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# EFFECT OF SALINITY ON THE SURVIVAL, GROWTH AND IMMUNITY RATE OF JUVENILE SEA CUCUMBERS (*Holothuria scabra*)

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**ABSTRACT**

Sea cucumber (*Holothuria scabra*) is important species as food and medicine, especially for Chinese market. It is a stenohaline and osmoconforming organism with a low level of tolerance to salinity change. Salinity of the medium is one of the environmental factors that affect the physiology and survival of juvenile sea cucumbers. This study was aimed at assessing the effect of different salinities on the growth, survival and immunity rate of juvenile sea cucumbers (*Holothuria scabra*) and also determining the suitable salinity level for the optimal growth, survival and immunity rate of the sea cucumbers. This experiment used a completely randomized design with 5 salinity treatments: 24, 29, 34, 39, and 44 ppt with 3 replications using fifteen 30 L-fiber glass tanks. The juvenile sea cucumbers measured 4.4 ± 0.2 cm in total length and 5.6 ± 0.3 g in body weight. The juveniles were raised at 15 individuals/tank, fed with cultured fresh benthos once a day in the afternoon. Coelomate was taken from the sea cucumber juveniles from each tank and used to determinie the immunity rate and also for the osmolality. The data were analyzed using ANOVA followed by Tukey's test at 5% level of significance. The salinity of the medium significantly affected ($P < 0.05$) 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 the 44 ppt salinity has resulted in not only a low survival rate (55.6%), but also had 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

The marine invertebrate *Holothuria scabra*, belonging to Phylum Echinoderm, is a sea cucumber with a low level of tolerance for changes in salinity and is called a "stenohaline" and "osmoconformer" organism (Geng *et al.* 2016). Among the abiotic factors, temperature and salinity play an important role in the growth and survival of the juvenile sea cucumbers (Wang *et al.* 2014; Li & Li 2010; Anderson *et al.* 2011) very low or very high salinities can stress the juveniles.

Some research showed that changes in salinity occuring at a certain level may not affect the growth and survival of aquatic organisms (Bai *et al.* 2015). However, other studies on Holothuroidea showed that changes in salinity had a negative effect on the sea cucumber juveniles. *Holuthuria scabra* farmed in an open-sea fish pen cage suffered from ulcers in their bodies and finally died when the sea water salinity rate decreased to 20 ppt (Lavitra *et al.* 2009). When *Apostichopus japonicus* was 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% under a low salinity (15 ppt) or a high salinity (40 ppt) (Russell 2013). However, *A. japonicas* adapted gradually to a salinity range of 20 - 39 ppt (Zang *et al.* 2012).

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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 their cellular and humoral immunity response, the sea cucumbers use the coelomic liquid in their bodies (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 is, the lower will the osmotic pressure be. Every aquatic biota has an optimal range of salinity in order to survive. Environmental conditions beyond the tolerable range of organisms may cause stress, disturbances on the growth and reproduction processes, and even death (Seeruttum *et al.* 2008). Hence, this research was conducted to determine the effect of salinity on the growth, survival and immunity rate of the sea cucumber, *Holothuria scabra* and to find the optimal range of salinity which is suitable for the species. This study becomes more important related to population of sea cucumber in nature is declined sharply, so culture or restocking is one of possibility to fulfill the market demand.

## MATERIALS AND METHODS

### Animal Tests and Treatments

This study was done at the Institute for Mariculture Research and Fisheries Extension (IMRAFE) Gondol, Bali from 21 August to 26 September 2017 using the completely randomized design consisting of 5 treatments and 3 replications. The treatments applied, A (24 ppt), B (29 ppt), C (34 ppt), D (39 ppt) and E (44 ppt), were based on Lavitra *et al.* (2009). Fifteen 30 L fiber glass tanks, each filled with 25 L of sea water were used as experimental tanks. The tanks were put in a wet laboratory (indoor) and were equipped with aeration as oxygen supplier. To maintain the salinity, every day 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 the water volume was adjusted to the initial level yet maintaining appropriate water salinity. Before adding water, the salinity was checked in each treatment and water supply tanks.

