



Biochemical response and production performance of spiny lobster *Panulirus homarus* seed reared with different shelter materials

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ABSTRACT

Provision of shelters during nursery rearing of the spiny lobster *Panulirus homarus* seed is expected to reduce stress and generate higher production performance. In the present study, three types of shelter materials viz, polyvinyl chloride (PVC) pipes, polyethylene (PE) nets and concrete blocks with three replications were used as treatments. The results showed that pipe shelter was the best among the three treatments with better yield, higher survival rates and lower stress level indications. Examination of haemolymph for total cholesterol, total protein, total haemocyte count (THC) and glucose level showed that pipe shelter treatment exhibited the lowest stress response compared to the other treatments. In terms of lobster weight and carapace length, the application of pipe shelters resulted in the best growth of seeds. Furthermore, the highest daily weight gain and survival rate of 1.41 ± 0.04 and $72.67 \pm 1.53\%$ respectively were recorded in seeds reared in pipe shelter, while the lowest values were recorded in concrete block shelter.

Keywords: Glucose, Lobster, Shelter, THC, Total cholesterol, Total protein

Introduction

Global trade of lobsters declined by more than 1000 t in 2019 compared to the previous year which reached 131,634 t. The same year, North Atlantic lobster industry also faced a supply shortage. Studies from the University of Maine predicted that lobster landings would tend to decline in the next decade due to climate change (FAO, 2020). As a solution to meet lobster market demands, Australian Centre for International Agricultural Research (ACIAR) led attentive research on lobster fattening for commercial purpose and methods for *Panulirus ornatus* larval rearing. However, the efforts on lobster hatchery activities have not been so successful (Sachlikidis *et al.*, 2010). The poor availability of lobster seeds is one of the obstacles to lobster cultivation. During the hatchery phase, larval mortality is very high (Thuy and Ngoc, 2004). According to Phillips *et al.* (2003), the larval mortality rate of *Panulirus cygnus* in the wild is estimated to be about 80-98% when the seed settles at the bottom.

The effect of shelter application in many lobster culture studies showed positive results. According to James *et al.* (2002), shelter application during lobster rearing could enhance survival rate of lobsters, although there is no significant effect on lobster growth. The application of PE nets shelter on *P. cygnus* puerulli revealed that shelter could reduce stress during moulting, lessen physical

contact among lobster seeds and also raise their growth and survival rate (Johnston *et al.*, 2006). Adiyana *et al.* (2014) had conducted preliminary research on the effect of different shelters on stress responses in *Panulirus homarus* during nursery stage. The type of shelter used was found to affect total haemocyte count (THC) and haemolymph glucose levels. PVC pipe shelter could be used to reduce stress levels, produce better growth and survival in *P. homarus* rearing (Adiyana *et al.*, 2014; Djai *et al.*, 2017; Supriyono *et al.*, 2017; Subhan *et al.*, 2018).

Stress response is the most important physiological variable which influences survival rate of lobster seeds. Stress response can be evaluated subjectively using behaviour observations or quantitative measurement in several physiological variables such as oxygen use level, pH, blood composition, haemocytes, ions and hormones (Lorenzon *et al.*, 2007). In crustaceans, THC can be used as a stress indicator (Adiyana *et al.*, 2014; Verghese *et al.*, 2007; Powell *et al.*, 2017). Glucose level in crustacean haemolymph has been reported to increase as a stress response during handling, emersion, salinity divergence, disease and pollutants (Lorenzon *et al.*, 2007; Takahashi *et al.*, 2014; Powell *et al.*, 2017). Several metabolic variables such as total protein and cholesterol can be used to monitor crustacean physiological condition in response to stress (Mercier *et al.*, 2006).

Currently, there is less information about the correlation between the use of various shelter types on lobster seed growth and survival rate (Chau *et al.*, 2008). Moreover, studies on various shelter materials for lobster growth have not been done and the present investigation was undertaken to analyse different types of shelter materials that can provide the lowest stress response and highest productivity of lobster seeds.

