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Health status of spiny lobster *Panulirus homarus* with sub-mersible net cage system in the different depths at kepulauan seribu, DKI Jakarta

Status kesehatan lobster pasir *Panulirus homarus* pada sistem karamba jaring tenggelam dengan kedalaman berbeda di kepulauan seribu, DKI Jakarta

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ABSTRACT

The *Panulirus homarus* lobster culture has been currently performed in a sub-mersible net cage system at a certain depth to obtain optimal temperature, light, and water pressure. This study aimed to evaluate the sand lobster *P. homarus* health status maintained in a sub-mersible net cage system at 250 cm × 272 cm × 135 cm size with 6 m and 8 m depths on Semak Daun Island waters, Kepulauan Seribu, Jakarta, Indonesia. The average size of lobster seeds used was 93.23 ± 0.99 g/lobster and stocked at 4 lobsters/m2. Lobsters were fed trash fish, molluscs, and crustaceans twice a day at 07.00 WIB (30%) and 17.00 WIB (70%), following the lobster biomass weight. This study used a completely randomized design with the two depth treatments as mentioned above and three replications. Total haemocyte count, differential haemocyte count, phenoloxydase activity, respiratory burst phagocytic activity, and hepatopancreatic histology were observed twice every 14 days. Based on the observation results, maintenance depth treatmen has no effect on the immune response as there were no visible damage to the cells and tissues of the lobster hepatopancreas.

Keywords: haemolymph, histology, lobster culture, sea, sub-mersible net cage system

ABSTRAK

Budidaya lobster *Panulirus homarus* kini dilakukan pada karamba jaring tenggelam pada kedalaman tertentu guna medapatkan suhu, cahaya, dan tekanan air yang optimal. Tujuan penelitian ini adalah mengevaluasi status kesehatan lobster pasir *Panulirus homarus* yang dipelihara dalam karamba jaring tenggelam berukuran 250 cm × 272 cm × 135 cm dengan kedalaman 6 m dan 8 m di perairan Pulau Semak Daun Kepulauan Seribu DKI Jakarta. Benih lobster yang digunakan berukuran rata-rata 93.23 ± 0.99 g/ekor dengan kepadatan 4 ekor/m2. Lobster diberi pakan ikan rucah, moluska dan krustasea, dengan frekuensi dua kali sehari pada 07.00 WIB 30% dan 17.00 WIB 70% dari biomassa lobster. Penelitian ini menggunakan rancangan acak lengkap dengan dua perlakuan kedalaman tersebut di atas dan tiga ulangan. Pengamatan total haemocyte count, differential haemocyte count, aktivitas phenoloxydase, respiratory burst aktivitas fagositik serta histologi hepatopankreas lobster dilakukan dua kali setiap 14 hari. Berdasarkan pengamatan tersebut di atas kedalaman tidak memengaruhi respons imun tidak terlihat kerusakan sel dan jaringan organ hepatopankreas lobster.

Kata kunci: budidaya lobster, hemolim, histologi, karamba jaring tenggelam, laut

INTRODUCTION

High market demand and limited supply due to reduced natural stock with expensive price trigger the lobster culture effort in several countries, including Indonesia (Effendi *et al*., 2022). The global lobster market reached US$ 6.3 billions (about IDR 91.5 trillions) in 2021 and is estimated to reach US$ 11.1 billions (about IDR 161 trillions) in 2027 (IMARC 2022). Indonesia has a great opportunity to become the world's lobster producer with national economical increase potential. The lobster seed potential in Indonesia is estimated >20 times more than Vietnam, which can produce up to 12.500 ton consumed lobsters, when being maintained for aquaculture development (Priyambodo *et al*., 2020). Vietnam is the world's main cultured lobster, although has currently been suffering from high mortality level, disease outbreak, and Chinese market dependence (Hai & Speelman, 2020). Several studies have been performed to increase the lobster culture productivity, such as artificial feed improvement (Slamet *et al*., 2020), culture system (Mustafa, 2013), and maintenance technology (Adiyana *et al*., 2014; Adiyana *et al*., 2015; Adiyana *et al*., 2020).

