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THE EFFECT OF SALINITY ON SURVIVAL, GROWTH AND IMMUNITY RATE OF SEA CUCUMBER (*Holothuria scabra*) JUVENILES

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Running title: Effect of salinity on sea cucumber juveniles

ABSTRACT

Sea cucumber is a stenohaline and osmoconforming organism with a low level of tolerance to salinity change. Salinity of the medium is one of the physiological factors that affect the growth and survival of sea cucumber juveniles. This study was aimed at finding out the effect of different salinities on growth, survival and immunity rate of sea cucumber juveniles (*Holothuria scabra*) and finding the most suitable salinity level for optimal growth, survival and immunity rate of sea cucumber. This study used a laboratory experimental method and completely randomized design with 5 salinity treatments: 24, 29, 34, 39, and 44 ppt with 3 replications. This study used fifteen 30 L-fiber glass tanks. The sea cucumber juvenile were 4.4 ± 0.2 cm in total length and 5.6 ± 0.3 g in body weight. The juvenile densities were 15 individuals/tank, fed with fresh benthos from a culture once a day in the afternoon. Coelomate was taken from the sea cucumber juveniles from each tank and used to find out the immunity rate dan also the for osmolality. The data collected were analyzed using ANOVA that was followed by Tukey's test at 5% level of significance. The results showed that the salinity of the medium has a significant effect ($P < 0.05$) on the growth, survival rate and immunity rate of sea cucumber juveniles. The 24 - 34 ppt salinity can support survival rate up to 100%, high growth (6.47 – 7.10 g) and immunity rate (27 – 76 x 10^4 phagocytic cell/mL), while at the 44 ppt salinity has an effect not only on a low survival rate (55.6%), but also on a bad effect on osmolality (303 ± 3.5 mOs-mol kg^{-1}), growth (3.12 ± 0.34 g), and immunity (209 x 10^4 phagocytic cell/mL).

Keywords: Growth, Immunity, Salinity, Sea Cucumber, Survival rate

INTRODUCTION

Sea cucumber is one of the marine invertebrate which belongs to Echinoderm Phylum. Echinoderm is an organism with a low level of tolerance of changes in salinity called a "stenohaline" and "osmoconformer" organism (Geng *et al.* 2016).

Controlling biotic and abiotic parameters in nurturing juvenile sea cucumbers is very important. Among the abiotic factors, temperature and salinity play an important role in the growth and survival of the sea cucumber juveniles (Wang *et al.*, 2014). Then, according to Bai *et al.*, (2015), changes in salinity that occur at a certain level may not affect the growth and the survival of aquatic organisms.

However, some research results on Holothuroidea showed that changes in salinity had a negative effect on the sea cucumber juveniles. *Holuthuria scabra* farmed in a fish pen cage in the sea will suffer from ulcers in their bodies and will finally die when the sea water salinity rate decreases to 20 ppt (Lavitra *et al*., 2009). When *Apostichopus japonicus* cultured in low salinity (20 ppt) or high salinity (40 ppt), its mortality rate reaches up to 20% (Dong *et al*., 2008: Meng *et al.,* 2011). *Holothuria spinferra* also had a mortality rate up to 25% in a low salinity (15 ppt) or a high salinity (40 ppt) (Russell, 2013). Zhang *et al.,* (2012) also reported that *A. japonicas* can adapt gradually to a salinity range of 20 - 39 ppt.

Water quality is one of the factors that have a very important effect on the growth and health of sea cucumber juveniles. This is related to the factor of stress of the sea cucumber juveniles as the effect of changes on water quality parameters, including salinity (Li & Li, 2010; Anderson *et al*., 2011). Bad environmental condition reduces antibody production in such a way that it reduces the immunity of the sea cucumber juveniles and makes them susceptible to infection from diseases (Wang *et al.*, 2008).

Sea cucumbers do not have an adaptive immunity response and only rely on innate immunity produced by the sea cucumber's body (Dong *et al*., 2013). As cellular and humoral immunity response, coelomic liquid in their bodies can be used (Xia *et al.,* 2013).