Materials and methods

P. homarus seeds (11.16 ± 0.03 mm; 2.09 ± 0.04 g) used for the study were acclimatised for seven days in the adaptation pool, before stocking in the treatment pool. This study used three types of shelters *viz.* PVC pipe, PE nets and concrete block (Fig. 1). The ratio of shelter volume with tank bottom surface area in each treatment was $7900 \text{ cm}^3 \text{ m}^{-2}$.

Experimental design

Lobster seeds were stocked in a recirculation system with eight plastic treatment tanks measuring $1.2 \times 0.9 \times 1.0$ m and water treatment in the recirculation system used biofilter and protein skimmer. Seed stocking density in each treatment pool was $100 \text{ lobsters m}^{-3}$. Minced anchovies were fed to lobsters once a day at 10% of their total body weight and were reared for 70 days. Proximate composition of anchovies used as feed is presented in Table 1. The study followed completely randomised design (CRD) with 3 treatments (PVC pipe, PE nets and concrete shelter).

Sampling for analyses

Temperature, salinity, pH and dissolved oxygen (DO) parameters were recorded daily in each treatment pool. Ammonia, nitrite and nitrate levels were analysed every seven days (APHA, 2012). Total protein, THC, haemolymph glucose and total cholesterol of haemolymph as physiological response parameters were recorded every 7 days. THC analysis followed Blaxhall and Daishley (1973), glucose analysis as per Wedemeyer and Yasutake (1977), total cholesterol

according to Mercier *et al.* (2006) and total protein was estimated as per Lowry *et al.* (1951). Biometric measurements in terms of body weight and carapace length were recorded every 7 days (Solanki *et al.* 2012). Seed survival rates were recorded at the end of the experiment.

Statistical analysis

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

Results

Water quality

Dissolved oxygen, nitrite and ammonia levels in water are known as limiting factors for lobster growth and will influence seed growth and survival rate. Water quality parameters recorded during the study period are shown in Table 2.

Haemolymph parameters

THC: Different types of shelter treatments resulted in varied responses in lobster THC, which ranged between 102.25 ± 37.74 to $506.77 \pm 23.96 \times 10^4 \text{ cells ml}^{-1}$ during the study (Fig. 2a). THC recorded during the study period was the highest ($533.66 \pm 23.96 \times 10^4 \text{ cells ml}^{-1}$) at the beginning of the experiment. Haemolymph THC tended to decline between the 7th and 28th day and then became relatively stable until the end of the experiment. From the 7th until

Table 1. Overview of anchovy proximate composition

Parameters (%)	Mean $\pm$ SD
Water content	78.49 ± 0.27
Protein	12.92 ± 0.23
Carbohydrate	0.70 ± 0.06
Lipid	2.28 ± 0.16
Ash	5.61 ± 0.14

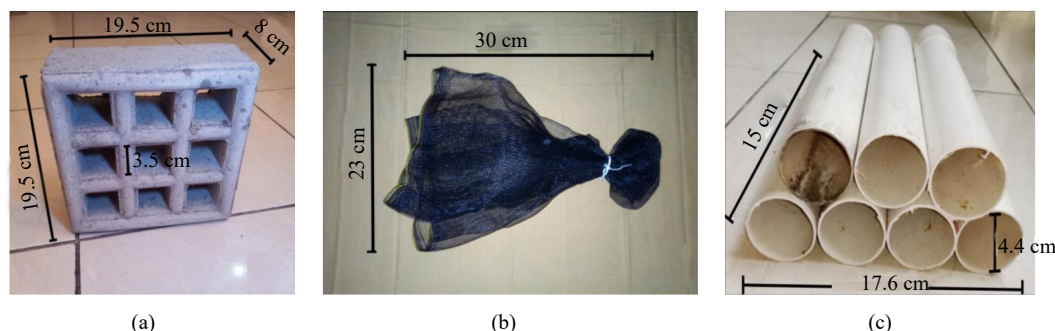


Fig. 1. Shelter types used in the treatments: (a) Concrete block, (b) PE nets and (c) PVC pipe