Lobster culture is commonly conducted in a floating-net cage, pen cage, sub-mersive net cage, or tank as performed in several countries (Rao *et al*. 2010, Daniels *et al*., 2015), while Indonesia commonly uses floating-net cage system (Priyambodo & Sarifin, 2009). Currently, the lobster culture in Indonesia has been conducted using a sub-mersive net cage system, following Vietnam with various frame materials, such as iron, polyvinyl chloride (PVC), and high-density polyethylene (HDPE) (Effendi *et al*., 2022). Lobsters live in the sea with coral and sandy substrates less than 16 m depth, approximately 4-12 m depth or 4-10 fathoms (DeBruin, 1969). Depth is part of hydrodynamical-oceanographic parameters related to light intensity, temperature, wave, current speed and direction, dissolved oxygen, salinity, hydropressure, hydrodensity, etc. (Saenuddin *et al*., 2020; Saenuddin *et al*., 2021). This condition is soon associated with the lobster physiology, biochemistry, stress, health status, growth, survival rate, meat texture and taste, and body color (Jimenez *et al*., 2012; Vanderstichel *et al*., 2016; Prama *et al*., 2017). Body color, meat texture, and meat taste will impact on the economical value of the lobster commodities.

Environmental change can affect the body immune system of organisms. The most important environmental factor for lobsters is water temperature, which directly influences the oxygen consumption, metabolism, moulting, growth, and survival rate (Prastowo *et al*., 2020). Temperature variation often causes stress for crustacea, resulting in a low immune function based on the decreased total haemocyte, proPO activation, phagocytic activity, and free-oxygen radical release (Le Moullac *et al*. 1998). Previously, Le Moullac and Haffner (2000) explained that various pro-phenoloxydase (proPO) depended on the temperature condition. Therefore, temperature variation response on crustacea will provide an ecologically important information. This study aimed to evaluate the health status of sand lobster *Panulirus homarus* maintained in a sub-mersive net cage at 6 and 8 m depths on Kepulauan Seribu, Jakarta, Indonesia.

MATERIALS AND METHODS

Place and period

This study was performed on December, 2021, mainly on the West season, which occurs on December – March marked by rain and relatively strong wind and current (Effendi *et al*. 2016). Lobsters were maintained in Sea Farming Center, Coastal and Marine Resource Study Center (PKSPL), Kepulauan Seribu, Jakarta, Indonesia (Figure 1). This location has 1-14 m seawater depth, with the water quality condition at 1-2 m depth: 28.6-29.7°C temperature, which is relatively even from the surface to the base, 32.0 ppt salinity, 0.10-0.63 NTU turbidity, 6.9 m light intensity, < 8 mg/L suspended solid, 34-85 mg/L dissolved solid, 7.4-9.4 mg/L dissolved oxygen, 0.122-0.145 mg/L nitrite, 1.209- 2.349 mg/L nitrate, and 1.021- 2.123 mg/L phosphate (Effendi *et al*. 2016). The total haemocyte count (THC), differential haemocyte count (DHC), phenoloxydase activity (PO), respiratory burst (RB), and phagocytic activity (AF) tests were performed in the Laboratory of Aquatic Organism Health, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University Bogor.

Experimental design

This study used a completely randomized design, containing two treatments and three replications. Treatments were applied as two different depths, namely 6 and 8 m, measured from the water surface until the upper part of sub-mersive net cage (surface).

Procedures

Sub-mersive net cage preparations

The sub-mersive net cage used was at 250 cm × 272 cm × 135 cm size, with high density polyethylene (HDPE) frame and net manufactured by the national private company in Bandung, Indonesia. The sub-mersive net cage was drowned, depending on its weight in different depths at 6 m and 8 m hung on 100 L HDPE-cylinder buoys (Figure 2). Each cage unit was equipped with four buoys. The cage was lifted or lowered through catamaran boat assistance and pulley. The sub-mersive net cage was initially cleaned and checked its quality to validate the net condition, before setting it in appropriate size. The net cage was also equipped with lifter tool and feeding funnel for lobsters.

Lobster preparations and acclimatizations

The lobster seeds used in this study were the sand lobster seeds (*Panulirus homarus*), obtained around the Western Java waters. The lobster seeds had an average size of 93.23 ± 0.99 g/lobster at 25 ekor/cage density. The lobster seeds were initially acclimatized in a sub-mersive net cage for seven days to improve the lobster seeds' adaptability against the environment and feed. Lobsters were acclimatized in a plastic pocket for 5-6 minutes, then the lobsters were allowed to move themselves into the cage underneath by slightly tilting the plastic pocket.

Lobster maintenance

The acclimatized lobster seeds were sorted to obtain a conformed weight with a good health condition and no defective body parts. Furthermore, the seeds were stocked on each depth treatment. During the experimental period, lobsters were fed with trash fish and mollusks twice a day at 07.00 WIB (30%) and 17.00 WIB (70%). The feed was weighed at 80 g in the morning (55 g trash fish and 25 g mollusks,

