis* sp was started. Before treatment running out, the *Artemia* cyst were hatched earlier in 50 L of conical fiber tank filled with 40 L of sea water salinity 28 ppt and it was given highly aerated. LED lamp 6 watt was set up near surface water in that tank to accelerate *Artemia* nauplii hatched. After 24 hours aerated, *Artemia* nauplii was hatched, then harvested, rinsed with the saline water, and stocked at the density of 500 ind./mL in the basin volume 5 L filled with 2 L of sea water salinity 28 ppt and aerated. Two liter of *Nannochloropsis* sp from the 5 L of *Nannochloropsis* sp culture stock in laboratory was added to *Artemia* nauplii in the basin to enrichment. The *Nannochloropsis* sp density was 636 x10^4 cell/mL. The enrichment was conducted during 5 hours, then *Artemia* nauplii was harvested and fed to the different stages of mangrove crab, *S. olivacea* larvae, such it was discribed below as the treatment in this research :

a). *Artemia* nauplii enriched using *Nannochloropsis* sp at the density 636 x10^4 cell/mL was given to the larvae zoea-3 stage until larvae develop to megalop stage.

b). *Artemia* nauplii enriched using *Nannochloropsis* sp at the density 636 x10^4 cell/mL was given to the larvae zoea-4 stage until larvae develop to megalop stage.

c). *Artemia* nauplii enriched using *Nannochloropsis* sp at the density 636 x10^4 cell/mL was given to the larvae zoea-5 stage until larvae develop to megalop stage.

d). *Artemia* nauplii unenriched using *Nannochloropsis* sp, was given to the larvae zoea-3 until larvae develop to megalop stage.

Each treatment was three replications. *Artemia* nauplii were given to the zoea-3 stage larvae at the density of 3 ind./mL and it increased to 5 ind./mL at megalop stage. The feeding regime for the larvae was showed in Table 1.

Table 1. Feeding regimes for mangrove crab, *S. olivacea* larvae

Stadium	Feeding frequency	Rotifer density (ind./mL)	*Artemia* nauplii density (ind/mL)
Zoea-1	1	20	-
Zoea-2	1	20	-
Zoea-3	1	20	3,0
Zoea-4	1	10	4,0
Zoea-5	1	10	4,0
Megalopa	1	-	5,0

Addition of sea water in the larvae rearing tanks was conducted after 5 days larvae rearing at about 10% of total water volume and exchanged water conducted after 8 days of culture at the 10% of total water volume. However, after zoea-5, the exchanged water increase to about 50% of total water volume and conducted at every two days. Larvae population from zoea-1 to zoea-5 were monitored using chamber volume 50 mL for zoea-1 to zoea-3 and volume 250 mL for zoea-4 and zoea-5 by inserted that chamber to the different places of surface water in the tank. The larvae included in the water were counted manually. Larvae density were calculated in ind./L. Whereas, larva development were monitored by sampled of 20 ind. of larvae in each replication of each treatment, then the status of larvae stage was also identified.

2.1 Larvae Development Index

The Larvae Development Index (LDI) is the value which described the expression of the fast of larvae development in the culture rearing tank. The LDI was calculated by the score to the each stage of larvae such zoea-1 = 1, zoea-2 = 2 , zoea-3 =3 .... to megalop = 6 (Truong *et al*., 2007b). In example of 20 ind. consisted of 5 ind. was zoea-3, 8 ind. was zoea-4 and 7 ind. was zoea-5, that mean the LDI= (5x3+8x4+7x5) /20 = 4,1

2.2 Megalop Occurence Indexes

At the day of culture (DOC) 20 larvae started metamorphosis to the megalop stage. Megalops population were monitored by calculated Megalop Occurence Index (MOI). MOI is the value which described the expression a numbers of megalops stage available in the culture tank. MOI was determined by calculated the occurence of megalops at determined numbers of larvae zoea-5 stage. In example from 100 individuals of larvae were counted only 5 individuals of megalops included, then the MOI value was 0.05. The MOI value would increase during 2-4 days, when the zoea-5 gradually metamorphosis to the megalop stage, then the MOI value decreased after megalop lay down in the bottom of the tank to metamorphose to the crablet instar 1.

