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Effect of Rotifers (*Branchionus plicatilis*) Replacement with Predigested Artificial Feed on Survival and Larvae Development of Blue Swimming Crab (*Portunus pelagicus*)

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Abstract

Agung Sudaryono, Widyawati Alik T, Siti Aslamyah, and Yushinta Fujaya. 2015. Effect of Rotifers (*Branchionus plicatilis*) Replacement with Predigested Artificial Feed on Survival and Larvae Development of Blue Swimming Crab (*Portunus pelagicus*). *Aquacultura Indonesiana, 16 (2): 63-68*. One of the problems faced in blue swimming crab hatchery business is the reliance on the use of natural feed. This research aimed to study the effect of rotifer feed replacement by predigested artificial feed on survival rates, growth rates of the larval stages and to determine the best feeding period of predigest artificial feed 100% to replace rotifers. The study was conducted in May-June 2015 in a household scale hatchery location at Bojo Village, Mallusettasi District, Kabupaten of Barru. There were five replacements of rotifers by predigest artificial feed namely: A. Control (feeding with rotifer up to stadia zoea 4), B (feeding with rotifer up to stadia zoea 3), C (feeding with rotifer up to stadia zoea 2), D (feeding with rotifer only at stadia zoea 1), E (No feeding with rotifer). Predigest artificial feeds were fed for a rearing period of larvae in all treatments. The results showed that the survival of the blue crabs larvae that did not consume rotifers as a feed source were significantly lower than the crab larvae consuming rotifers. The larvae with no feeding rotifer survived only at stadia zoea-2 while those feeding with rotifers survived until zoea-4. The rate of stadia changes did not differ among treatments getting rotifers. Use of predigest artificial feed on crab hatchery can be an alternative to rotifers in the future. Feeding predigest 100% can be started at stadia zoea-2.

Key words: Blue swimming crab; Larvae; *Portunus pelagicus*; Predigest artificial feed; Rotifers

Introduction

Blue swimming crab (*Portunus pelagicus*) is one of seawater resources having a high economic value. This crab is liked many people and traded widely around the world. In accordance with high demands and declining fishing production for the blue crab, so that the production through sustainable aquaculture will be the main alternative for the future. Unfortunately, up to now the blue swimming crab hatchery has not been commercially produced yet.

A high mortality during the larval rearing period of *P. pelagicus* is the main problem in the development of blue swimming crabs. This high mortality was due to some factors such as water quality, nutrition, disease, and canibalism (Fujaya *et al.*, 2014). Availability of rotifer depending on season also becomes the main gap.

One of solutions to reduce the larval crab mortality is by feeding with artificial feed as early as possible to reduce the use of natural feeds such as rotifer. However, low digestive system function of the early larvae stadia becomes the problem in introducting the artificial feeds. Nikhlani (2013) reported that the digestive organs of *Portunis* sp. can properly work after stadia of Zoea 3. At that stadia, the digestive endogenous anzymes already have been produced.

To solve this problem, it is necessary to feed the crab larvae with artificial feeds containing more digestible and simple nutrition composition. So that although the available endogenous digestive enzymes is limited, it is expected that the nutritions from artificial feeds can be more effectively utilized by *Portunis* sp. larvae (Aslamsyah, 2006). Simplying complex feed composition can be conducted through a predigestion process by using bacteria probiotic. In this case, hope that the enzymes produced by bacteria probiotic can assist to enhance digestive system function of the larvae by stimulating secretion of the endogenous enzymes.

According to Susanti (2002), in general *Bacillus* sp. can be found in anywhere including in digestion tracts of *Cyprinus carpio*, *Oreochromis* sp. and *Portunis* sp. The superior of *Bacillus* sp. are no toxin content, easy growth, low cost substrate, high tolerant in high temperature, and no metabolic waste residu

(Linggarjati *et al.,* 2013). Fardiaz (1992) added, *Bacillus* sp. are proteolitic heterotroph bacteria that can decompose protein to be amino acids, enhance dietary protein quality content, lower fibre content, decompose complex sugars to be simple sugars and have pectinolitic charateristics to produce a complex carbohydrate (William and Westhoff, 1989).