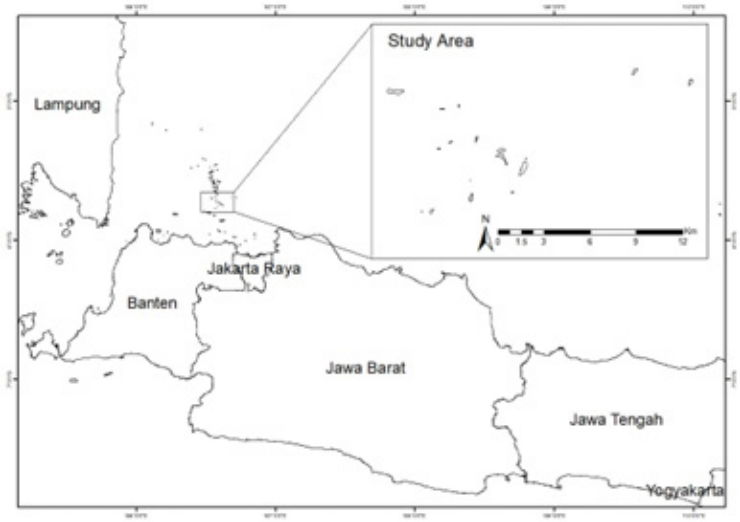


Figure 1. Lobster maintenance position in PKSPL IPB, Kepulauan Seribu, Jakarta based on satellite image from google maps software application.

Figure 2. Sub-mersive net cage marker location on the sea surface.

namely green mussels) and 240 g in the afternood (160 g trash fish and 80 g green mussels). Feeding was prepared by cutting the trash fish with scissors and crushing the mussels, then feed was placed in a feeding funnel equipped on the net cage. Parameters were observed after every 14 days, containing THC, DHC, PO, AF, RB, while the water quality parameters observed contained temperature, salinity, pH, and dissolved oxygen (DO).

Haemolymph sampling

Haemolymph was taken between pereiopods and abdomen (Figure 3) using a 1 ml syringe filled with anticoagulant solution (EDTA 10%), before collecting in a sterile microtube and preserving in a cool box. Haemolymph was taken on the 0-th and 14-th days after feeding (Ismawati *et al*. 2019).

Parameters

Total haemocyte count (THC)

The THC level was determined as a stress indicator, whereas THC was observed on the 0-th and 14-th days. The haemolymph sampling was performed using a syringe rinsed with an anticoagulant. The ratio of anticoagulant and haemolymph for THC analysis was 2:1 (0.4 mL anticoagulant for 0.2 mL haemolymph). The THC calcualtion used a haemocytometer. The THC analysis was performed following the Blaxhall and Daisley (1973) method. The THC was calculated following the equation:

$$\text{THC} = \text{Total average cells} \times \frac{1}{\text{Big box volume}} \times \text{Dilution factor}$$

Differential haemocyte count (DHC)

The DHC was determined by calculating the proportion of granular, semi-granular, hyaline cells in the haemolymph samples. The sample slides were prepared through fixation with methanol for 5 minutes, before dropping the 25 µL haemolymph and fixating the slide with methanol for 10 minutes, while being air-dried in the laboratory. Furthermore, staining was performed by dipping the slide in Giemsa solution for 15 minutes and washing the slide with aquadest. The dried DHC was observed under a microscope. The DHC was calculated using the following equation:

$$\text{DHC} = \frac{\text{Total differentiated hemocytes}}{\text{Total counted hemocytes}} \times 100$$

Phenoloxydase activity (PO)

The phenoloxydase activity (PO) was measured by mixing the 0.2 mL haemolymph and 0.4 mL anticoagulant, before being taken at 150 µL, added with a cocodylate-citrate buffer solution, and centrifuged for 5 minutes at 5000 rpm speed. Then, the supernatant was removed and the pellet was added with 50 µL tripsin as an activator (1 mg mL^{-1} in cocodylate buffer) and 100 µL cocodylate buffer, then incubated at 25-26ºC for 10 minutes. Furthermore, the mixture was homogenized with vortex and stood for 10 minutes. Then, the 50 µL L-DOPA (3 mg mL^{-1} cocodylate buffer) was added and stood for 5 minutes. Furthermore, the 800 µL cocodylate buffer was added and mixed once again, before taking 200 µL into each microplate titer well. The optical density (OD) was measured using a spectrophotometer at 490 nm wavelength.