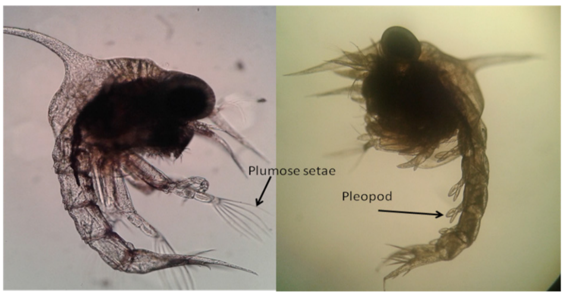
2.3. Water Quality and Total Vibrio sp Monitoring

Sampled of 300 mL of water was taken from the conical fiber tank of each replication in each treatment at the day 10 and 20 then brought to the laboratory to analyze water quality parameters such as ammonium, nitrite and Total Organic Matter (TOM) and 50 mL of water sample was also taken using dark, sterile bottle sample in each treatment to analyze of total *Vibrio* sp. To know the effect of the treatment to the larvae population, megalop survival and crablet production, the data obtained were compared and tested usinqg Complete Randomized Design Analysis, and following by LSD test to know which treatment was significant different each others. The SPSS (*Statistical Product Service Solution)* program was used to help an analysis for those data.

3. Results and Discussion

3.1 Larvae Development

The fast of mangrove crab larvae development was determined by the health of larvae, feed quality, quantity and suitability of environmental parametric for larvae life and development. The DHA and EPA content in the feed influenced to the larvae development and survival rate at the late stage (Ribeiro and Jones, 2000: Hamasaki *et al.*, 2002; Davis, 2004). Truong *et al*. (2007a) reported that the high ratio of DHA/EPA in rotifer and *Artemia* nauplii is better to accelerate the development of *S. paramamosain* larvae. The different stage of larvae was determined by total number of plumose setae and pleopod performance in the larvae (Figure 1).



A **B**

Figure 1. Larvae zoea-2 with 6 plumose setaes (A) and larvae zoea-5 with long of pleopod (B)

The present study showed that development of larvae from zoea-1 with 4 plumose setaes to the zoea-2 stage with six of plumose setae was required 6 days. The zoea-2 stage to zoea-3 stage was required 4 days. At the zoea-3 stage with 8 plumose setaes develop to zoea-4 was required 3 days. Zoea-4 stage was indicated with bud of pleopod showing in the abdomen and developed to zoea-5 was required 4 days. Zoea-5 developed to megalop stage was required 5 days. While megalop develop to the crablet stage need 7 days. The complete *S. olivacea* larvae development to the crablet stage was required about 29 days (Table 2). It seemed to be longer than the *S. transquebarica* in India that it was reported only required 22 days to completed larvae develop to the crablet stage (Thirunavukkarasu *et al.,* 2014). Hamasaki (2003) reported between 21-42 days for *S. serrata* larvae complete develop to the crab stage when rearing at water temperature 23-32°C. The different species also different water temperature of rearing, feeding schedule and dosage may responsible to the different time required to complete larvae development to the crablet stage. The larvae of *S. transquebarica* fed *Brachionus rotundiformis* and *Artemia* nauplii at libitum twice per day (Thirunavukkarasu *et al.,* 2014), while in this research the *S. olivacea* larvae only fed *Brachionus* sp at the density of 20 ind./mL and *Artemia* nauplii at the density 3-4 ind./mL and it was given at once per day. This feeding schedule may resulted longer development the larvae in this research. When the larvae zoea-5 fed artemia naupli only at the density 0.5 ind./mL resulted more larvae mortality caused by canibalisms (Suprayudi *et al*., 2002).

