Jurnal Ilmu dan Teknologi Kelautan Tropis Vol. 12 No. 1, Hlm. 297-308, April 2020
p-ISSN : 2087-9423 http://journal.ipb.ac.id/index.php/jurnalikt
e-ISSN : 2620-309X DOI: http://doi.org/10.29244/jitkt.v12i1.30753

ADDITION OF SHELTERS TO CONTROL THE PHYSIOLOGICAL RESPONSES AND PRODUCTION OF MUD CRAB *Scylla serrata* IN RECIRCULATION AQUACULTURE SYSTEM

PENAMBAHAN SHELTER UNTUK PENGENDALIAN RESPONS FISIOLOGI DAN PRODUKSI KEPITING BAKAU Scylla serrata PADA SISTEM RESIRKULASI AKUAKULTUR

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ABSTRACT

The availability of shelters in a specific density is expected to increase the production of mud crabs (*Scylla serrata)* in a recirculation system. Shelter, as one of the abiotic factors, plays a pivotal role in reducing death caused by cannibalism of crab and suppressing the stress levels of biota. Recirculation Aquaculture System (RAS), with the culture box capacity 60 L supported by the shelters, is predicted to produce the best physiological and growth responses of mud crabs. This present study aims to evaluate the effect of shelter addition in the environmental recirculation system on the physiological responses and production of mud crabs *S. serrata* with a density of 10 crabs per one culture box containing 60 L of seawater. The research was set up with three treatments of shelter addition, i.e., two shelters (S2), four shelters (S4), six shelters (S6), and control without shelter (C). Results showed that S6 was the best treatment with a survival rate of 73.33 ± 5.8%, a specific growth rate of 0.886 ± 0.014%, the growth rate of carapace width 0.024 ± 0.004 cm/day, and the lowest feed conversion ratio than those of other treatments. S6 treatment significantly influenced the total hemocyte count of crabs at the early cultivation (P <0.05). Addition of six shelters could optimize the growth of mud crabs with a stocking density of 10 crabs in one culture box.

Keywords: growth responses, mud crabs, physiological responses, RAS, shelters

ABSTRAK

Ketersediaan shelter (tempat perlindungan) pada kepadatan spesifik diharapkan dapat meningkatkan produksi kepiting bakau (Scylla serrata) dalam sistem resirkulasi. Shelter sebagai salah satu faktor abiotik berperan penting dalam mengurangi kematian yang disebabkan oleh kanibalisme kepiting dan menekan tingkat stres biota. Sistem Resirkulasi Akuakultur (RAS) dengan bak kotak kultur berkapasitas 60 L yang didukung oleh shelter diperkirakan menghasilkan respons fisiologis dan pertumbuhan kepiting bakau yang paling baik. Penelitian ini bertujuan untuk mengevaluasi dampak penambahan shelter pada lingkungan dengan sistem resirkulasi terhadap respons fisiologis dan produksi kepiting bakau S. serrata dengan kepadatan 10 kepiting per satu bak kultur yang berisi 60 L air laut. Penelitian ini dilakukan dengan tiga perlakuan penambahan shelter, yaitu 2 shelter (S2), 4 shelter (S4), 6 shelter (S6), dan kontrol tanpa shelter (C). S6 adalah perlakuan terbaik dengan tingkat kelangsungan hidup 73,33±5,8%, laju pertumbuhan spesifik 0,886±0,014%, pertumbuhan lebar karapas 0,024±0,004 cm/hari, dan rasio konversi pakan terendah dibandingkan dengan perlakuan lain. Perlakuan S6 secara signifikan mempengaruhi jumlah hemosit total kepiting pada awal budidaya (P <0,05). Penambahan 6 shelter dapat mengoptimalkan pertumbuhan kepiting lumpur dengan kepadatan 10 kepiting dalam satu bak kotak kultur.