Phagocytic activity (AF)

The lobster haemolymph sample was taken at 25 µL and mixed with *Staphylococcus aureus* (10^7 CFU/mL) at 25 µL, before being incubated for 20 minutes. The 10 µL of the mixture was dropped onto the object glass for slide preparation. This

Figure 3. Haemolymph sampling from lobsters.

slide was fixated with methanol 100% for 5 minutes and stained with Giemsa for 20 minutes, before rinsing with aquadest. The slide was observed under a microscope at 400 times magnification. The phagocytic activity was measured based on the phagocytic cell presentations that presented a phagocytosis activity (Immanueal *et al*. 2012). The phagocytic activity was measured following the equation (Suleman *et al*. 2019):

$$AF = \frac{\text{Total phagocytic cells that perform phagocytic}}{\text{Total phagocytic cells}} \times 100$$

Respiratory burst (RB)

The 30 µL mixture of haemolymph and anticoagulant was incubated at room temperature for 30 minutes. The incubation product was added with 100 µL nitroblue tetrazolium (NBT) and stood for an hour, before being centrifuged at 5000 rpm for 5 minutes. The supernatant from the centrifuged product was removed, while the pellet was added with 100 µL absolute methanol, before re-centrifugarion at 5000 rpm for 5 minutes. The pellet was rinsed twice with methanol 70%, before adding 120 µL KOH and 140 µL dimethyl sulfonil oxide (DMSO). Furthermore, the mixture was homogenized with vortex and dropped into a microplate at 200 µL, before measuring in a microplate reader with 630 nm wavelength.

Survival rate (SR)

The survival rate was calculated to identify the percentage of living lobsters until the final maintenance period. SR was calculated using the formula of Solanki *et al*. (2012):

$$SR = \frac{Nt}{No} \times 100$$

Note:
SR = Survival rate (%)
Nt = Total lobsters at the final maintenance period (lobster)
No = Total lobsters at the initial maintenance period (lobster)

Histopathological slide preparations

The normal lobster samples were taken their hepatopancreas for histological observation. The lobster samples were taken from the net cage. Each cage was taken one hepatopancreas sample. The histological slides were prepared following the Lightner and Redman (1977) method. This process was started from tissue sample fixation using a Davidson's solution for 48 hours. Then, the tissue was dehydrated, cleared, impregnated, embedded, and blocked, before being cut and stained with hematoxylin-eosin. Later, the slide was covered with a cover glass.

RESULTS AND DISCUSSIONS

Results

The total haemocyte count (THC) and differential haemocyte count (DHC) results can be shown respectively in Figure 4 and 5. From the THC value on the 0-th day, the results were relatively similar between the 6 m and 8 m depth treatments, however, a slight increase was occurred on the 14-th day compared to the 0-th day. The DHC values were relatively similar between the treatments, before tending to slightly increase for hyaline cells and decrease for granular cells on the 14-th day.

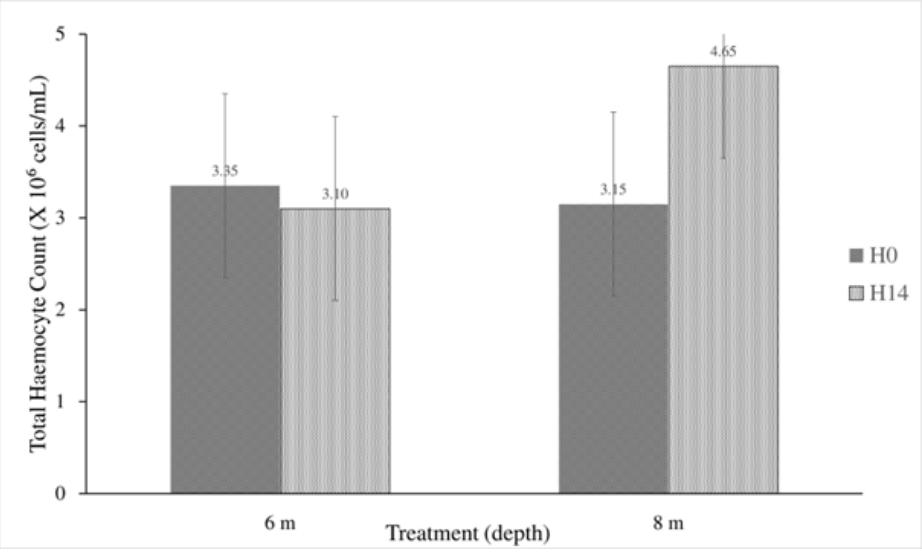


Figure 4. Total haemocytes of lobsters maintained in different depths on 0- and 14-th days.

Figure 6 presents the morphological differences among granular, semigranular/agranular, and hyaline cells. Each haemocyte was found in all observed samples with different shapes.

The phenoloxydase activity (PO), phagocytic activity (AF), and respiratory burst (RB) parameters are shown in Table 1 below. For these parameters, the results were similar without any significant differences between the treatments applied.

