Effect of shelter net sizes on growth, survivability, and health of scalloped spiny lobster, *Panulirus homarus* (Linnaeus 1758) reared in fiberglass tank

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Abstract. Scalloped spiny lobster (*Panulirus homarus*) is an export commodity with high economic value in Asian-Pacific markets. Addition of artificial shelter for spiny lobster grow out reduces cannibalism while addition of net in spiny lobster rearing tank can increase mobility space. This study aimed to determine the optimum of shelter net size on growth, survival rate and health of spiny lobster grow out. This study used completely randomized design with three treatments and three replications; analyzed by ANOVA. The rearing was conducted using 9 fiberglass tank 1000 liter in volume. This research used scalloped spiny lobster, with average body weight (BW) 8.56±1.23 g and total length (TL) 7.39±0.114 cm, in stocking density 50 lobster/tank. Three shelter net sizes as treatments, i.e.: A: shelter net size of 4 time of bottom area (4 m\(^2\)), B: size of 2 time of bottom area (2 m\(^2\)) and C: without net addition. Feeding were twice a day, with dry pellet 1% biomass/day and trash fish and mussel meat (2:1) 10% biomass/day. The sampling of survival rate, total length and body weight were conducted every 15 days; while total haemocyte (THC) and BRIX index on the end of experiment. For supporting data was observed of water quality parameters: salinity, temperature, pH, DO, nitrite and ammonia. The result show that the survival rate on experiment during 75 days rearing, was significant different (P<0.05) among treatments. The best survival rate was on treatment A (4 time of bottom area) with survival rate (93.94±2.62%), followed by treatment B (2 time of bottom area) (80.56±2.88%) and treatment C (without net addition) (68.78±4.67%). The grow of body weight and total length show that highest on treatment B with body weight and total length in the end of experiment was 37.77±1.896 g and 10.30 ±0.361 cm; followed by treatment A (35.58±0.405 g and 10.15 ±0.146 cm) and lowest in C (35.34±2.061 g and 10.04 ±0.265 cm); but from statistic analyze not significant different among treatment (p>0.05). Addition for shelter net size of 4 time of bottom area net more suitable for scallop spiny lobster reared in fiberglass tank; because can increased the survival rate and reduced the cannibalism.

1. Introduction

Scalloped spiny lobster (*Panulirus homarus*) is an export commodity and high economic value in Asian-Pacific markets. Demand for sea lobster needs in the market international reach 2000 - 2500 metric tons/years, while the supply of lobster in the market, no continuously available [1]. In some locations, spiny lobsters have shown overfishing [2, 3, 4, 5]. There are 6 species of spiny lobster in...
Indonesia i.e., scalloped spiny lobster (Panulirus homarus), ornated lobster (P. ornatus), bamboo lobster (P. versicolor), Pakistan lobster (P. polyphagus), rock lobster (P. penicillatus) dan red lobster (P. longipes). Scalloped spiny lobster was potential for aquaculture because of high value and supported by availability of wild fry [6]. Lobster cage culture has great potential to develop in Indonesia. Indonesia has 5.8 million km² of marine area, 17,504 islands and 81,000 km of coastline, including many lagoons and bays that are suitable for sea cage culture. Usually lobster culture in sea cage occurs in combination with other species, primarily grouper, sea bass and snapper.

The main problem of spiny lobster aquaculture is high mortality because of cannibalism during monting [7, 8, 9, 10] and disease caused by handling. High mortality during nursery of post puerulus is also a problem of spiny lobster aquaculture in Indonesia [11]. Scalloped spiny lobster culture (P. homarus) in India starting from 70-100 g body weight with high survival rate is about 70-75% [12].

Grow out of scalloped spiny lobster can be conducted in the rearing tank. [13, 14], reported for scalloped spiny lobster nursery in concrete tank with low survival rate of 10-30%. Addition of artificial shelter on spiny lobster grow out can reduce cannibalism. Addition of net in spiny lobster rearing tank can increase the space for moving [15]. The choice of shelter, feeding density, and feeding frequency is critical to the survival rate of post larvae and juveniles [16, 17, 18, 19]. Several artificial material shelters, including cement, plastic plates, PVC tubes, and wood have been used [18, 19]. The purpose of this study was to determine the best size of net for shelter of spiny lobster grow out.