Kata kunci: *kepiting bakau, RAS, respons fisiologis, respons pertumbuhan, shelter*

I. INTRODUCTION

The export volume of crabs in Indonesia reaches 34,173 tons in 2013 and drops to 28,091 tons in 2014 (KKP, 2016). The development of cultivation technology is a great effort to improve production without disturbing their natural population. Aquaculture system for mud crabs production has not obtained optimum results due to the absence of proper technique to accommodate their life behavior. Mud crab living habits are naturally solitary and cannibals when kept in high density (Cholik, 1999; Herlinah *et al.*, 2010). To deal with cannibalism, "one mud crab in one culture box" technique has been still applied. However, the effort should also consider economic feasibility, thus the rearing system with more crabs in one container needs to be develop.

Modified culture box which is different from the natural habitat potentially causes physiological stress. Physiological stress response of crustacean is commonly observed in the haemolymph and other tissues through several physiological parameters, such as glucose level and haemocyte count. The physiological stress may affect growth responses of crab, leading to a reduction in their survival (Stoner, 2012). Therefore, a technology adapting the natural habitat of crab is required. The integrated recirculation system is considered to support the optimal production of crab and control the environmental conditions for crabs' survival.

Implementation of shelter in the recirculation aquaculture system (RAS) may control mud crab's behaviors, which are solitary and cannibals. The presence of the shelters in high-density cultivation containers is essential to improve the productivity of mud crab. In addition, shelters are able to function like muddy soil holes that are naturally used by the crabs for their protection against other crab attacks. Provision of suitable shelters is recommended to diminish cannibalism of mud crabs (Genodepa, 2017).

The success of cultivation activities is also determined by several other factors, one of which is water quality. Water quality parameters, including dissolved oxygen (DO), the concentration of inorganic nitrogen compounds (nitrate, nitrite, and ammonia), salinity, pH, and temperature, are crucial to mud crabs cultivation in the recirculation aquaculture system. Salinity is a limiting factor for determining the survival and growth of mud crab (Pedapoli and Ramudu, 2014). Temperature is an abiotic factor that affects various crustacean activities, such as appetite, oxygen consumption, and metabolic rate (Nurdiani and Zeng, 2007). The optimum condition for mud crabs cultivation in RAS is a salinity of 25 ppt, pH 7, and temperature 29 ^{o}C (Hastuti *et al.*, 2015; Hastuti *et al.*, 2016; Hastuti *et al.*, 2019). Different cultivation container design in aquaculture environments might produce different activity and survival of culture biota. Hastuti *et al*. (2017) reported that different shelters number resulted in different water quality and bacterial activities involved in ammonia and nitrite oxidation. The purpose of the present study was to assess the effect of shelter addition in the water environment with recirculation aquaculture system on the physiological responses and production of mud crabs *S. serrata*.

II. MATERIALS AND METHODS

2.1. The Experimental Animals

The experiment was carried out at the Environmental Laboratory, Department of Aquaculture, IPB University, Bogor, for 60 days. Mud crabs *S. serrata* was obtained from natural habitat in Banjarmasin, South Kalimantan, Indonesia. Mud crabs were transported in containers with high air humidity from Banjarmasin to the laboratory.

2.2. The Experimental Protocols

Mud crabs cultivation was performed in a recirculation aquaculture system (RAS). We used a culture box with a size of 80 cm x 60 cm (L x W) completed with 20 W and 60 W pumps for water recirculation. Mud crabs with a density of 10 crabs (71.89±6.33 g/individual) were reared in a culture box containing 60 L of seawater (Hastuti *et al.*, 2017). Culture medium used for crab cultivation was seawater (salinity 25 ppt) acquired from Jakarta Bay, Ancol, North Jakarta. Sterilized freshwater was used to maintain a salinity of 25 ppt (Hastuti *et al.*, 2015) and pH 7 (Hastuti *et al.*, 2016). The artificial crab shelter used in this study was a box with a size of 10 cm x 10 cm x 10 cm (L x W x H) made from polyvinyl chloride (PVC), whose number was adjusted to the treatments (Figure 1).

2.3. Experimental Design

This study was set up using a completely randomized design consisting of three treatments and control, i.e., S2 (completed with two shelters), S4 (completed with four shelters), S6 (completed with six shelters), C (without shelter as control). All treatments were carried out at three replicates of crab culture.