The hepatopancreatic histological condition of lobsters also obtained no significant differences between the 0- and 14-th days, as shown respectively in Figure 7 and 8. Based on the histopathological observation, the hepatopancreatic cells present several normal cells (R-, B-, F-, and E-cells)

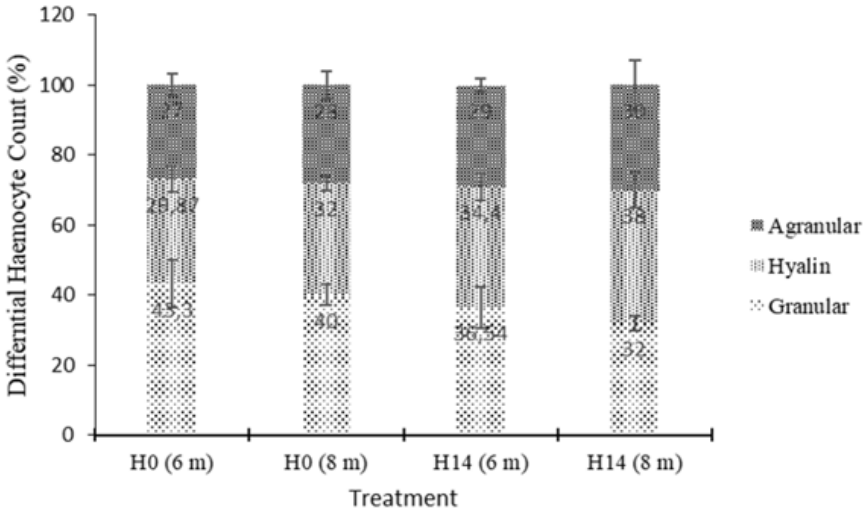


Figure 5. Haemocyte differentiation of lobsters maintained in different depths on 0- and 14-th days.

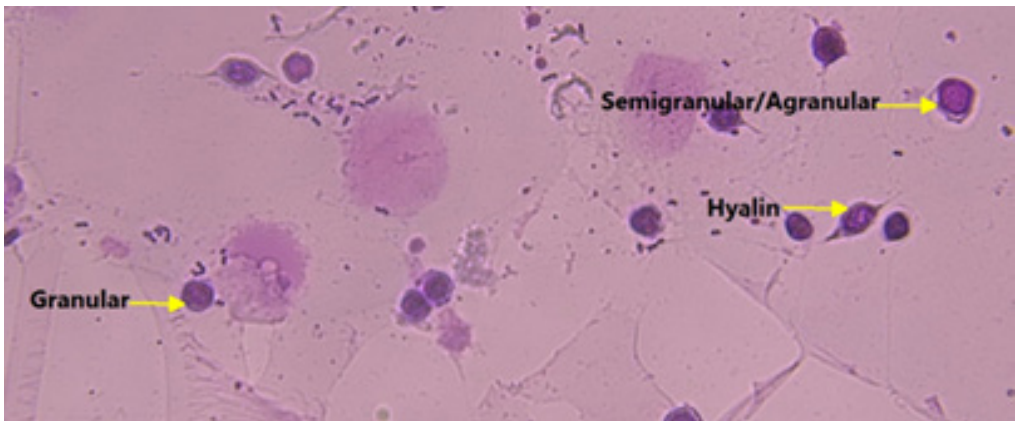


Figure 6. Morphological differences of granular, agranular, and hyaline cells in lobsters.

Table 1. Phagocytic activity, phenoloxydase activity, and respiratory burst of lobsters maintained in sub-mersible net cage at different depths.

Activity	Sampling (-th day)			
	0		14	
	Depth (m)		Depth (m)	
	6	8	6	8
Phagocytic (AF) (%)	26.63 ± 3.35[a]	36.63 ± 6.65[a]	22 ± 4.04[a]	24.53 ± 2.14[a]
Phenoloxydase (OD 490 nm)	0.410 ± 0.247[a]	0.289 ± 0.035[a]	0.069 ± 0.003[a]	0.073 ± 0.010[a]
Respiratory burst (OD 630 nm)	0.287 ± 0.037[a]	0.365 ± 0.066[a]	0.337 ± 0.251[a]	0.362 ± 0.270[a]

[a] Different superscript letters in the same row indicate a significant difference value.

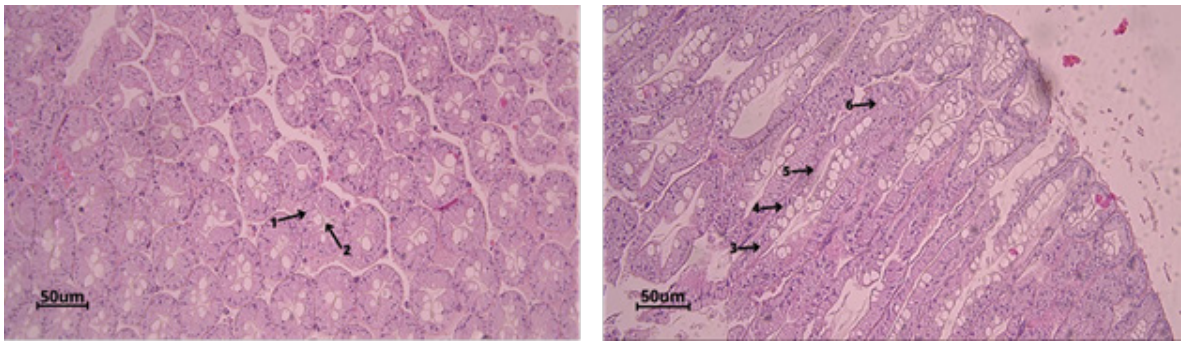
identified between the 6 m and 8 m treatments on the 14-th day without any cell damages found.

