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Original Research

Physiological response and growth performance of spiny lobster (*Panulirus homarus*) juvenile rearing in recirculating aquaculture system with various shelter type

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ABSTRACT

Spiny lobster nursery is done to produce more adaptive and uniform juvenile lobsters quality. Shelters used in spiny lobster nursery served to reduce physical contact among lobsters in the rearing tank. The purpose of this study was to analyze the effect of different shelter types on physiological response and growth of spiny lobster (*Panulirus homarus*) juvenile rearing in recirculating aquaculture systems. Lobsters with an average weight of 50.07 ± 2.89 g were reared for 60 days. They were fed once a day with trash fish. The daily feeding rate was 3-4% of total weight. This study used four types of shelter as treatments with two replications. PVC pipe shelter as control (K), individual shelter square shaped (IS ■), individual shelter triangle shaped (IS ▲), and individual shelter tube shaped (IS ●). The weight and length of the lobster carapace improved with the duration of the research in all treatments. Throughout the trial, glucose levels in controls were generally greater than those in specific shelf treatments. The reaction of lobster hemolymph total protein to different shelters is highly variable. Overall, the usage of individual shelters had a considerable positive influence on grown lobsters in this study. This is because individual shelter eliminates contact between lobsters, eliminating the possibility of cannibalism in the cultivation container. This study concludes that IS ■ used in rearing *Panulirus homarus* showed a lower stress response than the other treatments in terms of glucose and total protein lobster hemolymph during the study. IS ■ is the best because it reduced stress levels and yielded better total biomass among the other treatments.

1. Introduction

Spiny lobster (*Panulirus homarus*) is a high market value fishery commodity. Demand for sea lobster consumption continues to increase from year to year. According to FAO (2017), demand for sea lobster in the international market reached more than 200,000 tons year-1. Meanwhile, the supply of lobsters in the market is not available continuously. Efforts to culture lobster (nursery and grow out) have been made as an attempt to meet the demand for lobster.

One of the obstacles in the rearing of lobsters is the low survival rate of cultured seeds (Thuy and Ngoc, 2004). The survival rate of lobster enlargement in the area of Lombok and Sukabumi is only 30-50% (Lesmana, 2013). High mortality in the rearing of lobster, commonly caused by cannibalism. Efforts to prevent cannibalism in the rearing system of lobster can be made with the provision of an artificial hideout (shelter) (Musbir *et al.*, 2014).

Various research on shelter application to improve production performance of rearing lobster has been done, among others, using PVC pipe shelter, compartment system, and housing system. The effectiveness of PVC pipe shelter utilization on *P. homarus* lobsters resulted in a survival rate of 65.26 ±1.41% and a daily growth rate of 1.38 ±0.04% day-1 (Adiyana *et al.*, 2014). The use of a compartment system in floating net cages with the lowest density of 25 lobster m^{-2} yielded an 84% survival and a daily growth rate of 0.77 ± 0.014% day^{-1} (Lesmana, 2013).

Applications of conventional shelter that are commonly used by lobster farmers, such as seaweed, plastic sack (pocong technique), bamboo pieces (Suastika *et al.*, 2008), rocks, wood, or nets (Nguyen *et al.*, 2009), are

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not yet optimal in improving lobsters. The use of shelter not only minimizes contact between lobster seeds but also is suspected to be less effective in overcoming cannibalism. According to Irvin & Williams (2009), lobster rearing individually results in a better survival rate than compared to communal systems.

