

The stress and growth responses of spiny lobster *Panulirus homarus* reared in recirculation system equipped by PVC shelter

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Abstract. The use of shelter in recirculation system aims to minimize contact between lobsters. This study analyzed and determined the shelter's ratio in the intensive nursery which can minimize the stress response and provide good seed production. This study used completely randomized design with 4 treatments and two replications. The treatment's type were the ratio between shelter's number and the lobster's amount inside the tank, among others 1:6; 1:3; 1:1.2 and 1:1. This research used lobster juvenile with average body weight 42.86 ± 6.22 g lobster⁻¹ in stocking density 40 lobsters m⁻² which reared in 1 m³ water tank for 30 days. The results of hemolymph test (total hemocyte count, total protein and cholesterol) during the study showed that all of lobsters experienced stress and it wasn't significantly different ($p > 0.05$) between all treatments. Water quality conditions throughout the study were still in accordance with the lobster culture standards. The growth was increased but also not significantly different ($p > 0.05$) between treatments. The 1:3 shelter ratio generated the best survival rate between treatments ($p < 0.05$) that was $56.25 \pm 5.84\%$. The lack of playground extents outside the shelters is supposed to influence the high stress experienced by the lobster.

Key Words: spiny lobster, *Panulirus homarus*, shelter, stress, hemolymph.

Introduction. Lobster is a valuable fisheries commodity which is always highly demanded in the worldwide commercial fish market. The demand and consumption of the lobster continue to increase year by year. According to Drengstig & Bergheim (2013), the lobster demand in international market reached 2000 to 2500 tons year⁻¹, while the lobster supply in the market are not available continuously. It caused by diminishing carrying capacity of lobster in the nature and the climate effect that make fishing activities more difficult.

In Pelabuhan Ratu-Indonesia, lobster culture activities are conducted in floating net cages, to raise the seed which have size less than 50 g/individual. In addition to the lobster's rearing activity, the seed used was not through the process of acclimatization beforehand. It caused the seeds mortality rate remained high. The variation of seed size could potentially lead to higher cannibalism. The mortality rate during the puerulus phase was also very high, mainly caused by cannibalism (Johnston et al 2006). Therefore, efforts should be made to produce more adaptable lobster seeds in uniform size for rearing activities.

Sea lobster nursery activities can be done in indoor. According to James (2007), activities in the indoor nursery have advantages over outdoor, including lower operating costs and infrastructure. Lobster rearing activity indoor aimed to acclimate the seed. The process of separating the lobster aims to provide an opportunity for the seed lobster to adapt to a new environment, so that the produced seeds will be more adaptive to the environmental changing conditions (Rao et al 2010), and can reduce the death rate of seeds (Mohammed et al 2010). The previous research which have been done to reduce the high rate of cannibalism was shelter utilization in the lobster maintenance container. According to Adiyana (2014), the analyses of the efficiency of the use of various shelters types including PVC pipe, brick vent and net on juvenile lobster rearing activity, showed that the PVC pipe shelter for lobsters weighing 2.12 ± 0.02 g gives the best results with a

specific growth rate of $1.38 \pm 0.04\% \text{ day}^{-1}$ and $65.26 \pm 1.41\%$ survival rate. Study for lobster *Panulirus homarus* rearing activity in the larger size is very important and has never been done. Therefore, this study aimed to examine the efficiency of PVC pipe shelter on the larger size of lobster seed with finding the most effective ratio of PVC pipe and the lobster amount.

Material and Method. The research was conducted from November to December 2015, in the Laboratory of Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Pasir Putih 1 Street East Ancol, North Jakarta.

Containers and media research. The acclimation pond has $4 \times 1 \times 1.5 \text{ m}^3$ dimension, with 0.5 m water level depth. Acclimation pond was equipped with a physical filter containing a foam sponge array, bioball, bioblock and shelters in the form of PVC pipe in size of 3-5 inches. There were 8 treatment tanks with $1 \times 1 \times 1 \text{ m}^3$ dimension, 1 filtrate container tank and 1 circular tank which has 1.5 m diameter and 0.75 m of height as a water reservoir from the outlet.

