Effect of Salinity on Osmoregulation and Growth of Batik Lobster (*Panulirus longipes*)

Muhammad Haikal Abdurachman¹, Yushinta Fujaya^{*2} and Dody Dharmawan Trijuno³

¹Program Studi Ilmu Perikanan, Universitas Hasanuddin, Jl. Perintis Kemerdekaan Km 10 Tamalanrea, Makassar 90245.

^{*2}Corresponding author; Fakultas Ilmu Kelautan dan Perikanan, Universitas Hasanuddin, Jl. Perintis Kemerdekaan Km 10 Tamalanrea, Makassar 90245

³Fakultas Ilmu Kelautan dan Perikanan, Universitas Hasanuddin, Jl. Perintis Kemerdekaan Km 10 Tamalanrea, Makassar 90245

Corresponding Email: muh.haikal.a@gmail.com

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Abstract

Muhammad Haikal Abdurachman, Yushinta Fujaya and Dody Dharmawan Trijuno. 2020. Effect of Salinity on Osmoregulation and Growth of Batik Lobster (*Panulirus longipes*). *Aquacultura Indonesiana*, 21 (1): 14 - 23. Salinity is one of the environmental factors that influence the body's physiological processes primarily in the osmoregulation process and impacts on growth. The purpose of this study was to see the impact of salinity on osmoregulation and growth and to determine the optimum salinity for the maintenance of *Panulirus longipes*. This research was conducted in September 2019-January 2020, at Balai Udang Mata Seed City, Kendari City, Southeast Sulawesi, Indonesia. The salinity tested in this study were 28, 31, and 34 ppt. The results showed high salinity had an impact on the level of osmotic pressure which was higher, but the diameter of granulocytes and hyalinocytes was getting smaller. Male lobsters had better adaptability to salinity than females, where the salinity range of 28-34 ppt could still be tolerated and did not affect growth. Conversely, females were strongly affected by salinity and the optimal salinity to support growth was 31 ppt

Keywords: growth, hemocyte diameter, homeostatis, lobster, osmotic pressure, salinity

Background

Seawater lobster (*Panulirus* spp.) is one of Indonesia's export commodities, but in recent years production has declined. Based on statistical data, there was a decrease in exports from 1,243 tons in 2018 to 713 tons in 2019. The decline in catch was due to overfishing ranging from seed size to consumption size, in addition to changes in its habitat and environment that also had an impact on the number of successful lobsters captured (Erlania et al., 2017 and Damora et al., 2018)) This can be seen from the decrease in the size and number of catches in several regions of Indonesia such as Yogyakarta (Larasati et al., 2018); Sukabumi (Rombe et al., 2018); Bali (Kembaren et al., 2015). This condition also happened to the undeveloped outdoor lobster farming in several regions of Indonesia (Erlania et al., 2017).

Lobster culture has now become a world trend in efforts to meet market demand. However, the potential for cultivation in some countries such as Indonesia had not fully developed yet (Erlania et al., 2017 and Shanks & Jones, 2010). Lobster culture systems that are often used are floating net cages, step net cages, and basic confinement (Shanks & Jones, 2010). The system often gets a large influence from changes in water quality in different seasons to ultimately affect the growth and number of lobster production (Jones, 2010 and Haikal et al., 2017). In contrast to the culture in indoor system where monitoring of various factors can be more intensively carried out, this is considered the best effort in lobster culture (Shanks & Jones, 2010).

Indoor scale lobster farming has been carried out by paying attention to various

quality factors such as salinity (Erlania et al., 2017). Salinity can influence the body's metabolism through osmoregulation mechanisms (Jones, 2009 & Vidya & Joseph, 2012). This condition is affected by several changes in osmotic pressure (Henry et al., 2012; Jiménez et al., 2012 and Bazer et al., 2016) and hemocyte size (Romano & Zeng, 2012). Other impacts can be seen from the activity of the Na^+ enzymes, K^+ , and ATPase that cause changes in the body's energy allocation (Shock et al., 2009; Romano et al., 2014 and Romano & Zeng, 2012).