2.4. Management of Water Quality and Feeding

We conducted shiponication before and after feeding to obtain the optimum water quality. Shiponication was carried out to discard any unwanted elements in the bottom of the culture box. Water quality parameters, including temperature, pH, and dissolved oxygen (DO), were controlled every day. While other parameters, consisting of alkalinity and turbidity, were weekly measured (APHA, 2005). The filter was used as a place and substrate for microbes in the RAS environment. Mud crabs were fed with fresh fish using the restricted method. Feeding was performed twice a day at 9 am and 5 pm at a feeding rate of 5% in the week 1–5 and 4.5% in the week 6-9 (Hastuti *et al*., 2015).

2.5. Physiological Responses

Physiological responses of mud crabs measured in this study were blood glucose level, blood cholesterol level, and total hemocyte count (THC). The measurements of physiological responses were performed at the beginning and the end of the mud crabs' cultivation period. We measured three mud crabs for each treatment (n=3).

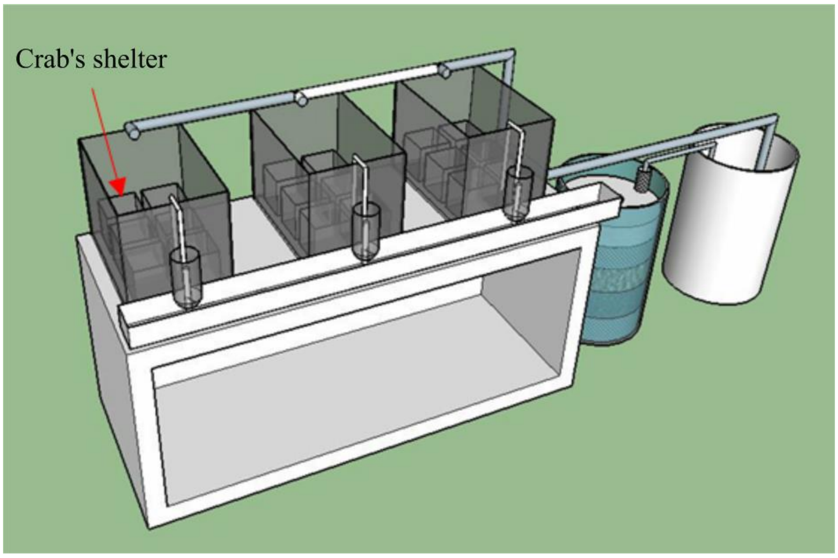


Figure 1. Design shelter of mud crab reared in a recirculation aquaculture system.

2.5.1. Glucose Levels

The analysis of blood glucose level was done using CHOD-PAP (enzymatic-colorimetric test for glucose with deproteinization) with glucose liquid color kit (HUMAN Diagnostics, Wiesbaden, DE). The blood glucose level is calculated based on sample and blank absorbent.

2.5.2. Cholesterol Levels

Measurement of total plasma cholesterol, consisting of triglycerides, HDL cholesterol (High-Density Lipoprotein), and LDL cholesterol (Low-Density Lipoprotein), was conducted using the colorimetric enzymatic principles. The fat level was analyzed using the Soxhlet Weibull method (Indonesian National Standard [SNI] 01-2891-1992). Fat digestibility was calculated from the consumption of reduced fecal fat (%) divided by the concentration of fat produced.

2.5.3. Total Hemocyte Count (THC)

THC was enumerated according to Blaxhall and Daishley's (1973) method. A 0.1 mL of crab blood (hemolymph) was taken from the arthrodial portion of the crab legs using a 1 mL syringe containing 0.1 mL of 3.8% Na anticoagulant. The mixture was homogenized. Then the first drop was removed, while the subsequent drop was placed on the hemocytometer and observed under a light microscope at 400× magnification.

2.6. The Change of Growth

Growth responses were observed at once a week by weighing the crab and measuring the crab carapace width for 60 days rearing period. The growth responses, including survival rate (SR), specific growth rate (SGR), and feed conversion ratio (FCR), were also determined.

