The effect of differences in feed protein raw materials on the glycogen content, metamorphosis rate of mangrove crab larvae (*Scylla olivacea*) and feed price

Haryati, Y Fujaya and E Saade

Department of Fisheries, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia

Email: haryati_fikpunhas@yahoo.com

Abstract. Feed is one of the factors that determine the success of rearing mangrove crab larvae (*Scylla olivacea*). The use of artificial feed in the form of micro diet can guarantee higher availability and flexibility of feed. The artificial feed generally used is commercial produced and relatively expensive; therefore research is needed to produce good quality artificial feed at low prices. This study aimed to determine feed with certain protein raw materials that can replace commercial feed in terms of glycogen content of larvae, metamorphosis rate and feed price. The experimental design to determine the treatment effect on larval glycogen content was a completely randomized design of eight treatments with three replicates. The treatments were: A) 100% Artemia meal, B) 100% fish meal, C) 100% squid meal, D) 50% Artemia meal, 50% fish meal, E) 50% Artemia meal, 50% squid meal, F) 50% fish meal, 50% squid meal, G) 35% fish meal, 35% squid meal, 30% Artemia meal as raw material for feed protein and H) commercial feed. The rate of metamorphosis and price of feed were analysed descriptively. Results of the variance analysis showed that the difference in feed protein raw material had a significant effect (p<0.05) on the glycogen content of the megalopa stage larvae. The glycogen content in treatments C and E was not significantly different (p>0.05) but was higher and significantly different (p<0.05) than other treatments. Mangrove crab larvae in treatments A, C, E and F have reached the megalopa stage at the age of 18 days, while other treatments have only reached the new megalopa stage when the larvae were 19 days old. The lowest production cost of making the low budget feed is feed with protein raw material in the form of 100% fish meal and the most expensive one is feed with 100% raw material *Artemia* meal. Based on the result of this study, feed with protein raw material in the form of 100% squid meal can be used as a substitute for commercial feed, besides providing a better response to glycogen content and metamorphosis rate, the price of this feed is also cheaper than commercial feed.

1. Introduction

Feed is one of the success determinants of an aquatic hatchery business. The use of artificial feed in micro form (micro diet) can guarantee availability, lower production costs and higher flexibility [1]. Research results [2] have shown that mangrove crab larvae (*Scylla olivacea*) can be given artificial feed that had been pre-digested with the addition of papain enzyme with a dosages of 4.5% starting at zoea two stage. The use of commercial feed is relatively expensive (currently Rp. 250,000/per kg), therefore research is required to develop artificial feed that has a suitable quality in accordance with the requirements of the larvae at a more affordable price than commercial feed. Micro-bound diets are
one type of artificial feed that is widely applied in nutritional studies on crustacean larvae. The quality of artificial micro-bound diets is determined by the raw material protein sources [3]. Raw protein ingredients will affect the quality of feed.

The purpose of this study was to test a number of combinations of raw materials as artificial feed protein after hydrolysis using the enzyme papain, in order to determine the material which produced the best glycogen content and metamorphosis rate, as well as low feed prices compared to commercial feed.

2. Research Methodology

The feed manufacturing process and hydrolysis of feed ingredients were carried out in the Laboratory of Nutrition and Feed Technology, Faculty of Marine and Fisheries Sciences, Hasanuddin University, Makassar, Indonesia. Analysis of glycogen content was carried out in the Animal Husbandry Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The papain enzyme used was Newzime which is produced by the Jepara Brackish water Aquaculture Centre. Artificial feed used was powdered commercial feed.

The experimental design used was a completely randomized design with eight treatments and three replicates. The treatment in this study is the difference in feed protein raw materials:

A. 100% Artemia meal
B. 100% fish meal
C. 100% squid meal
D. 50% Artemia meal, 50% fish meal
E. 50% Artemia meal, 50% squid meal
F. 50% fish meal, 50% squid meal
G. 30% Artemia meal, 35% fish meal, 35% squid meal
H. Commercial feed

In addition to the protein raw materials, ingredients used were 5% fish oil, 5% vitamin-mineral mix and 5% tapioca flour as a binder. The feed was hydrolysed using 4.5% of papain.