Salinity affects the body's metabolism through osmoregulation mechanisms that have a crucial impact on the body of a lobster (Shock et al., 2009; Jones, 2009 and Vidya & Joseph, 2012). High and low salinity can cause the use of energy for the body to be ineffective, this condition certainly has an impact on lobster growth and cultivation. The

purpose of this study was to see the effect of salinity on osmoregulation and growth of *P. longipes*. The usefulness of this research is as a source of information in determining the optimum salinity as an effort in rearing lobster in indoor culture system.

Material and Method

Time and Place

This research was conducted from, September 2019 to January 2020. The rearing of lobster was carried out at UPTD Balai Benih Udang Mata, Kendari City, Southeast Sulawesi, Indonesia (Figure 1). Cell diameter measurements were carried out at the Basic Biology Laboratory of Halu Oleo University, Kendari, while measurements of osmotic pressure were carried out at the Maros Freshwater Engineering Center

Figure 1. Map of the research location: the red zone is the cultivating location and the blue zone is the lobster capture area.

Test Animals

Test animals that used in this study were male and female batik lobster, weighing 240 ± 50 g, carapace length of 8.2 ± 1 mm, and carapace width of 4.73 ± 0.54 mm (Fig. 2). The lobsters were obtained from the catch of fishermen in the seawaters of North Buton, Southeast Sulawesi. The lobsters were then

kept in a net of 90 x 90 x 120 cm and placed in a tub of 2 x 4 x 150 cm. Each net contained pair of male and female lobsters with a total of 12 test animals/tanks. The feed given was brown sugar (*Modiolus modulaides*), while the feeding referred to research of Haikal et al., (2017) on ad libitum 3 times a day (06.00; 17.00 & 23.00 WITA).

Figure 2. Batik lobster (*P. longipes*) and the dimensions of the measurement

The experimental design

The study used a factorial randomized block design (RBD), consisting of 2 factors: three levels of salinity (28, 31 and 34 ppt) and sex (male and female). Salinity treatment was obtained by diluting seawater with initial salinity of 36 ppt. Dilution of salinity used the following equation:

$$S2 = \frac{a \times S1}{n + a}$$

Note: S2 = Target salinity (ppt); S1 = Diluted Salinity (ppt)

a = Volume of diluted seawater (L); n = Freshwater volume (L)

Test Parameters

Variables observed in this study consisted of osmotic pressure rate, cell diameter (granulocytes and hyalinocytes) while growth parameters were absolute growth and specific growth rates.

Osmotic Pressure

Osmotic pressure rate was measured using Fiske Model 210 Micro-Osmometer, where the collection of hemolymph entered in appendorf tube of 1.5 ml and stored in cooling cabinets. The analysis begins with hemolymph centrifuge with a speed of 5000 rpm for 3 minutes which then forms a supernatant layer. The supernatant layer of hemolymph is then taken as much as 20 µl and put in a disposable osmometer for analysis. Whereas to find out the value of osmotic pressure rate an equation of Lignot et al., (1999) was used :

Description: OP = Osmotic Pressure (mOsm.L⁻¹); OM = Media Osmolarity (mOsm.L⁻¹)

OH = Hemolymph Osmolarity (mOsm.L⁻¹)

Hemocyte Cell diameter

Measurement of hemocyte cell diameter was performed on granulocytes and hyalinocytes by taking hemolymph using a 0.5 ml syringe between prosoma and lobster opisthosoma. The syringe contained a Na-citrate solution. The ratio between Na-citrate and hemolymph is 1: 1. The hemocyte fluid was then dripped sufficiently on top of the glass preparations and flattened using other glass preparations. Glass preparations containing hemocytes were then soaked in methanol for 10 minutes, followed by gyms staining for 15 minutes and rinsed using distilled water. Differences in the shape of hemocyte cells referred to the explanation of Factor, (1995); Matozzo & Mrin, (2010) and Hong et al., (2013) that granulocytes have irregular shapes that have granular resembling bubbles at the edges of cells, whereas hylinocytes have perfectly rounded shapes. Cell size was analyzed using the Image-J application according to the instructions of Rifano, (2014) and Adeogun et al., (2015). Cell diameter was measured using the equation:

$$OP = OM - OH$$

$$D = \frac{C}{\pi}$$

Description: D = Diameter (μm); C = cell area (μm)

$$\Pi = 3.14$$

Growth

There are two growth categories measured, absolute growth and specific growth rate. Absolute growth is the difference between the final weight and the initial weight of lobsters. The absolute growth measured using the formula.