This study used shelters from PVC pipe with 2-inch diameter with 25 cm length. This study used a recirculation system operated by two submersible pumps which have 5500 L h^{-1} capacity. The aeration system used 2 aerator diffusers for each treatment tank. The filter was composed from several substrates, among others: sponges and bioblock as the degrading microorganism growth media and also protein skimmer.

Lobster seed. This study used spiny lobster *Panulirus homarus* (Linnaeus, 1758) juvenile as an animal object with average length of $10.51 \pm 0.03 \text{ cm}$ /individual and average body weight of $42.86 \pm 6.22 \text{ g}$ /individual. The lobster seeds were obtained from wild capture in Pelabuhan Ratu, West Java, Indonesia.

Experimental design. This study compared the ratio of shelter number with lobster amount in a tank. It used completely randomized design (CRD), consisting of 4 treatments and 2 replications as described in Table 1.

Table 1

Research layout

Code	Treatment ratio
A	7 PVC pipes : 40 lobsters (1:6)
B	14 PVC pipes : 40 lobsters (1:3)
C	35 PVC pipes : 40 lobsters (1:1.2)
D	40 PVC pipes : 40 lobsters (1:1)

Tank preparation and acclimatization. This research used 8 plastic tanks with 1000 L capacity, and also 2 filtration tanks which were cleaned and disinfected before using chlorine 5 mg L^{-1} doze. Then the tanks were washed, then drained and filled with 600 L of water. Lobster acclimatization process was conducted for 7 days in the acclimatization tank.

Feed management. The feed used is the fish *Selaroides leptolepis* from Muara Angke area. The feed given with 3% feeding rate (FR) of the biomass. The feeding frequency was once in a day, at 5:00 pm.

Water quality management. This study used a recirculation system with a physical filter consisting of sponges and bioblock. To maintain the water quality, the solid dirt and residual feed of the lobster were eliminated. Water exchanged 20-30% of total volume daily.

Water quality parameters. Daily water quality measurements were conducted according to APHA (1990) consisting of water pH, salinity, temperature and dissolved

oxygen (DO). Water chemical parameters analyzed included ammonia, nitrite, nitrate done on day 0 then every 10 days until the end of the study.

Stress response parameters. Total protein measurement was done according to Lowry et al (1951), cholesterol after Frings & Dunn (1970) and total hemocyte count (THC) hemolymph according to Blaxhall & Daisley (1973). These measurements were performed at day 0 then every 10 days until the end of the study.

Growth and survival parameters. Lobster biometric measurement done every 10 days, covered weight, carapace length and the total length. Observation on specific growth rate was done every 10 days during the maintenance. The lobster survival rate was evaluated at the end of the study.

Data analysis. This study used a completely randomized design (CRD). Data of THC, glucose, total protein, cholesterol, survival, length and weight growth, were statistically analyzed using analysis of variance (ANOVA) F-test with 95% confidence interval, used Ms. Excel 2013 and Minitab 16.0. If it was significantly different, then it continued by a further test of Tukey to see the differences between treatments. Water quality data were analyzed descriptively.

Results and Discussion

Water quality. Water quality is one of the most supporting factor of lobster life. The water quality parameters recorded during this study are presented in Table 2.

Table 2
Average value of water quality parameters during maintained time of *P. homarus*

Parameters	Treatment				Optimum range
	A	B	C	D	
pH	6.9-8.1	6.9-8.1	6.9-8.1	6.9-8.1	7.07-7.86 (Adiyana 2014)
Temperature (°C)	26.9-28.4	27.0-28.4	26.9-28.3	27.0-28.3	28°C (Phillips & Kittaka 2000)
Salinity (g L ⁻¹)	30-32	30-32	30-32	30-32	30-40 g L ⁻¹ (Verghese et al 2007)
DO (mg L ⁻¹)	6.6-8.3	6.9-8.5	6.6-8.5	6.9-8.5	2.7-5.4 mg L ⁻¹ (Phillips & Kittaka 2000)
Nitrite (mg L ⁻¹)	0.02-0.89	0.02-0.92	0.02-0.95	0.02-1.00	< 5 mg L ⁻¹ (Drengstig & Bergheim 2013)
Nitrate (mg L ⁻¹)	0.05-0.86	0.05-1.40	0.05-1.68	0.05-0.54	< 100 mg L ⁻¹ (Phillips & Kittaka 2000)
Amonia (mg L ⁻¹)	0.001-0.007	0.001-0.006	0.001-0.007	0.001-0.006	< 0.1 mg L ⁻¹ (Phillips & Kittaka 2000)