Analysis of glycogen content was carried out at the end of the experiment, at the megalopa stage. Determination of glycogen levels was carried out using all parts of the body of the larvae because it was difficult to separate the hepatopancreas from other parts of the body.

Glycogen content was calculated using the following formula:

\[
glycogen (mg/g\ sample) = \frac{abs.\ spl}{abs.\ std} \times kons.\ std \times fp \times \frac{1}{1000}
\]

where:

- \(abs.\ spl\) = sample absorbance at \(\lambda\) 670 nm
- \(abs.\ std\) = standard absorbance
- \(kons.\ std\) = standard concentration (500 \(\mu g/mL\))
- \(fp\) = dilution factor (5X)
- \(1/1000\) = conversion factor from micrograms to milligrams

To determine the rate of larval development, 10 larvae were taken every day at 6:00 a.m. and the larval morphology was observed. Larval development rates were calculated using the Larval Stage Index (LSI) referring to the method used by [4]. To calculate LSI, the larval stage index is presented in Table 1. The LSI was calculated based on [4] (modified method):

\[
LSI = \frac{(S_t \times n_t) + (S_{t-1} \times n_{t-1})}{N}
\]

where:

- \(LSI\) = Larval Stage Index
- \(S_t\) = LSI stage \(t\)
- \(n_t\) = number of larvae at stage \(t\)
- \(S_{t-1}\) = LSI stage \(t-1\) (before stage \(t\))
\[ n_{t-1} = \text{number of larva stage } t-1 \]
\[ N = \text{number of samples (individuals)} \]

### Table 1. Table of LSI values for each stage of larval development

| Larva Stage       | Larva Stage Index Value | LSI Class |
|-------------------|-------------------------|-----------|
| Zoea 1 (Z-1)      | 1 – 1.5                 | 1         |
| Zoea 2 (Z-2)      | 1.6 – 2.5               | 2         |
| Zoea 3 (Z-3)      | >2.5 – 3.5              | 3         |
| Zoea 4 (Z-4)      | >3.5 – 4.5              | 4         |
| Zoea 5 (Z-5)      | >4.5 – 5.5              | 5         |
| Megalopa          | >5.5                    | 6         |

To determine the effect of differences in feed protein raw material and commercial feed on the glycogen content, analysis of variance (ANOVA) was used. If the treatment had a significant effect, the W-Tukey post-hoc test was used to determine the feed protein raw material that produced the best glycogen content. To determine the effect of different feed protein and commercial feed raw materials on the rate of metamorphosis and feed prices, descriptive analysis was applied.

### 3. Results

#### Mangrove crab larvae glycogen content

Larval glycogen content is presented in Table 2. Results of the Analysis of Variance showed that the difference in feed protein raw material had a significant effect (p<0.05) on the glycogen content of mangrove crab larvae (*Scylla olivacea*) at the megalopa stage.

### Table 2. Glycogen content (mg/g) of mangrove crab (*Scylla olivacea*) larvae at the megalopa stage

| Treatments                  | Glycogen content\(^1\) |
|-----------------------------|------------------------|
| A. 100% *Artemia* meal      | 11.3167 ± 0.0121\(^c\) |
| B. 100% fish meal           | 10.4200 ± 0.0240\(^ae\) |
| C. 100% squid meal          | 11.7200 ± 0.0392\(^ab\) |
| D. 50% *Artemia* meal, 50% fish meal | 10.9233 ± 0.0019\(^e\) |
| E. 50% *Artemia* meal, 50% squid meal | 11.9433 ± 0.0035\(^a\) |
| F. 50% fish meal, 50% squid meal | 11.5000 ± 0.0042\(^bc\) |
| G. 30% *Artemia* meal, 35% fish meal, 35% squid meal | 10.2533 ± 0.0067\(^e\) |
| H. Commercial feed          | 10.6367 ± 0.0049\(^d\) |

\(^1\)Different subscript letters indicate significantly different values (p<0.05)

The glycogen content was highest in larvae fed with the combination of 50% *Artemia* meal and 50% squid meal (treatment E), while the 100% squid meal treatment (C) was not significantly different (p > 0.05) from E and was higher than other treatments. Glycogen content in larvae fed with a combination of 30% *Artemia* meal, 35% fish meal and 35% squid meal (G) was the lowest and significantly different (p<0.05) with other treatments except 100% fish meal (B) (p > 0.05).