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

### Parameters Measured

The parameters measured were growth, survival, osmolality and immunity of the sea cucumber juveniles, while the water quality was measured in terms of 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 during the experiment period. The average weight of the sea cucumbers from every experiment unit was used to obtain data on Specific Growth Rate (SGR) that indicates the increasing body weight during the experiment and was calculated as follows:

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

Where:
SGR = Specific growth rate (%/day)
Wo = Initial weight (g)
Wt = Final Weight (g)
t = Duration of rearing (day)

The survival rate of the sea cucumber juveniles was based on their number at the start and end of the research and was calculated as follows (Effendie 1997):

SR = (Nt/No) x 100% (2)

Where:
SR = Survival (%)
No = the number of sea cucumbers at the beginning of the experiment
Nt = the number of sea cucumbers at the end of the experiment

*Osmolality*

To find out the relationship between the use of feed energy for growth or for maintaining osmosis balance of the juvenile sea cucumber in different salinity treatments, osmolality was measured at the beginning and end of the research by extracting a substance in the form of coelomic liquid with Fiske-Osmometer according to Herlinah and Septiningsih (2014).

Coelomate was taken from the right ventral part of the respiratory organ (Coelom) of the juvenile sea cucumbers from each tank using a 26 g x 1/2"needle 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°C).

The osmolality was measured 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 removed using the pipette and transferred into a new tube. To analyze the osmolality, 20 µL supernatant was put into disposable tubes of Osmometer and then measured. Before doing the analysis for the next sample, the tube was cleaned using the probe cleaner and left to stay until it dried.

*Coelomate and Phagocyte*

Coelomate was also used to assess the immunity rate of sea cucumber juveniles (Smith *et al.* 2010). Coelomate functions as immune affector cell in Echinoderm in a manner that, 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 water quality was observed and analyzed every month at the Nutrition and Chemistry laboratory at Institute for Mariculture Research and Fisheries Extension (IMRAFE). The temperature was measured using thermocouple and the dissolved oxygen was measured using electrode membrane method/DO meter.

### Data Analysis

The data on growth, survival, osmolality, and the number of coelomates and phagocytes were analyzed using ANOVA and Tukey's test.

## RESULTS AND DISCUSSION

### Growth Rate

The highest absolute weight growth (AWG) and specific growth rate (SGR) of the juvenile sea cucumbers was reached at treatment B (29 ± 2 ppt), 7.10 g and 0.16% a day, respectively. The lowest rate was obtained at treatment E (44 ± 2 ppt), 0.34 g and -0.393% a day, respectively (Table 1). Statistical analysis showed that the salinity of the rearing medium significantly affected (P<0.05) the weight growth and SGR of the juvenile sea cucumbers.

Table 1 Absolute weight 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$^{a}$ |

Note: *means with different superscript in a column significantly differ from each other

Tukey's test showed that the weight growth rate and SGR of the sea cucumber juveniles reared at salinities 24 ± 2 ppt; 29 ± 2 ppt and 34 ± 2 ppt were significantly higher than those reared at salinities 39 ± 2 ppt and 44 ± 2 ppt. But in this experiment, in low salinity treatment (24 ppt), the sea cucumber juveniles showed lower specific growth rate compared to salinity treatments (29 & 34 ppt) because more energy is needed to maintain its iso-osmotic activities. *Holuthuria scabra* suffered from ulcers when the sea water salinity decreased to 20 ppt and at high salinity it experienced a higher mortality rate (Russel 2013). Furthermore, at high salinity earthen pond (> 46 ppt), *H. scabra* still survive (Giri *et al.* 2019), however, at high salinities (39 & 44 ppt), sea cucumber juveniles showed negative specific growth rate and reduced body weight at the end of the experiment. 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; Rhodes-Ondi & Turner 2009). Moreover, 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 (Abdel-Raheem 2015).

**Survival**

Salinity has significantly affected the survival of sea cucumber (P<0.05). At salinities 24 ± 2 ppt, 29 ± 2 ppt and 34 ± 2 ppt, the survival rate of the sea cucumber juveniles was 100.0%, while at salinity 39 ± 2 ppt it 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 rates of the sea cucumber juveniles reared at salinities 24 ± 2 ppt, 29 ± 2 ppt, 34 ± 2 ppt and 39 ± 2 ppt did not significantly differ (P>0.05), but were significantly higher than those reared at salinity 44 ± 2 ppt (P<0.05).