2.6.1. The Survival Rate (SR)

The survival rate of the mud crabs was determined by comparing the number of mud crabs that survived at the beginning and the end of the experiment (Goddard, 1996) as follows:

$$SR = \frac{Nt}{No} \times 100 \qquad (1)$$

where, *SR* was the survival rate (%), *Nt* was the total number of surviving crabs in the end of experiment, and *No* was the total number of crabs in the beginning of the experiment.

2.6.2. The Specific Growth Rate (SGR)

Specific growth rate of the mud crabs was calculated using this following equation (Schulz *et al.*, 2005):

$$SGR = \left[\sqrt[t]{\frac{Wt}{Wo}} - 1\right] \times 100 \qquad (2)$$

where, *SGR* was specific growth rate (%), *Wt* was average bodyweight in certain time (g), *Wo* was initial average bodyweight (g), *t* was rearing period (days).

2.6.3. The Growth Rate of Carapace Width

The growth rate of carapace width was measured according to (Putro *et al.*, 2015). The growth of mud crab was calculated based on the ratio of the average carapace width at the final to the initial observation, as follows:

$$CW = \frac{Wt - Wo}{t} \qquad (3)$$

where, *CW* was the growth rate of carapace width (cm/day), *Wt* was average carapace width in certain time (cm), *Wo* was initial average carapace width (cm), *t* was rearing period (days).

2.6.4. The Molting Percentage

Molting is one of the natural activities of animals related to skin replacement as a growth indicator. Molting percentage was calculated by comparing the number of molting crabs to the initial number of crabs (Aslamsyah and Fujaya, 2010).

2.6.5. Feed Conversion Ratio (FCR)

The feed conversion ratio was only evaluated on the last day of the experimental period based on Goddard (1996). FCR was calculated by comparing the amount of feed given to the crab from the start until the final day to the increase in crab biomass.

$$FCR = \frac{F}{(Wt+Wd)-Wo} \quad \text{.........................(4)}$$

where, *FCR* was feed conversion ratio, F was the amount of feed (g), *Wt* was final mud crabs biomass (g), *Wd* was biomass of dead mud crabs during the experimental period (g), *Wo* was initial mud crabs biomass (g).

2.7. Statistical Analysis

All data of physiological and growth responses were analyzed using analysis of variance (ANOVA) methods at a significance level of 0.05 by SPSS 16.0. The variance of their significance was verified using the Duncan test. Descriptive analysis was used to analyze the water quality parameters. Then, data were presented using Ms. Excel.

III. RESULTS AND DISCUSSION

3.1. Water Quality During the Cultivation Period

During the 60 days rearing period, water temperature ranged from 26.5 to 27.9°C (Table 1), while pH ranged from 4.41 to 8.25 (Table 1). Alkalinity reflects the effectiveness of CO_3 content in the cultivation system against pH. HCO_3^- and $CaCO_3^-$ are closely related to the acidity in the system. The finding was supported by the fact that alkalinity ($CaCO_3$) decrease in week 5 corresponded to pH decline (pH 5). Temperature plays an important role in the vital optic component activity, appetite, oxygen consumption, and metabolic rate of crustacean (Nurdiani and Zeng, 2007). An increase in temperature leads to higher oxygen consumption of an aquatic organism (Boyd, 2012). Dissolved oxygen (DO) ranged between 3.7 and 6.9 mg/L (Table 1), following the standard for mud crabs culture set by FAO (2011) and FAO (2014). The salinity was maintained at 25 ppt as the optimal salinity for growth and survival of mud crab, contributing to a high growth rate and survival rate (Hastuti *et al.*, 2015). Meanwhile, turbidity showed the lowest range in S6 treatment achieved 4.60-11.70 NTU. Presumably, the number of shelters directly affects the crab's metabolic process, influencing residual products and turbidity value. This result was supported by the growth rate and FCR value of mud crabs (Figure 3; 5). Optimal environmental conditions can provide suitable condition for the growth and activity of macro and microorganisms in aquaculture containers. The optimal microbial activity in the culture environment is able to generate inorganic compounds that are safe for mud crab survival.

Table 1. The value range of physical and chemical parameters of water quality during the cultivation period.