Changes in salinity will cause changes in osmotic pressure of an organism through osmoregulating process, in which the lower the salinity the lower the osmotic pressure will be. Every aquatic biota has an optimal range of salinity to survive. Environmental conditions beyond the tolerable range may cause stress, disturbance on growth and reproduction, even death (Seeruttum *et al.*, 2008). Hence, this research was conducted with the aim of finding out the effect of salinity on growth, survival and immunity rate of sea cucumber, *Holothuria scabra* and of finding the range of salinity which is suitable for the species.

MATERIALS AND METHODS

Animal Tests and Treatments

This study was done at Institute for Mariculture Research and Fisheries Extension (IMRAFE) Gondol, Bali from 21 August to 26 September 2017. The experiment design used was completely randomized design consisting of 5 treatments and 3 replications. The treatments in these experiments were different salinities: A (24ppt); B (29ppt); C (34ppt); D (39ppt) and E (44ppt). The treatments was decided based on Lavitra *et al*., (2009) *Holuthuria scabra* suffer from ulcers when the sea water salinity rate decreases to 20 ppt while at high salinity could affect on higher mortality rate (Russel, 2013). But in our experiment in high salinity earthen pond (> 46 ppt), *H. scabra* still survive (Giri *et al*., 2017). Fifteen 30 L fiber glass tanks, each filled with 25 L of sea water were use as experiment tanks. The tanks were put in a wet laboratory (indoor) and were equipped with aeration as oxygen supplier. To maintain the salinity, everyday seawater which matched the salinity of the treatment was prepared in 200 L fiber tank equipped with aeration to homogenize the salinity. To obtain the 39 and 44 ppt salinity, salt was added while to obtain the 24 and 29 ppt salinity, fresh water was added. Siphoning the bottom of the tank was done every day and then adjusted the water volume into the initial level by adding appropriate water salinity. Before adding the water, water salinity was checked in each treatment and water supply tanks.

Sea cucumber juveniles used in this research were produced at IMRAFE, Gondol. They were 4.4±0.2 cm in total length and 5.6±0.3g in body weight. The sea cucumber juveniles were nursed at the density of 15 individuals/tank. During the experiment, the sea cucumbers were fed with fresh benthos, from a culture tank, harvest by filtering using plankton net, squeezed to get rid water contain and then feeding to juvenile. The benthos was consisted of phytoplankton of Diatoms class and *Melosiraseace*, *Naviculaceae*, *Nitzschiaceae* families and zooplankton of *Acartiidae* family (Sembiring *et al*., 2015). The dosage of the feed was 4% of biomass weight/day given once a day in the afternoon (Sembiring *et al*., 2017).

Parameters Observed

The parameters observed were growth, survival, osmolality and immunity of the sea cucumber juveniles while the water quality parameters observed were temperature and dissolved oxygen.

Growth and Survival Rate of Sea Cucumber Juveniles

The measurement of the sea cucumber juveniles was done every 14 days or four times measurement during the experiment. The average weight of the sea cucumbers from every experiment unit was used to obtain data on Specific Growth Rate (SGR). SGR indicates the increasing body weight during the experiment and was calculated using the following formula:

$$SGR = 100\ (Ln\ Wt - Ln\ Wo)\ /t \qquad (1)$$

Where:

SGR : Specific growth rate (%/day)

Wo : Initial weight (g)

Wt : Final Weight (g)

t : Duration of rearing (day)

Sea cucumber juvenile survival rate was based on the number at the end of the research and was calculated using the following formula (Effendie, 1997):

$$SR = (Nt/No) \times 100\ \% \qquad (2)$$

Where:

SR : Survival (%)

Nt & No : the number of sea cucumbers at the end and at the beginning of experiment

Osmolality

To find out the relationship between the use of feed energy for growth or for maintaining osmosis balance of sea cucumber juvenile in different salinity treatments, osmolality was measured at the beginning and the end of the research. The osmolality was measured by using a substance in the form of coelomic liquid with Fiske-Osmometer.

The measurement method for osmolality according to Herlinah & Septiningsih (2014). Coelomate was taken from the sea cucumber juveniles from each tank. Coelomate was taken from the ventral part at the right side of respiratory organ (Coelom) using a 26 g x 1/2”nedle and a 1 mL syringe containing cold anticoagulant compound (2% NaCl, 0.1 M glucose, 30 mM Na citrate, 26 mM citrate acid, 10 mM EDTA). Before the measurement was performed, the coelomate was stored in the freezer (-80 ^oC).