2. Material and methods
The rearing was conducted by using 9 fiberglass tanks with capacity of 1000 liter in volume. All tanks received flow-through ambient temperature seawater at a flow rate of 200 % water volume per day. This study used completely randomized design with three treatments and three replications. Scalloped spiny lobster used for this research were obtained from the catch of fishermen in Lombok; with initial body weight 8.56 g and total length 7.39 cm, was reared at density of 50 ind./tank. Three treatments, i.e.: A: net size of 4 time of bottom area (4 m²), B: net size of 2 time of bottom area (2 m²) and C: without net addition. Feeding were twice a day, with dry pellet 1% biomass/day and trash fish + mussel meat (2:1) 10% biomass/day. Dry pellets used in this study had a protein content of 46.51%, a fat content of 7.07%, an ash content of 14.82% and a water content of 7.57%. Every 15 days the number of lobsters is counted and samples are taken of 10 lobsters per tank for observation of total length and body weight. Body weight of lobsters was measured using an electronic balance to the nearest 0.1 g after blotting excess water with absorbent towel. Total length is measured at mid-back from the dorsal transverse between the frontal spines to the tail end using vernier calliper to the nearest 0.1 mm. The calculation of survival rate (SR) refereed to the method of [20]; with formula: Survival rate = (Lobster number day-t/Lobster number day 0) x100%. The calculation of body weight gain (g), body weight increments (g per day), Daily total length increments (mm per day) referred to the method of [20] with formula: Body weight gain (g) = (Final mean lobster weight - initial mean lobster weight); Wt-W0. Body weight increments (g per day) = (final body weight - initial body weight)/Number of days; (Wf-W0)/t. Daily total length increments (mm per day) = (final total length – initial total length)/ Number of days; (TLf - TL0 )/t. The calculation of Feed conversion ratio (FCR) refereed to the method of [21]; with formula: Feed Conversion Ratio = Total Feed Consumed/Body Weight Gain. 

At the end of the study, haemolymph samples were taken for observation of total haemocyte count (THC) and BRIX index of 10 lobsters in each tank. For each lobster, 0.2 ml haemolymph samples were taken for THC analysis and 0.1 ml for the BRIX index. Haemolymph samples were taken from The 3rd walking leg through a 1 ml disposable syringe containing anticoagulant solution at pH 4.6, and stored at 4°C. THC calculations were carried out under microscope using haemocytometer. Calculation of total haemocyte count (THC) haemolymph was done according to [22]. Measurement of BRIX index using a portable digital BRIX meter Atago USA with 0-32% scale and 1% accuracy. For supporting data every week was observed of water quality parameters: temperature, ammonia, pH,
Nitrite, DO and salinity. Daily water quality measurements were conducted according to [23] consisting of water temperature, pH, salinity, and dissolved oxygen (DO); with a portable probe unit YSI 556, Ohio, USA; for ammonia and nitrite, done on day 0 then every 10 days until the end of the study, with HACH DR1900-01H, Love land, USA. Data of survival, length and weight growth, were statistically analyzed using analysis of variance (ANOVA) F-test with 95% confidence interval, used Ms. Excel. 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.

3. Results and discussion

The data of survival rate, total length, and body weight of scalloped spiny lobster reared in fiberglass tanks for 75 days, with different net size for shelter, is shown in Table 1. Data of survival rate of scalloped spiny lobster (*Panulirus homarus*) fry, reared at fiberglass tank for 75 days with different net size for shelter is shown in Figure 1. From data survival rate on experiment during 75 days rearing (Table 1 and Figure 1) show that there was significant difference (P<0.05) among treatments. The best survival rate was on treatment A (4 time of bottom area) with survival rate (93.94±2.62%), followed by treatment B (2 time of bottom area) (80.56±2.88%) and treatment C (without net addition) (68.78±4.67%).

**Table 1.** Survival rate, total length, total length gain, body weight, body weight gain and food conversion ratio of scalloped spiny lobster (*Panulirus homarus*) reared at fiberglass tank for 75 days with different net size for shelter

| Parameter                        | Net size for shelter                      |
|----------------------------------|------------------------------------------|
|                                  | 4 time of bottom area (A) | 2 time of bottom area (B) | without net addition (C) |
| Survival Rate (%)                | 93.94±2.62<sup>a</sup>              | 80.56±2.88<sup>b</sup> | 68.78±4.67<sup>c</sup> |
| Initial total length (cm)       | 7.39±0.114                          | 7.39±0.114               | 7.39±0.114               |
| Final total length (cm)         | 10.15 ±0.146                        | 10.30 ±0.361             | 10.04 ±0.265             |
| Total length gain(cm)           | 2.66                                 | 2.91                     | 2.81                     |
| Daily total length growth (mm/day) | 0.355                       | 0.388                    | 0.375                    |
| Initial weight (g)              | 8.56±1.23                           | 8.56±1.23                | 8.56±1.23                |
| Final weight (g)                | 35.58±0.405                         | 37.77±1.896              | 35.34±2.061              |
| Body weight gain (g)            | 27.02                                | 29.21                    | 26.78                    |
| Daily weight growth (g/day)     | 0.360                                | 0.389                    | 0.357                    |
| Food conversion ratio           | 3.19                                 | 3.28                     | 3.85                     |
Figure 1. Survival rate of scalloped spiny lobster (*Panulirus homarus*) reared at fiberglass tank for 75 days with different net size for shelter (A: 4 time of bottom area, B: 2 time of bottom area, C: without net addition).