Table 2. Water quality parameters recorded during the experimental period

Parameters	Shelter treatment			Optimum range
	Concrete block	PVC pipe	PE Nets	
Temperature (°C)	26.80±0.10-28.47±0.06	26.67±0.15-28.40±0.10	26.50±0.10-28.27±0.15	25-30 (Philips and Kittaka, 2000)
Salinity (‰)	31.12±0.09-33.45±0.06	31.15±0.05-33.81±0.12	31.12±0.11-33.77±0.08	32-36 (Wickins and Lee, 2002)
pH	7.92±0.08-8.35±0.05	7.95±0.05-8.38±0.08	7.98±0.04-8.30±0.11	7.8-8.5 (Wickins and Lee, 2002)
DO (mg l ⁻¹)	5.45±0.10-6.71±0.06	5.61±0.15-6.75±0.10	5.46±0.10-6.68±0.15	Minimum 2,7-5,4 (Philips and Kittaka, 2000)
Ammonia (mg l ⁻¹)	0.00±0.00-0.06±0.01	0.00±0.00-0.05±0.01	0.00±0.00-0.06±0.01	<0.1 (Wickins and Lee, 2002)
Nitrate (mg l ⁻¹)	0.01±0.00-1.77±0.11	0.01±0.00-1.35±0.08	0.01±0.00-1.50±0.14	<100 (Wickins and Lee, 2002)
Nitrite (mg l ⁻¹)	0.02±0.01-0.37±0.03	0.02±0.00-0.40±0.02	0.02±0.00-0.38±0.03	<5 (Drengstig and Bergheim, 2013)

the 28th day, the PVC shelter and PE net shelter had similar influence on THC ($p>0.05$), but the THC of lobsters from PVC shelter was greatly more affected ($p<0.05$) than the concrete block shelter. THC levels of seed reared in PVC shelter tended to be most stable compared to other shelter treatments.

Glucose: The overall glucose concentration was in the range of 5.10±1.81 to 22.06±1.64 mg dl⁻¹ (Fig. 2b). Glucose concentration was highest on day 0 (22.06±1.64 mg dl⁻¹) compared to the experimental period. On the 7th day, glucose concentration in all treatments decreased to the range of 8.09±1.01 to 9.53±0.83 mg dl⁻¹. Haemolymph glucose concentration of seed reared in PVC shelter treatment was the lowest and most stable than in other treatments. PVC shelter treatment had significant influence on haemolymph glucose concentration ($p<0.05$) as compared to other treatments.

Total protein: Overall, total protein concentration was in the range of 13.65±1.38 to 40.03±3.04 mg ml⁻¹ (Fig. 2c). Total protein concentration was highest (40.03±3.04 mg ml⁻¹) on day 0. Total protein concentrations in all shelter treatments (day 7 to 70), although fluctuating, showed the tendency to decline during the experimental period. Total protein concentration of PVC shelter treatment was the lowest and stable compared to the other treatments during the study. The analysis of variance showed that the treatments did not significantly ($p>0.05$) affect lobster haemolymph total protein.

Total cholesterol: Lobster haemolymph total cholesterol response to various shelters tended to fluctuate, in the range of 6.10±0.63 to 14.31±0.88 mg dl⁻¹ (Fig. 2d). On day 0, total cholesterol concentrations showed the highest level (14.31±0.88 mg dl⁻¹). Total cholesterol concentrations in all treatments decreased on the 7th day and during day 14 until day 70, showed fluctuating patterns. Generally, total cholesterol in treatment using modified PVC shelters showed the lowest propensity and most stable compared to the other treatments. Similar to glucose concentration, total cholesterol concentration data towards the end of the study period showed that PVC shelter treatment significantly influenced total cholesterol

($p<0.05$) as compared to PE net shelter. However, concrete block shelter and PVC shelter treatment had similar effects ($p>0.05$) on total cholesterol of haemolymph.