The lobster conditions maintained in different depths are closely related to the water quality of each depth. Table 2 presents several water quality measurement results at 6 m and 8 m depths to evaluate the relationship of lobster and health status and depths for 14 days.

Discussions

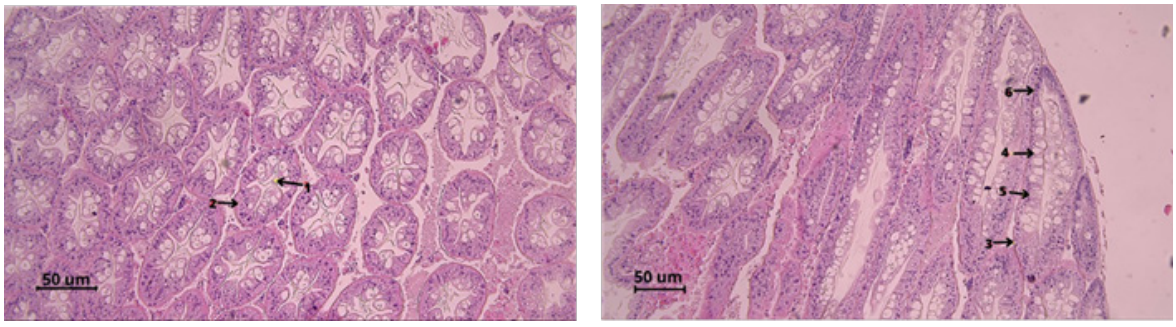
Tropical lobster culture, namely *P. homarus*, *P. ornatus*, etc. has currently been developed to fulfill the market demand that tends to increase, as the lobster supply from catch production tends to decrease (Effendi *et al*., 2022; Priyambodo *et al*. 2020). Various studies to support the production system development and business technology have been conducted, containing culture tank condition, water quality and culture environment, nutrients and feeding management, disease handling, harvesting, and post-harvesting management aspects (Hai *et al*., 2020; Koesharyani *et al*., 2021; Nankervis & Jones, 2022; Pozhoth & Jeffs, 2022; Ren *et al*., 2022; Rodríguez-Viera *et al*., 2022).

Lobster culture was firstly conducted in a floating-net cage that produced financial profit to the culturists (Solanki *et al*., 2012; Daniels *et al*., 2015; Rao *et al*., 2020, Dinesh *et al*., 2022). This commodity has also been maintained indoor in the land, namely in the plastic tank with recirculation system (Adiyana *et al*., 2020). The lobster culture effort in a sub-mersive cage is being



(a) (b)

Figure 7. The hepatopancreatic tubules and newly-growing hepatopancreatic tubules at 6 m depth. (a) Cross section of hepatopancreatic tubules: 1. Lumen 2. Tubules; (b) Cross section of the newly-growing hepatopancreatic tubule.



(a) (b)

Figure 8. The hepatopancreatic tubules and newly-growing hepatopancreatic tubules at 8 m depth. (a) Cross section of hepatopancreatic tubules: 1. Lumen 2. Tubules; (b) Cross section of the newly-growing hepatopancreatic tubules: 3. R cells, 4. B cells, 5. F cells, 6. E cells.

Table 2. Water quality of lobster maintenance media.

	Treatment		
Parameters	6 m	8 m	Optimum Range
Temperature	29.0 ± 0.2 [a]	28.7 ± 0.1 [a]	22-31° C (Jones 2009)
pH	8.1± 0.1 [a]	8.2 ± 0.1 [a]	7.8-8.2 (Mojjada *et al*.2012)
Salinity	30.3 ± 0.58 [a]	32.0 ± 1.00 [a]	25-35 g/L 9 (Vidya and Joseph 2012)
Dissolved oxygen	6.8 ± 0.2 [a]	7.2 ± 0.1 [a]	>5 mg/L (Rathina *et al*.2014)

[a] Different superscript letters in the same row indicate a significant difference value.

applied currently to provide more appropriate environment for this species physiologically (Fitzgibbon *et al*., 2017). This cage system has been applied in Vietnam to support the production and business performances of this commodity (Hai & Speelman, 2020; Priyambodo *et al*., 2020). Cage placement in several meters deep under the seawater will surely impact on the environmental condition of the containers used, such as light intensity, temperature, hydropressure, and dissolved oxygen. However, there are still less information regarding the lobster health related to the sub-mersive cage system application.