The osmolality measurement stage was as follows: anticoagulant was added into the ceolomic sample with the ratio 4:1. One mL of the mixture was taken using 1 mL syringe, and then the sample was put into a 1.5 mL tube and centrifuged at 5000 rpm for 3 minutes. One mL supernatant was taken with pipette and transferred into a new tube. To analyze the osmolality, 20 µL supernatant was put into disposable tubes of Osmometer and the measurement was performed. Before doing the analysis for the next sample, the tube was cleaned using probe cleaner and it was left to stay until it dry.

Coelomate and Phagocyte

Coelomate was also used to find out the immunity rate of sea cucumber juveniles (Smith *et al*., 2010). Coelomate functions as immune affector cell in Echinoderm, when the sea cucumber suffers from stress, the total number of coelomates and phagocytes will increase. The calculation of the total coelomates and phagocytes for every individual was done by using Hemocytometer under a light microscope with a twenty-fold magnification.

Water Quality

During the research, the observation of water quality was carried out every month. The analysis of water quality was done in the Nutrition and Chemistry laboratory at IMRAFE. The temperature was measured using thermocouple and the dissolved oxygen was measured using electrode membrane method/ DO meter.

Data Analysis

The data of the research obtained were growth, survival, osmolality, and the number of coelomates and phagocytes and were analyzed using a variety test (ANOVA) that was followed up with Tukey's test to know the differences between the treatments.

RESULTS AND DISCUSSION

Growth Rate

The results showed that the highest absolute weight growth and specific growth rate (SGR) of sea cucumber juveniles was reached in treatment B (29± 2 ppt), at 7.10 g and 0.16% a day consecutively. The lowest rate was obtained in treatment E (44± 2 ppt), at 0.34 g and -0.393% a day consecutively (Table 1). Statistical analysis showed that the salinity of the rearing medium had a significant effect (P<0.05) on weight growth and SGR of sea cucumber juveniles.

Table 1 Absolute growth, survival, and Specific growth rate of *H. scabra* juveniles reared in different salinities.

Treatments (ppt)	Absolute growth (g)	Survival rate (%)	Specific growth rate (%)
24 ± 2	6.47 ± 0.30^{c}	100.0^{b}	0.097^{c}
29 ± 2	7.10 ± 0.30^{c}	100.0^{b}	0.160^{c}
34 ± 2	6.80 ± 0.20^{c}	100.0^{b}	0.137^{c}
39 ± 2	4.67 ± 0.27^{b}	97.8^{b}	-0.120^{b}
44 ± 2	3.12 ± 0.34^{a}	55.6^{a}	-0.393^{a}

Mean values noted by different letters are significantly different from one another

The result of Tukey's test showed that the growth rate and SGR of sea cucumber juveniles reared at salinities 24 ± 2 ppt; 29 ± 2 ppt and 34 ± 2 ppt were higher and significantly different from those reared at salinities 39 ± 2 ppt and 44 ± 2 ppt. At low salinity treatment (24 ppt), sea cucumber juveniles showed slow growth rate because more energy is needed to maintain its iso-osmotic. On the other hand, at the high salinity (39 & 44 ppt), sea cucumber juveniles showed negative specific growth rate and reduced the body weight at the end of the experiment. According to Rhodes-Ondi & Turner (2009), changes in salinity will cause changes in osmotic pressure in coelomic liquid and will also cause changes in protein synthesis in sea cucumbers (Niu *et al*., 2008). Then Abdel-Raheem (2015), also states that the optimal growth in sea organism will be reached in iso-osmotic salinity condition, where the organism does not need a lot of energy for osmoregulator process so that more energy can be used for growth.

Survival

The result showed that salinity has a significant effect on sea cucumber survival (P< 0.05). The survival rate of the sea cucumber juveniles reared in the salinities of 24 ± 2 ppt; 29 ± 2 ppt and

34 ± 2 ppt were 100.0%, while at salinity 39 ± 2 ppt was 97.8% and the lowest was at salinity 44 ± 2 ppt with only 55.6% of survival rate (Table 1). Further analysis with Tukey's test showed that the survival rate of the sea cucumber juveniles reared at salinities 24 ± 2 ppt; 29 ± 2 ppt; 34 ± 2 ppt; and 39 ±2 ppt did not significantly different ($P>0.05$), but was higher and significantly different from reared at salinity 44 ± 2 ppt ($P<0.05$).