Production performance

Average weight of lobsters at the beginning of the study was 2.09±0.04 g. The highest lobster weight on day 70 was obtained with modified PVC shelter treatment (5.60±0.16 g) and the lowest was in concrete block treatment (4.95±0.13 g) (Table 3). PVC pipe shelter achieved the highest lobster daily growth rate (SGR_w) (1.41±0.04%), while the lowest was obtained with concrete block shelter treatment (1.24±0.04%). Daily growth in terms of weight of lobsters was the highest ($p<0.05$) in PVC pipe shelter treatment compared to other treatments.

Initial average carapace length of lobsters was 11.16±0.03 mm and the highest carapace length (18.61±0.04 mm) was obtained for PVC shelter and the lowest for concrete block (16.88±0.04 mm). The specific growth rate (SGR_{CL}) in terms of lobster carapace length was found to be highest (0.73±0.01%) in PVC shelter treatment, while the lowest was recorded in concrete block treatment (0.59±0.00%). SGR_{CL} values significantly varied with treatments ($p<0.05$).

The highest survival rate was obtained with modified PVC pipe shelter treatment (72.67±1.53%), while the lowest in concrete block treatment (63.00±2.00%). Survival rate with PVC shelter was the highest ($p<0.05$) compared to concrete block and PE nets shelter.

Feed conversion ratio (FCR) of lobsters recorded during the study was in the range 7.81±0.17-8.81±0.16 (Table 3). The lowest FCR (8.53±0.16) was found in PVC pipe shelter treatment, while the highest (9.33±0.16) was found in concrete block shelter treatment. The FCR of PVC pipe shelter treatment was lower ($p<0.05$) than concrete block and was almost similar ($p>0.05$) to PE nets shelter treatment.

Biochemical composition of flesh

The proximate composition of lobsters reared in various shelters at the end of the study is summarised in Table 4. The