Parameters	C	S2	S4	S6
Temperature (°C)	26.5-27.7	26.6-27.7	26.5-27.7	26.5-27.9
pH	5.52-8.25	4.41-7.09	4.29-7.89	4.70-7.53
DO (mg/L)	3.7-6.6	4.1-6.9	3.9-6.7	4.3-6.8
Turbidity (NTU)	3.80-19.80	4.10-14.50	5.50-15.10	4.60-11.70
Alkalinity (mg $CaCO_3$/L)	10.70-53.50	5.35-42.80	10.70-53.50	5.35-42.80

C: control (without shelter), S2: 2 shelters, S4: 4 shelters, S6: 6 shelters

Table 2. Physiological conditions of mud crab in RAS at each treatment of shelter.

Treatments	Glucose (mg/dL)		Cholesterol (mg/dL)		THC (cell/mm)	
	Initial	Final	Initial	Final	Initial	Final
C	8.71±2.19[a]	3.90±0.54[a]	13.85±4.19[a]	3.96±1.17[a]	1.85±0.03[a]	1.17±1.01[a]
S2	5.99±3.19[a]	1.27±0.86[a]	9.82±2.88[a]	4.07±1.00[a]	1.91±0.04[ab]	1.76±0.06[a]
S4	12.47±8.73[a]	3.58±3.31[a]	13.65±10.89[a]	8.38±7.68[a]	1.97±0.04[b]	1.96±0.06[a]
S6	6.20±2.26[a]	0.86±0.62[a]	11.13±6.71[a]	3.15±2.39[a]	2.13±0.06[c]	2.07±0.09[a]

Different superscripts following the values in the same treatment indicate significant differences at a significance level of 5% (DMRT) (P <0.05). C: control (without shelter), S2: 2 shelters, S4: 4 shelters, S6: 6 shelters. The values are presented as mean ± standard deviation.

Ammonia produced by the activity of ammonia-oxidizing bacteria affects the growth of *S. serrata* larvae, even though in small amounts around 0–0.5 mg/L (Neil *et al.*, 2005). It has been previously reported that S6 produce a relatively higher percentage of ammonium-oxidizing bacterial activity reached 74.01% compared to S4 which only around 33.59% (Hastuti *et al.*, 2017).

3.2. Physiological Responses

Physiological responses of culture biota can be described through varying levels of stress, i.e., primary, secondary, and tertiary levels (Iwama *et al.*, 1999). Primary responses is the initial neuroendocrine/ endocrine response to the body's, such as crustacean hyperglycemic hormone (CHH). Then, this leads to secondary responses which is the changes in the body caused by metabolic changes, including increased haemolymph glucose, lactate and pH, decreased glycogen, impaired immune function changes, and osmoregulation. Tertiary responses represent whole-animal changes, such as declined growth, immune response, disease resistance and behavioral alterations (Stoner *et al.*, 2012).

Glucose is one of the metabolic energy sources for osmoregulatory purposes. However, excess blood glucose leads to inappropriate osmoregulation and metabolic performance. Our data showed that shelter addition had no significant effects on glucose levels in both the early and end of experimental periods (P >0.05) (Table 2). The initial measurement showed higher glucose levels, and tend to decline in the subsequent measurements. The metabolic activities might cause it after an adaptation period to environmental changes. Indirectly, this condition triggers more energy use for adaptation, contributing to metabolic activity increment and blood glucose utilization. Decrease in blood glucose levels can also enhance their ability to consume oxygen from the cultivation media, which is in line with the stocking density of mud crab in the culture medium. The density of the biota may affect environmental conditions (Hastuti *et al.*, 2016). Quantitatively, we found that S6 treatment resulted in the attenuation of blood glucose levels from 6.20 mg/dL to 0.86 mg/dL (Table 2). Loss of glucose was probably used for the growth of culture mud crab. The results show that S6 is the most desirable treatment for mud crab cultivation. Also, S6 treatment is associated with low-stress levels in comparison with other treatments, which is indicated from low glucose and cholesterol levels, and high total hemocyte (Cann and Shelley, 1999). Not only for crabs, but environmental conditions also affect microbial communities in the aquaculture environment. Based on the kinetic analysis of the Michael-Menten mechanism, a higher number of shelters (in

S6 treatment) can also support higher activity and abundance of beneficial bacteria (e.g., nitrifying and denitrifying bacteria) in the cultivation environment compared to the control (Hastuti *et al*., 2017).