In this research the zoea-1 were stocked in the conical fiberglass tank at the density of 400 ind./L. The larvae density of zoea-2 stage was dropped to the 360-366 ind./L. The decreasing of the larvae population continues up to the zoea-3 stage, where their population was 343.0±15.6 ind./L (A), 320.3±17.6 ind./L (B), 334.01±1.7 ind./L (C) and 321.03±7.15 ind./L (D). The zoea-4 stage started obtained at the day 11, where at the day 14 the larvae population was dropped to 161.7±38.9 ind./L (A), 144.0±34.2 ind/L (B), 172.0±13.0 ind./L (C) and 149.0±39.6 ind./L (D). The dropped sharply in zoea-3 to the zoea-4 stage may caused by limiting of *Artemia* naupli supplied to the larvae in the tank. In this research the artemia naupli was given to the zoea-3 at the density of 3-4 ind./mL. Truong *et al.* (2007b) reported that after larvae develop to zoea-3 until zoea-5 stage the feed should adjusted depending on

Table 2. Identification of larvae development and period required by each stage

Stadium	Specification	Period required by larvae in the rearing tank (days)
 Zoea-1	Total number of plumose setae 4 Sessile eyes, Abdomen 5 segmented	6
 Zoea-2	Total number of plumose setae 6 Sessile eyes, Abdomen 5 segmented	4
 Zoea-3	Total number of plumose setae 8, eyes stalked. Abdomen 6 segmented.	3
 Zoea-4	Total number of plumose setae 10, Pleopod bud on abdomen segmen 2-6	4
 Zoea-5	Pleopod in abdominal segment well developed	5
 Megalop	First periopod modified into cheliped. Four pair of leg, where swimming leg stil undevelop	7
 Crablet	A pair of cheliped, three walking legs and a pair of swimming leg, 9 anterolateral spines	

Table 3. Population of mangrove crab *S. olivacea* larvae fed *Artemia* nauplii enriched and unenriched using *Nannochloropsis* sp

Treatments	Larvae population (ind./L) from zoea-1 to zoea-5 stage				
	Day- 1	Day-7	Day-11	Day-14	Day-21
	Z-1 (ind/L)	Z-2 (ind/L)	Z-3 (ind/L)	Z-4 (ind/L)	Z-5 and megalopa (ind/L)
A.	400	377.3±19.6[a]	343.0±15.6[a]	161.7±38.9[a]	54.3± 6.0[a]
B.	400	355.81±19.0[a]	320.3±17.6[a]	144.0±34.2[a]	44.0±6.92[a]
C.	400	388.73±6.2[a]	334.01±1.7[a]	172.0±13.1[a]	57.3±2.30[a]
D.	400	362.3±12.7[a]	321.03±7.15[a]	149.0±39.6[a]	45.7±12.4[a]

Notes : A). Artemia nauplii enriched using Nannochloropsis sp was given to the larvae zoea-3 stage until develop to megalop stage. B). Artemia nauplii enriched using Nannochloropsis sp was given to the larvae zoea-4 stage until develop to megalop stage. C). Artemia nauplii enriched using Nannochloropsis sp was given to the larvae zoea-5 stage until develop to megalop stage. D). Artemia nauplii without enriched using Nannochloropsis sp was given to the larvae zoea-3 until develop to megalop stage.

the species, larvae stage, larvae status, prey size, rearing system and techniques, the rotifer should be increase from zoea-1 to zoea-2 at the density 40 ind./mL, while *Artemia* nauplii should be increased to 10-15 ind./ml after larvae reach stage zoea-3 to zoea-4.

In this research the zoea-5 stage was detected at the day 15. At this time, the larvae population was dropped to 54.3±6.0 ind./L (A), 44.0±6.92 ind./L (B), 57.3±2.30 ind./L (C) and 45.7±12.4 ind/L (D). That mean only about 11-13% of larvae zoea-1 develop to the zoea-5 stage. Epibiotic bacteria and larval mycosis most affected mortality to the mud crab larvae from zoea-1 to the zoea-5 (Truong *et al.*, 2007b). The megalop stage was detected at the day 21 of rearing and occurence of megalop in each treatment was performed in the Megalop Occurences Indices (MOI). The statistical analysis to the zoea population (zoea-1 to zoea-5 stage) among that treatments were not significant different (P>0.05) (Table 3).