Haemocyte has an important role in the immune system of crustacea, which can be used as a health assessment through immune characteristics and activities against infectious agents. The observation results showed that the depth treatments on 0-th and 14-th days (Figure 4), obtained normal number of haemocytes, following Marlina and Nursandi (2022), who stated that the total haemocytes was 2.10-7.80 × 10^6 cells/mL, which maintained in various stocking densities, namely 10 – 25 lobsters/ m^2. This condition was thought due to different genera between species, and may also due to different size. The THC value in a normal range on each treatment showed that the lobsters were in a stable condition and adaptable to the new environment for two weeks of maintenance. Moreover, the THC value occurred above the normal range can cause high stress condition in the new environment (6 m and 8 m depths), which surely can endanger the lobster health and be more vulnerable against pathogen attack. According to Ekawati *et al*. (2012), the high total haemocytes in haemolymph causes the body inadaptability to respond condition change, producing an immunostressor that decreases the lobster body immune system. The initial maintenance for two weeks was monitored, because unsuitable environmental conditions for the lobsters could be seen in the immune response and overall health status of the lobster. After more than two weeks of maintenance, the lobsters are expected to be more adaptive to its new environment.

Each cell proportion (granular, hyaline, and agranular) in lobster haemocytes has roles in lobster body immune system, as cell proportion can represent the lobster health condition. This condition occurs as these cells work automatically during pathogen infiltration into the lobster body. The DHC value (Figure 5) for initial sampling showed that the 6 m treatment obtained the highest granular cells at averagely 43.30%, followed by hyaline and agranular cells with a relatively similar DHC value, namely 27.00% and 29.87%. Different condition was occurred in the 8 m treatment, which obtained granular cells at 40.00%, hyaline cells at 28.00%, and agranular cells at 32.00%. Relatively similar results were occurred on the 14-th day between both treatments. These cell proportions, when compared to the previous study showed granular cells at 17-40%, hyaline cells at 60-93%, and agranular cells at 51-62% (Owens & O'Neill 1997). The hyaline, semigranular, and granular cells in California lobster *Panulirus interruptus* were 30%, 10%, and 10%, respectively (Day *et al*., 2019). The *Panulirus elephas* lobster had cell proportions of hyaline cells at 70%, semigranular cells at 4%, and granular cells at 26% (Filiciotto *et al*., 2014).

From these study results, the DHC value for granular cells was still in a normal range, following the previous study, that the agranular and hyaline cells were relatively lower than the previous study. The DHC proportion variety could cause amonge crustacean species, as thought due to internal factors (sex and molting cycle) and external factor (environment). This condition was occurred on the lobster condition in this study, performed in a field scale (in the sea), while the previous study was performed in a laboratory scale (in the tank). Each cell proportion in this study tended to be balanced, which may be due to no extreme condition in the environment with different depth treatments. Hyaline cells have small size with bigger nucleus than its cytoplasm, followed by available or unavailable granulocytes. The granular cells are haemocytes with the biggest size, as the nuclues is relatively smaller than the cytoplasm and more granulocyte presences. The agranular or semigranular cells are cells with hyaline and granular cell characteristics (Figure 6). Each haemocyte has different functions. Hyaline cells are functioned in phagocytic activity, while semigranular or agranular cells are functioned in encapsulation, proPO system activity, cytotoxicity, and limited phagocytic activity. The granular cells are functioned in cytotoxicity and proPO system activity (Day *et al*., 2019).

The phagocytosis activity parameter (Table 1) becomes the main parameter for immune system to attack pathogens entering the lobster body. The phagocytic activity on each treatment obtained 26.60% in the 6-m treatment and 36.30% in the 8-th treatment on the 0-th day (H0). The phagocytic activity on the 14-th day (H14) obtained 22% in the 6-m treatment and 24.53% in the 8-m treatment. The measurement

results of phagocytic activity were in a normal range, namely 2-28% of total haemolymph cells (Sang *et al*., 2014). The results of both sampling period in 14 days interval obtained a good lobster condition.