Cholesterol is an essential sterol for animals, appearing freely or binding to fatty acids in all cells. The observations indicated that the treatments had no significant effects on cholesterol levels (P >0.05) (Table 2). The first measurement showed higher cholesterol levels compared to the second measurement. The crabs were presumably stress in the early period of cultivation, and then they enable to adapt to new environmental conditions over the cultivation period. Cholesterol participates as a precursor for various physiological compounds, such as molting hormones, sex hormones, corticoid, bile acids, and vitamin D (Sheen, 2000).

Crustacean haemocytes play essential roles in the host immune response, performing functions such as recognition, phagocytosis, melanization, cytotoxicity and cell–cell communication (Johansson *et al*., 2000). The total haemocytes counts drops in pathogen-infected crab (Chain and Morado, 2001). We found that the presence of shelters indicated significant influences on the total hemocyte counts of crabs at the initial rearing period (P <0.05) (Table 2). In this study, mud crab seeds were obtained from their natural habitat. Initial rearing period did not result in similar haemocytes counts because of different adaptability of each mud crab natural habitat and container environment at each treatment. On the other hand, a contrary effect observed at the end of the rearing period, in which the total hemocytes counts declined in all treatments and showed no significant difference among treatments (Table 2). However, we observed that the total haemocytes counts in the control sharply decreased in comparison with other treatments (Table 2). This decrease indicated that the control tended to trigger physiological stress. According to Moullac and Haffiner (2000), the total hemocyte counts is an environmental stress indicator. Commonly, this result showed the effect of the shelter number on total haemocytes counts of cultured mud crab. As shown in Table 2, it can be observed that shelter utilization in RAS does not significantly affect the physiological condition of mud crab.

3.3. Production Responses of Mud Crabs

3.3.1. The Survival Rate

The analysis of survival rate showed that S2 and S4 treatment were significantly different from those of the control, reached 30.00 ± 10.0% and 43.33 ± 5.8%, respectively (P <0.05) (Figure 2). The results revealed that S6 treatment had the highest survival rate (73.33 ± 5.8%), which was significantly higher than the control (6.67 ± 5.8%) (P <0.05).

It suggests that proper artificial shelters can reduce cannibalism and improve survival rates of mud crabs. Mud crabs live in mangrove forests environment with sandy and muddy soil texture. Under that condition, mud crabs will immerse themselves in mud substrates or into a hole when molting process and the presence of an attack, such as cannibalism (FAO, 2011). In this study, the protector hole, as well as a nest for mud crabs, was replaced by shelters. Six shelters in S6 treatment could contribute as hiding places for mud crabs resulting in death declined. Sunaryo *et al*. (2007) reported that shelters play an important role in reducing the mortality rates of crustacean from cannibalism.

3.3.2. The Specific Growth Rate (SGR) and The Growth Rate of Carapace Width

The specific growth rate is the percentage of crab weight gain per day during the rearing period. We found that S6 treatment exhibited the highest SGR of carb (0.886±0.014%),

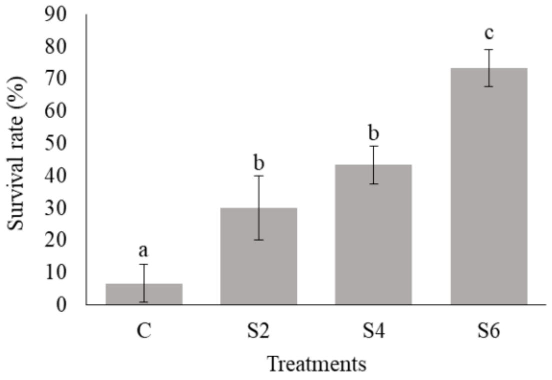
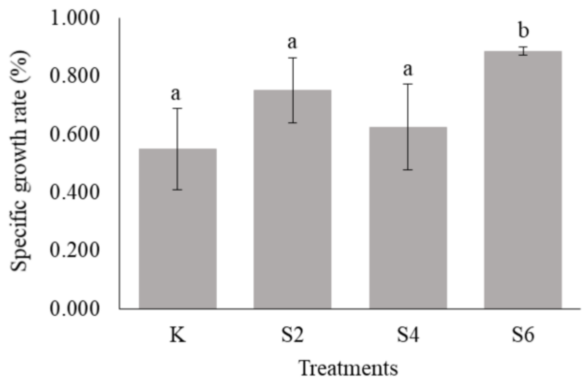


