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EFFECT OF THE USE OF *Gracilaria* sp. ON WATER QUALITY, PHYSIOLOGICAL AND GROWTH PERFORMANCE OF *Holothuria scabra* IN CULTURE TANK

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ABSTRACT

Sea cucumber *Holothuria scabra* was reared on a small scale with the addition of seaweed *Gracilaria* sp. as a phytoremediation agent. This research aimed to determine the effect of *Gracilaria* sp. on water quality, physiological response, and growth performance of *H. scabra*. Ten individuals of *H. scabra* with an initial length of 5 ± 0.09 cm and an initial weight of 7.6 ± 0.2 g were reared in a culture tank (20 cm x 30 cm x 20 cm) with 15 cm of water depth. *Gracilaria* sp. was floated on the culture tank at three weight levels with three replicates, i.e., low (15 g); medium (30 g); and high seaweed density (45 g), with the control (0 g), during the 30-day rearing period. Results showed no significant difference in water temperature, dissolved oxygen (DO), salinity, and total ammonia nitrogen (TAN) between all treatments except for pH. There were no significant differences in ammonia and nitrite concentrations and significant differences in nitrate concentration and total organic matter (TOM) between all treatments and the control. On day 30, the application of *Gracilaria* sp. exhibited a lower nitrate concentration than the control. *Gracilaria* sp. maintained the water quality in the culture tank within a tolerable range for *H. scabra*. On the physiological response of *H. scabra*, high seaweed density exhibited the lowest blood cholesterol and glucose levels on day 30 and the highest specific growth rate (SGR) in weight ($0.59 \pm 0.2\%$) and length ($1.16 \pm 0.09\%$). The survival rate of *H. scabra* in all treatments reached 100%, suggesting the indoor cultivation system in this experiment did not negatively affect the growth of *H. scabra*.

KEYWORDS: algae; environment; mariculture; phytoremediation; sandfish

INTRODUCTION

Sea cucumber is one of the main targets of capture fisheries due to its high economic value and benefits as food, cosmetics, and medicine (Choo, 2008). Sea cucumbers from Indonesia are exported to Hong Kong with an export volume of 1,231.6 tons in 2015, increasing from their export volume in 2014 by 1,153.2 tons (Ministry of Trade, Republic of Indonesia, 2016). In Indonesia, sea cucumber production increased from 4,390 tons in 2013 to 5,428 tons in 2014 (Sidatik, 2015). One of the three tropical sea cucumber species with a high economic value is *Holothuria scabra* (Purcell, 2014).

Unfortunately, the high demand for sea cucumbers on the global market has led to overfishing and habitat destruction, which slowly declined sea cucumber stock in its natural habitat and pushed it into extinction (Conand, 2017; Conand, 2018). In the natural habitat, sea cucumbers as a detritus organism play an important role as a bioindicator in a marine environment with high organic matter. The development of sea cucumber culture is a potential way to recover their natural population and meet the increased demand for sea cucumbers (Sicuro & Levine, 2011). Mariculture contributes to the improvement of fisheries stock in nature through aquaculture-based restocking or sea ranching (Bell *et al.*, 2008). In natural farming, sea cucumbers' cultivation using the stepping nets in ocean grow-out is highly vulnerable to predation, poaching, and escaping. Another sea cucumber cultivation system has also been carried out under onshore systems, yet it requires maximum

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water exchange and an increasingly large area for scaling up production. Sea cucumber production in ocean grow-out is preferred than in culture tanks. However, an indoor controlled cultivation system holds some advantages over the ocean grow-out system, i.e., more simple in rearing and harvesting, reducing sea cucumber's escape and predation by birds, gastropods, and crustaceans, and producing high product quality, quantity, and sustainability. Robinson *et al.* (2018) reported that the rearing of juveniles *H. scabra* on laboratory-scale tanks through manipulation of the C:N ratio increased their growth rate and biomass, and further optimization could increase biomass yield which can be developed in a commercial scale.