$$G = G_t - G_0$$

Description: G = Absolute Growth (g); G_t = Final weight (g)

G₀ = Initial weight (g)

Specific Growth Rate

Specific rate growth refers to the equation used by Cook et al., (2000):

$$SGR = \frac{\text{Lon Wt} - \text{Lon W0}}{t} \times 100$$

Description: SGR = Specific growth rate (% per day); W0 = initial weight (g)

W_t = final weight (g); t = Cultivating time (day)

Data Analysis

The effect of various salinity treatment levels on osmotic pressure, diameter of granulocytes, diameter of hyalinocytes and growth were analyzed using Multivariate Analysis of Variance (MANOVA) and followed by Duncan test using SPSS software version 23.0 to determine the best salinity.

Results

Osmotic Pressure

Osmotic pressure level of *P. longipes* those maintained at 28, 31, and 34 ppt show different values. The lowest value was at 28 ppt, both in the male range (813-869 mOsm.L-1), and females (795-874 mOsm.L-1). While the highest value was at 34 ppt, with the highest range dominated by female lobsters (1019-1348 mOsm.L-1), followed by males in the range (1031-1206 mOsm.L-1). Whereas at 31 ppt it shows in the range ± 900 mOsm.L-1 (Fig. 1 and 2).

Figure 1. Level of osmotic pressure of *P. longipes* male in different salinity (28, 31 & 34 ppt) during experiment days (0, 30, 60 & 90) (P < 0.05)

Figure 2. Level of osmotic pressure of *P. longipes* male on different salinity (28, 31 & 34 ppt) during experiment days (0, 30, 60 & 90) ($P < 0.05$)

Hemocyte Cell Diameter

The hemocyte cell diameter observed consisted of granulocytes and hyalinocytes. The size of the diameter of the granulocytes at 28 ppt day 0 for males was $7.68 \pm 1.53 \mu\text{m}$ and females $15.11 \pm 0.98 \mu\text{m}$. The size of the granulocytes increased proportional to the time of maintenance, on the 90th day: the size of the male granulocytes became $15.68 \pm 0.23 \mu\text{m}$, and the female $32.13 \pm 5.67 \mu\text{m}$. Whereas

at 31 and 34 ppt it consists of cells of smaller size in both males and females. The smallest diameter of granulocytes were found at 34 ppt. On day 0 granulocytes of male was $3.07 \pm 0.39 \mu\text{m}$ and female $4.07 \pm 1.89 \mu\text{m}$, then increased on the 30th day with male of $4.31 \pm 0.28 \mu\text{m}$ and female of $6.14 \pm 0.12 \mu\text{m}$, but decreased on 90th day; $3.65 \pm 0.43 \mu\text{m}$ for male and $5.67 \pm 0.11 \mu\text{m}$ for female (Fig. 3).

Figure 3. The diameter of granulocytes cells *P. longipes* male (M) and female (F) at different salinities (28, 31 & 34 ppt) in different days (0, 30, 60 & 90) ($P < 0.05$)

The diameter size of hyalinocytes also increased with the length of rearing time like granulocytes. The diameter of hyalinocytes at 28 ppt is the largest, on day 0 the size of male

hyalinocytes is $4.35 \pm 0.25 \mu\text{m}$ and female $4.30 \pm 0.23 \mu\text{m}$, then on the 60th day the size of hyalinocytes was male $5.32 \pm 2.04 \mu\text{m}$ and females $6.19 \pm 0.37 \mu\text{m}$. The size decreased on

the 90th day to $4.42 \pm 2.38 \mu\text{m}$ male and $5.96 \pm 0.58 \mu\text{m}$ female. Whereas at 31 and 34 ppt

decreasing size until the end of the maintenance that is shown in Figure 4.