A high salinity can 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 manifested with skin ulcers followed by liquid secretion from the body, changes in skin color that would finally cause death. In general, the symptoms observed at high salinities confirm the study results of Asha *et al.* (2011). The juveniles that suffer from skin ulcers showed a decreased metabolism that would affect the growth and finally would result in their death.

**Osmolality**

The highest average level of osmolality in sea cucumber juveniles 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$^{-1}$ (Fig. 1). Analysis of variance showed that salinity has significantly affected (P<0.05) the osmolality of these cucumber juveniles.

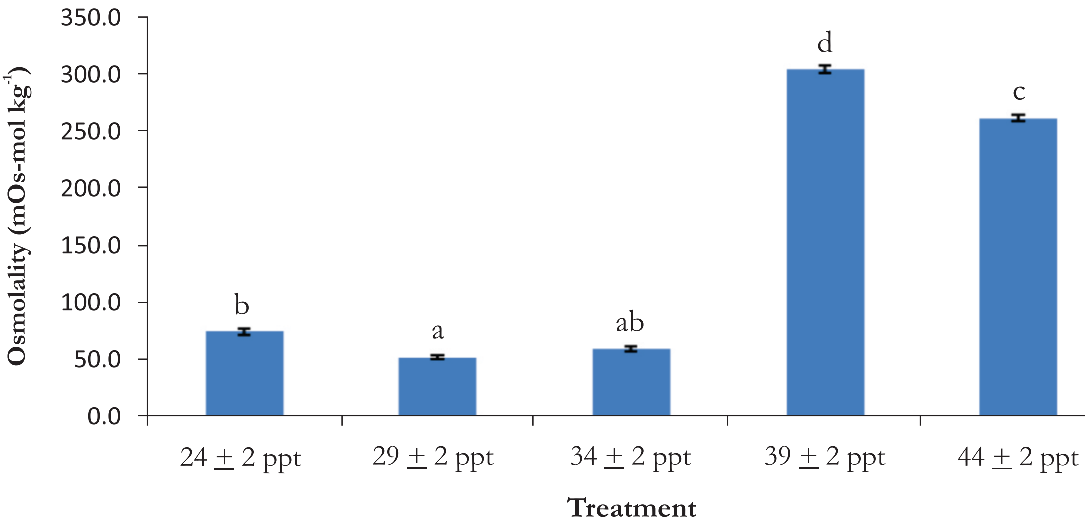


Figure 1 Osmolality of *H. scabra* juveniles reared at different salinities (superscripts with different letter showed significantly differ from each other)

Osmotic pressure and the release of coelomic liquid from sea cucumber juveniles significantly differed at varied salinities (Fig. 1). At high salinities, the coelomic liquid of the sea cucumbers was hyperosmotic to its external media. At 44 ppt medium, measurement of rearing medium osmolality was 1,175 mOsm/kg and in the coelomic liquid of juvenile was 914 mOsm/kg, so according to the formula applied, the osmolality of the juvenile was 260.66 mOsm/kg. In this condition, the coloemic liquid diffused out to the medium because the membrane of Echinoderm is relatively thin and highly permeable (Meng *et al.* 2011). Water exchanges occurred between the coelomic liquid in the sea cucumber body and the external media (Barker & Russel 2008). Then 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 (Freire *et al.* 2011). An 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 the skin and by producing hypo-osmotic urine through an excretive organ in the form of dermal branchia, caecum intestine and tuber legs in sea cucumbers (Bai *et al.* 2015; Rahmawati *et al.* 2012). When the coelomic liquid of sea cucumber juveniles is in hypo or hyper osmotic condition, the osmotic balance is difficult to attain or may even cause stress and reduced immunity. The sea cucumber juveniles at treatment 24 ppt are at hypo osmotic condition but are still able to reach iso-osmosis by using most of its energy and leaving only a small portion of this energy for growth. On the other hand, juveniles at 39 and 44 ppt suffered from hyper osmosis and from difficulties in reaching the iso-osmotic condition even by using all feed energy and its deposit energy. They also experienced low immunity, negative growth rate and disease infections. The juveniles at treatments 29 and 34 ppt are in iso-osmotic condition, and thus, need less energy for maintaining such iso-osmotic condition so that its energy obtain from the feed is mostly used for growth.

