RNA/DNA Ratio Performance of Blue Swimming Crab (*Portunus pelagicus*) Fed with Natural Food *Phronima* sp. and *Artemia salina* in Juvenile Phase

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Abstract

The availability and quality of fry is a major problem in blue swimming crab aquaculture, with the high mortality at the larval stage. RNA/DNA ratio is one of the parameters that can be used to evaluate the quality of crab fry, including health, nutrition, and growth conditions. This study aims to analyze the effect of Phronima sp. as a substitute feed for Artemia salina on the RNA/DNA ratio performance of blue swimming crab in juvenile phase (crablet 5) during rearing before cultivation in aquaculture ponds. This research was conducted at the Crab Hatchery Unit of the Brackish Water Aquaculture Fisheries Center (BPBAP) Takalar in February 2023. The study used a quantitative experimental completely randomized design (CRD) with five treatments and three repetitions. The ratio of Phronima sp. and Artemia sp. used were: Treatment A 100% Phronima sp; Treatment B 100% Artemia sp; Treatment C 75% Phronima sp and 25% Artemia sp; Treatment D 25% Phronima sp and 75% Artemia sp.; and E: Phronima 75%+Artemia salina 25%. The results showed that Artemia salina combined with Phronima sp. increased the RNA/DNA ratio of the crab compared to a single feed (100% Phronima sp. or Artemia salina 100%). The feed combination with the highest RNA/DNA ratio was shown in treatment E (Phronima sp. 25% + Artemia salina 75%) with an RNA/DNA ratio of 2.02 ± 0.032 ng/µL.

***Keywords**: Portunus pelagicus, Artemia salina, Crablet, Phronima sp., RNA/DNA ratio*

INTRODUCTION

Blue swimming crab (*Portunus pelagicus*) is the third largest export commodity in Indonesia after shrimp and tuna (Ministry of Marine Affairs and Fisheries, 2022). Blue swimming crab has a high economic value for the international market (Maryani *et al*., 2023; Redjeki *et al*., 2021). The production of blue swimming crab aquaculture from Indonesia are exported to America around 90% and various countries such as Malaysia, Singapore, Japan, and China (Laksono *et al*., 2023). The high demand for blue crabs is due to their nutrition, which contains highly digestible protein (Nanda *et al*., 2021). Crab has a high protein content ranging from 16-17 grams per 100 grams of crab weight (Izzah *et al*., 2019).

In order to achieve the successful cultivation of blue swimming crab, it is crucial to understand the aspects of biology and nutrition in the early stages of its growth (Jiang *et al*., 2023). The issue of seed quality is a major problem in Blue swimming crab cultivation (Huda *et al*., 2021). The mortality due to cannibalism during entry into the juvenile phase is one of the problems in aquaculture (Usman *et al*., 2019). The poor survival and growth rate of crab seeds produced indicates that the quality of crab seeds produced is relatively poor. The availability of appropriate feed at the required time is considered to be the reason for this problem (Fattah *et al*., 2014).

In the larval stage, blue swimming crabs are given with natural food such as *Artemia salina*. *Artemia salina* cultivation in Indonesia is still not widely practiced and still requires imports from several countries. This is because *Artemia salina* requires protein feed, which is also relatively expensive (Dharmawan *et al*., 2020). In addition, *Artemia salina* has a body size larger than the mouth of blue swimming crab in juvenile phase. This condition causes *Artemia salina* to be unsuitable as feed for

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blue swimming crab in juvenile phase (Muawanah *et al*., 2017). From several existing problems, it is necessary to find an alternative natural feed that can be given in juvenile phase.

The existence of *Phronima* sp. has the opportunity to substitute *Artemia* salina as natural food in terms of price and nutrition (Ratri, 2020). Phronima sp is an endemic microcrustacean commonly found in brackish waters in Suppa District, Pinrang Regency, South Sulawesi (Fattah *et al*., 2014). *Phronima* sp. can be cultivated massively, and the price is relatively cheaper than *Artemia salina* (Herawati *et al*., 2020; Pangestika *et al*., 2020). *Phronima* sp. has nutrients that are almost the same as the nutrients of *Artemia salina* (Ratri, 2020). *Phronima pacifica* contains nutrients such as protein ranging from 40%-50%, fat 10-15% and Eicosapentaenoic Acid (EPA) 7.52%, while *Artemia* sp. contains protein ranging from 45-55%, fat 10-12% (Herawati *et al*., 2021).