Hyalinocytes

Figure 4. The diameter of the cells hyalinocytes of *P. longipes* male (M) and female (F) at different salinities (28, 31, & 34 ppt) during experiment days (0, 30, 60 & 90) ($P < 0.05$)

Growth

The highest growth rate of male *P. longipes* at 34 ppt ($36.17 \pm 58.68 \text{ g}$) and lowtst values on 31 ppt ($25.17 \pm 20.87 \text{ g}$). Like on growth, the spesific rate growth have similar in

the highest values at 34 ppt ($0.15 \pm 0.27 \%$ per day) and the lowtst values at 31 ($0.11 \pm 0.09 \%$ per day) (Fig. 5).

Figure 5. Growth (g) and spesific rate growth (% per day) of male *P. longipes* at different salinity (28, 31 & 34 ppt) ($P > 0.05$)

Different with Male, the highest growth rate of female *P. longipes* at 31 ppt ($50.08 \pm 22.04 \text{ g}$) and lowtst values at 34 ppt ($0.70 \pm 0.009 \text{ g}$). Like on growth, the spesific

rate growth have similar in the highest values at 31 ppt ($0.21 \pm 0.09 \%$ per day) and the lowtst values at 34 ($0.009 \pm 0.15 \%$ per day) (Fig. 6).

Figure 6. . Specific growth rates of *P. longipes* of male (M) and female (F) reared in different salinity (28, 31 & 34‰) during rearing periods (0, 30, 60 & 90 days) ($P > 0.05$)

Discussion

Lobsters are crustaceans with osmoconformer capabilities. This is evident from the research. Salinity is very influential on the osmoregulation mechanism of batik lobster (*P. longipes*). This is characterized by changes in osmotic hemolymph pressure and hemocyte diameter in response to changes in salinity. As stated by Fujaya & Sudaryono (2016), salinity is an environmental factor that plays a major role in osmoregulation where the osmoregulation process generally involves the activity of Na^+ , K^+ , and ATPase in the gills to regulate the body's ions to remain stable (homeostatic).

Salinity is the amount of salt that dissolves in a body of water. This is usually measured in g salt/kg of sea water or part per thousand (ppt). Increasing the salinity level will directly increase the level of osmotic pressure proportionally (Henry et al., 2012). Likewise, batik lobster (*P. longipes*) in this study (Fig. 1 and 2). Batik lobster has an osmotic pressure of around 869-873 mOsm.L-1, 956-981 mOsm.L-1, and 1205-1348 mOsm.L-1, respectively at 28 ppt, 31 ppt and 34 ppt. Similar to *P. longipes*, osmotic pressure from various types of lobster has also been reported by some previous researchers, such as *P. elephas* having 679 mOsm.L-1 at 27 ppt (Lucu et al., 2000), *Homarus americanus* has 900 mOsm.L-1 at 31 ppt (Charmantier et

al., 1984), and *P. argus* has 1194-1212 mOsm.L-1 at 35 ppt, (Jiménez et al., 2012).

Male and female lobsters had different responses to the salinity. Female lobsters generally have a range of osmotic levels greater (Fig. 2) than males at the same salinity level (Fig. 1). According to Charmantier et al., (2001); Jiménez et al., (2012); Romano & Zeng, (2012); Romano et al., (2014) and Bazer et al., (2016), the function and work of Na^+ , K^+ and ATPase on the gills more stable in male lobster, it affects the body's ability to carry out the process of maintain osmotic pressure with its environment. Evans (2008), also found that female lobster tends to require a longer adaptation time to changes in salinity, this condition is related to the complexity of the energy allocation mechanism used to survive, grow and ovarian maturation. This information is important in determining the location and management of water salinity in the cultivation.