## Coelomate

The total number of coelomates and phagocytes of sea cucumber reared below and above 34 ppt salinity were significantly increased ($P<0.05$) (Fig. 2, 3). An increase in the number of coelomates and phagocytes are related to the effort of maintaining homeostasis caused by stress and increasing the immunity response of the sea cucumber juveniles.

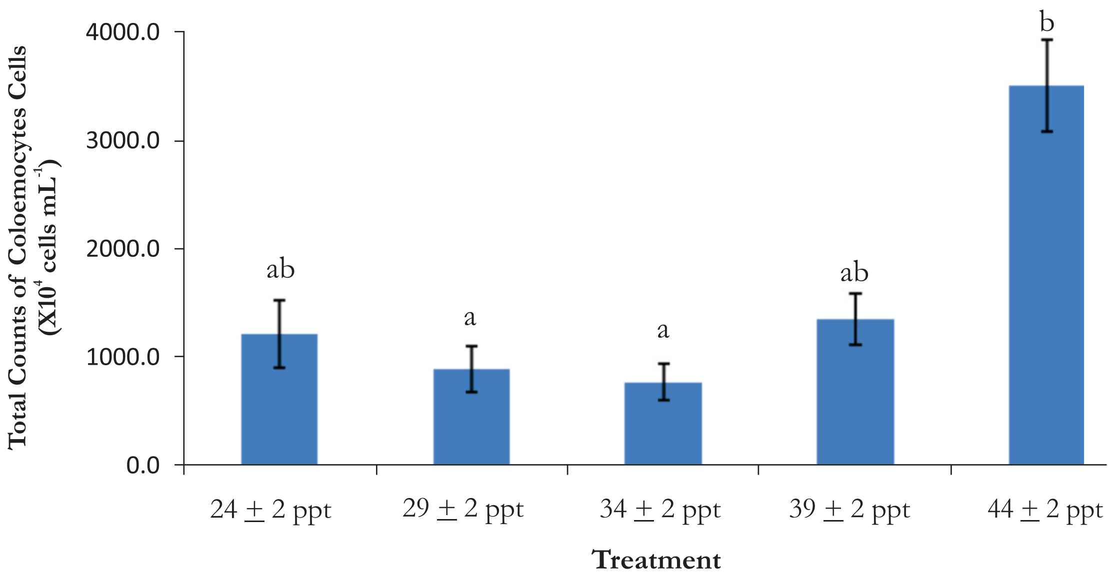


Figure 2 Total number of coelomate of *H. scabra* juveniles reared at different salinities (superscripts with different letter showed significantly differ from each other)