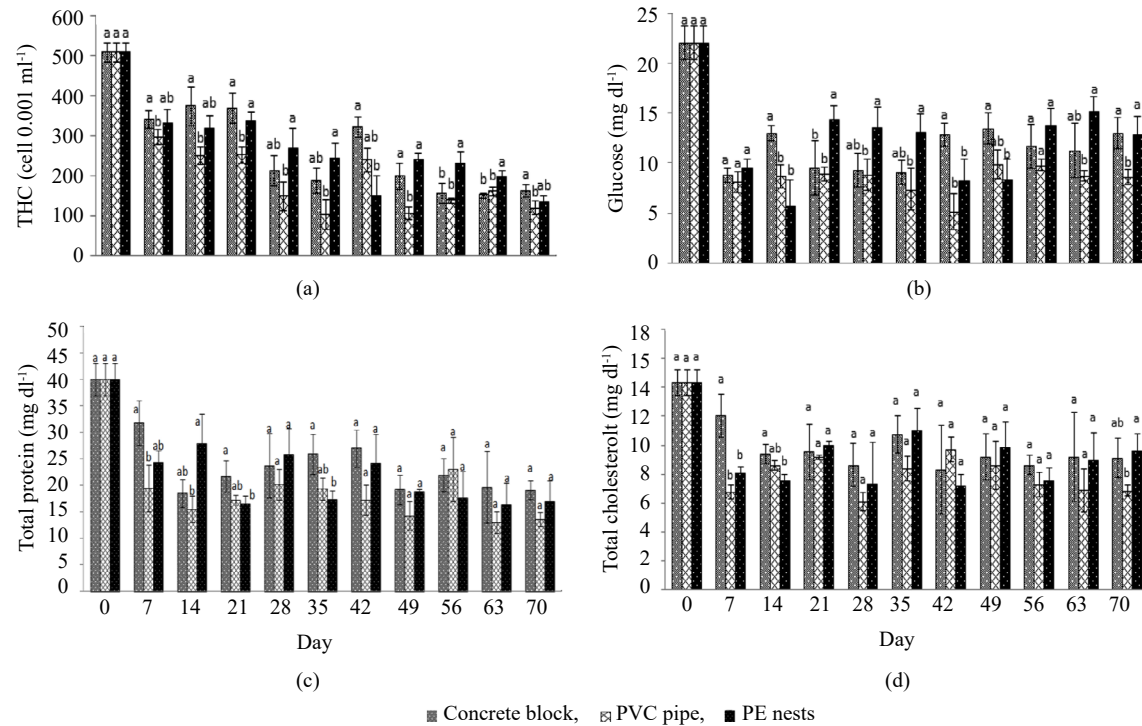


Fig. 2. (a) THC, (b) Glucose, (c) Total protein and (d) Total cholesterol in the haemolymph of lobsters recorded during the experimental period. Different lowercase letters in the graph indicates significant difference ($p < 0.05$)

Table 3. The production performance of *P. homarus* after 70 days of culture in different shelters

Production performance	Shelter treatment		
	Concrete block	PVC pipe	PE nets
Final weight (g)	4.95±0.13 ^b	5.60±0.16 ^a	5.12±0.06 ^b
Final carapace length (mm)	16.88±0.04 ^a	18.61±0.14 ^b	17.75±0.05 ^c
Survival rate (%)	63.00±2.00 ^b	72.67±1.53 ^a	66.67±1.15 ^b
SGR _w (% day ⁻¹)	1.24±0.04 ^b	1.41±0.04 ^a	1.29±0.02 ^b
SGR _{CL} (% day ⁻¹)	0.59±0.00 ^a	0.73±0.01 ^b	0.67±0.00 ^c
FCR	8.81±0.16 ^b	7.81±0.17 ^a	8.02±0.17 ^a

Table 4. Proximate composition of lobsters at the end of the study period

Parameters (%)	Treatments		
	Concrete block	PVC pipe	PE nets
Water content	73.13±0.03	73.1±0.05	73.14±0.06
Protein	15.41±0.10	15.52±0.12	15.51±0.08
Carbohydrate	3.92±0.06	3.92±0.03	3.85±0.04
Lipid	1.34±0.05	1.27±0.07	1.3±0.03
Ash	6.2±0.01	6.19±0.05	6.2±0.02

final proximate contents of lobsters (moisture, protein, carbohydrate, fat and ash content) in different treatments did not show significant differences. Protein content ranged between 15.35±0.06-15.59±0.06%, carbohydrates 3.845±0.05-3.945±0.05%, fat 1.25±0.08-1.345±0.06% and ash content 6.16±0.01-6.23±0.03%.

Discussion

Water quality parameters recorded during the study showed that they had met the optimal qualification for lobster seeds growth and all water quality parameters complied with standard requirements for lobster cultivation (Table 2). It can be concluded that the recirculation system used in this study was capable of maintaining water quality in the system, which provided low concentration of ammonia and nitrite in the water during the study and a sufficient DO level for lobster growth (DO > 5 mg l⁻¹). Water ammonia concentrations ranged from 0.001-0.06 mg l⁻¹, while nitrite concentrations ranged from 0.02-0.40 mg l⁻¹. Several lobster species cultured in recirculation system such as *Jasus edwardsii* (Phillips and Kittaka, 2000) and spiny lobsters (Wickins and Lee, 2002), grow healthy at low concentrations of ammonia at less than 0.1 mg l⁻¹. Drenstvig and Bergheim (2013), recommended water nitrite level lower than 5 mg l⁻¹ for *Homarus gammarus* reared in a recirculation system. Quite low ammonia and nitrite concentrations in water during the study indicated that nitrification process inside biofilter equipped in the recirculation system performed well.