Hemocytes are blood cells in lobster or other crustaceans and have the same function as blood cells in vertebrates as carriers of oxygen and nutrients needed for cell metabolism. Hemocyte size is strongly influenced by the body's osmotic pressure in response to salinity. Therefore, the size of the hemocytes is different between males and females at the same level of salinity as different body osmotic pressure responses

(Fig.3 dan Fig. 4). Another study informs that the size of hemocytes varies like as: *Carcinus aestuarii*, about $11.94 \pm 1.43 \mu\text{m}$ in granulocytes and hyalinocytes $7.88 \pm 1.6 \mu\text{m}$ (Matozzo & Mrin, 2010); *E. sinensis* have granulocytes sizes about $14.05 \pm 2.60 \mu\text{m}$ and hyalinocytes $6.02 \pm 0.88 \mu\text{m}$ (Hong et al., 2013); *Callinectes amnicola* have granulocytes cell about $19.37 \pm 2.76 \mu\text{m}$ and hyalinocytes $8.95 \pm 1.62 \mu\text{m}$ (Adeogun et al., 2015). Factor (1995) suggests that the size of the homarus hemocyte have a range of $15 \mu\text{m}$ for granulocytes, $11 \mu\text{m}$ for hyalinocytes and generally granulocytes is larger than hyalinocytes.

Changes in hemocyte size are an indicator of heavy performance in the osmoregulation process. If osmoregulation goes well, it is marked by no significant changes in the size of the hemocytes. Lobster hemocytes can respond to changes in water salinity, these conditions are closely related to changes in the level of osmotic pressure for to maintain body homeostasis (Jiménez et al., 2012; Fujaya & Sudaryono, 2016). According to Evans (2008) and Hong et al., (2013) in a hyperosmotic state, cells will experience degranulation and shrinkage due to the release of water through a diffusion process. Conversely in a hypoosmotic state, the cell will undergo lysis and detachment which results in excess water entering the cell and followed by the release of protein and amino acid components from the inside. Therefore, osmotic pressure can influence to change in the size of granulocytes and hyalinocytes, like in *Eriocheir sinensis*. Hong et al., (2013) argues that the high level of osmotic pressure in *E. sinensis* causes osmosis (diffusion) which results in the movement of water out of the cell so that the size of the cytoplasm is become smaller. Factor (1995), in contrast, the impact of low osmotic pressure on lobster causes absorption of water in cells is more dominant and can be inflict lysis on cell. Evans (2008) also explain that, low salinity can cause an increase in cell size, even lysis or rupture of hemocytes due to the large volume of water entering the cell. Conversely, in high salinity can cause shrinkage of cell size due to high Na^+ ions and K^+ in hemocytes, resulting in shrinkage of the epidermis due to moisture

content that disappears in the osmosis process. Changes of water transmission entering to the cell have influence on the activity of enzymes in the process of anabolism and catabolism in particular: asparagin, glutamine, proline, alanine, glycine, and serine. Osmolarity activity on the high salinity have effect to synthesis of free amino acids is high. While low salinity can reduce the cell osmolarity that triggers deliverance amino acids excreted to media through the posterior gills.

The results of this study indicate that high levels of osmotic pressure will have an impact on low growth in lobsters (Fig. 5 and 6). This condition is seen in male lobsters with relatively stable osmotic pressure so that the growth obtained is relatively higher and stable at all levels of salinity. this is inversely proportional to female lobster. As we know that the osmoregulation process requires metabolic energy to support ion transport in an effort to regulate the body's osmotics. Na^+ regulation and chloride cell activation are known to require amino acids to maintain permeable stability in the osmoregulation process (Bazer et al., 2016 and Romano et al., 2014). Therefore, if the osmoregulation activity takes place high, it will affect the energy expenditure for activation of Na , K , and ATPase so that it affects the growth (Lucu et al., 2000; Jones, 2009; Henry et al., 2012 and Vidya & Joseph, 2012).

Conclusion

Salinity levels greatly affect the osmoregulation mechanism in batik lobsters. Osmoregulation performance is clearly seen in this study, through changes in osmotic pressure and hemocyte size directly. Indirectly, high osmotic pressure will have an impact on low growth in lobsters. One interesting thing from the results of this study is that there are differences in the adaptability of batik lobster to salinity based on gender. Adaptability to salinity is higher in male than in females. Optimum salinity for females is in the range at 31 ppt.

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