Water quality parameters showed optimal conditions supporting lobster life during study. The condition of pH is relatively stable in the range of 6.86 to 8.15 (Table 2). According to Adiyana (2014) pH value within the range of 7.07-7.86 was still supported the life of *P. homarus* juvenile. The temperature condition during the study was in the range of 26.9-28.4°C (Table 2). The water temperature was also relatively stable throughout the study. Generally, *P. homarus*'s fastest growth can be generated at the water temperature of 28°C, so that it could be achieved in normal weather (Phillips & Kittaka 2000). The salinity during the study was between 30-32 g L⁻¹ (Table 2). Salinity conditions were still

appropriated and could support the lobster's life. *P. homarus* have a fairly broad salinity tolerance that was 30-40 g L⁻¹ (Verghese et al 2007). In this study, the salinity was maintained periodically in the range of 30-32 g L⁻¹ by diluting fresh water when adding sea water after evaporation. Extra water addition is performed around 2-3 days. And then the value of water DO (dissolved oxygen) during the study was ranged from 6.6 to 8.5 mg L⁻¹ (Table 2). The optimal conditions of dissolved oxygen to support lobster's life in aquaculture was ranged from 2.7 to 5.4 mg L⁻¹ (Phillips & Kittaka 2000). The condition during this study was above this concentration, thus the lobsters got an oxygen supply in sufficient quantities.

The water nitrite's concentration during the maintenance period was in the range 0.02 to 1.00 mg L⁻¹ (Table 2). The condition of nitrite in the water during the study remain relevant and could supported lobster's life. Nitrite concentration < 5 mg L⁻¹ was recommended for *Homarus gammarus* lobster's culture (Drengstig & Bergheim 2013). The trends of nitrite concentration increased in the first 10 days and declined consistently in the next maintenance period. This was in line with the nitrite converter bacteria colonies development in the recirculation systems, so that the nitrite conversion was not optimal yet. According to Tatari et al (2016) nitrification bacteria on biokinetic system for drinking water production with unknown number of starter colonist bacteria, its presence began to be identified by real-time quantitative PCR on the day 9 after the system ran. The nitrate concentration in the water during the maintenance period was in the range 0.05 to 1.68 mg L⁻¹ (Table 2). The nitrate concentration during the study is still relatively low, according to Phillips & Kittaka (2000) on lobster *Jasus edwardsii* in a recirculation system the nitrate concentrations should be < 100 mg L⁻¹. The first 10 days of maintenance was the adaptation period and development time for nitrifying microorganisms (Tatari et al 2016). With the inadequate bacteria amount, the nitrification process has not run optimally. If the nitrite and nitrate concentrations were increased in the first 10 days maintenance, means the conversion process was already running.

The ammonia concentration in the water during the maintenance period was in the range of 0.001–0.007 mg L⁻¹ (Table 2). The ammonia condition in the water was risen until day 20. The first 10 days was not risen significantly compared to the first day due to nitrification process, that was ammonia into ammonium and then converted into nitrites by *Nitrosomonas*, then nitrite to nitrate by *Nitrobacter*. On the day 20, the nitrification process was still going well. But at the sampling time it was assumed that what obtained in the water was the initial phase of nitrification, that is why the ammonia concentration was so high while the nitrite and nitrate still low. The conversion of ammonia to ammonium, then to be nitrite and nitrate takes time. According to Jenie & Rahayu (1993) nitrification process normally takes 10 hours or more, depending on the metabolism of microbes and the environmental conditions. In the sea water usually requires a longer time. Several factors that influence the nitrification process are nitrification bacteria concentration, retention time (regeneration time of microbes that will relate to the amount of energy required during the oxidation process), dissolved oxygen, temperature, pH and ammonia concentration. Although the graph on the day 20 showed an ammonia increase up to 0.007 mg L⁻¹ but the concentration was still below the lethal limit (< 0.1 mg L⁻¹) and still qualify for support the lobster's (Phillips & Kittaka 2000).

