Effect of calcium and enzyme involvement to survival rate and development of the early stage zoea *Portunus pelagicus*

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Abstract. The early stage of blue swimming crab (*Portunus pelagicus*) zoea requires calcium for calcification after molting which can be drawn from water and its diet. However, the digestive system of the early stages of the larvae is still not perfect, so the ability to digest food is limited. Calcium and enzyme inclusion in its formulated diet is important to aid the calcification process. Therefore, in this study, the effect of calcium and enzyme inclusion to survival rate and development of the zoea were examined. Zoeas were fed with nauplii *Artemia* and three levels of calcium Kalzana-D without enzyme Enzyplex inclusion (At, Bt and Ct) and with enzyme Enzyplex inclusion (AtE, BtE and CtE). There were three replicates in each treatment that had 100 zoeas in 2-L plastic tube on 30°C water bath. The result revealed that 50 mg calcium with enzyme involvement supported the early stage of zoea to molt and develop to the next stage. In addition, calcium inclusion with or without enzyme involvement promoted survival rate of the zoea.

1. Introduction

Rajungan or blue swimming crab (*Portunus pelagicus*) is one of the main fishery commodities in Indonesia. It export value rose 6.15% per year from 2012 to 2017 [1]. Between January – September 2018, Indonesia exported 21.570 ton of crab, that was valued up to USD 370,14 million, to the USA, Japan, China, Malaysia and Singapore. This contributed to 2.69% of fisheries export volume and 10.5% of fisheries export value of Indonesia [2]. Moreover, this increment of the export value indicates a high demand of the crab commodity that becomes the opportunity for Indonesia to increase its crab production.

Hatchery-based aquaculture that is an sustainable practice of aquaculture by using juveniles from hatcheries, can increase its production. In contrast, closing its life cycle and maintaining its larvae to produce its juveniles are still difficult to be achieved. Required rearing environment and nutrition should be provided in order to increase its juvenile's production. The larvae of blue swimming crab periodically shed its exoskeleton through the molting process to grow and develop [3].
The soft new exoskeleton will be hard and calcified in several hours after molting. This calcification process requires calcium that can be absorbed from water [4] or diet [5].

In addition, as theirs grow, their undeveloped digestive system also to be more complex and low enzyme activity [6, 7]. Previous studies revealed that zoea husbandry can be performed in the rearing containment in the laboratory, and feed with the nauplii Artemia and formulated diet. They can successfully reach the juvenile stage using this feed combination [8-10]. In this study would like to address the effect of calcium and enzyme in the formulated diet for the survival rate and growth of the early stage _P. pelagicus_ zoea.

### 2. Method

#### 2.1. The Larvae Source

The zoea of _Portunus pelagicus_ was obtained from the hatchery in Laboratorium Basah Budidaya Organisme Laut, Pusat Penelitian Oceanografi (P2O) LIPI, Ancol, Jakarta. Previously, a berried female _P. pelagicus_ was collected from Jakarta Bay. This female then was bathed in 50 ppm formaldehyde for 15 minutes to disinfect prior was placed into the 250-L hatching tank. Aeration was supplied to provide adequate level of dissolved oxygen (DO). Approximately 75% of rearing water was changed everyday to maintain water quality. Newly hatched zoeas then were transferred into larval rearing tanks.

#### 2.2. Larval Husbandry

At zoea 1, larvae were reared in 1,000-L tanks at 19 individual/L of stocking density. Seawater used is first sterilized through a process of filtration, chlorination, and UV sterilization, chlorination and UV sterilization process. 24-h light, heater and aeration were set to maintain environmental parameter at optimum level for the early stage larvae husbandry. Environmental variable, for instance, dissolved oxygen (DO), water pH and light intensity were measured. At zoea stage 2, larvae then were taken for this experiment.

#### 2.3. Experimental Containment and Husbandry

As a single replicate, 100 larvae at zoea stage 2 were reared in a single 3-L ecoplast tube that was filled with 2-L sterilized seawater as previously mentioned. Rearing containments then were placed in 32°C water bath under 24-h light (figure 1). In order to maintain water quality, a uneaten feed was siphoned, and water was exchanged every morning prior to feeding. After they were metamorphosed into zoea stage 3 in three days, all survived zoea at stage 3 were counted and put into formaldehyde. A body measurement of all zoea stage 3 was performed after three days the preservation.

![Figure 1. Larval zoea rearing diagram of Portunus pelagicus.](image-url)
Approximately 15,000 freshly hatched nauplii *Artemia* and experimental formulated feed were given in each replicate. Each formulated diet treatment had a different composition (Table 1.) that each of their ingredients was homogenized and then steamed cooked. 5 grams of dough were homogenized with 100 mL distilled water. 2 mL was taken and then poured into its rearing tube to feed the larvae based on the treatment.