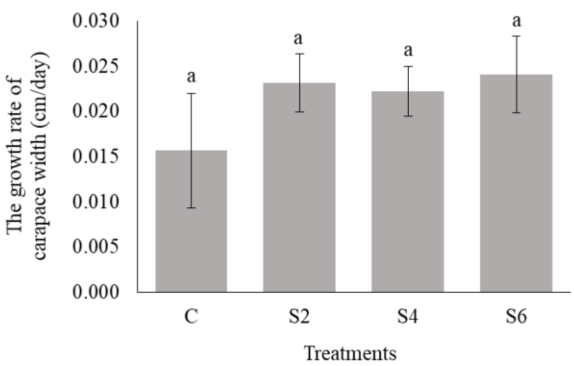
Figure 2. Survival rate (SR) of mud crab *S. serrata* during cultivation period with shelter addition. C: control (without shelter), S2: 2 shelters, S4: 4 shelters, S6: 6 shelters. The values are presented as mean ± standard deviation. Different letters above the bars denote significant differences between treatments at a significance level of 5% (P <0.05).

which was significantly different compared to the control (0.549±0.139%) (Figure 3a). The high SGR in S6 treatment suggests that shelters can provide similar conditions to the natural habitat and territorial limits of each crab. Also, the territorial boundaries formed by the shelters can make the crab consume feed optimally without any competition at the feed. According to Hastuti *et al.* (2015), the optimum physiological growth can be achieved when the energy from the feed is maximally utilized after its reduction by energy needs for other activities, such as stress responses and metabolism. The result of the specific growth rate was in accordance with the growth rate of carapace width (Figure 3b).

The growth rate of carapace width demonstrates an increase in carapace width throughout the mud crab-rearing period. The data revealed that the growth rate of carapace width was not significantly different between treatments (P >0.05) (Figure 3b). Although the statistical analysis did not show any significant differences, however, the S6 treatment yielded the highest growth rate of carapace width in comparison with other treatments. The growth rate of carapace width of S6, S4, S2, and control achieved 0.024±0.004 cm/day, 0.022±0.003 cm/day, 0.023±0.003 cm/day, and 0.016±0.006 cm/day, respectively, as presented in Figure 3b.



(a)



(b)

Figure 3. The specific growth rate (a) and the growth rate of carapace width (b) of mud crab *S. serrata* during cultivation period with shelter addition. C: control (without shelter), S2: 2 shelters, S4: 4 shelters, S6: 6 shelters. The values are presented as mean ± standard deviation. Different letters above the bars denote significant differences between treatments at a significance level of 5% (P <0.05).

3.3.3. The Molting Percentage

Crustacean growth occurs due to weight gain, which can be observed from the molting percentage. Our results showed that shelter treatments generated different effects on the molting percentage of crabs (*P* >0.05) (Figure 4). Figure 4 exhibits that the control had the highest molting percentage (96.67±5.773%), which was supported by higher cholesterol levels in this treatment compared to other treatments (Table 2). Besides, high molting percentage in the control was probably caused by high-stress levels. Low molting percentage is correlated with low biosynthesis of ecdysone (Sheen, 2000). Meanwhile, cholesterol is a vital metabolic precursor for ecdysone biosynthesis on crustaceans, especially during the molting process. Molting process in mud crabs usually because of two factors, i.e., growth and stress.