Lobster's physiological response. The THC concentration of the lobster during the study ranged from 3.63±1.41 x 10⁶ to 15.80±4.29 x 10⁶ cells mL⁻¹ (Figure 1). The concentration was higher in the day 0 because the lobster experienced stress due to transportation handling from the holding tank to the maintenance tank. This was in accordance with Fotedar et al (2006) which stated that the concentration of THC in *Panulirus cygnus* increased during arrest and transportation time then declined after 16-48 hours in the new media. Figure 1 shows that the concentration of THC declined after 24 hours, it indicated that the lobsters have adapted to the new environment. But in the following days THC concentration came to increase again and fluctuated until the end of the maintenance period. The increasing of the THC concentration indicated that lobster

exposed to stress. The stress response in the lobster can be caused by several things including environmental factors, handling and bacterial infections (Lorenzon et al 2013). According to Jusilla et al (1997), the THC concentration of *P. cygnus* in normal conditions was in the range of $5.6 \pm 0.7 \times 10^6$ cells mL^{-1} , therefore if the THC concentration was beyond these range, it can be assumed that lobster is in unstable condition.

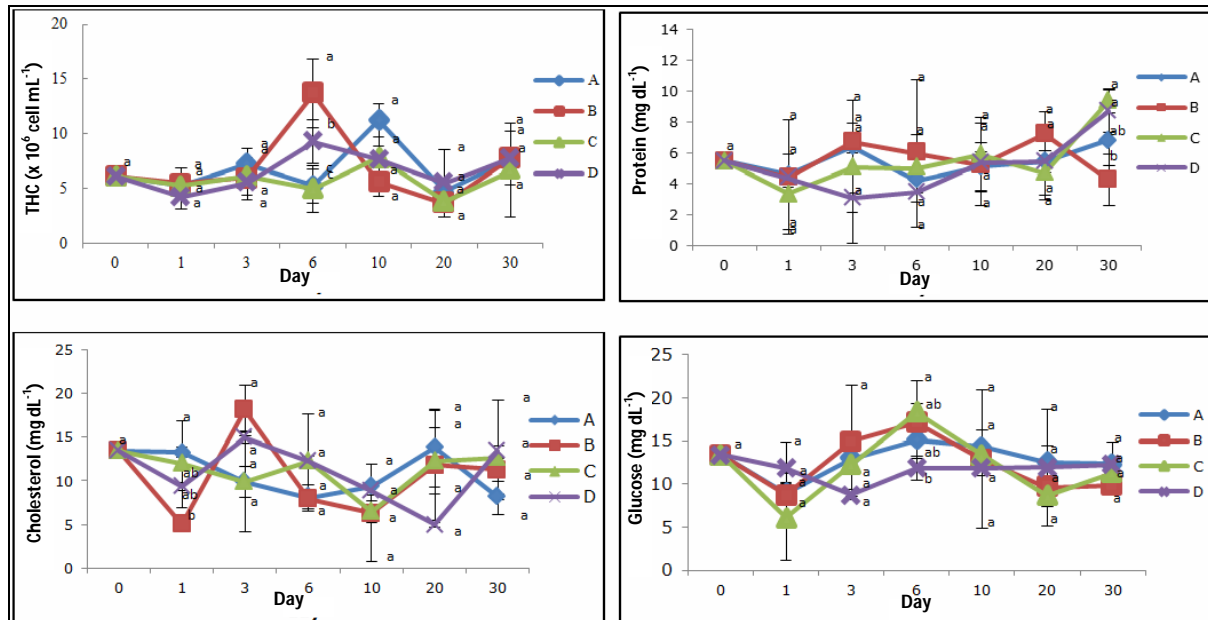


Figure 1. Overview of total hemocyte, protein, cholesterol and glucose of the hemolymph during the study: (A) shelter PVC ratio of 1: 6; (B) PVC shelter ratio of 1: 3; (C) PVC shelter ratio of 1: 1.2; (D) shelter PVC ratio of 1:1. Different small letters on the graph show the significant difference ($p < 0.05$).

