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## Blue swimming crab (*Portunus pelagicus*) megalopa stage seed feed enrichment with beta carotene

Andi Yusneri <sup>1</sup>, Hadijah <sup>2</sup> and Sutia Budi<sup>2</sup>

<sup>1</sup>Aquaculture Study Program, Faculty of Agriculture, Bosowa University, 90231, South Sulawesi, Indonesia

<sup>2</sup>Postgraduate Program, Aquaculture Study Program, Bosowa University, Makassar, 90231, Indonesia

Email: hadijahmahyuddin@gmail.com

**Abstract.** This study aimed to increase the nutritional value of natural rotifer and *Artemia* feed by adding beta carotene sourced from carrots. This research was conducted in May 2020 at the hatchery unit of the Brackish Water Aquaculture Fishery Centre (BPBAP) Takalar. The research design used was a completely randomized design (CRD) with 4 treatments and 3 replications. The experimental animals used were small blue swimming crab larvae (*Portunus pelagicus*) in the megalopa stage, which were stocked with a density of 5 individuals/L, and kept until they entered the crab stage. The expected output of this research is natural feed enrichment technology for rotifers and *Artemia* using beta carotene from carrots. The results showed that providing the right frequency of feed after enriching with beta carotene could increase the survival and growth of small blue swimming crab larvae. The optimum feeding frequency for megalopa stage blue swimming crab larvae was 3 times/day.

### 1. Introduction

The blue swimming crab (*Portunus pelagicus*) is an important Indonesian fishery export commodity, the 3rd largest after tuna and shrimp (<https://www.apri.or.id/coordination-meeting-of-blue-swimming-crab-fishery-in-indonesia/>). In 2017 the export value of crab meat from Indonesia was nearly USD 411 million [1]. Up to the present time, the export market still relies on wild caught blue swimming crabs [2] fishing pressure is continuous, with even berried crabs being caught and processed [3] There is a concern that this will cause a decrease in the stock of blue swimming crabs in nature [4,5]. As a result of the increase in fisheries catch, the wild crab population is decreasing significantly, especially in areas with densely populated fishing communities, such as in Java and South Sulawesi. To reduce the exploitation of blue swimming crabs through fishing efforts, there needs to be an attempt to increase production through pond aquaculture. The increasing demand for blue swimming crab meat to meet the needs of export or local destinations demands sufficient and sustainable resource availability that could be met through intensive cultivation [6].

Based on the afore-mentioned situation, efforts are required to produce crab seeds of appropriate quality, in sufficient quantities, and in a timely manner, by leveraging various factors that are considered to have a positive effect, including the use of feed that meets nutritional standards. According to Budi (2017) [7] feed is the main component needed by juvenile crabs to survive and grow. Complete nutrition in the feed is absolutely necessary



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to ensure the normal growth of the crab seed. One of the key factors in larval rearing is feed nutrition. Therefore, larvae must be fed with appropriate and balanced nutrition to obtain optimal survival rates (Ariyati, 2018 [8]; Nikhlani et al, 2017 [9]). Enrichment is the addition of essential nutrients for larval development to the feed [10], and one way of doing this is by using beta carotene.

## 2. Research methods

This research was conducted at the crab hatchery unit, Brackish Water Aquaculture Fishery Centre (BPBAP) Takalar. The research was conducted in May 2020 over a period of one month. The first research stage was to obtain experimental animals in the form of blue swimming crab broodstock that were nurtured at BPBAP Takalar and then to breed crab larvae.

The containers used in this study were 12 black plastic basins with a volume of 30 L, which had been filled with seawater (34 ppt salinity). The containers were equipped with aeration equipment in order to maintain a stable temperature. The containers were placed randomly. To maintain the dissolved oxygen levels in the culture medium (water), each container was gently aerated using a hose connected to a Pasteur pipette. The feed used in this study was natural feed consisting of rotifers and *Artemia* nauplii. Before being fed to the larvae, the rotifers and *Artemia* were immersed in water enriched with 10 ppm  $\beta$ -carotene extract for 2-3 hours [11].