3.2 Larvae Development Index

Larvae Development Indexes (LDI) is the value which indicates to the larvae development. At the day 18 of culture it was observed that 50% of larvae in treatment A has developed to the zoea-5 stage, it was also obtained in larvae of treatment C and D, while larvae in treatment B seemed to be late develop. At the day 21, most of the larvae has developed to the zoea-5 in treatment A and C. There are a few numbers of zoea-5 succeed metamorphosis to the megalop stage. This situation was also observed in treatment C. The zoea-4 develop to zoea-5 stage attained to 86% and 92% of the larvae population in treatment B and D, meanwhile the megalop stage was still unobserved in treatment B and D. That means the larvae in treatment B and D were late to develop to the next stage compared than those of larvae in treatment A and C. It was due to the larvae in treatment D only fed *Artemia* nauplii with lower of DHA and EPA, not any addition of DHA and EPA from *Nannochloropsis* sp enriched to the *Artemia* nauplii, thus resulted late of larvae development and low of larvae vitality (Matthew *et al.*, 2006). Futhermore Hamasaki *et al*. (2002) also reported that the mud crab *S. serrata* larvae morphogenesis was accelerated according to the HUFA content in their natural feed.

In the 24 days of culture showed that 25% of larvae zoea-5 stage in treatment A had developed to the megalop stage. That mean the larvae in treatment A continuous develop to megalop stage in the next day. Larvae in treatment B were also continuously developing from zoea stage to megalop stage. It can be seen from the LDI value at 4.86 on the day 21, then develop to 5.18 on the day 24. While in treatment C the LDI value was seen decreased namely from 5,09 on the day 21 to 4,92 on the day 24. The decreasing LDI value also was observed in treatment D from 4.92 on the day 21 decreased to 4.67 on the day 24 (Table 4). That means in treatment C and D the larvae development was unaccelerated, even thought in the treatment C, *Artemia* nauplii was enriched using *Nannochloropsis* sp, but it was given to the larvae zoea-5. While in treatment D the *Artemia* nauplii fed to the larvae zoea-3 stage but was not enriched using *Nannochloropsis* sp. Besides that larvae also given commercial of artificial feed at the dosage of 1 mg/L. Meanwhile, this treatment did not impact to the accelerating larvae development. That phenomenon seemed to be concluded that *Artemia* nauplii which enriched using *Nannochloropsis* sp would increase DHA/EPA and then fed to the larvae zoea-3 stage was more effective to accelerated the larvae development to the megalop stage. The contrary, the larvae zoea-5 stage fed *Artemia* nauplii enriched *Nannochloropsis* sp (treatment C) or larvae zoea-3 fed *Artemia* nauplii unenriched using *Nannochloropsis* sp but added artificial feed (treatment D) seemed to be less developed to the next stages (Table 4). Anuar *et al.* (2011) reported that larvae *S. serrata* fed unenrich *Artemia* nauplii showed

Table 4. Larvae Development Indexes of mangrove crab *S. olivacea* larvae fed *Artemia* nauplii enriched and unenriched using *Nannochloropsis* sp

DOC (day)	**1**	**7**	**9**	**11**	**14**	**18**	**21**	**24**
Larvae Stage	Z-1	Z-2	Z-3	Z-3	Z-4	Z-5	Z-5 and Megalop	Z-5 and Megalop
Treatment								
A	1	1.7±0.07[a]	2.1±0.1[a]	2.6±0.03[a]	3.2±0.03[a]	4.5±0.05[a]	5.09±0.47[a]	5.25±0.2[a]
B	1	1.7±0.07[a]	2.2±0.2[a]	2.6±0.1[a]	3.3±0.03[a]	4.3±0.06[a]	4.86±0.03[a]	5.18±0.5[a]
C	1	1.7±0.1[a]	2.1±0.1[a]	2.6±0.03[a]	3.3±0.03[a]	4.5±0.03[a]	5.09±0.01[a]	4.92±0.1[b]
D	1	1.5±0.07[a]	2.1±0.1[a]	2.5±0.1[a]	3.2±0.03[a]	4.5±0.20[a]	4.92±0.04[a]	4.67±0.4[b]