During the maintenance period the THC concentration fluctuated between treatments but was not significantly different ($p > 0.05$) between treatments, except on day 6. Overall the THC concentrations tend to remain high and fluctuated. It indicated that the lobster experienced stress during the maintenance period. Total protein response of the lobster during the maintenance period ranging between 3.09 ± 2.92 to 9.35 ± 0.76 mg dL^{-1} (Figure 1). Although fluctuated, the total protein concentration during the study showed that the results were not significantly different ($p > 0.05$) between treatments. The trend of concentration graphs increased relatively high, showing that the lobster was exposed to stress during maintenance, in accordance with Lorenzon et al (2007) which stated that normal hemolymph protein concentration in lobster *Homarus americanus* was 4.45 mg dL^{-1} and the concentration during study was above it. The proteins concentration of hemolymph was influenced by several factors, including growth, moulting, reproduction, infection and hypoxia.

At the beginning of the maintenance period, lobster stricken by milky hemolymph disease, it looked from the lobster's bottom abdomen which became white, while the normal lobster should have a limpid abdomen muscle. This disease was caused by Rickettsia-like bacteria (RLB), which suspected inadvertent brought from fresh fish feed that was given to the lobsters. According to Hoang et al (2008) this disease could be caused by feed and environment that carry the plague bacteria. However, due to the maintenance used a recirculation system, then most likely the cause is feed because it comes from the outside maintenance system. The implication of this disease is seen in THC concentration which increased at days 6 and 10. The THC concentration in treatments A and B increased at day 6, which indicates there was an immune response to defend themselves from disease while in treatments C and D concentration decreased, which means it failed to maintain immunity. Milky hemolymph disease caused mortality in lobster, lobster treatments A and B showed a better resistance so that the survival rate was higher than treatments C and D.

The concentration of cholesterol in the blood was always changing every time, cholesterol tend to increase when lobster experienced stress. The cholesterol production inside the body could be triggered by stress hormones, that was adrenaline and cortisol. Cortisol served to regulate the blood pressure and immune system of the organism. If the lobster experience stress, that hormone triggers the sugar production inside the body, but body can't utilize the sugar optimally. So that the body will convert sugars into fatty acids and triglycerides to produce cholesterol, therefore the hemolymph cholesterol concentration will increase (Lorenzon et al 2008). Lobster hemolymph cholesterol levels during the study fluctuated in range of 4.94 ± 0.23 to 18.09 ± 2.90 mg L⁻¹. The analysis of variance showed no significant difference ($p > 0.05$) in cholesterol concentration between treatments except on day 1. On day 1 the cholesterol concentrations relatively decreased after being transported from the acclimatization tank to the maintaining tank. This showed the lobster adaptation response into a new environment for 24 hours early. But the next day, the concentration gained up, it was estimated that the stress experienced by the lobster made the cholesterol concentration increase. According to Lorenzon et al (2008) cholesterol concentrations will increase if crustacea are exposed to the stress of transport or environmental factors. The increase in the concentration of cholesterol can also be caused by high energy needs after the lobster fasted.