From data survival rate on experiment during 75 days rearing (Table 1 and Figure 1) show that the highest survival rate is in treatment A, followed by treatment B and lowest on treatment C. This result shows that net addition for shelter at size of 4 times the bottom area net is more suitable for scallop spiny lobster reared in fiberglass tank; because it can increased the survival rate and reduced the cannibalism of scallop spiny lobster.

Increased the net shelter area, can increase lobster survival rate. This is because of the net shelter addition can increase the space for lobsters to move and reduce their contact between the lobsters so as to reduce the level of cannibalism during rearing and increase survival rate. Net shelter addition can extend the escape area when it attacked by the other lobsters. There was a big probability that the weak lobster which was injured or molting, was attacked by another lobster. According to [24], increasing shelter area can reduce mortality, especially when molting can reduce cannibalism attacks by other lobsters. Contacts between lobsters increased the risk of cannibalism, especially when molting time. Molted lobster would be more interested for another lobster because it released an interesting aroma. When attacked by another lobster, molting lobster could not escape because of the lobster amount in the bottom of the tank was quite high [25].

The survival rate during nursery (Figure 1) showed that the survival rate was still 100% in the first two week, and then decreased in the next sampling. This is caused because of cannibalism when molting, during first 2 weeks all most lobster not molt yet. This is evidenced by not found dead lobster carcasses in the tank. The molting frequency is related to the size of the spiny lobster; the younger spiny lobster with higher molting frequency and cannibalism than the larger size [26]. Spiny lobster grow out by battery system cannibalism can be reduced to 100% [14]. The result of survival rate of fry in this experiment (68.78-93.94%) is still good value compared with another result. The growth of *P. homarus* start from 85 g body weight size by feeding with trash fish during 5 months in bottom cage with survival rate 75-85% [9]. On ornate spiny lobster (*Panulirus ornatus*) grow out start from 40 g body weigh size, using dry pellet as food [27], with result of survival about 84%.

The survival rate In treatment C (without net shelter addition), the resulting lobsters were lowest (68.78±4.67%). This is because still high contact between another lobsters. Contact between the lobsters will cause the level of lobster cannibalism to be high, resulting in lower survival rate. Similar results were obtained by [28], 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 treatment C (net shelter size at 4 time of bottom area) (93.94±2.62%) compared to treatment B (net shelter size at 4 time of bottom area) (80.56±2.88%). This suggests
lower levels of lobster seed stress. According to [29], stress can lead to a decreased immunological ability to disease, growth disturbances, poor reproductive performance, and lower survival rate.

Body weight growth of scalloped spiny lobster (Panulirus homarus) reared in fiberglass tanks with different net size for shelter are shown in Figure 2. From data body weight on experiment during 75 days rearing (Table 1 and Figure 2) show that highest on treatment B (2 time of bottom area) with body weight in the end of experiment was 37.77±1.896 g; followed by treatment A (4 time of bottom area) (35.58±0.405 g) and lowest on treatment C (without net addition) (35.34±2.061 g); but from statistic analyze not significant different among treatment (p>0.05). Growth is a change in shape and size, either length, weight, or volume within a certain time frame [2]. Growth is also an increase in biomass as a process of transforming matter from feed energy into body mass. In crustaceans, body weight occur periodically after molting [30]. Lobsters in the treatment B had the highest Daily weight growth (0.389 g/day) compared on treatment A (0.360 g/day) and treatment C (0.357 g/day). This is because of larger cannibalism so that lobsters get additional intake apart from the feed given. 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 [31], lobsters with low stocking density will be more efficient in utilizing the feed given.

**Figure 2.** Body weight grow of scalloped spiny lobster (Panulirus homarus) reared at fiberglass tank for 75 days with different net size for shelter (A: 4 time of bottom area, B: 2 time of bottom area, C: without net addition).