Table 1. Formulated diet composition of *P. pelagicus*.

| Ingredients             | At  | Bt  | Ct  | AtE | BtE | CtE |
|-------------------------|-----|-----|-----|-----|-----|-----|
| Chicken eggs (mL)       | 25  | 25  | 25  | 25  | 25  | 25  |
| Cod liver oil (mL)      | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 |
| Multi-vitamin (mL)      | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Spirulina powder (gr)   | 2   | 2   | 2   | 2   | 2   | 2   |
| Enzyplex (caplet)       | 0   | 0   | 0   | 0.5 | 0.5 | 0.5 |
| Calcium Kalzana-D (100mg/caplet) | 0 | 0.5 | 1 | 0 | 0.5 | 1 |

2.5. Morphometry and Survival Rate

Body measurement was performed for the length of cephalothorax, abdomen and telson [11] under the microscope. Calibrated ocular micrometer and 10 x 10 magnification were utilized during these body parts measurements. In addition, zoea stage of each larva was determined and the number of them was calculated. Mean of survival rate and body measurement between treatments were calculated.

Figure 2. Zoeal larva showing limits for length measurements of cephalothorax and body [11] a: cephalothorax; b: abdomen; c: telson.
3. Result and Discussion

3.1. Survival Rate

This current study revealed that 50 mg calcium inclusion without enzyme addition promoted the highest survival rate of the zoea rather than the highest calcium involvement at 100 mg (figure 2). Previous studies in shrimp and prawn suggested that dietary calcium inclusion improved survival rate due to increase hemocytes number and phagocytic activities [12, 13]. Moreover, crustaceans require calcium for hardening their exoskeleton after molting. Calcium will be deposited into their exoskeleton as calcium carbonate during the post-molt period [5, 14, 15]. During pre-molt, calcium uptake occurs through gills and stomach but then increases during postmolt [14, 16]. However, they will lose around 87% of their calcium during ecdysis as they shed their old exoskeleton [14].

![Figure 3. Zoea larva survival rate of Portunus pelagicus.](image)

Current studies show that enzyme inclusion increases survival rates by increasing the level of calcium involvement level. Digestive enzymes, for instance amylase, cellulase, protease, lipase and trypsin, are required to digest dietary carbohydrate, protein and fat. Those digestive enzymes can also be exogenous that obtained from diet [17, 18]. Due to undeveloped digestive system of the larvae, those enzymes should be obtained from their diet [7, 18]. Thus, enzyme involvement can aid the larvae digestive system to digest the formulated diet and then increase nutrient assimilation. Moreover, increase calcium content in this diet also increase calcium assimilation by the larvae [5].

3.2. Body Size and Zoea Stage

In this study, the result revealed that 100 mg calcium without enzyme inclusion promoted the highest cephalothorax length of the larvae almost 500 µm (table 2). On the other hand, with addition the enzyme inclusion, 50 mg calcium with enzyme involvement produced the highest cephalothorax length almost 500 µm as well. Previous study suggested that zoea stage 2 of P. pelagicus has cephalothorax length between 500 – 700 µm [19]. Moreover, low survival rate in those two treatments that had the highest cephalothorax length might indicate that the majority of zoeas in those two treatments were newly molting of zoea stage 2.
During molting, cannibalism is likely occurred in crustaceans which might possibly cause high mortality in those two treatments [20, 21]. In addition, newly molt zoea are slightly colorless which was observed during the examination under the microscope [20]. In contrast, short cephalothorax length but high survival rate in 50 mg calcium without enzyme inclusion suggested that zoeas in this treatment suffered from the lengthening of intermolt period. That is because of the absence of enzyme inclusion limited feed digestion and then nutrient assimilation [22, 23], therefore the composition of the stage in each treatment becomes an important indicator. More and more zoea stadia composition further indicates growth and the molting process is proceeding rapidly.

The composition of the stage of each treatment in this study is presented in figure 4. The picture shows the effect of calcium and enzimplex supplemented diet on Zoea I and Zoea II ratio. The highest ratio Zoea I and Zoea II reached on BtE diet (calcium and enzimplex (50mg:50mg) formulated diet. Increasing calcium concentration on diet without increasing enzimplex result in reducing that ratio. It might be the higher calcium concentration could not be digested by larvae effectively.