#### Metamorphosis rate

The metamorphosis rate of mangrove crab larvae fed artificial feed with a variety of protein raw materials which have been pre-digested using 4.5% papain enzymes from zoea stage 1 to megalopa were presented in Table 3. Mangrove crab larvae in treatments A, C, E and F had already reached the megalopa stage at the age of 18 days; in other treatments, the megalopa stage was reached when the larvae were 19 days old.
Table 3. Mean values of the Larval Stage Index (LSI) of *Scylla olivacea* larvae reared with different feed protein raw materials and larval stages (LS) reached by the larvae on treatment days 1 to 19 *a*

| T  | LSI  | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  |
|----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A  | LSI  | Z1  | Z1  | Z2  | Z2  | Z2  | Z2  | Z3  | Z3  | Z3  | Z3  | Z4  | Z4  | Z4  | Z4  | Z4  | Z5  | Z5  | Z5  | Z5  |
| B  | LSI  | Z1  | Z1  | Z2  | Z2  | Z2  | Z2  | Z3  | Z3  | Z3  | Z3  | Z4  | Z4  | Z4  | Z4  | Z4  | Z5  | Z5  | Z5  | Z5  |
| C  | LSI  | Z1  | Z1  | Z2  | Z2  | Z2  | Z2  | Z3  | Z3  | Z3  | Z3  | Z4  | Z4  | Z4  | Z4  | Z4  | Z5  | Z5  | Z5  | Z5  |
| D  | LSI  | Z1  | Z1  | Z2  | Z2  | Z2  | Z2  | Z3  | Z3  | Z3  | Z3  | Z4  | Z4  | Z4  | Z4  | Z4  | Z5  | Z5  | Z5  | Z5  |
| E  | LSI  | Z1  | Z1  | Z2  | Z2  | Z2  | Z2  | Z3  | Z3  | Z3  | Z3  | Z4  | Z4  | Z4  | Z4  | Z4  | Z5  | Z5  | Z5  | Z5  |
| F  | LSI  | Z1  | Z1  | Z2  | Z2  | Z2  | Z2  | Z3  | Z3  | Z3  | Z3  | Z4  | Z4  | Z4  | Z4  | Z4  | Z5  | Z5  | Z5  | Z5  |
| G  | LSI  | Z1  | Z1  | Z2  | Z2  | Z2  | Z2  | Z3  | Z3  | Z3  | Z3  | Z4  | Z4  | Z4  | Z4  | Z4  | Z5  | Z5  | Z5  | Z5  |
| H  | LSI  | Z1  | Z1  | Z2  | Z2  | Z2  | Z2  | Z3  | Z3  | Z3  | Z3  | Z4  | Z4  | Z4  | Z4  | Z4  | Z5  | Z5  | Z5  | Z5  |

*a T = Treatment; LSI = Larval Stage Index; LS = Larval stage (Z1 to Z5, see Table 1)*
**Feed Cost**
The cost to produce the feed formulations (treatment A to G) and the price of commercial feed (treatment H) are presented in Table 4. Based on the production cost per kilogram of feed, the most expensive feed was the feed with 100% *Artemia* meal, and the cheapest was the feed with 100% fish meal as its protein source.

| Treatments               | Rp.  |
|--------------------------|------|
| A- *Artemia* meal        | 1,200,000 |
| B- Fish meal             | 85,000  |
| C- Squid meal            | 130,000 |
| D- 50% *Artemia* meal, 50% fish meal | 645,000  |
| E- 50% *Artemia* meal, 50% squid meal | 665,000  |
| F- 50% fish meal, 50% squid meal | 110,000  |
| G- 30% *Artemia* meal, 35% fish meal, 35% squid meal | 475,250  |
| H- Commercial feed       | 250,000 |

4. **Discussion**
The success of feeding is determined by the quality and quantity of feed given. The quality of feed from a chemical aspect is determined by the protein, lipid (fat) and carbohydrate content. Protein requirement at the *Scylla serrata* megalopa stage is 79.4% according to [5], whereas according to [6] the protein requirement of juvenile *Scylla serrata* ranges from 34.2% - 51.8%. Feed protein content in the various combinations of feed protein raw materials and the commercial feed ranged from 38.67% - 72.17%. The commercial feed had the lowest protein content, while feed with 100% squid flour had the highest protein content [7]. Based on protein content, the feed quality should be adequate for the needs of mangrove crab larvae.