Several studies have examined *Phronima* sp. as a substitute for *Artemia* as a natural feed for aquaculture. Fattah *et al*. (2014) showed that *Phronima* sp. can be used as a natural food substitute for *Artemia salina* in Tiger Prawn Cultivation. Ratri's (2020) research found that adding *Phronima* sp. as a natural feed can reduce the requirement of *Artemia* sp. for vaname shrimp post-larvae (*Litopenaeus vannamei*). In addition, Ahmad *et al*. (2020) also studied the utilization of *Phronima* sp. combined with *Artemia nauplii* as a natural food for juvenile seahorses (*Hippocampus barbouri*). However, studies that discuss *Phronima* sp. as a substitute of *Artemia* as a natural food for blue swimming crabs have not been widely discussed.

In evaluating the nutrition of feed given to crustaceans such as blue swimming crabs, the RNA/DNA ratio is one of the parameters used (Roessler *et al*., 2020). This is due to the important role of DNA and RNA in cellular metabolism in blue swimming crabs. DNA acts as a carrier of genetic information in protein synthesis. The increased protein synthesis that occurs will increase the amount of RNA in the cell. Thus, the RNA/DNA ratio can be used as a parameter of protein synthesis activity, which ends in the form of weight gain (Mutmainnah, 2019). Therefore, this study aims to analyze the effect of feeding *Phronima* sp. as a substitute for *Artemia salina* on the RNA/DNA ratio of blue swimming crab in juvenile phase (crablet 5) during rearing before cultivation in aquaculture ponds.

MATERIALS DAN METHODS

This research was conducted at the Crab Hatchery Unit of the Brackish Water Aquaculture Fisheries Center (BPBAP) Takalar. RNA/DNA Ratio analysis was conducted at the BPBAP Takalar Test Laboratory. The test animals used in this study were blue swimming crab in juvenile phase or crablet 5 (C5) with an initial weight of 0.0121 g and a width of 3.707 mm. The crablets were placed in 15 containers as a basin with a volume capacity of 30 liters. Each container was filled with 20 liters of sterile seawater with a salinity of 31-32 ppt and 20 crablets (C5). Crablets were grown for 15 days with daily *Phronima* sp. and *Artemia salina* feeding daily. The density of each type of food was 50 ind/liter with different ratios according to the treatment. Media water replacement and residual feed removal were carried out daily by 30-50%.

This research is based on experiments with quantitative methods. This research uses a complete randomized design (CRD), which is carried out with three replications of each treatment. The feed used in this study is two natural feed types: *Phronima* sp. with protein content of 40.26%, fat 5.14%, ash 30.20%, crude fiber 5.93%, EPA 7.52%, and DHA 4.19% and *Artemia salina* which contains EPA 4.05%, DHA 1.23%, protein 48.87%, fat 9.28%, and ash 13.9%. The nutritional content of the two natural feeds has been adjusted to the protein needs of crustaceans within the range of 30-60%, as stated by Saputra *et al*. (2019). The study used a quantitative experiment with a completely randomized design (CRD) consisting of five treatments and three replications. The ratio of *Phronima* sp. and *Artemia salina* for each treatment used in this study can be seen in Table 1.

RNA/DNA ratio analysis was conducted on crablet 5 (C5) that lived until the end of the experiment to ensure that the test sample tissue had not been damaged before analysis. The RNA/DNA

Table 1. Natural feed composition of treatment

Treatment	Composition of Natural Feed
A	*Phronima* sp. 100%,
B	*Artemia salina* 100%
C	*Phronima* sp. 50% + *Artemia salina* 50%
D	*Phronima* sp. 75% + *Artemia salina* 25%
E	*Phronima* sp. 25% + *Artemia salina* 75%