Total length grows of scalloped spiny lobster (Panulirus homarus) reared at fiberglass tank with different net size for shelter are showed in Figure 3. From data total length on experiment during 75 days rearing (Table 1 and Figure 3) show that highest on treatment B (2 time of bottom area) with total length in the end of experiment was 10.30 ±0.361 cm; followed by treatment A (4 time of bottom area) (10.15 ±0.146 cm) and lowest on treatment C (without net addition) (10.04 ±0.265 cm); but from statistic analyze not significant different among treatment (p>0.05). Lobsters in the treatment B had the highest Daily total length (0.388 mm/day) compared on treatment A (0.355 mm/day) and treatment C (0.375 mm/day). In crustaceans, long growth occur periodically after molting [30]. Growth is affected by several factors, namely genetic traits, conditions, physiological, and environmental maintenance media [32].
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Figure 3. Total length grows of scalloped spiny lobster (*Panulirus homarus*) reared at fiberglass tank for 75 days with different net size for shelter (A: 4 time of bottom area, B: 2 time of bottom area, C: without net addition).

From Table 1, Figure 1, 2 and 3, showed that the best result (survival rate, body weight and total length growth) was in treatment treatment A (4 times of bottom area), followed by treatment B (2 time of bottom area) and treatment C (without net addition).

Spiny lobster juvenile more interested in shelter darker and less gaps. Usually, lobster juvenile has spends more time to take shelter inside the shelter than adult lobsters [33]. The shelter addition on lobster rearing can increased the survival rate and stress levels in lobsters [34]. Shelters in lobster grow out aims to reduce cannibalism; however, size, material, and appearance can affect the final survival rate; lobster will change choice of shelter as lobster grows [35]. When choosing shelters, most studies use artificial material shelters, such as cement, plastic plates, PVC tube, or wood [34, 35].

The result on total haemocyte count (THC) dan BRIX index of spiny lobster (*P homarus*) reared with different net size for shelter, shown in Table 2. The result on observation of total haemocyte and BRIX index was (268±26) x 10^4 cel per ml and 16.7±3.56 % in treatment A, (259±29) x 10^4 cel per ml and 17.3±4.14 % in treatment B and (246±32) x 10^4 cel per ml 18.4±4.33 % and in treatment C; almost same among treatment.

Table 2. Total haemocyte count (THC) dan BRIX index of spiny lobster (*P homarus*) reared with Different Net Size for Shelter

| Treatment                        | THC (x 10^4 / ml) | BRIX index (%) |
|----------------------------------|-------------------|---------------|
| A (4 time of bottom area)        | 268±26^a          | 16.7±3.56^a   |
| B (2 time of bottom area)        | 259±29^a          | 17.3±4.14^a   |
| C (without net addition)         | 246±32^a          | 18.4±4.33^a   |

From the data of total haemocyte was (268±26) x 10^4 cel per ml in treatment A, (259±29) x 10^4 cel per ml in treatment B and (246±32) x 10^4 cel per ml in treatment C; almost same among treatment. Total Haemocyte Count (THC) is one of the parameters that can be used as an indicator of stress in crustaceans [24]. THC level is one of the physiological parameters that indicate the level of stress in crustaceans [36, 37, 38, 39]. According to [40], the number of haemocytes incrustacean hemolymph showing reaction against environmental stressors and disease, so that it can be an indicator of health.
status crustaceans and the presence of environmental stressors. Amount haemocytes is one of the parameters of haemolymph the most sensitive and constant to conditions stress on shrimp culture [41]. Determination of the number of haemocytes can be early indicator for the condition of juvenile vitality crustaceans early [42]. Analysis of variance showed that the addition of net shelter showed the value of THC not significantly different (p>0.05). The THC concentration is almost same in all treatment (A, B and C). According to [43], an increase in the amount of THC indicates there is a stress response due to handling and response to environmental changes that occur. Thing this shows the stress level of lobster on control treatment is higher than other treatments. Higher stress levels in the control treatment caused by lobster has less space to move than other treatments. Narrower range of motion cause contact between lobsters and levels of cannibalism is greater, so the stress level higher and survival is lower. The use of nets produces a level of stress the lower one. This is in addition to wider range of motion, use of materials dark colors produce stress levels inferior lobster. According to [33], juvenile lobsters prefer shelters that are made of darker colored material.

Haemocytes are important in the crustacean immune system, which can be used in the assessment of health conditions and body resistance [44]. Stress conditions in crustaceans were indicated by the highest increase in glucose levels reaching 20.80 mg/dl in P. homarus [43] 14.41 mg/dl in Helix pomatia [45], 13,20 mg/dl in Astacus leptodactylus [46] . The increase of the THC concentration indicated that the lobster was exposed to stress. Increased amount of THC indicates an increased cardiovascular activity due to stress from handling and response to environmental change [47].The stress response in the lobster can be caused by several things including environmental factors, handling and bacterial infections [48]. The THC concentration of P. cygnus in normal conditions was in the range of 5.6±0.7 x 10^6 cells ml^-1, therefore if the THC concentration was beyond this range, it can be assumed that lobster is in unstable condition [49].