Lipids are known to play an important role as a source of energy, maintaining the structural integrity of biological membranes and function as important steroid precursors [3]. The effect of dietary lipid content on the growth of mangrove crab juveniles was investigated by [8]; the results showed that lipid content from 3.3% to 13.8% was sufficient for juvenile mangrove crabs. Meanwhile, lipid requirements ranging from 6% to 12% for juvenile *Scylla serrata* [6], and 6% at the megalopa stage [8] have been reported. The lipid content of feed in the seven combinations of feed protein raw materials and commercial feed used in this study ranged from 2.87% - 15.91%. The fat content of the commercial feed is lower than the reported requirements of mangrove crab larvae, while the feed with 100% *Artemia* meal had a fat content higher than the ranges previously reported as meeting the requirements of these larvae [7]. The fat content of the other six treatments was within the range reported as meeting the needs of mangrove crab larvae.

Nitrogen Free Extract (NFE) content of the feeds used as treatments ranged from 1.27 – 22.54% wet weight corresponding to 1.45% - 27.81% dry matter [7–9] suggested that the carbohydrate requirement for juvenile *Scylla serrata* ranged from 13.5% to 27% dry matter. The carbohydrate requirements of mangrove crab larvae were unknown. The result of the research by [7] showed that the NFE content of feed with 100% *Artemia* meal and a combination of 50% *Artemia* meal and 50% squid meal, were respectively 12.31% and 11.32% of relative dry ingredients and met the needs of mangrove crab larvae. The NFE content in commercial feed (29.97% dry matter) was higher than the requirement based on [7], while in the seven feed formulations (A to G) the carbohydrate content should be increased.

If glucose derived from food is not used as a source of energy, then it will be stored, either in the form of fat, as triglycerides, or in the form of glycogen. Through the process of glycogenesis, by glycogen synthetase, glucose is a monosaccharide that will undergo a metabolic process to produce glycogen. This glycogen will then function as an energy reserve. According to [10], an increase in glycogen levels indicates the presence of glucose remaining in the blood after the metabolic energy
requirement are met, which is immediately converted to glycogen and then stored in the muscles and liver. Glycogen content in larvae fed with a combination of 50% Artemia meal and 50% squid meal and those fed with 100% squid meal were higher than other treatments. The higher glycogen content shows that the protein and carbohydrate content needs were met, and therefore the carbohydrates that were not used to produce energy could be stored in the form of glycogen. The lower glycogen content in larvae fed with a combination of 30% Artemia meal, 35% squid meal and 35% fish meal and those fed with 100% fish meal was most likely due to the low carbohydrate content (4.20 - 4.21%), so that the carbohydrates contained in the feed were mostly used as a source of energy. The glycogen content produced in this study was higher than the results of research by [2]. The glycogen content of mangrove crab larval (Scylla olivacea) megalopa stage fed pre-digested commercial feed using 4.5% papain enzyme was only 6.518 ± 0.527 mg/g.

Most animals can synthesize sterols from acetate, but crustaceans such as arthropods do not have the ability to synthesize sterols de novo [11]; therefore, cholesterol is essential for the growth and survival of crustaceans. In crustaceans, cholesterol is the precursor for a number of compounds, including moulting hormones. A study on S. serrata juveniles observed significantly higher growth in juveniles fed with 0.50% and 0.79% cholesterol content [9]. In Penaeus vannamei, high growth occurred when given a diet with 0.5% cholesterol content [12]. In mud crab larvae, the survival rate to the megalopa stage was highest in larvae fed with a 1% cholesterol content [5]. The study conducted by [7] found the cholesterol content was highest in the feed with 100% squid meal (0.547%), followed by the combination of 50% squid meal, 50% fish meal (0.251%) and the combination of 50% squid meal and 50% Artemia meal (0.241%). The lowest cholesterol content was in the commercial feed (0.170%). The cholesterol content seems to cause the relatively fast rate of metamorphosis observed under the treatments in this study. The duration of metamorphosis starts from zoea stage 1 to megalopa was achieved within 18 to 19 days. According to [13], the duration of the metamorphosis of mangrove crabs from the zoea stage to megalopa generally takes around 17-26 days, where the time required for each zoea stage is generally 3-5 days. The duration of metamorphosis process of mangrove crabs (Scylla olivacea) fed pre-digested feed by using the enzyme papain 4.5% from the zoea stage 1 to megalopa stage was also 18 days [2].