The stress response is the most important physiological variable which influences lobster seeds' survival rate. The stress response can be evaluated subjectively using behavior observations or quantitative measurement in several physiological variables such as oxygen use level, blood composition, pH, hormones, ions, and hemocytes (Lorenzon *et al.*, 2007). According to Lorenzon *et al.* (2007); Yildiz *et al.* (2004), hemolymph glucose can be used as a stress indicator in crustaceans. Glucose concentration level in lobster hemolymph increased as stress response during handling, emersion, salinity divergence, disease, and pollutants. Several metabolic variables such as total protein and cholesterol can be used to monitor crustacean's physiological condition to stress (Mercier *et al.*, 2006)

According to Drengstig and Bergheim (2013), application technology Recirculating Aquaculture System (RAS) on lobster nursery activities can be used to maintain water quality and minimize the risk of disease attack. This lobster nursery process aims to provide an opportunity for lobster seed to adapt to the new environment so that the resulting seed is more adaptive to changes in environmental conditions and can reduce the rate of seed death (Syda-Rao *et al.*, 2010; Mohammed *et al.*, 2010). This study aims to analyze the physiological response and growth performance of juvenile sand lobster (*Panulirus homarus*) against the use of different shelters.

2. Materials and Methods

2.1 Lobster seeds

The advanced lobster juveniles used in the study were spiny lobster *Panulirus homarus* with an average weight of 50.07 ± 2.89 g. The stocking densities of each treatment is 35 lobster m^{-2}. The lobster seeds are kept in the treatment basin for 60 days.

Table 1. Overview of trash fish proximate

Parameters (%)	Trash fish	
	Wet weight	Dry weight
Water Content	78.62±0.17	-
Protein	12.83±0.23	60.04±0.58
Carbohydrate	0.71±0.09	3.30±0.40
Fat	2.31±0.22	10.79±1.11
Ash	5.53±0.07	25.87±0.13

2.2 Feed

The feeding in this study used fresh feed by using the trash fish pieces obtained from fishermen around Ancol area. The feed was given once in the afternoon at 17.00 pm. The feeding rate used in this study was 3 to 4% of lobster weight (Djai, 2017). Trash fish proximate content can be seen in Table 1.

2.3 The shape of Individual Shelter

Individual shelter (IS) that is used in this research consists of various forms, i.e., a modified tube of PVC pipe, a modified triangle of PVC water gutters, and a modified square of commercial plastic basket. The IS shape and size used can be seen in Figure 1.

2.4 Recirculation system used in research

Lobsters were reared in an indoor recirculation system. It has eight plastic tanks for lobster rearing, one plastic tank for filtrate storage, and one fiber tank for collecting outlets from all rearing tanks, and storage for a protein skimmer. The plastic tank's dimensions were 1.2×0.95×1 m, while the fiber tank has a diameter of 1.5 m and 0.75 m in height. The recirculation system used three submersible pumps with 12000 L $hour^{-1}$ capacity for filtrating, skimming, and water distribution.

2.5 Research design

In this research, we used a complete randomized design, consisting of 4 treatments and duplicate. The treatment were rearing lobster with various shape of

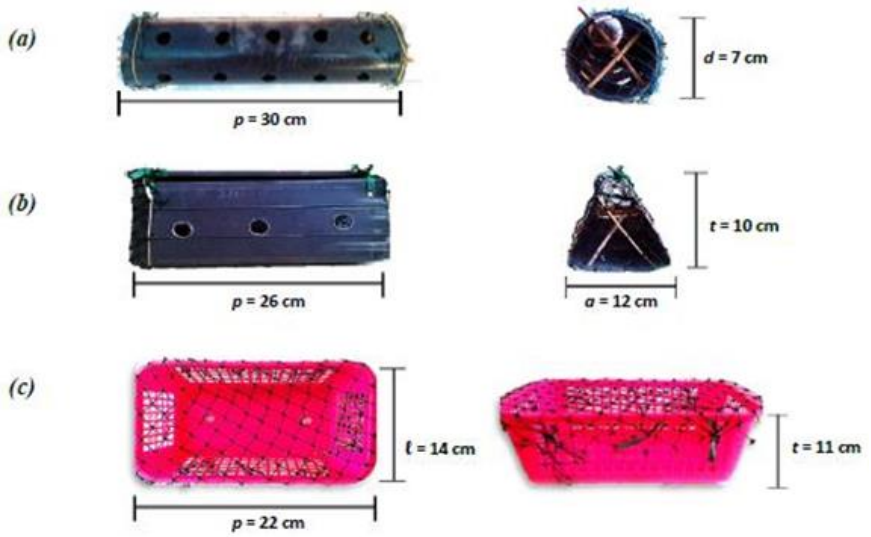

Figure 1. Various types and dimensions of individual shelter (IS) applied in this study: (a) IS tube shaped, (b) IS triangle shaped, and (c) IS square shaped