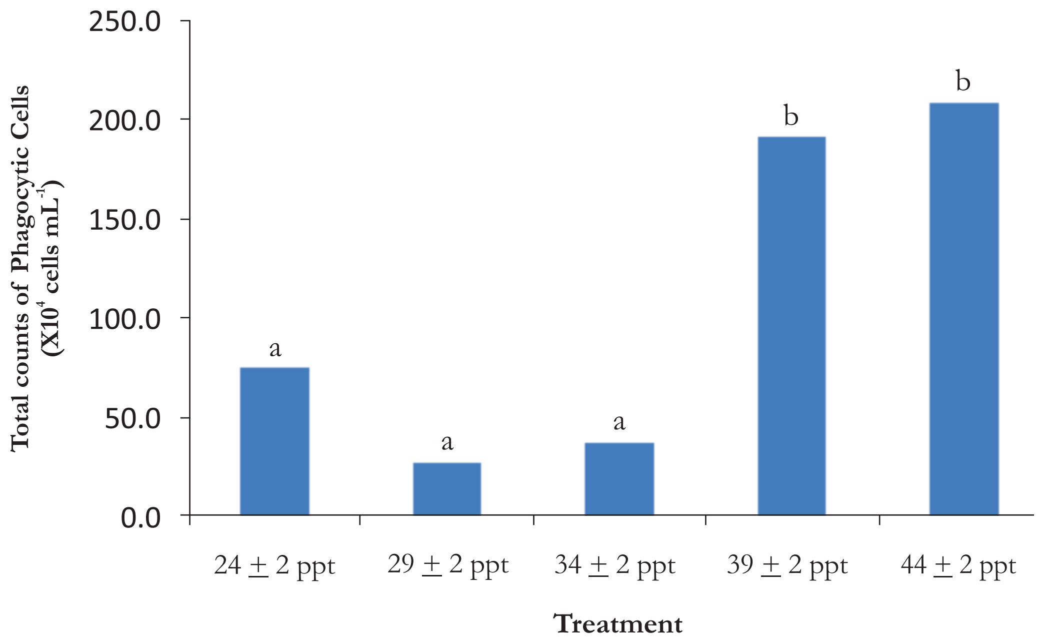


Figure 3 Total number of phagocytocic cells of *H. Scabra* juveniles reared at different salinities (superscripts with different letter showed significantly differ from each other)

Changes in the environmental factors can decrease/lower the body immunity so that resistance to infections and survival of the sea cucumber juveniles also decrease (Gowda *et al.* 2008). At a low salinity (24 ppt) and high salinities (39 and 44 ppt), the total number of coelomates reached 1,200-3,400 x $10^4$ cell/mL (Fig. 2). Obviously, the sea cucumber juveniles are in a stressed condition while the sea cucumber juveniles at salinities 29 and 34 ppt with low coelomates at 700-800 x $10^4$ cell/mL, are in normal condition.

At salinities 24, 29 and 34 ppt, the phagocytes were at 27-76 x $10^4$ cell/mL; lower than those at salinities 39 and 44 ppt with values of 190-209 x $10^4$ cell/mL. These results proved that in stressed condition, the sea cucumber juveniles showed high coelomic and phagocytic values as their response to salinity changes. In addition to its function as the main affector cell in the immunity system of Echinoderm (including Holothuridae), coelomates also function in the transport and storage of nutrients and oxygen, pigment biosynthesis and excretion, so that when juveniles suffer from stress, the total number of coelomates and phagocytesis affected (Meng *et al.* 2011). Hence, increase in phagocytes is influenced by changes in salinity (Wang *et al.* 2008).

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

## Water Quality

Water quality gives an important effect on the growth and survival of organisms living in water (Lavitra *et al.* 2010). Temperature is predominant for growth while salinity and dissolved oxygen tend to affect on survival rate. Optimum salinity and temperature will promote good feeding rate, in contrary, when salinity and temperature is too low or too high, sea cucumber tend to dwell deep in the mud and stop feeding and even cause mortality.

Table 2 Average values of water quality parameters 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 ranges of water temperature and dissolved oxygen during the experiment were still within the tolerance limit of sea cucumber juveniles (Table 2). The range of temperatures in the morning and afternoon were 26.7-30.2ºC with dissolved oxygen at 6.62-6.78 ppm. Some researches reported that sea cucumbers can survive at 25-30ºC (Giraspy & Ivy 2005; Therkildsen & Petersen 2006). If the temperature is higher than 30ºC, the sea cucumber's body becomes inactive, but its tentacles can still move (Hu *et al.* 2010). The optimum dissolved oxygen content in the sea cucumber rearing media was between 6-8 ppm (Yusron & Pramudji 1987 *in* Louhenapessy 2013) and the value in this research was still in this range.

## CONCLUSION

Different salinities in the culture media of the sea cucumber (*H. scabra*) juveniles have significantly affected their growth and survival rates, osmolality and immunity. The range of salinities which can support growth, survival and immunity rate in sea cucumber juveniles is 29-34 ppt. At treatment 24 ppt, the sea cucumber juveniles were able to reach iso-osmotic capacity by using most of its energy for survival. Salinities at 39 and 44 ppt are not recommended because at this point it is very hard for the juveniles to reach iso-osmotic capacity and for them to survive.

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