Notes: A). *Artemia* nauplii enriched using *Nannochloropsis* sp was given to the larvae zoea-3 stage until develop to megalop stage. B). *Artemia* nauplii enriched using *Nannochloropsis* sp was given to the larvae zoea-4 stage until develop to megalop stage. C). *Artemia* nauplii enriched using *Nannochloropsis* sp was given to the larvae zoea-5 stage until develop to megalop stage. D. *Artemia* nauplii without enriched using *Nannochloropsis* sp was given to the larvae zoea-3 until develop to megalop stage.

clear sign of essensial fatty acid defisiency, prolong intermolt period, low survival and reduce swimming activity.

DHA and EPA has been known to be important thing in the development of mud crab larvae. The DHA and EPA content in *Artemia* nauplii was low. Enriched *Artemia* nauplii using *Nannochloropsis* sp wil be increased the content of EPA, DHA and omega fatty acid in *Artemia* nauplii (Hamasaki *et al.*, 2002; Okauchi, 2004; Truong *et al.*, 2007a; Jean and Hur, 2011; Suprayudi *et al.*, 2012; Gunarto and Herlinah, 2013). That mean the feed quality for larvae will increases after enriched using *Nannochloropsis* sp (Churchil, 2003). Besides an enhancing of DHA/EPA rasio in rotifer, *Brachionus* sp and *Artemia* nauplii enriched with *Nannochloropsis gaditana*, it would also increasing of protein, lipid and carbohydrate content (Ferreira *et al.*, 2009). Larvae will increase vitality and viability after fed artemia naupli enriched using HUFA (Karim, 2006). The ratio of DHA and EPA in life feed is the key factor responsible to the success of mangrove crab larvae develop to the next stages (Truong *et al.*, 2007a; Sui *et al.*, 2007).

The DHA/EPA ratio in rotifer and artemia nauplii after enriched using HUFA and *Nannochloropsis* sp. in this research showed in Table 5. The DHA/EPA ratio in rotifer after enriched using HUFA at 20 mg/L during 1 hour was 1.210. Gunarto and Herlinah, (2015) obtained that the DHA/EPA ratio in rotifer without enriched using HUFA was 0.063 That mean the DHA/EPA ratio was increased to 1820%. Artemia nauplii after enriched using HUFA at 50 mg/L during 5 hours, the DHA/EPA ratio in this research was 0.221 and there was increased to 0.294 after artemia nauplii also enriched using *Nannochloropsis* sp at the density of 636 x10^4 cell/mL. That mean the DHA/EPA ratio was increased to 33.032%. Earlier research the DHA/EPA ratio in rotifer after enriched using HUFA at 20 mg/L during three hours was only increased to 69.23%, while in artemia nauplii after enriched using HUFA at 300 mg/L during five hours was increased to 28.72% (Gunarto and Herlinah, 2015).

Table 5. The DHA/EPA ratio in rotifer and artemia nauplii after enriched using HUFA and *Nannochloropsis* sp.

Sample	EPA	DHA	DHA/EPA
	(mg/100g)	(mg/100g)	Ratio
Rotifer + HUFA	50.2	61	1.21
Nauplius Artemia + HUFA	37.5	8.3	0.221
Nauplius Artemia + HUFA +*Nannochloropsis* sp	44.1	13,0	0.294