individual shelter (IS) i.e. IS tube shaped, IS triangle shaped, IS square shaped and PVC pipe shelter as control. In control, lobsters were rearing in plastic tank with PVC pipe communal shelter.

Several parameters such as hemocyte glucose and total protein hemolymph were recorded at 0, 3, 10 days, then every ten days, until the end of the study period. Glucose analysis refers to Wedemeyer and Yasutake (1977) method, while total protein analysis refers to Lowry *et al.* (1981). Lobster biological performance is recorded every ten days (body weight, carapace length, and total length). The survival rate was observed on the last day of the experiment. Biometric measurements refer to Solanki *et al.* (2012).

Data recorded during the study were statistically analyzed using variance analysis (ANOVA) with an F test at 95% confidence interval, using Minitab Statistical Software 16. If the result was significant, we used further tests using Tukey's method to see the differences between treatments.

3. Results

3.1 Hemolymph Glucose

The lobster glucose response to various shelters tends to fluctuate. Overall glucose concentration in the range of 3.80 ± 0.64 to 27.53 ± 1.37 mg dL^{-1}. At the beginning of the study (day 0), the glucose concentration has to be a higher tendency when compared with glucose concentration during the study. On the third day, glucose concentration in all treatments decreased in the range of 14.18 ± 2.15 to 20.11 ± 3.49 mg dL^{-1}.

The results of variance analysis on glucose level was also recorded on the tenth day. The tenth day analysis indicated that all individual shelter treatments were significantly different ($p < 0.05$) with control. Overall, glucose levels in the controls during the study tended to be higher when compared with individual shelf treatments. The condition of lobster glucose level can be seen in Figure 2.

3.2 Hemolymph Total Protein

The response of lobster hemolymph total protein on various shelters tends to have fluctuated. Overall total protein concentration in the range of 9.45 ± 0.65 to 39.17 ± 1.48 mg mL^{-1}. On the first day (day 0), total proteins concentration showed higher propensity (39.17 ± 1.48 mg mL^{-1}). From the first day until the 7th day, the total protein concentration tends to decrease, then increase on the 10th day. Total protein concentration at IS ■ treatment revealed lower and more stable compared to other treatments. On the 20th day, the total protein concentration observed on all individual shelters was significantly different ($p < 0.05$) with controls. While on the last day (60th day), although the protein concentration recorded at the modified PVC shelter indicated lower and stable, but not significantly different ($p > 0.05$) with other treatments. The total condition of lobster hemolymph protein can be seen in Figure 3.

3.3 Growth Response

The weight and length of the lobster carapace in all treatments increased with the length of study. The highest weight of lobster at the end of the study there was control at 73.74 ± 2.15 gram with carapace length 53.56 ± 2.52 mm. The highest daily lobster weight (SGR) growth rate was in control, which was 0.65 ± 0.04%, while the lowest was in the IS ● treatment of 0.23 ± 0.045% (Figure 4). The use of IS ■ and control treatment resulted in significantly different daily weight growth rates ($p < 0.05$) with IS ▲ and IS ● treatment. The weight, length of carapace, total length, and lobster growth rate can be seen in Figure 4.

3.4 Survival Rate

The highest lobster (SR) survival rate was found in IS ■ (92.85 ± 2.02%), while the lowest was in control of 74.28 ± 4.04%. The results of the variance analysis showed that the synthesis on the control treatment was significantly different ($p < 0.05$) from the treatment of individual shelter. The survival rate of lobster in this study can be seen in Figure 5.