One of humoral immune responses in crustacea is phenoloxydase (PO) formation in haemolymph during the inactive condition. PO has roles in recognizing the foreign matters entering the lobster body and attacking the pathogens. The measurement results showed PO value at 0.410 in the 6-m treatment and 0.289 in the 8-m treatment on the initial sampling period (H0). Meanwhile, the measurement results on the second sampling period in two weeks later (H14) showed PO value at 0.069 in the 6-m treatment and 0.073 in the 8-m treatment on the initial sampling period (H0). The PO value in both sampling periods (Table 1) was in a normal range, namely 0.020 – 1.278 (Jimenez *et al*., 2012). The PO value was connected to the RB value and proportional to the THC value, which showed a normal range value. The initiation and non-self recognition processes are performed by the pattern recognition proteins (PRPs) against the microbial cell components. This complex will induce the serine protease (SP) cascade that will soon convert pro-phenoloxydase (proPO) to active phenoloxydase (PO) through proteolysis process. The active phenoloxydase will oxidize phenol to become quinone, producing mellanin. Mellanin is dark chocolate pigment functioned in trapping and preventing the pathogen contact with the host (Amparyup *et al*., 2013).

The respiratory burst (RB) measurement in the 6-m and 8-m treatments obtained relatively similar results (Table 1). The initial sampling (H0) in the 6-m treatment obtained the RB value at 0.287 and 8-m treatment at 0.365. After 14-th day sampling, the 6-m treatment obtained the RB value ay 0.337 and 8-treament at 0.362. The measurement results of RB value between both parameters were still in a normal range, namely 0.080 – 0.370 (Rosas *et al*., 2004). RB activity is a series of phagocytic microbial particle destructions, involving the release of degradative enzymes into phagosomes and production of reactive oxygen intermediates (ROI).

Hepatopancreas is a highly-sensitive organ against environmental change in crustacea. The histological condition of hepatopancreas in lobster maintained in different depths was in a good condition (Figure 7), due to closed-tubuli arrangements without being thickened. The hepatopancreas size in the lobsters is different, following the lobster growth. The hepatopancreas of lobsters contains tubuli with columnary cells that alters during feeding activity. Tubuli contain lumen and epithelial cells with different functions based on the cell types, namely E-, F-, B-, and R-cells. The E-cell has roles in molting intensity level, F-cell has roles in digestive enzyme synthesis, B-cell has roles in a blister-like vacuola closed to the F-cells, and R-cell has roles in absorbing nutrients from lumen through active transportation, besides preserving and metabolizing the glycogen and lipid contents in hepatopancreas (Ihsan *et al*., 2017).

Different depth treatments on the immune response observed from haemolymph condition obtained similar results between treatments. Immune response becomes an indicantor of stess response in lobsters maintained in different depths. The 6-m and 8-m treatments had no significant differences on immune response parameters based on the haemolymph condition. Depth changes can affect the environmental condition. An environmental change can affect the homeostatic adaptability level that becomes part of non-specific biological phenomenan, known as stress, which will affect the aquatic physiological process of the biota such as lobsters, either physical damage or death.

Water quality is one of the important factors that influences the lobster maintenance in a submersive net cage. The water quality condition in the study location is presented in Table 2. Temperature, salinity, pH, and dissolved oxygen values were commonly in a good condition to support the lobster maintenance. The temperature measurement resulted in a value of 29 ^{o}C at 6-m treatment and 28.7 ^{o}C at 8-m treatment. Based on Jones (2009), water temperature for lobster maintenance is 22-31 ^{o}C. The pH condition in the field obtained a value of 8.1 at 6-m treatment and 8.2 at 8-m treatment. According to Mojjada *et al*. (2012), the optimal pH value for lobsters is 7.8-8.2. The dissolved oxygen level (DO) in the 6-m and 8-m depth treatments was 6.8 and 7.2 mg/L, respectively. The optimal value of dissolved oxygen levels in lobster maintenance is >5 mg/L (Rathina *et al*.2014). The salinity measurement results in the 6-m and 8-m treatments were 30.3 and 32 g/L. The good salinity range for lobsters is 25-35 g/L (Vidya & Joseph 2012).

Stress response is caused by the environmental condition change marked by the existence of physiological change in short- or long-term that causes the energy resource alteration for vital process, as this process can damage or threat the homeostatic condition. Under stress condition, there is a reallocation of metabolic energy from investment activities (such as growth

and reproduction) to homeostatic improvement activities, such as respiration, movement, hydromineral regulation, and tissue repair. Water quality at different depths showed results within the normal range that could be tolerated by lobsters, so the water quality parameters, such as temperature, pH, dissolved oxygen, and salinity had no significant effect on lobster conditions.

CONCLUSIONS

The crustacean health status becomes an indicator for stress response of lobsters maintained in different depth treatments. Different depth treatments had no effect on total haemocytes, differential haemocytes, phagocytic activity, phenoloxydase activity, respiratory burst activity, and hepatopancreatic histological profiles.

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