Based on the above facts, the diet replacement from natural feeds to predigested artificial feeds by assistance of *Bacillus* sp. is expected increasing artificial feed efficiency utilisation and reducing natural feed consumption. So that this can result in a better quality of blue swimming crab seeds. Rotifer replacement by predigested artificial feeds was conducted at every stadia development to determine the right time to replace rotifer with artificial feeds. Hope that information from this reserach will be able to reduce the dependency of rotifer as the main feed source for *Portunis* sp. larvae.

Material and Method

This research was conducted in May to June 2015 in householе scale hatchery, Bojo Village, Mallusettasi District, Barru Regency.

Tested Animal

The tested animal used in the study was blue swimming crabs (*Portunis pelagicus*) larvae at zoea-1 stadia obtained from the hatchery of wild broodstock catched from Makassar Strait. The only healthy and active swimming larvae toward light were used in the study. Before stocking, the larvae were desinfected by using 1 mg/L elbazin solution to protect against pathogen bacteria. Stocking density of the larvae was 50 larvae/L. The larvae were placed in 12 L cone containing desinfected 30 ppt seawater. The water were desinfected by using 150 mg/L chlorin and then the water were netralized by 25 mg/L $Na_2S_2O_3$ and aerated for 24 h.

Feed

Feeds used in the study were natural feeds of rotifers (*Branchionus plicatilis*) and naupli Artemia salina. Rotifers were obtained from massal culture and Artemia were obtained from the hatchery of artemia cysts. Rotifers and Artemia were fed at 35 rotifers/mL/d and 2 Artemia/mL/d, respectively. Artificial feed used was a commercial shrimp feed containing 48% crude protein and 8% crude lipid.

A daily feeding frequency was 8 times (2 times with natural feeds and 6 times with artificial feed). Feeding doses followed the development of larvae stadia.

Table 1. Feeding regimes

Time	Type of feeds
06.00	Artificial feed
09.00	Rotifer and artemia
12.00	Artificial feed
15.00	Artificial feed
18.00	Artificial feed
21.00	Rotifer and artemia
00.00	Artificial feed
03.00	Artificial feed

Table 2. Predigested Artificial Feed Doses

Stadia	Artficial feed doses (mg/L/d)
Zoea-1	2.5
Zoea-2	5.0
Zoea-3	7.5
Zoea-4	10.0
Megalopa	15.0

Predigestion

Probiotic bacteria used were pure cultures of *Bacillus subtilis* obtained from Research Institute and Brackishwater Aquaculture Development, Maros, South Sulawesi. The bacteria cultured at Microbiology laboratory, Faculty of Marine Science and Fisheries, Hassanuddin University. The bacteria cultures were conducted by taking 10 mL pure cultures of *B. Subtilis* bacteria and entered into a 250 mL sterile TSB medium. Then the bacteria were incubated in the shaker incubator for 1x24 h at 30°C. After that, the bacteria were ready to applied to artificial feed with a dose of $2x10^8$ CFU/g.

Predigestion to artificial feed was carried out by spraying homogenly the bacteria solution on the surface of the feed. Incubation was conducted for 13 h based on the final phase period of *Bacillus* sp. exponential growth (Aslamyah, 2006).

Treatment and Research Design

The research design used was a completely randomized design with 5 treatments and 3 replications (Table 3).

Parameters Measurement

Parameters measured in the study were stadia survival and exchage rates. Water quality was used as supporting data consisted of salinity, temperature, pH, DO and ammonia. Survival of zoea larvae at the end of the experiment was calculated using the formulation as follows:

$SR = N_t/N_0 \times 100$

Where :
SR = Survival (%)
N_t = total amount of live larvae at the end of the experiment (individual)
N_o= total amount of live larvae at the beginning of the experiment (individual)

The development larvae were observed everyday using a light microscop. The rearing period and the morphology of larvae were noted to determine the Larval Development Index (LDI). The morphology characteristics of each larvae stadia followed Kosiilen *et al*. (2004) (Table 4) and the LDI was determined according to the method suggested by Ikhwanuddin *et al*. (2011).

$$LDI = \frac{[(Z_0 \times n_0) + (Z_{0+1} \times n_{0+1})]}{N}$$

Where:
Z_0 = Initial stadia value
n_0 = Total amount of initial stadia larvae
Z_{0+1} = Value of one the next stadia
n_{0+1} = Total amount of the larvae on the next stadia
N = Total amount of the sampling larvae

Analisis Data

Data obtained were analysed using a analysis of variance (ANOVA). A significant data was continued by W-Tukey Test.