3.3 *Megalop Occurence Indexes and Crablet Production*

The value of Megalop Occurence Indexes (MOI) at the first day (DOC 21) was very low due to only a few of zoea-5 succced metamorphosis to the megalop stage. At the second days (DOC 22) the highest MOI was observed in C (0.14), followed D treatment (0.11), In earlier study Gunarto *et al*. (2016) was obtained MOI value of *S. serrata* at the second day was 0.13, where the larvae started from the zoea-3 stage also was fed with artemia naupli enriched using *Nannochloropsis* sp at the density of 636 x 10^4 sel/mL. At the third day (DOC 23) the highest of MOI value was obtained in A treatment (0.242), then followed by C treatment (0.208), whereas B and D treatment the MOI value relative stagnant namely 0.034 and 0.123. At the fourth day (DOC 24) the highest of MOI value still in A treatment (0.294), then followed by C (0.242), D (0.105) and B treatment (0.118). it can be concluded that the peak of MOI value obtained at the fourth day in all treatments (Table 6). After five days, the megalop become benthic and stayed in the bottom of the tank to start metamorphosis to the crablet stage. At the sevent days after occurences of megalop, the crablet instar-1 was observed in the bottom of the tank. From the MOI value development in all treatments, it was indicated that larvae in A treatment was more simultant develop to the megalop stage, even was only 29% of larvae population after 4 days from megalop appearance in the culture rearing of mud crab larvae. Then followed by larvae in the C (24%), B treatments (22%) and the smalest simultant was obtained in D treatment (10%), This situation was proved that enriched *Artemia* nauplii using *Nannochloropsis* sp was affected to the more synchronizing of zoea-5 metamorphosis to the megalop stage.

The highest of D-10 crablet production with various size namely, 0.003-0.02 g/ind. was obtained in A treatment (191±15.6 ind./tank), then followed by C treatment (190.5±13.4 ind./tank), B treatment (165.5±7.1 ind./tank) and those were significant different (P<0.05) with the lowest of crablet production was obtained in D treatment (121.5±2.1 ind./ tank). The various size of crablet production was represented that larvae developement stage in the same tank was different. Thereafter, even in the tank was find out the crablet, but the megalops were still observed swimming or megalop as a benthic in the bottom of the tank to initiate metamorphosis to the crablet stage. The bigger size of crablet that mean the larvae fast develop to crablet stage. It was due to unsyncronizing of larvae development, there mean the canibalisms oportunity become the bigger mostly by megalop to the zoea-5 stage or by the crablet to megalops stayed in the tank bottom. Thus the unsynchronizing of zoea-5 metamorphosis to the megalop stage would result the lower crablet production.

The next reseach could be focused to the aspect of how to minimize of unsyncronizing larvae development in the rearing tank. The optimum number of larvae stock-

Table 6. The Megalop Occurence Indexes (MOI) of mangrove crab *S. olivacea* larvae fed *Artemia* nauplii enriched and unenriched using *Nannochloropsis* sp

Treatments	**Megalop Occurence Indexes (ind. of megalop/100 ind. of larvae) and crablet production in each treatment**				
	First day (DOC 21)	Second day (DOC 22)	Third day (DOC 23)	Fourth day (DOC 24)	Crablet-D10 (ind./tank)
A	0.03^a	0.053^a	0.242^a	0.294^a	191 ± 15.6^a
B	0.01^a	0.034^a	0.034^b	0.218^a	165.5 ± 7.1^a
C.	0.008^a	0.140^b	0.208^a	0.242^a	190.5 ± 13.4^a
D.	0.02^a	0.115^b	0.123^b	0.105^b	121 ± 2.1^b

Notes : A). *Artemia* nauplii enriched using *Nannochloropsis* sp was given to the larvae zoea-3 stage until develop to megalop stage. B). *Artemia* nauplii enriched using *Nannochloropsis* sp was given to the larvae zoea-4 stage until develop to megalop stage. C). *Artemia* nauplii enriched using *Nannochloropsis* sp was given to the larvae zoea-5 stage until develop to megalop stage. D). *Artemia* nauplii without enriched using *Nannochloropsis* sp was given to the larvae zoea-3 until develop to megalop stage.

ing density and the feeding regimes presumably to be important factors influenced to the syncronizing of larvae development. By syncronizing larvae development in the rearing tank that mean the larvae almost in the same stage development, therefore, the canibalism in megalop and crablet stage able to minimizing and finally, will be resulted the higher number of crablet production.