THC has an important task in crustacean immune system. Harrington *et al.* (2019), stated that changes in water temperature outside their normal habitat will lead

to increase in THC value in *Homarus americanus*, which indicates their stress response to environmental change. According to Maharani *et al.* (2009), haemolymph composition can be measured and used for crustacean health assessment.

At the beginning of the study, THC concentration levels were higher as the lobster seeds experienced stress because they had not adapted to previous handling, on-land holding time and human touch when being transferred from acclimatisation pond to treatment pool. Environmental changes caused stress on *P. homarus* and affected their immune response, which lead to change in THC, phenoloxidase activity and phagocytic activity (Verghese *et al.*, 2007). The increasing value of THC briefly at the beginning of the study, indicated a stress response due to handling. In *P. cygnus* THC concentration increased during handling and transporting due to stress, then decreased after being reared for 16-48 h in holding tank (Fotadar *et al.*, 2006). THC in lobsters from modified PVC shelter treatment was lowest and most stable compared to the other treatments.

On the first day of the experiment, haemolymph glucose level was relatively higher when compared to glucose levels during subsequent period of the study. It can be attributed to stress experienced by lobster seeds through initial handling before stocking in treatment pools. Stress can occur due to changes in environmental conditions (pond, water and temperature). According to Hastuti *et al.* (2003) stress causes an increase in blood glucose levels (hyperglycemia). Subhan *et al.* (2018), stated that glucose concentration in haemolymph is affected by neuropeptide hyperglycemic hormone (CHH) of crustaceans, neuropeptide moulting inhibitory effect hormone (MIH) and mandibular organs inhibitory hormone (MOIH). These hormones are produced by sinus glands of crustaceans and regulate reproduction, osmotic regulation and moulting mechanism.

On the 7th day, glucose concentration in all treatments decreased which is in agreement with the observations of Lorenzon *et al.* (2007) who reported a significant decrease in haemolymph glucose levels of 0.85 mg dl⁻¹ to 0.4 mg dl⁻¹ during the 24 h monitoring in the lobster *H. americanus* exposed to stress. The haemolymph glucose concentration in lobsters from PVC tube shelter treatment during the study period, was relatively low and stable than other treatments, since the other treatments showed slightly high and unstable glucose levels. This indicated that PVC pipe shelter was effective in reducing stress in lobsters than other shelter types.

At the beginning of the study (day 0), the total protein concentration in haemolymph tended to be

high, which indicated stress on lobsters. Stress leads to increased metabolic activity in response to improved homeostasis. Increased metabolic activity increases the need for oxygen transport. In times of stress, the amount of haemocyanin in haemolymph increases. Haemocyanin enhancement is associated with its main function in oxygen transport, which carries 94% oxygen from cells to body tissues (Lorenzon *et al.*, 2007). Particularly in decapods, haemocyanin is dissolved in blood plasma. Haemocyanin proportion accounted for more than 60% and in some species of crustaceans they reach more than 93% of the total protein concentration in the haemolymph (Sladkova and Kholodkevich, 2011). Overall, the trend of the level of total protein in lobsters from PVC pipe shelter treatment was found lowest and most stable compared to the other shelters.

The high concentrations of total cholesterol at baseline indicated stress in lobsters (Lorenzon *et al.*, 2007). Stress causes the progressive increase of energy to improve homeostasis. Increased fat levels in the haemolymph of crustaceans occur because of gluconeogenesis mechanism to meet the energy needs during stress (Schmitt and Santos, 1993; Castiglioni *et al.*, 2010). Based on the observation during the experiment, total cholesterol concentration in lobsters from PVC tube shelter treatment was lowest than other treatments, indicating most stable condition.

Specific growth rate in terms of lobster weight (SGR_w) during the study ranged from 1.24±0.04% (concrete block shelter) - 1.41±0.04% (PVC pipe shelter). According to Johnston *et al.* (2006), the use of PE net shelters on puerulus of *P. cygnus* fed with wet meal yielded the highest SGR_w of 1.25±0.03%. The results of other studies on juveniles of *J. edwardsii* with shelter and feed wet meal resulted in SGR_w 1.32% (Phillips and Kittaka, 2000).