Growth and survival. The lobster initial average body weight was 42.86 ± 6.22 g individual⁻¹ and increased until the end of the study period in the range of 46.93 ± 1.40 to 48.15 ± 5.87 g individual⁻¹ (Figure 2). Nevertheless the results of analysis of variance showed the growth was not significantly different ($p > 0.05$) between treatments. Analysis of specific growth rate (SGR) variance was also not significantly different ($p > 0.05$) between treatments (Figure 2). The utilization of PVC shelter aimed to minimize the level of stress on a lobster and as a daily protection during lobster moulting period. But after observed during the maintenance period, for a lobster weighing 40-50 g turns more activities outside the shelter. When the lobster moulted, it prefers to change their skin outside the shelter. The lobsters with that size assumed were prefer to moult outside the shelter due to it would be easier to escape from the shell outside than inside the shelter. This is consistent with the statement of Rossong et al (2011) where lobsters with a carapace length > 35 mm will spend more time outside the shelter. It was also observed during the study that mortality is very high at the moulting time. It is caused by cannibalism, many lobsters died just because of being attacked by another lobster when moulted.

The average total length of the lobsters at the beginning of the study was 10.51 ± 0.03 cm, and increased during maintenance to 11.26 ± 0.40 – 11.59 ± 0.29 cm. Although it has increased, the total length of the lobster was not significantly different ($p > 0.05$) between treatments (Figure 2). Then for carapace length which have an average initial length of 3.95 ± 0.09 cm increased to 4.09 ± 0.16 – 4.28 ± 0.14 cm at the end of the study (Figure 2).

The lobster survival rate (SR) during study was quite low but significantly different ($p < 0.05$) between treatments (Figure 3). Treatment B generated the best SR but not significantly different ($p > 0.05$) with treatment A. It showed that 1:3 ratio was the most effective than the others. It estimated that shelter formation of treatment B was the most suitable for the maintenance media due to the shelters were spread in each corner of the tank and just only consist of single stage compartment. It was different with treatment C and D which each have double and triple stage of compartment. Based on direct observation during research, lobster were rarely occupied or live in the second or third stage of the shelter. It is suspected that the lobster was difficult to climb, and got in to the higher stage of shelter rather than the bottom one. In the first shelter stage, it was also observed that 2-3 lobsters were found in one shelter hole.