Other factors that might cause various body length between treatments was the water quality. The temperature might greatly affect metabolic rate of the crustaceans as an adaptation response to cope with environmental change [24]. However, current study utilized and maintained water quality parameters between treatments under their optimum range to live and develop (table 3). Thus, the effect of different water quality on survival rate and body length could be negligible.
Table 3. Water quality on rearing larvae *Portunus pelagicus*.

| Parameter       | Unit | Present Study | Previous Study                                                                 |
|-----------------|------|---------------|--------------------------------------------------------------------------------|
| Temperature     | °C   | 29 – 30       | 26 – 30 (Gong *et al.*, 2015); 23 – 32 (Hamasaki, 2003)                         |
| Dissolved Oxygen| mg/L | 5.80 – 6.44   | 5.20 – 6.03 (Juwana & Permadi. 2014a)                                          |
| pH              |      | 7.99 – 8.03   | 7.80 – 8.00 (Juwana & Permadi. 2014a)                                          |
| Light Intensity | Lux  | 1400 – 1500   | 1100 – 3000 (Juwana & Permadi. 2014b)                                          |

4. Conclusion

In this current study, 50 mg calcium with enzyme inclusion in formulated diet encouraged larval molting to the next stage also boosted larval survival rate at the end of rearing. In addition, Enzimplex supplemented diet influence larval growth by increasing Zoea I and Zoea II ratio or larval molting and reach to the next stage (Zoea I to Zoea II).

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6. References

[1] Perikanan KKd *Produktifitas Perikanan Indonesia* 2018 (Jakarta: Kementerian Komunikasi dan Informatika) p 49
[2] Direktorat Jenderal Penguatan Daya Saing Produk Kelautan Dan Perikanan *Kinerja Ekspor Produk Perikanan Indonesia Tahun 2018* 2018
[3] Gaude A R, Anderson J A 2011 *Southern Regional Aquaculture Center (SRAC)* 4306
[4] Perry H, Trigg C, Larsen K, Freeman J, Erickson M, Henry R 2001 *Aquaculture* 198 197–208.
[5] Zanotto F P, Pinheiro F, Sa MG 2009 *Sér Zool* 99 (3) 295-300
[6] Jantrarotai P, Siriinsiruwanch K, Pripanapong S, Chayarat C 2006 *Kasettsart Journal (Natural Science)* 40 (2) 507-16
[7] Serrano A E J, Traifalgar R F O 2012 *AACL Bioflux* 5 (3) 101-11
[8] Juwana S, Ruyitno, Alfiansyah Y R, Sujono 2010 *Oseanologi dan Limnologi di Indonesia* 36 (3) 259-79
[9] Juwana S, Permadi S 2014 *Oseanologi dan Limnologi di Indonesia* 40 (1) 43-54
[10] Juwana S, Permadi S 2015 *Oseanologi dan Limnologi di Indonesia* 41 (2) 181-203
[11] Juwana S, Aswandy I, Pangabean M 1987 *Marine Research in Indonesia* 26 29-50
[12] Anuta J D, Buenello A, Patnaik S, Lawrence A L, Mustafa A, Hume M E 2011 *J. of the World Aquaculture Society* 42 (6) 834-44
[13] Moss A S, Ishikawa M, Koshio S, Yokoyama S, Dawood M A O 2019 *American Fisheries Society* 81 (1) 55-66
[14] Norum U, Bodgaard M, Pedersen TV, Bjerregaard P 2005 *Aquatic Toxicology* 72 29-44
[15] Zanotto F P, F Pinheiro, Brito L A, Wheatly M G 2004 *Int. Congress Series* 1275 89-95
[16] Bondgaard M, Bjerregaard P 2005 *Aquatic Toxicology* 72 17-28
[17] Anand P S S, Kohli M P S, Roy S D, Sundaray J K, Kumar S, Sinha 2013 *Aquaculture* (59-68) 392-5
[18] Serrano A E J 2013 *European J. of Zoological Research* 2 (2) 10-4
[19] Juwana *S. Petunjuk praktis pembenihan rajungan (Portunus pelagicus) di Pusat Penelitian Oseanografi LIPI* 2006 (Jakarta: Pusat Penelitian Oseanografi LIPI)
[20] Heasman M P, Fielder D R 1983 *Aquaculture* 34 303-16
[21] Anh N T N, Ut V N, Wille M, Hoa NV, Sorgeloos P 2011 *Aquaculture Nutrition* 17 (2) 549-58.
[22] Baylon J C 2009 *Aquaculture* 288 190-5
[23] Gong J, Shu K Y L , Ye H, Li S, Zeng C 2015 *Journal of Experimental Marine Biology and Ecology* 464 11-7
[24] Cuzon G, Lawrence A, Gaxiola G, Rosas C, Guillaume J 2004 *Aquaculture* 235 513-52