SGR in terms of carapace length (SGR_{CL}) during the study ranged from 0.59±0.00% (concrete block shelter) to 0.73±0.01% (PVC pipe shelter). According to Thomas (1972), SGR_{CL} level of *P. homarus* puerulus cultured for 62 days in rocks with pieces of asbestos shelter was 0.46%. Broadly, application of the PVC pipe shelter resulted in better lobster growth when compared with other treatments, as PVC pipe shelter reduced stress levels and hence the energy diverted for regaining homeostasis was low. The low energy diverted for homeostasis during stress, can be used as a metabolic energy investment for growth (Hastuti *et al.*, 2004).

Stress response in each type of shelter can be related to the response of lobster behaviour to shelter object. According to Rossong *et al.* (2011), juvenile lobsters preferred shelter made from materials that have a darker

colour or are non-transparent. In addition, juvenile lobsters spend more time to take refuge in the shelter than adult lobsters. According to Kari and Spanier (2007), *Scyllarides elisabethae* and *S. aequinoctialis* lobsters preferred shelter that resembles a cave hole in the rock or coral in its natural habitat, with two driveways at the end of an open shelter. In another study, lobsters preferred artificial substrates derived from plastic material that has rough surface. PVC pipe shelter was found to be the best shelter, because it has two sides of open surface holes at the end of the shelter and dark in colour which is made from a thermoplastic polymer.

The high survival rate of lobsters with the use of PVC pipe shelters ($72.67 \pm 1.53\%$), indicated a low level of stress in lobsters. According to Adiyana *et al.* (2014); Fotedar *et al.* (2006) and Verghese *et al.* (2007), stress causes immunological decline, impaired growth, poor reproductive performance and low survival.

The feed conversion ratio in lobsters is commonly influenced by lobster feed types. In this experiment, fresh anchovies were used as lobster feed. According to Phillips and Kittaka (2000), feeding juvenile lobsters with wet feed will result in FCR of 3-9. Factors which can affect feed conversion efficiency are feed type, age of lobsters, body size, feeding level, salinity and water temperature.

Dry weight percentage of protein, carbohydrates, fat and ash in fresh anchovies were $60.08 \pm 0.42\%$; $3.27 \pm 0.29\%$; $10.60 \pm 0.85\%$ and $26.05 \pm 0.31\%$ respectively (Table 1). According to Johnston *et al.* (2007), there are several optimal feed nutrient requirements for lobster growth, as for *P. cygnus* (50% protein, 6% fat), *P. ornatus* (53% protein, 10% fat) and *J. edwardsii* (31% protein, 27% carbohydrate, 13.5% fat). Williams (2007) also stated that, the optimal percentage ranges of carbohydrates and fat for *J. edwardsii* were 16-51 and 3-19%, respectively. In general, protein and fat contents in fresh anchovies used as feed for lobsters during the study were found suitable for optimal lobster growth.

Final lobster proximate contents (moisture, protein, carbohydrate, fat and ash), among all treatments were not significantly different. Proximate composition of lobsters at the beginning of the study and at the end of the study period, also did not vary significantly. It can be concluded that application of different shelter materials during lobster rearing would not influence lobster final proximate contents.

From the analysis of THC, glucose, total protein and total cholesterol, PVC pipe shelter was found to reduce stress on lobster seeds compared to other treatments. Concrete block shelter treatment resulted in the lowest survival rate. This was due to higher levels of stress

caused by frequent physical contact among lobster seeds, which will reduce the level of survival. This is consistent with research conducted by Lorenzon *et al.* (2007), that shelters could lessen body contact among lobster seeds, reduce stress during moulting, maximising growth and thus resulting in higher survival rates.

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