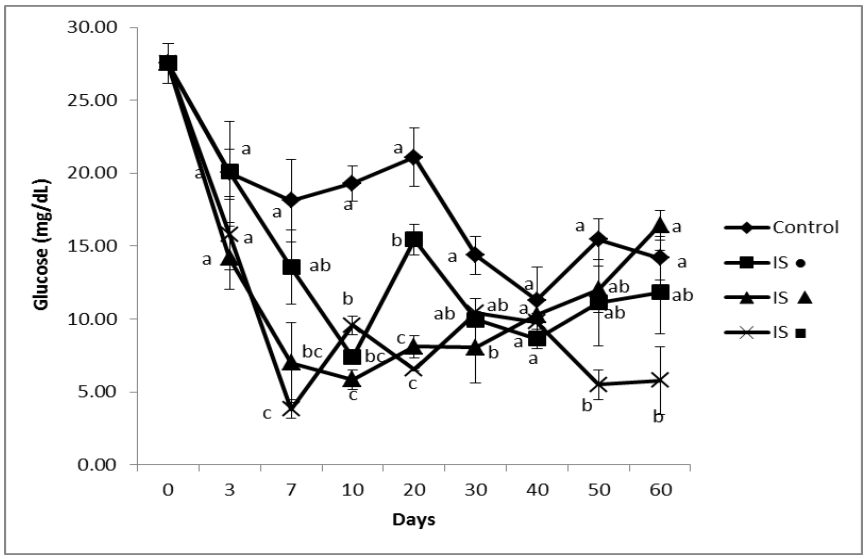

Figure 2. Hemolymph glucose of lobster at various shelters throughout the study. (IS●) individual shelter tube shaped, (IS ▲) individual shelter triangle shaped, (IS ■) individual shelter square shaped. Different lowercase letters in graph indicates significantly difference ($p < 0.05$)

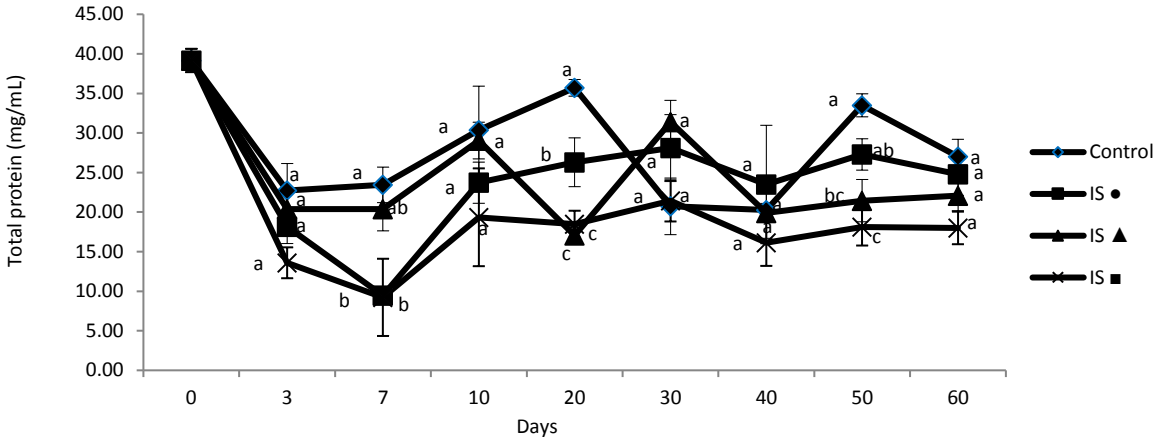

Figure 3. Hemolymph total protein of lobster at various shelters throughout the study. (IS●) individual shelter tube shaped, (IS ▲) individual shelter triangle shaped, (IS ■) individual shelter square shaped. Different lowercase letters in graph indicates significantly difference ($p < 0.05$).