5. Conclusion
Glycogen content was higher in larvae fed with 100% squid meal or a combination of 50% Artemia meal and 50% squid meal than other protein raw materials and commercial feed. The metamorphosis rate from zoea stage 1 to megalopa was relatively similar, ranging from 18 to 19 days. In terms of production costs per kg of feed, the most expensive feed was the formulation with 100% Artemia meal and the cheapest was the feed with 100% fish meal. Based on the larval glycogen content in the megalopa stage, the metamorphosis rate and the feed production costs, artificial feed with 100% squid meal as protein can be used for rearing mangrove crab larvae (Scylla olivacea) to the megalopa stage.

Acknowledgements
The authors acknowledge funding for this research from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia through the Applied Higher Education Research scheme 2018 budget.

References
[1] Gatesoupe F and Luquet P 1991 Practical diet for mass culture of the rotifer Brachionus plicatilis: application to larval rearing of sea bass, Dicentrarchus labrax Aquaculture 22 149–63
[2] Haryati, Fujaya Y, Saade E and Fajrianti D 2018 Effectiveness of addition papain enzyme in Artificial diet on the metamorphosis rate and glycogen content of mangrove crab larvae (Scylla olivacea) Torani 1 31–9
[3] May-Helen H 2008 Towards development of formulated diet for mud crab (Scylla serrata)
larvae, with emphasis on lipid nutrition (PhD thesis, James Cook University)

[4] Redzuari A, Azra M N, Abol-Munafi A B, Aizam Z A, Hii Y S and Ikhwanuddin M 2012 Effects of feeding regimes on survival, development and growth of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) larvae *World Appl. Sci. J.* 18 472–8

[5] Genodepa J, Southgate P C and Zeng C 2004 Preliminary assessment of a microbound diet as an Artemia replacement for mud crab, *Scylla serrata* megalopa *Aquaculture* 236 497 – 509

[6] Catacutan M R 2002 Growth and body composition of juvenile mud crab, *Scylla serrata*, fed different dietary protein and lipid levels and protein to energy ratio. *Aquaculture* 208 113–23

[7] Haryati, Fujaya Y and Saade E 2018 The effect of differences feed protein raw materials to the dissolved protein content, the degree of protein hydrolysis and feed nutrient content of mud crab larvae feed (*Scylla olivacea*) *Asian J. Aquat. Sci.* 1 52 – 57

[8] Sheen S S and Wu S W 1999 The effect of dietary lipid levels on the growth response of juvenile mud crab, *Scylla serrata* *Aquaculture* 93 121–34

[9] Sheen S S 2000 Dietary cholesterol requirement of juvenile mud crab *Scylla serrata* *Aquaculture* 189 277–85

[10] Handayani H and Widodo W 2011 *Fish Nutrition* (Malang: UMM press Malang)

[11] Sheen S S, Liu P C and Chen S N . 1994 Cholesterol requirement of juvenile tiger shrimp (*Penaeus monodon*) *Aquaculture* 125 131 – 137

[12] Pedroza-Islas R, Gallardo P, Vernon-Carter E J, Garcia-Galano T, Rosas C, Pascual C and Gaxiola G 2004 Growth, survival, quality and digestive enzyme activities of larval shrimp fed microencapsulated, mixed and live diets *Aquacult. Nutr.* 10 167–73

[13] Kasry A 1996 *Budidaya Kepiting Bakau dan Biologi Ringkas* [Mangrove crabs and their biology, an overview] (Jakarta: Bharata)