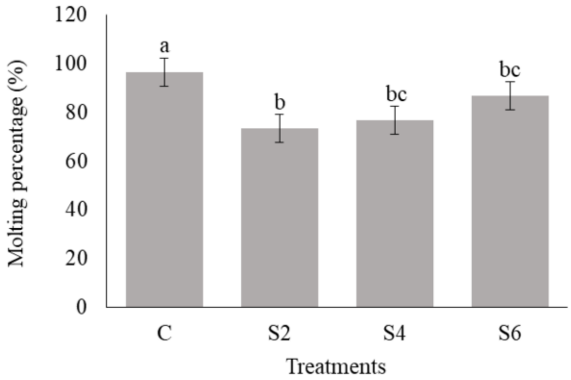


Figure 4. The molting percentage of mud crab *S. serrata* cultivation period with shelter addition. C: control (without shelter), S2: 2 shelters, S4: 4 shelters, S6: 6 shelters. The values are presented as mean ± standard deviation. Different letters above the bars denote significant differences between treatments a significance level of 5% (*P* <0.05).

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3.3.4 Feed Conversion Ratio (FCR)

FCR of all treatments (ranging from 6.5 to 14) was significantly different from the control (*P* <0.05) (Figure 5). The amount of feed given based on mud crab biomass can result in varying average FCR. Lower FCR means more efficient feed consumption by mud crabs. FCR at the control had a better value compared to the treatment with two shelters (S2) and four shelters (S4). It was probably due to the low survival rate of mud crabs in the control that affects the comfort level and stress responses of the biota. S6 treatment had the lowest FCR value with the highest survival rate in comparison with other treatments, suggesting that shelters addition has a good effect on FCR value which is proportional to their survival rate.

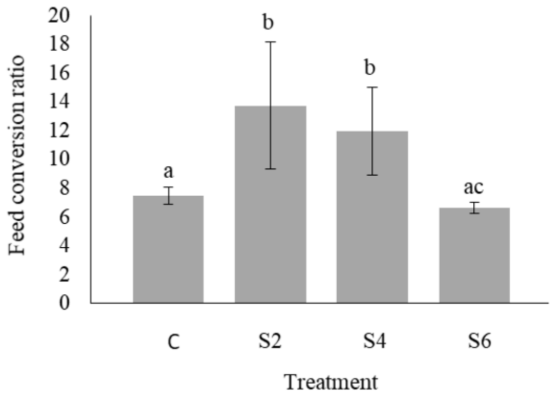

Figure 5. The feed conversion ratio of mud crab *S. serrata* during cultivation period with shelter addition. C: control (without shelter), S2: 2 shelters, S4: 4 shelters, S6: 6 shelters. The values are presented as mean ± standard deviation. Different letters above the bars denote significant differences between treatments a significance level of 5% (*P* <0.05).

The exploitation of mud crabs *S. serrata* in natural ecosystems intimidates their population. Sustainable management of the mud crabs is highly recommended (Alberts-Hubatsch *et al.*, 2015). Cultivation techniques of this species are still being developed to maintain their populations. Based on the results of this study, mud crabs are greatly possible to be cultivated in one culture box with shelter addition. Subsequently, proper shelter design is needed for the mud crabs cultivation, so they can control their physiological responses, increase the production responses, and control the cost requirement.

IV. CONCLUSION

A recirculation aquaculture system (RAS) of the mud crab *S. serrata* with the addition of six shelters on the cultivation of 10 crabs in one culture box (S6 treatment) was able to produce the most desirable result according to physiological response and the best production compared to other treatments. S6 treatment resulted in the expected growth responses, such as an increase in survival rate, specific growth rate, the growth rate of carapace width, and the lowest feed conversion ratio. Physiological responses, including glucose level, cholesterol level, and total hemocyte count, did not present any significant difference between treatments and the control. Also, water quality remained at the tolerable levels for the growth of mud crabs. The addition of six shelters could optimize the mud crab cultivation under a recirculation aquaculture system.

ACKNOWLEDGMENT

Authors thank the Ministry of Research, Technology and Higher Education, the Center for Fish Quarantine, Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia for funding and supporting this study.

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Received : 03 March 2020
Reviewed : 04 April 2020
Accepted : 25 April 2020