A high salinity was reported to cause the decrease in viability and high mortality rate in some organisms belonging to Echinoderm phylum (Santos *et al*., 2013). At 44 ppt salinity, sea cucumber juveniles suffer from stress with ulcers on the skin followed by liquid secretion on the body, changes in skin color which finally will cause death. In general, the juvenile symptom at the high salinity confirms the results of observation conducted by Asha *et al*., (2011), juveniles that suffer from ulcers on the surface of the skin cause a decrease in metabolism process and will affect the growth and finally will cause death.

Osmolality

The highest average level of osmolality in sea cucumber juveniles was occured at salinity 39 ± 2 ppt, at 303 ± 3.5 mOs-mol kg^{-1}, and the lowest was at salinity 24 ± 2 ppt at 51.3 ± 1.5 mOs-mol kg^{-1} (Figure 1). Analysis of variance showed that salinity has significant effect ($P<0.05$) on osmolality in sea cucumber juveniles.

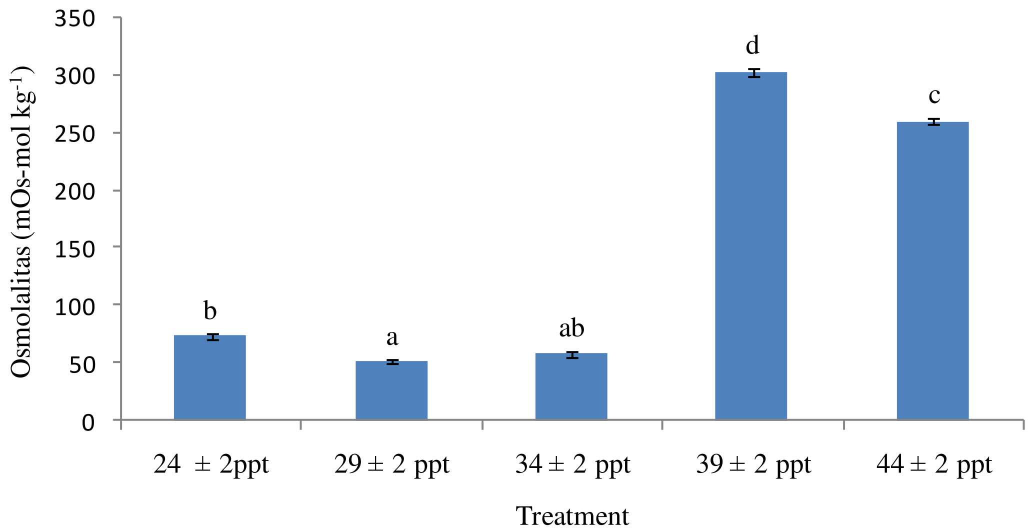

Figure 1 Osmolality of *H. scabra* juveniles reared in different salinities (Mean values noted by different letters are significantly different from one another)

In figure 1 it is seen a significant relation in osmotic pressure observed between coelomic liquid of sea cucumber juveniles reared on different salinities. In the high salinity, the coelomic liquid of the sea cucumbers was hyperosmotic to its external media. In 44 ppt medium, osmolality measurement of rearing medium and coloemic liquid were 1,175 and 914 mOsm/kg consecutively, so that the osmolality was 260.66 mOsm/kg. In this condition, coloemic liquid diffused out to medium because the membrane of Echinoderm is relatively thin and highly permeable (Meng *et al*., 2011). Water exchange was occured between the coeloemic liquid in the sea cucumber body and the external media (Barker & Russel, 2008). Then, Freire *et al*., (2011), stated that the ions from the body liquid tends to diffuse outside the body and this process will stop when osmotic-balanced (iso-osmotic) condition has been reached. This is in line with Rahmawati *et al.,* (2012); who stated that aquatic organism will try to maintain the osmolality of its body liquid through hyper osmotic regulation mechanism, that is, by increasing ion (salt) absorption from external media through skin and producing hypo-osmotic urine through an excretive organ in the form of *dermal branchia*, *Caecum intestin* and tuber legs in sea cucumbers (Bai *et al*., 2015). When coloemic liquid of sea cucumber juveniles in hypo or hyper osmotic, it will hard to reach osmotic balance or even caused stress and reduce its immunity. For sea cucumber juveniles at treatment 24 ppt faced hypo osmotic but still able to reach iso-osmotic by using most of its energy and only small portion of energy is available for growth. On the other hand, juveniles at 39 and 44 ppt faced hyper osmotic and very hard to reach iso-osmotic even by using all feed energy and its deposit energy and also faced low immunity and caused negative growth and diseases infection. Sea cucumber at treatments 29 and 34 ppt, seems in iso-osmotic condition, less energy needed for maintaining iso-osmotic so that its energy obtain from feed mostly used for growth.