BRIX index of spiny lobster (P homarus) reared with different net size for shelter (Table 2) almost same among treatment in the range of 16.7 – 18.4 %. Degrees BRIX is the sugar content of an aqueous solution. An extensively studied haemolymph parameter is the refractive index , which is commonly measured as a BRIX value [50]. The refractive index and Brix value are highly correlated with the haemolymph total protein concentration [51, 52, 53], BRIX indexes can be used to determine stress levels in lobsters. Under stress conditions, reallocation occurs metabolic energy such as growth and reproduction into activities to improve homeostasis, such as respiration, movement, hydro mineral regulation and repair network. [54] identified the BRIX index as the most accurate and practical for health condition spiny lobsters. Stress conditions in crustaceans can be indicated by an increase in haemolymph glucose levels [37, 55, 38]. Increased haemolymph glucose levels can indicate increased stress levels in lobsters [56].Glucose is a metabolism variables such as , can be used for monitoring the physiological condition of crustaceans due to stress [57].

Energy requirements to repair homeostasis during stress are replaced by processes glycogenolysis and gluconeogenesis which will produce glucose [58, 59]. Stress treatment research on lobster Homarus americanus, after 24 hours observations, showed a decrease in haemolymph glucose levels from 0.85 mmol/l to 0.4 mmol/l [60]. The BRIX index in lobster shows the amount of sugar contained in the haemolymph, and is highly correlated with protein levels in the haemolymph. Currently, the BRIX index is used by researchers and the industry to determine the health status of lobsters and determine decisions in handling and shipping lobsters [61]. The high BRIX index in lobsters indicates that the protein level in the lobster haemolymph is high. The high BRIX index also correlates with the molting process. Healthy lobsters with a high BRIX index value will spur the success of the lobster molting process. The BRIX index will reach its maximum point just before molting, then it will decrease after the molting process occurs [62].

Results of water quality analyze (temperature, pH, salinity, ammonia, phosphate and nitrite) showed in Table 3. Data water quality analyze i.e., temperature, pH, salinity, ammonia, phosphate and nitrite were still good value and almost the same among treatments; because of the running water (500-600% per day).
Table 3. Water quality on reared scalloped spiny lobster (Panulirus homarus) at fiberglass tank for 75 days with different net size for shelter.

| Parameters       | net size for shelter |
|------------------|----------------------|
|                  | A (4 time of bottom area) | B (2 time of bottom area) | A (without net addition) |
| Temperature (°C) | 28.5-29.6             | 28.4-29.6                  | 28.6-29.6                  |
| Ammonia (ppm)    | 0.0113-0.0315          | 0.0115-0.0366              | 0.028-0.0349               |
| pH               | 8.1-8.2                | 8.1-8.2                    | 8.1-8.2                    |
| Nitrite (ppm)    | 0.0325-0.0689          | 0.0325-0.0545              | 0.036-0.0424               |
| DO (ppm)         | 5.5-6.2                | 5.6-6.1                    | 5.5-6.3                    |
| Salinity (ppt)   | 34-35                 | 34-35                      | 34-35                      |

Data of water quality during experiment i.e. temperature, DO, salinity, ammonia, phosphate and nitrite; still in the range of good value for spiny lobster grow out. The scalloped spiny lobster grows well in water conditions at 25.5-29.5 °C, pH 7.5-8.5, DO 4.5-7.5 and salinity 25-35 ppt [26]. Spiny lobster P. homarus grow well at salinity of 30-35 ppt [63]. Water quality parameters showed optimal conditions supporting lobster life during study. The condition of pH is relatively stable in the range of 8.1-8.2 (Table 3). According to [48] 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 28.4-29.6°C (Table 3). 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 [64]. The salinity during the study was between 34-35 (Table 3). Salinity conditions were still appropriated and could support the lobster’s life. P. homarus have a fairly broad salinity tolerance that was 30-40 ppt [65].

Overall, the addition of net shelter in this study proved to have a significant positive effect on cultivated lobsters. This is because, with net shelter addition, contact between the lobsters can be reduce, so cannibalism in the cultivation container can be reduce. Net shelter addition of 4 time of bottom area 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.

4. Conclusion
The net addition for shelter at size of 4 time of bottom area was more suitable for scalloped spiny lobster reared in fiberglass tank; because it increased the survival rate and reduced the cannibalism of scalloped spiny lobster.

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