Results and Discussion

Survival

The survival of blue swimming crabs fed by predigested artifical feeds to replace rotifer can be seen in Table 5.

Table 3. Feeding regimes at different stadia and treatments

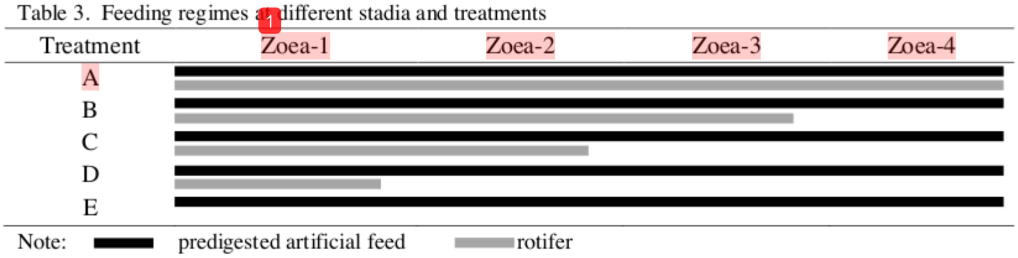

Table 4. Morphological characteristics and Larval Development Index (LDI) of blue swimmer crab

Stadia	Morphological Characteristics	LDI
Zoea-1 (Z1)	Eyes sessile, 5 segmented abdomen with a fork resembles a telson	1
Zoea-2 (Z2)	Eyes stalked, abdomen and telson are in similar condition with the ones in the previous stage	>1-2
Zoea-3 (Z3)	Eyes stalked, 6 segmented abdomen, lateral spines on somites 3-5 longer, paired pleopodbuds at ventral posterior end of somites 2-5. telson similar to previous stage	>2-3
Zoea-4 (Z4)	Eyes stalked, 6 segmented abdomen, pleopod buds well developed telson similar to that ofzoea-3 except for additional short seta on inner margin	>3-4
Megalopa (M)	More like crabs than zoea, the carapace gets rounded according to its length, first pereiopod appears in the tip of the upper arm, abdomen in the posterior is still long	>4-5

Tabel 5. Average survival (%) of blue swimming crab (*Portunus pelagicus*) on zoea-1 – zoea-1 stadia

Treatment	Survival (5)
A (rotifer fed up to the zoea 4 stadia)	0.76 ± 0.16^b
B (rotifer fed up to the zoea 3 stadia)	0.98 ± 0.32^b
C (rotifer fed up to the zoea 2 stadia)	0.77 ± 0.21^b
D (rotifer fed up to the zoea 1 stadia only)	0.72 ± 0.19^b
E (No feeding with rotifer)	0.11 ± 0.13^a

Note: The values with the different superscripts are significant (P<0.05)

Results of variance analysis showed that substitution of rotifer with predigested artificial feed resulted in a significant effect ($P<0.05$) on the blue crab larvae. After W-Tuckey test showed that survival of the larvae reared without feeding rotifer was a lower significant than those with feeding rotifer. However, no different significances on survival of the larvae (zoea-1, zoea-2, 3, up to zoae-4) were found when they fed with rotifer.

Based on this study, it can be concluded that the predigested artificial feeds have not been able to totally replace rotifer as feed source. There is a superior in nutrition of rotifer as a natural feed. Rotifer is also able to spread more evenly in the water coloum so that the larvae relatively have a higher opportunity to feed the rotifer (Rimandi, 2015). In addition, the natural feed contain a ready nutrient unit to utilize in digestive tract when they were consumed by the blue crab larvae. Although the nutrients of *predigested* artificial feed have been already in the simple forms, but the nutrients are susceptible to leaching out by the maintenance media so that it could result in a decrease of water quality (Genodepa *et al*., 2004; Aslamyah, 2006). The *predigested* artificial feeds also tended to spread less evenly in the water coloum. It will cause to waste and settle in the bottom waters. This is indicated that predigested artificial feeds could not be able to well replace the natural feeds.