extraction process was carried out using the silica extraction method using the Silica-Extraction Kit. The crablets samples of 20 mg were placed in a 1.5 mL eppendorf tube and added with 900 µL of GT Buffer solution. The crablets were crushed and centrifuged at 12,000 rpm for 3 minutes. The 600 mL of supernatant was transferred into a new eppendorf tube and added with 40µL of silica. The mixture was then centrifuged at 12,000 rpm for 15 seconds. The supernatant was removed, and the silica was washed with 500 µL of GT buffer. Then, the mixture was centrifuged at 12,000 rpm for 15 seconds. Furthermore, the supernatant was removed, and the silica was washed with 1 ml of 70% ethanol. The mixture was centrifuged at 12,000 rpm for 15 seconds. Ethanol was removed, and added with diethyl pyrocarbonate (DEPC) ddH_2O solution. The mixture was incubated at 55 °C for 10 minutes, vortexed, and centrifuged at 12,000 rpm for 2 minutes. The supernatant of 500 µL was transferred into a new eppendorf tube for measurement of the RNA/DNA ratio of the test sample by the NanoDrop method (Moruf & Adekoya, 2021).

RNA and DNA ratio measurements were calculated by dropping 1-2 µL of each test sample from each extracted genome on a UV-Vis Spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific) directly connected to a computer. The application will read the nucleic acid concentration in ng/µL (Moruf & Adekoya, 2021). The absorbance was read at a wavelength of 260 nm (Parenrengi *et al*., 2013; Sarnecka *et al*., 2019). To calculate the concentration of DNA and RNA ratio, the formula used is as follows (Gusmiaty *et al*., 2021):

[DNA] = Å260 x 50 x Dilution factor
[RNA] = Å260 x 40 x Dilution factor

Note: Å260 = optical density value at 260 nm; 50 = A solution with an optical density value of 1.0 is equivalent to 50 ug/ml of double stranded DNA (dsDNA); 40 = A solution with an optical density value of 1.0 is equivalent to 40 ug/ml single stranded RNA (ssRNA).

Data Analysis

Data analysis included data tabulation using Microsoft Excel, and the effect of treatment on RNA/DNA ratio was analyzed using analysis of variance (ANOVA) and W-Tuckey test (Ahmad *et al*., 2020). Statistical test tools used in this study is SPSS software 23.0 version.

RESULT AND DISCUSSION

RNA/DNA ratio analysis is one of the parameters that can be used to evaluate the quality of crustaceans, including blue swimming crabs. The measurement of the RNA/DNA ratio in crablets 5 that have been reared by feeding *Phronima* sp. and *Artemia salina* with different ratios can be seen in Table 2. The results of the analysis of variance showed that the feeding of *Phronima* sp. and *Artemia salina* to crablet 5 with different ratios had a significant effect on the RNA/DNA ratio ($p<0.05$). Based on the W-Tuckey test results, treatment A, B, C, and treatment D showed significant differences ($p>0.05$). Similarly, treatments A, B, D, and E also showed significant differences ($p>0.05$), but treatments C and E did not show significant differences ($p>0.05$). The results of the average RNA/DNA ratio can be seen in Figure 1.

Table 2. RNA/DNA Ratio Measurement Results on Crablet 5 for each treatment

Treatment	RNA/DNA Ratio (ng/µL)
A (*Phronima* sp. 100%)	0.81 ± 0.034[a]
B (*Artemia salina* 100%)	1.02 ± 0.011[b]
C (*Phronima* sp. 50%+*Artemia salina* 50%)	1.96 ± 0.062[c]
D (*Phronima* sp. 75%+*Artemia salina* 25%)	0.93 ± 0.013[d]
E (*Phronima* sp. 25%+*Artemia salina* 75%)	2.02 ± 0.032[c]