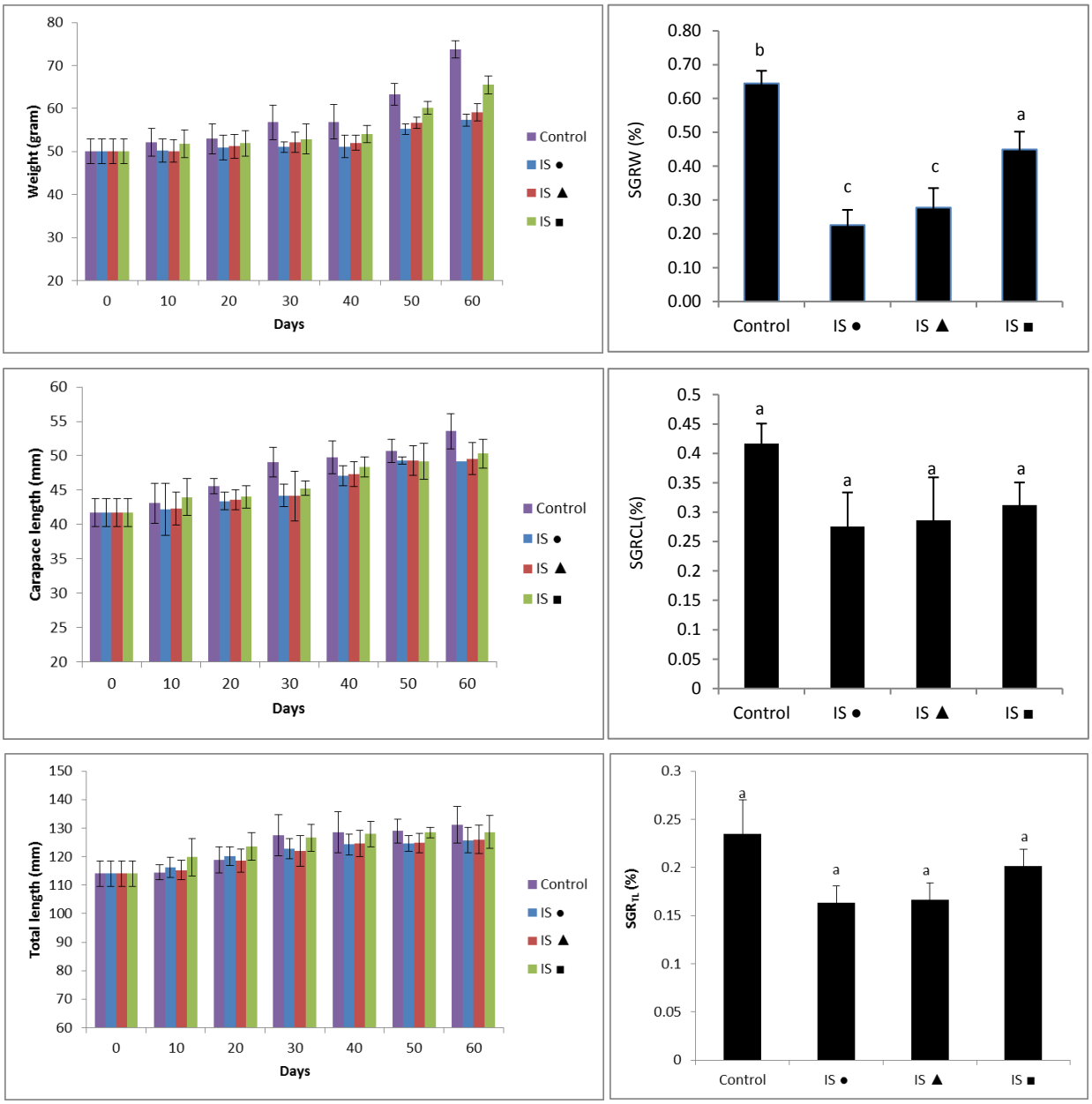

Figure 4. Growth response of lobster in various individual shelters during the study. (IS●) individual shelter tube shaped, (IS ▲) individual shelter triangle shaped, (IS ■) individual shelter square shaped. Different lowercase letters in graph indicates significantly difference ($p < 0.05$).