The stocking density of the megalopa larvae was 5 individuals/litre. According to Marjono, et al (2002) [12], one factor that can cause a low survival rate in blue swimming crab larvae (megalopa stage) is the cannibalistic instinct. To reduce cannibalism between the crab larvae, the initial stocking density was adjusted to 3-5 larvae/litre. The feeding consisted of 4 feeding frequency treatments with 3 replicates. Feeding in treatment A was carried out 2 times a day, in treatment B it was carried out 3 times a day, in treatment C it was carried out 4 times a day and in treatment D it was carried out 5 times a day. Research parameters measured were:

Survival Rate According to Effendi (2002); Hadijah (2015); Hadijah (2017) [13 - 15], survival rate is the percentage of cultured organisms surviving at a given time, calculated using the following formula:

Survival Rate

$$SR = \frac{N_t}{N_0} \times 100\%$$

where:

SR= Survival rate (%),

$N_t$  = The number of live experimental animal at time t (the end of the study)

$N_0$ = The number of live experimental animal at the beginning of the study

### 2.1. Growth

To determine the growth rate of the blue swimming crab seeds, the absolute growth in weight (weight gain) over the study period was measured. The larvae were weighed at the beginning and end of the study using analytical scales with a precision of 0.0001 grams. Weight gain was calculated according to the method in Effendi (1979) [16] using the following formula:

$$h = W_t - W_o$$

where:

h = Absolute weight growth (gram)

$W_t$  = The average weight (g) of experimental animals at time t (the end of study)

$W_o$  = The average weight (g) of experimental animals at the beginning of the study

### 2.2. RNA/DNA Ratio

DNA concentration was calculated using the formula used by Indahsari (2019) [18] as follows:

$$[DNA] = A_{260} \times 50 \times \text{Dilution factor}$$

where:  $A_{260}$  = Absorbance value at 260 nm

50 = A solution with an absorbance value of 1.0 is equal to 50  $\mu\text{g}$  of double strand DNA per ml (dsDNA)

$$[RNA] = A_{260} \times 40 \times \text{Dilution factor}$$

where: 40 = 40  $\mu\text{g}/\text{ml}$  single strand RNA (ssRNA)

#### Data Analysis

The research data were analysed using analysis of variance (ANOVA). If there was a significant effect, a post-hoc W-Tukey test was performed [17]. Statistical tests were implemented in SPSS version 20.

### 3. Result and Discussion

Survival rate was obtained based on the ratio between the number of crab larvae in the megalopa stage alive at the end of the study and the number of larvae stocked at the beginning of the study. It took six days of research before all crab larvae in the megalopa stage turned into the crablet stage. Data on the average survival rate of small crab larvae can be seen in Table 1.

**Table 1.** The average survival rate of small crab larvae

Feed Frequency	Survival rate (%)
2	34,00 $\pm$ 1,76
3	41,56 $\pm$ 1,68
4	38,00 $\pm$ 1,76
5	30,44 $\pm$ 1,68

**Table 2.** the average growth rate of small crab larvae

Feeding frequency	Growth rate
2	5,00 $\pm$ 0,01
3	4,73 $\pm$ 0,03
4	3,82 $\pm$ 0,02

The highest larval survival rate was obtained in treatment B, at 3 times/day (41.56%) feeding frequency, then at the feeding frequency of 4 times/day (38.00%) followed by 2 times/day (34.00%), and the lowest survival rate was from feeding 5 times/day (30.44%). The high survival rate obtained in treatment B is considered to be due to the availability of sufficient but not excessive feed, which means that environmental variables could be maintained within suitable ranges in the rearing medium. This could support a high survival rate for the crab larvae. According to Effendi et al. (2005) [15], sufficient feed facilitates the larvae in finding and eating the feed provided, so that a high survival rate can be maintained. Meanwhile, according to Budi et al (2017) [7] and Budi et al (2018) [20], one of the factors that influence survival is the biotic factor of adaptability, and the adaptability is influenced by the feed consumed by crab larvae.

The low survival rate under treatment D (5 times/day) was thought to be because of the excessive feeding frequency. According to Mutmainnah et al. (2019) [21], feed given to crab larvae in excessive amounts causes inefficient use of the feed. Meanwhile, the low survival rate in treatment A (2 times/day), was due to the frequency of feeding being too low compared to treatment B and C, causing cannibalism to occur between the crab larvae,



resulting in a reduced survival rate. This was emphasized by Marshall et al (2005) [22], who stated that variation in the survival of crab larvae is influenced by the cannibalistic properties crab larvae that will prey on other larvae, greatly affecting the percentage survival rate. Furthermore, Marjono et al. (2002) [12] and Romano & Zen (2017) [23] also stated that the low survival rate of crab larvae was caused by cannibalism between crab larvae.

The survival rate results did not show that higher survival rates resulted in greater weight gain and growth of the crab larvae. It can be seen in Table 2 where the highest survival rate (41.56%) was under treatment B and in Table 3 where the highest weight gain (5.00%) was obtained from treatment A. This was caused by the dose of natural feed which is one of the external factors in supporting growth.