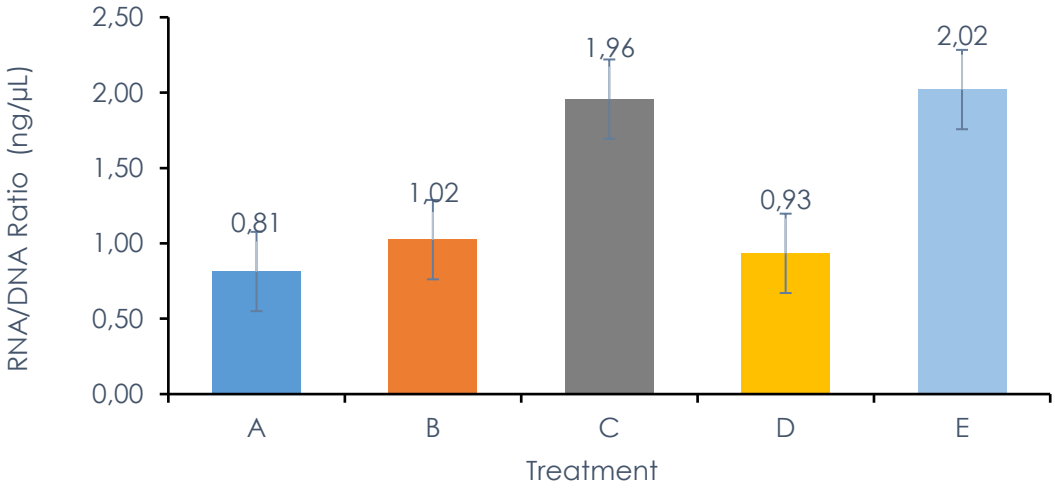

Figure 1. The RNA/DNA ratio of crablet 5 fed with different ratios of *Phronima* sp. and *Artemia salina*.

The results of RNA/DNA ratio measurements due to the feeding of *Phronima* sp. and *Artemia salina* to crablet 5 with different ratios are shown in Figure 1. The highest results were found in treatment E (*Phronima* sp. 25% + *Artemia salina* 75%) at 2.0 ± 0.032 ng/µL. In addition, the results of treatment C (*Phronima* sp. 50% + *Artemia salina* 50%) amounted to 1.96 ± 0.018 ng/µL, treatment B (Artemia salina 100%) amounted to 1.02 ± 0.011 ng/µL, treatment D (*Phronima* sp. 75%+*Artemia salina* 25%) amounted to 0.93 ± 0.013 ng/µL and treatment A (*Phronima* sp 100%) amounted to 0.81± 0.034 ng/µL. The high RNA/DNA ratio in treatments C and E proves that those compositions of *Phronima* sp. and *Artemia salina* are the most optimal for the growth of crablet 5. The high ratio of RNA/DNA indicates protein synthesis activity that occurs. With the addition of *Phronima* sp. by 25% and 50% in crab feed, it can provide maximum nutritional intake. This indicates that Phronima sp. and *Artemia salina* can complement each other as a natural feed for crablet 5. The content of nutrients, especially protein, in both natural feeds is not much different, which makes them complement each other.

In Figure 2, it can be seen that an increase in the percentage of Artemia salina between each treatment given to crablets was followed by an increase in the value of the RNA/DNA ratio as in treatments C (*Phronima* sp. 50% + *Artemia salina* 50%) and E (*Phronima* sp. 25% + *Artemia salina* 75%). Similarly, treatment D (*Phronima* sp. 75% + *Artemia salina* 25%), which has a lower percentage of *Artemia salina*, also showed a lower RNA/DNA ratio. These results are caused by the higher nutrition of *Artemia salina* and its swimming behavior, making it easier for the crabs to eat it (Morgana *et al*., 2018). However, *Phronima* sp. tends to hide in shelters and move fast when threatened, making it more difficult for crabs to eat. This behavior is due to the original habitat of *Phronima* living as parasites in the body of marine animals and quickly adapting to the environment (Bagheri *et al*., 2022).

The lowest RNA/DNA ratio measurement results were obtained in treatment A (*Phronima* sp. 100%) as a single feed at 0.81 ± 0.034 ng/µL. This condition is due to the content of nutrients such as protein, fat, and some other nutrients in *Phronima* sp., which is lower than Artemia salina, causing a lack of nutrient intake from the feed consumed (Herawati *et al*., 2020). This condition affects protein synthesis in crablets. In addition, the consumption of *Phronima* sp. as a single feed becomes less due to crablet crab difficulty in preying on *Phronima* sp. (Bagheri *et al*., 2022).