Table 2. The condition of water quality during study

Parameters	Value	Standard	References
Temperature (°C)	27.55 –29.30	25.00-30.00	Phillips dan Kittaka, 2000
Salinity (ppt)	33.10– 34.50	32.00-36.00	Wickins dan Lee, 2002
pH	7.90– 8.30	7.80-8.50	Wickins dan Lee, 2002
Alkalinity (mg/L)	45.12– 246.88	40.00-200.00	Chen *et al.*, 2006, Biesterfeldet *et al.*, 2003
Dissolved Oxygen (mg/L)	4.21– 7.00	2.70–5.40	Phillips dan Kittaka, 2000
Ammonia (mg/L)	0.00– 0.06	<1.00	Wickins dan Lee, 2002
Nitrite (mg/L)	0.02– 0.44	<5.00	Drengstig dan Bergheim, 2013
Nitrate (mg/L)	0.01– 6.78	<100.00	Wickins dan Lee, 2002
Total Organic Matter (mg/L)	29.90– 115.30	14.70–225.10	Budiardi *et al.*, 2007

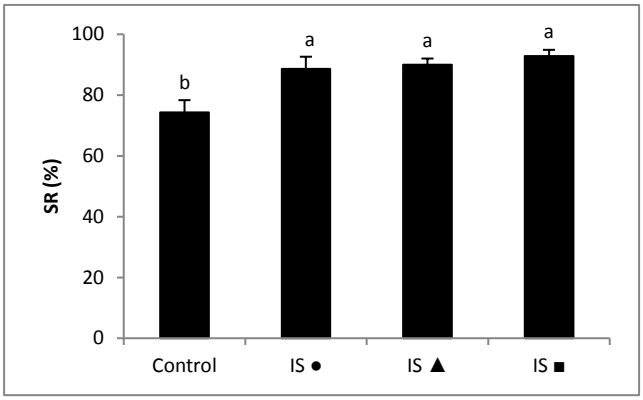

Figure 5. Survival rate of lobsters in the end of study. The different small letters on the graph show significantly different (p<0,05)

4. Discussions

Based on Table 2, overall water quality during the study still meets the standards for lobster rearing. Recirculation systems with filters and skimmer proteins are proven to maintain water quality at optimal conditions during lobster rearing.

Stress causes reallocation of metabolic energy for investment activity (growth and reproduction) into haemostatic improvement activity include respiration, movement, hidromineral regulation, and tissue repair. Energy sources for haemostatic improvement during stress were fulfilled by glycogenolysis and gluconeogenesis process that produces glucose (Hastuti *et al.*, 2004; Ocampo *et al.*, 2003).

On the first day of treatment, haemolymph glucose level was relatively high when compared with glucose conditions during the study. It was due to lobster seed experience stress through initial handling before being stocked in treatment pools. Stress can occur due to changes in environmental conditions (difference pond, water, and temperature). According to Hastuti *et al.* (2003), stress causes an increase in blood glucose levels (hyperglycemia), with the following mechanisms: (1) solving liver and muscle glycogen through glycogenolysis to produce glucose, this process was metabolic effects of catecholamines; (2) Proteins and lipids breakdown through gluko-neogenesis were metabolic effects of stress hormones; (3) Insulin inactivation was metabolic effects of stress hormones that inhibit cells from using glucose.