Coelomate

The total number of coelomates and phagocytes showed a significant increase ($P<0.05$) at low salinities (Figures 2 and 3). An increase in the number of coelomates and phagocytes are related to the effort at maintaining homeostasis caused by stress and to increase immunity response in the sea cucumber juveniles.

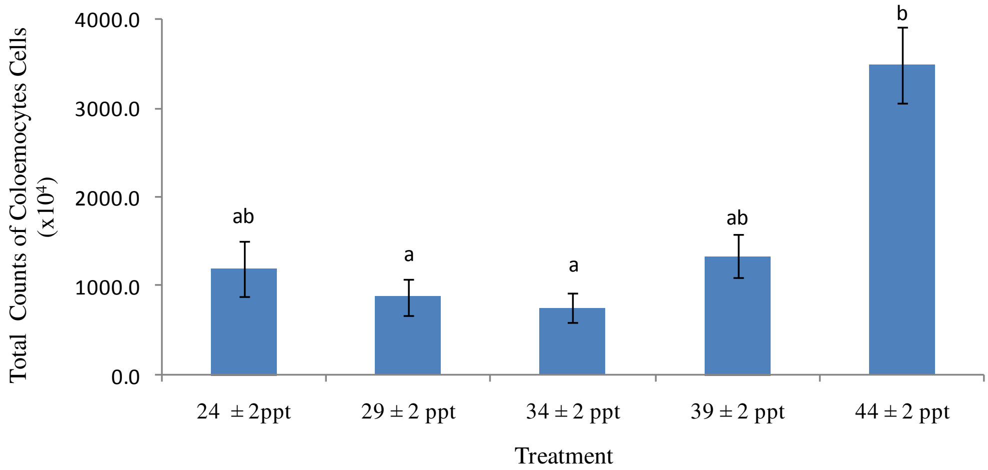

Figure 2 Total coelomate of *H. scabra* juveniles reared in different salinities (Mean values noted by different letters are significantly different from one another)

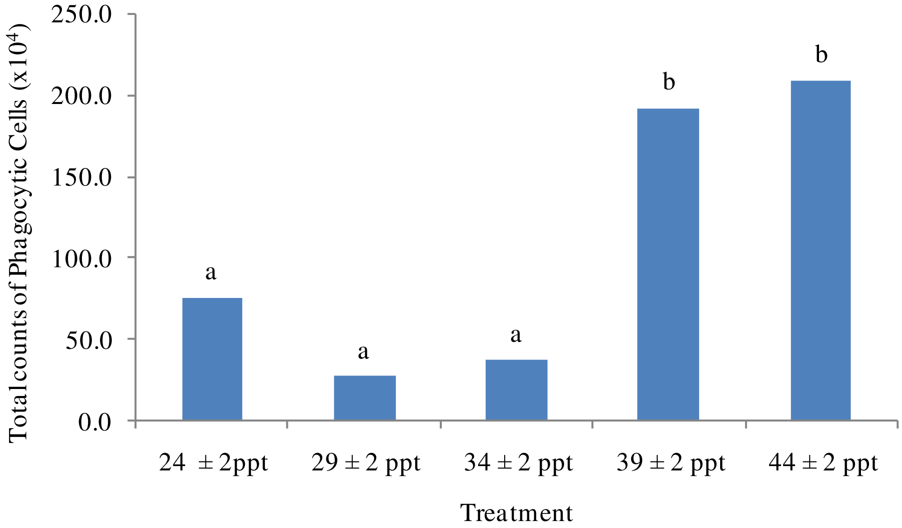

Figure 3 Total phagocytocic of *H. scabra* juveniles reared in different salinities (Mean values noted by different letters are significantly different from one another)