### 3.1 Growth

Affandi and Tang (2017) [24] suggested that growth is a process of changing size over a certain period of time. Data on average growth in small crab larvae can be seen in Table 2.

The results show that the highest growth rate was at 2 times of feeding (5.00), then at 3 times of feeding (4.73), then at 5 times of feeding (4.37) and the lowest was at 4 times of feeding (3.82). The high level of growth at 2 times of feeding is thought to be due to cannibalism. This point was described in Moller et al. (2008) [25], that cannibalism can directly accelerate growth, as larger larvae usually occupy a higher trophic level compared to smaller larvae. Thus, it is possible for cannibalism to occur if the size of the larvae is not uniform. stated that growth of juvenile crabs is influenced by the quantity and size of food available, age, organism size, and water quality parameters.

### 3.2. RNA/DNA Ratio

The average RNA/DNA ratio of small crab larvae that had been given feed enriched with beta carotene can be seen in Table 3.

**Table 3** The average RNA/DNA ratio of small crab larvae

Feeding Frequency	RNA/DNA Ratio
2	0.84 ± 0.04
3	0.78 ± 0.02
4	0.75 ± 0.04
5	0.76 ± 0.04

Table 3 shows that the RNA/DNA ratio obtained was highest under the treatment of 2 times daily feed frequency (0.84), then 3 times daily feed frequency (0.78) then 5 times daily feed frequency (0.76), and finally, the lowest was in treatment 4 times daily feed frequency (0.75). The RNA/DNA ratio can be used as a parameter to assess the quality of larvae to be cultured. Budi (2017) [7] stated that one of the indicators of growth and development of organisms is the RNA/DNA ratio. This is supported by several research results as reported by Buckley (1979) [26], that there was a positive correlation between the growth rate of Atlantic cod larvae (*Gadus morhua*) and the RNA/DNA ratio, where the RNA/DNA ratio increased with increasing growth rate.

### 3.3. Water Quality

Viable culture medium (water) quality is very important for blue swimming crab larvae. Several physical and chemical parameters can be considered important variables, including temperature, salinity, PH, dissolved oxygen (DO) and ammonia. The water quality parameter values during the study can be seen in Table 4.

**Table 4.** The water quality parameter values during the study

Parameter	Feed Frequency (times/day)			
	2	3	4	5
Temperature(°C)	28 – 30	29 – 31	29 – 30	28 – 30
Salinity (ppt)	32 – 33	32 – 33	32 – 33	32 – 33

pH	8.2 - 8.3	8.2 - 8.3	8.2 - 8.4	8.2 - 8.4
DO (mg/L)	4.9 - 5.5	4.8 - 5.7	4.9 - 5.5	5.0 - 5.6
Ammonia (mg/L)	0.004 - 0.017	0.004 - 0.018	0.005 - 0.019	0.005 - 0.019

The water quality and feed are important criteria in crab culture: if these components are unsuitable, it is likely that the larvae will develop diseases that can cause mortality. Water temperature greatly affects the survival of crabs and other marine organisms where changes in temperature are very influential on the metabolism rate and the activity of other organisms. Changes in environmental factors such as temperature, dissolved oxygen, salinity and other environmental parameters will affect the frequency of moulting and size increase in crustaceans [27].

The water temperature during the study ranged from 28-30 °C, this is a relatively optimum range for the survival of crab larvae, as supported by the opinion of Budi et al (2017) [7] that the optimal temperature for the megalopa phase of crab larvae ranges from 28-34°C. According to Zaidin et al (2013) [4] temperature affects the solubility of gases in water and crab metabolism processes. According to Jamal et al (2019) [10] the optimum temperature for crabs is 28-30 °C [4].

The pH value of the water during the study ranged from pH 7-8. This range is considered optimal for growth and survival of blue swimming crab larvae. pH of 7.0-8.5 is within normal limits for the survival of crab larvae in the megalopa stage [21,28].

The ammonia content measured during the study ranged from 0.004-0.019 ppm. This range is within the optimal limit for the survival and growth of blue swimming crab larvae. According to Talpur et al et al. (2013) [29], the optimal ammonia content for the survival and growth of crab larvae is <0.02 ppm.

#### 4. Conclusion

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

1. Providing the right frequency of feed after enriched with beta carotene can affect both the survival and growth of blue swimming crab larvae
2. The optimum feeding frequency for blue swimming crab larvae to support the survival rate was 3 times/day. It is suggested that natural feed for blue swimming crab (*Portunus pelagicus*) larvae should be enriched first with beta carotene then given 3 times/day.

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