On the third day, glucose concentration in all treatments decreased. According to Hastuti *et al.* (2003), the decline in glucose levels, caused by lobster adaptation to a new environment, the side process of protein catabolism in amino acids form will increase in blood. The amino acid activates the insulin so as to carry out the transport of glucose, causing blood glucose concentration to decrease toward normal. The lobster glucose concentration in treatment IS ■ during the study period, relatively low and stable when compared with other treatments that tend to be high and volatile. It shows that IS ■ is able to reduce stress than other shelters.

Hemolymph protein is one of the main three proteins forms found in lobster blood; the protein is an amino acid, colloidal form in blood plasma. Blood proteins function for the recirculation process of fat molecules, hormones, vitamins, iron, protease inhibitors, precursors, regulatory activity, non-functional at the cellular defense system (Rustam *et al.*, 2013). Stress causes changes in physiological responses in the body. Some metabolic variables such as glucose, total protein, lactate, hemocyanin, osmoregulation capacity, total fat, triglycerides, and cholesterol can be used for monitoring the physiological condition of crustaceans due to stress (Mercier *et al.*, 2006).

At the beginning of the study (day 0), the total protein concentration tends to be high. This shows the stress on the lobster. Stress leads to increased metabolic activity in response to improve homeostasis. Increased metabolic activity increases the need for oxygen transport. In times of stress, the amount of hemocyanin in the hemolymph increased. Hemocyanin enhancement is associated with the main function of oxygen transport, which carries 94% oxygen from cells to tissues (Lorenzon *et al.*, 2007). In decapod, hemocyanin is dissolved in blood plasma. Hemocyanin proportion accounted for more than 60%, even in some species of crustaceans more than 93% of the total protein concentration in the hemolymph (Sladkova

and Kholodkevich, 2011). Overall, the trend of the level of total protein in IS ■ looks lower and more stable when compared to other shelters.

Growth is a change in shape and size, either length, weight, or volume within a certain timeframe (Hargiyatno *et al*., 2013). Growth is also an increase in biomass as a process of transforming matter from feed energy into body mass. In crustaceans, long growth and body weight occur periodically after molting (Bianchini and Ragonese, 2007).

Lobsters in the control treatment had the highest SGR (0.65 ± 0.04%) compared with other treatments. This is due to the control treatment of larger cannibalism so that lobsters get additional intake apart from the feed given. It can be seen that the control treatment synthesis is the lowest that is equal to 74.28 ±4.04%. The cannibalism factor will reduce the lobster population, so the competition in the grab for feed will decrease, and the lobsters can use the feed better. According to Cokrowati *et al*. (2012), lobsters with low stocking density will be more efficient in utilizing the feed given.

In control, the resulting lobsters were lowest. This is because the control treatment still allows contact between the lobsters. Contact between the lobsters will cause the level of lobster cannibalism to be high, resulting in lower severity. Similar results were obtained by Irvin and Williams (2009), who found that the lobster *Panulirus ornatus* cultured using individual systems had a higher survival rate (89%) than the communal system (72%). The highest survival rate of lobsters is found in IS ■ usage (92.85 ± 2.02%). This suggests lower levels of lobster seed stress maintained by IS ■. According to Fotedar *et al*. (2006); Verghese *et al*. (2007), stress can lead to a decreased immunological ability to disease, growth disturbances, poor reproductive performance, and lower survival.

Overall, the use of individual shelters in this study proved to have a significant positive effect on cultivated lobsters. This is because, with the use of the individual shelter, contact between the lobsters does not exist, so there is no cannibalism factor in the cultivation container. IS ■ is the best treatment for the nursery of rearing lobster because it has the lowest stress response and the highest total final lobster biomass when compared with other treatments.

5. Conclusion

Based on the study results, it can be concluded that IS ■ used in *Panulirus homarus* rearing showed lower stress response than the other treatments, in terms of several parameters glucose, and total protein lobster hemolymph during the study. IS ■ is the best shelter because it reduced stress levels and yielded better total biomass among the other treatments.

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Conflict of interest

The authors declare no known conflict of financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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