Changes in the environmental factors will have a bad effect on the body immunity so that resistance to the infection and survival of the sea cucumber juveniles decrease (Gowda *et al.*, 2008). In a low salinity (24 ppt) and a high salinities (39 and 44 ppt), the total number of coelomates reaches 1,200-3,400 x 10^4 cell/mL, these results clearly showed that sea cucumber juveniles in stress condition. While sea cucumber juveniles at salinity 29 and 34 ppt with low coelomic at 700-800 x 10^4 cell/mL, is in normal condition.

The result found for treatment on salinity 24, 29 and 34 ppt, the phagocytes value was 27-76 x 10^4 cell/mL, lower than salinity 39 and 44 ppt with value of 190 -209 x 10^4 cell/mL. These results proved that in stress condition, sea cucumber juveniles showed high coelomic and phagocytic values as the response to the changes in salinity. According to Meng *et al.*, (2011) in addition to its function as the main affector cell in the immunity system of Echinoderm (including Holothuridae), coelomate also functions as storage and transportation of nutrition and oxygen, pigment biosynthesis and excretion, so that when juveniles suffer from stress this will affect the total number of coelomates and phagocytes. Furthermore, Wang *et al*., (2008), also stated that increase in phagocytes is influenced by environmental factors, especially changes in salinity.

The result of research showed that the range of salinities which can support growth, survival and the immunity rate of sea cucumber juveniles were 24-34 ppt. Although Purcell *et al*., (2009), stated that some species from Holothuroidea are susceptible to low salinity, but *H. scabra* juveniles reared at 24 ppt salinity (treatment A) still have a high growth and survival rate and not significantly different with treatments (B) 29 and (C) 34 ppt. According to Lavitra *et al*., (2009), an ideal salinity for sea cucumber is ranged between 28 and 31 ppt. Then, Lavitra *et al*., (2010), stated that in nature, when the salinity is low *H. scabra* will hide into the substrate to balance ion concentration in its coelomic liquid.

Water Quality

Water quality gives an important effect on growth and survival of organisms living in water (Lavitra *et al*., 2010). The result on water quality measurements can be seen in Table 2.

Table 2 Average values of water qualities during the experiment.

Treatments	Salinity (ppt)	Temperature (ºC)		Dissolved oxygen (ppm)
		Morning	Afternoon	
A	24 ± 2	26.9 ± 0.49	30.1 ± 0.68	6.68 ± 0.54
B	29 ± 2	26.8 ± 0.96	30.2 ± 0.83	6.77 ± 0.49
C	34 ± 2	27.0 ± 0.53	30.2 ± 0.90	6.73 ± 0.53
D	39 ± 2	26.7 ± 0.88	30.2 ± 0.85	6.62 ± 0.54
E	44 ± 2	26.9 ± 0.79	30.2 ± 0.85	6.78 ± 0.49

The range of water temperature and dissolved oxygen during the experiment was still in the tolerance limit of sea cucumber juveniles. The range of temperatures in the morning and afternoon were 26.7ºC - 30.2ºC with dissolved oxygen at 6.62 - 6.78 ppm. Some research reported that sea cucumbers can survive at 25ºC - 35ºC. If the temperature is higher than 35ºC, the sea cucumber body becomes inactive, but its tentacles can still move (Hu *et al.*, 2010). According to Yusron & Pramudji (1987) *in* Louhenapessy (2013), the optimum dissolved oxygen content in the sea cucumber rearing media is between 6 - 8 ppm and the dissolved oxygen in this experiment was still in good.

CONCLUSION

Different salinities in sea cucumber juveniles, *H. scabra* culture media have significant effects on growth and survival rate, osmolality and immunity. The range of salinities which can support growth, survival and immunity rate in sea cucumber juveniles is between 29-34 ppt. The sea cucumber juveniles at treatment 24 ppt able to reach iso-osmotic by using most of its energy for survival. At 39 and 44 ppt is not recommended because very hard to reach iso-osmotic and not able to support survival.

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