Natural feed becomes a main choice in meeting the nutrition requirement of initial blue crab larvae stadia. Superiority of natural feeds in meeting nutrition requirements of the larvae is closely related to exogenous digestive enzymes availability. The larvae with lack of digestive tracts development will obtain a benefid of available exogenous enzymes. Where a efficient combination of exogenous and endogenous enzymes will result in an increase in abaility to digest the feeds (Soundarapandian *et al*., 2007).

Larvae Development Rates

Results of the blue crab larvae development rates during the study presented in Table 6. The results showed that the daily larvae development rates during a rearing period for all treatments were relatively similar. The larvae fed with rotifer were able to achieve and survive to be megalopa stadia. These megalopa larvae were firstly found on the 11th day. However, the larvae without feeding rotifer (treatment E) were only able to achieve up zoea-2.

The results showed that a period of zoea-1 development for all treatments was 2 d. The period of zoea-1 larvae development in the study was different with the previous tudy conducted by Josileen and Menon (2004), Arshad *et al*. (2006) and Redzuari *et al*. (2012) reported a period of the zoea-1 larvae development to zoea-2 was for 3-4 d. Zoea-1 stadia larvae started to require natural feed intake when they were in transition from endogenous feeding to *exogenous feeding*. Rotifer are very good natural feeds for this stadia due to their relative small size and slower swimming speeds so that they are easier to catch by the larvae (Godfred *et al*., 1997).

On the rearing observation showed that the larvae started to be zoae-4 stadia on the day-9 and to be megalopa on the day-11. This showed that the feeding schedule treatments A, B, C and D did not significantly affect on larvae development rates during the zoae stadia. Delay in the larvae turnover only happened on B treatment. This indicated that predigested feeds have not been suitable yet to replace rotifer.

Dependency of the blue crabs larvae on available natural feeds could be minimized by application of predigested feeds (Rimandi, 2015). However, based on the results of this study, predigested feeds could not totally substitute natural feeds to improve the larvae growth. In fact, the availability of natural feeds during the zoae-1 phase is essential.

Water Quality

Water quality parameters significantly affected on the larvae development and survival rates of the crabs larvae during the rearing period. Water quality parameters such as temperature, salinity, dissolved oxygen and ammonia were measured every 3 d. Water quality data showed that during a period of the study, water quality were within a good range for the blue crabs larvae rearing (Table 7).

Temperature was one of water quality parameters that significantly affected on survival of the larvae in this study. During the study, although a range of temperature was within limited tolerances, however this high daily temperature fluctuation ranged 2-3^{o}C was suspected to be a cause of the mortality larvae. According to Boyd (1990), if the temperature fluctuated in a range of 3-4^{o}C within a fast period so that it will result in stress and mortality. Bryars and Havenhard (2006) said that larvae rearing under low temperature can result in decreasing feeding activity and affecting the larvae survival.

Table 6. The blue crab larvae development rates based on larvae development index.

Treatment		Larvae age (d)									
		1	2	3	4	5	6	7	8	9	10
A	IPL	1.0	1.0	1.9	2.0	2.0	2.7	3.0	3.0	3.8	4.0
	Stadia	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4
B	IPL	1.0	1.0	1.8	2.0	2.0	2.8	3.0	3.0	3.7	4.0
	Stadia	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4
C	IPL	1.0	1.0	1.7	2.0	2.0	2.6	3.0	3.0	3.6	4.0
	Stadia	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4
D	IPL	1.0	1.0	1.6	2.0	2.0	2.5	3.0	3.0	3.7	4.0
	Stadia	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4
E	IPL	1.0	1.0	1.6	2.0	2.0	0.0	0.0	0.0	0.0	0.0
	Stadia	Z1	Z1	Z2	Z2	Z2	-	-	-	-	-

Table 7. Water quality parameters observed during the study

Parameter	Range
Temperature (°C)	28-33
Salinity (ppt)	32-34
pH	7.0-7.7
Dissolved Oxygen (mg/L)	4.1-7.3
Ammonia (mg/L)	1-2

Based on the research results, it can be concluded that although predigested artificial feeds can be an alternative feed of rotifer, however, it is still essentially required for the blue crab larvae rearing (at least on stadia zoea-1 larvae). Feeding with 100% predigested artificial feeds can be conducted after stadia zoea-2 larvae without any adverse in a decrease of the survival.

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