The results of this study in treatment B (*Artemia salina* 100%) as a single feed showed an RNA/DNA ratio of 1.02 ± 0.011 ng/µL. This result was lower than treatments C and E, which had a lower percentage of *Phronima* sp. This result was due to the absence of variation in the source of nutrients consumed by crablets. Nofiyanti *et al*. (2014) stated that a good feed composition has a varied source of nutrients. The variety of natural feed types with different sizes and nutritional content will make it easier to fulfill the nutrients needed by crustaceans such as crabs. According to Han *et al*. (2021), feed with high protein content can positively affect protein synthesis in mud crab larvae. An increased rate of protein synthesis will usually contribute to better growth and development in the larvae. With a high rate of protein synthesis, cellular activity in the crab also increases. This condition results in a higher RNA/DNA ratio. The Increasing RNA/DNA ratio in crabs can increase growth and improve the quality of the seeds produced (Wu *et al*., 2022). The RNA/DNA ratio reflects protein synthesis activities that occur, which increases the number of cells (hyperplasia) and cell size (hypertrophy). The number of cells can be estimated from the DNA concentration in the tissue, while the concentration of RNA can be used to estimate the cell size. The DNA content is relatively constant in cells, while the RNA concentration will fluctuate depending on the protein synthesis (Yusneri *et al*., 2020).

RNA/DNA ratio analysis has become a widely used parameter in marine organism research, especially in the quality assessment of creatures such as fish and crustaceans. This metric serves as an indicator of quality by shedding light on various aspects of an organism's growth, which is influenced by multiple external factors, such as the environment, and internal ones, such as the feed given and the biosynthesis that occurs. The quantity of RNA in an organism essentially signifies the level of expression of genes responsible for growth, which respond to environmental conditions. Simultaneously, the amount of DNA provides insight into the number of tissue cells in the animal's body. For this reason, the RNA/DNA ratio has recently become an efficient indicator of growth potential, primarily driven by the capacity of the organism to biosynthesize proteins (Chang *et al*., 2021; Kou *et al*., 2022).

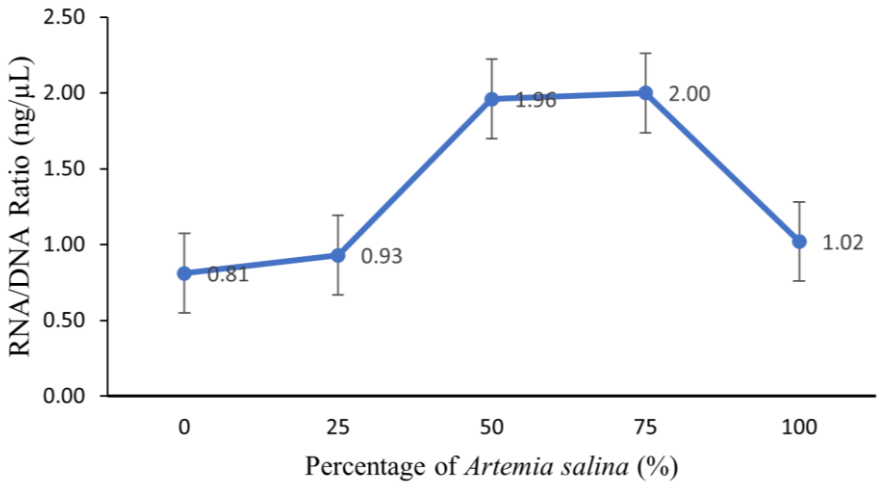

Figure 2. The Relationship of RNA/DNA Ratio with Various Percentages of *Artemia salina* given to Crablet 5

The quality assessment of seeds based on RNA/DNA ratio characters has been conducted on African catfish by Dewi & Tahapari, (2017). The results of their research stated that the increase in RNA/DNA ratio is in line with the good growth of the African catfish. In addition, research related to seed quality based on RNA/DNA ratio was also conducted on crustaceans such as tiger shrimp (Parenrengi *et al*., 2013), mud crab lavae (Misbah, 2018), king crab (Jamal, 2019) which also stated that the RNA/DNA ratio increased with increasing growth rate.

CONCLUSION

Based on the results of the study, it can be concluded that feeding Artemia salina combined with Phronima sp can increase the RNA/DNA ratio of crab compared to single feed (100% Phronima sp. or 100% Artemia salina). The feed combination with the highest RNA/DNA ratio result is shown in treatment E (Phronima sp 25% + Artemia salina 75%) with RNA/DNA ratio 2.02 $\pm$ 0.032 ng/µL. The results of this study can be applied to crab entrepreneurs in reducing the cost of purchasing Artemia salina that must be imported for crab feed. In addition, crab entrepreneurs can minimize capital and increase profits due to reduced costs for crab feed but still maintain the quality of crab growth. Future research is expected to focus on survival rates, measuring physical growth rates (weight and carapace width), and bioeconomic value to advance crab quality and production in Indonesia.

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