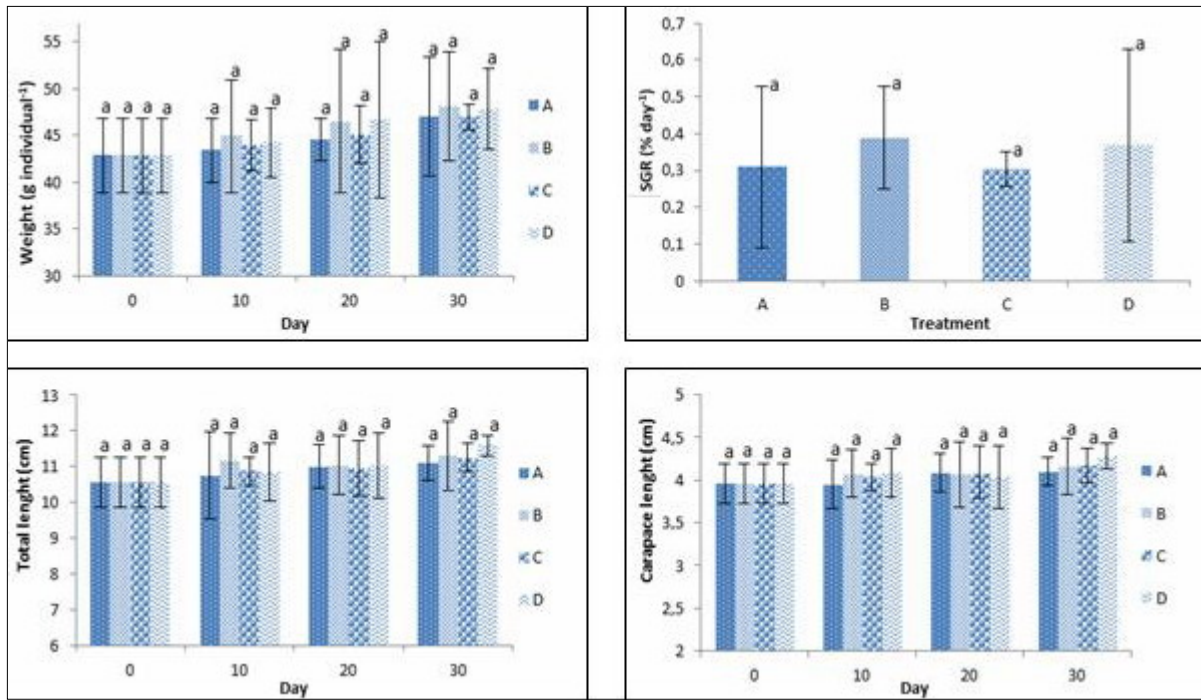


Figure 2. Lobster growth graph during the study. (A) shelter PVC ratio of 1:6; (B) PVC shelter ratio of 1:3; (C) PVC shelter ratio of 1:1.2; (D) shelter PVC ratio of 1:1. Different small letters on the graph shows the significant difference ($p < 0.05$) between treatments.

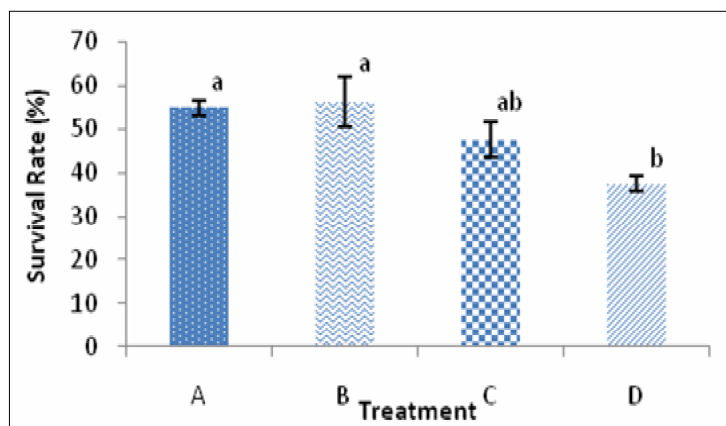


Figure 3. Lobster survival graph during the study. Different small letters on the graph show the significant difference ($p < 0.05$) between treatments.

The excessive amount of shelter narrowing the space and caused the lack of playground area for the lobster outside the shelter, so that the contact between lobsters was still quite high. Contacts between lobsters increased the risk of cannibalism, especially when moulting time. Moulded lobster would be more interested for another lobster because it released an interesting aroma (Moore 2015). When attacked by another lobster, molting lobster could not escape because of the extent of the maintenance tank was 1 m² and the lobster amount in the bottom of the tank was quite high for those areas.

Feeding at the restricted area outside the shelter lead to contact between lobsters while foraging. There was a big probability that the weak lobster which was injured or moulting, was attacked by another lobster. Here it is known that the playground area was also important for the lobster to minimize the contact with other lobsters, creating a comfortable living environment due to territorial character even extend the escape area when it attacked by the other lobsters. The high intensity of contact between lobsters due to activity outside the shelter increased cannibalism which could trigger stress and it implicated a low growth. It was in terms of the physiological parameters such as the high rates of THC, total protein concentration, glucose and cholesterol fluctuations (Lorenzon

et al 2008). Stress caused less growth energy allocation because organisms used it for homeostasis, so the growth stunted (Hastuti et al 2004). According to Kulmiye & Mavuti (2005) lobster which experienced stress due to got injured have a smaller carapace growth than a normal one. In some cases even the injured lobster still molting although showed no growth in carapace length.

Conclusions. The different shelters ratio treatment in maintenance tank of lobster *Panulirus homarus* did not show significantly different ($p > 0.05$) growth and survival between treatments but significantly different ($p < 0.05$) in survival rate. The shelter ratio 1:3 (B) generated the best production between treatments. Physiological parameters analysis showed not significantly different ($p > 0.05$) result, which mean that lobster experienced stress during maintenance time and it implicated to low growth. To exam the effectiveness of the shelter is necessary to conduct a study using smaller size juveniles in lower stocking density, so that the growth trend will be more visible and hopefully can reduce the level of cannibalism. An innovative layout to optimize the use of the shelter is also worth to try.

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