3.4 Water Quality and Vibrio sp Population

The range value of water quality parameters in mud crab larvae rearing during this study was presented in Table 7. Larvae reared at the water temperature 27-29°C, salinity 28 ppt, dissolved oxygen (6.5-7.2mg/L). Gunarto and Widodo (2012) find out higher survival rate of *S. olivacea* larvae reared at the water temperature 30-31,5°C compared than that larvae reared at the water temperature 28-29.5°C. Baylon (2011) claimed that *S. olivacea* larvae development was delayed for 5-10 days on zoea-3 stage after reared at salinity 25-35 ppt and water temperature 20°C. Ammonium concentration in the larvae rearing tank was relatively high (A = 0.11 mg/L; B= 0.08 mg/L, C=0,12 mg/L; D : 0.20 mg/L) at the day 6 of culture. The water excanged in larvae rearing was only 10% of total water volume started at the day 6 of culture At the day 20, when larvae started metamorphosis to the megalop stage, the highest of ammonium concentration was observed in D treatment (0.24 mg/L). While the other treatment the ammonium concentration was relatively in lower concentration, namely A (0.13 mg/L), B (0.14 mg/L) and C (0.13 mg/L) (Table 6). That mean addition of artificial feed in D treatment could be responsible to increase the ammonium concentration. Nell *et al*. (2005) found LC-50 during 24 hours of *S. serrata* stadia larvae zoea-1 and zoea-5 to the amonia unionized were 4,05 mg/L and 6,45 mg/L. respectively. Based to that data can be concluded that mortality of the larvae during this research was not caused by ammonium concentration in the tank. It is because of the ammonium concentration still far from the LC-50 value. The lowest of nitrite concentration was detected at the day 6 of culture, but at the 20 day of culture, the nitrite concentration was relatively high at B (2.24 mg/L) and D treatment (1.21 mg/L) and the lowest concentration found at A treatment (0.47 mg/L). The LC-50 during 96 hours on zoea-1, 2, 3, 4, 5 of *S. serrata* larvae namely ; 41,58; 63,04; 25,54; 29,98; and 69,93 mg/L respectively (Mary *et al.*, 2007).

The Total Organic matter (TOM) concentration was relatively high in all treatments (>50 mg/L) and the peak obtained in D treatment (56 mg/L) and that it was resulted high population of total *Vibrio* sp in all treatments namely 10^3 cfu/mL at the 6 days of culture, while at the day 20 of culture, the *Vibrio* sp population decreased to 10^2 cfu/mL, except in D treatment the *Vibrio* sp population was still at the level of 10^3 cfu./mL. This situation was proved that the addition of artificial feed would influence to the increase of *Vibrio* sp population in larvae rearing in tank. Zhenguo *et al*. (2010) claimed that some factors most influenzing to the crablet production consist of micro environment, water quality, rearing. Technique, total *Vibrio* sp and feed quality and quantity were given to the larvae.

Table 7. The range value of water quality parameters in mud crab larvae rearing

Treatments	Amonium (mg/L)	Nitrite (mg/L)	TOM mg/L	*Vibrio sp*	*Vibrio sp*
				(log cfu/mL)	*(log cfu/mL)*
				At zoea-2 stage	*At megalop stage*
A	0.11-0.13	0.41-0.47	53,2-56,9	*3.81+0.19*	*2.71+0.03*
B	0.08-0.14	0.63-2.24	51,5-52,5	*3.77+0.05*	*2.82+0.01*
C	0.12-0.13	0.51-0.58	50,1-52,5	*3.59+0.01*	*2.91+0.11*
D	0.20-0.24	0.15-1,21	56,3-56,9	*3.78+0.22*	*3.77+0.23*

Notes : A). Artemia nauplii enriched using Nannochloropsis sp was given to the larvae zoea-3 stage until develop to megalop stage. B). Artemia nauplii enriched using Nannochloropsis sp was given to the larvae zoea-4 stage until develop to megalop stage. C). Artemia nauplii enriched using Nannochloropsis sp was given to the larvae zoea-5 stage until develop to megalop stage. D). Artemia nauplii without enriched using Nannochloropsis sp was given to the larvae zoea-3 until develop to megalop stage.

4. Conclusion

Larvae zoea-3 to megalop stage fed unenriched *Artemia* nauplii, showed the lower of crablet production compared than that of larvae fed *Artemia* nauplii enriched using *Nannochloropsis* sp was given to the larvae zoea-3, 4 and 5 untill develop to the megalop stage.

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