Sea cucumber aquaculture is able to produce waste products containing organic and inorganic compounds, such as ammonium/ammonia, nitrite, and nitrate. If not well processed, organic and inorganic compounds can reduce water quality, which is a crucial parameter for the growth of sea cucumber (Meirinawati *et al.*, 2020). The waste treatment has an important effect on the process and yield of aquaculture activities. An innovative technology in aquaculture waste treatment is phytoremediation using plants or seaweeds. A controlled phytoremediation application can reduce mortality of culture biota, maintain water quality, and increase the sea cucumbers growth (Robinson *et al.*, 2018).

Phytoremediation technology not only plays a role in the management of aquaculture environments, physically, chemically, and biologically, but this technology can also function as a supporting feed for several aquatic organisms which are detritivores and scavengers, such as the Holothuridae and Stichopodidae groups. Thus, the improvement of water quality and cultured biota production can be achieved (Thayer *et al.*, 2005). Inorganic nitrogen compounds in water can be utilized directly as an energy source for plants or seaweed, and also as substrates for beneficial bacteria, such as nitrifying and denitrifying bacteria. A seaweed species, *Gracilaria* sp. can reduce the number of inorganic nitrogen compounds in water, including 68.44% ammonium, 13.04% nitrite, and 23.03% nitrate, and uses it as nutrients for their growth (Capillo *et al.*, 2015). Polyculture of *H. scabra* separately with *Gracilaria* sp. and *Gracilaria arcuata* under pen-culture in Southeast Maluku and Kupang, Indonesia, result in a survival rate of sea cucumber reached 92.5% and 100%, respectively (Tomatala *et al.*, 2019; Tel, 2018). This seaweed is presumably potential as a phytoremediation agent in sea cucumber cultivation under a controlled system. The use of *Gracilaria* sp. was considered to improve the quality of rearing

water and sea cucumber growth. This present study aimed to investigate the effect of seaweed *Gracilaria* sp. on the water quality, physiological responses, and growth of sea cucumber *H. scabra* in a controlled rearing system.

MATERIALS AND METHODS

Experimental Design

The experimental design used in this study was a completely randomized design which consisted of three densities of seaweed *Gracilaria* sp., i.e., control (without seaweed), low (15 g or 0.00167 g cm⁻³), medium (30 g or 0.00334 g cm⁻³), and high (45 g or 0.005 g cm⁻³), with three replicates. We used a total of 12 sterile culture tanks (four treatments x three replicates) with a size of 20 cm x 30 cm x 20 cm arranged using a double bottom system with the addition of filters in the culture tank in order to stabilize the water quality for 30-day rearing period without water exchange (Figure 1A). The substrate contained a mixture of 3 kg sand and 1 kg nitrogen-phosphor-potassium (NPK) fertilizer (3:1). Fertilizer was applied to promote algae and diatoms growth as natural food for sea cucumber *H. scabra* at the beginning of the cultivation period. In addition, ammonia-contained fertilizer was applied as an initial nutrient for the growth of *Gracilaria* sp. Each culture tank was filled with the substrate at the bottom part, followed by seawaters until a height of 15 cm with a water volume of 5 L. A heater was used to maintain a stable water temperature in the culture tank.

Seaweeds *Gracilaria* sp.

The weight of *Gracilaria* sp. was previously measured according to the treatments, i.e., 0 g (control), 15 g (low), 30 g (medium), and 45 g (high). Seaweeds were tied with a distance of 5 cm between clumps and were bound to each culture tank following the treatments (Figure 1B).

Sea Cucumber *H. scabra* Stocking

H. scabra with an average initial length of 5 ± 0.09 cm (mean ± SE) and an average weight of 7.6 ± 0.2 g (mean ± SE) were obtained from the Institute for Mariculture Research and Fisheries Extension (IMRAFE), Gondol, Bali, Indonesia. After *H. scabra* transported to the study site, the sea cucumbers were acclimatized in a stock aquarium with a size of 100 cm x 40 cm x 30 cm for seven days as an initial adaptation process and given 1 g of feed in the form of artificial pellets containing *Spirulina* once every two days (Xia *et al.*, 2012). Then, sea cucumbers were moved to experimental tanks with a density of 10

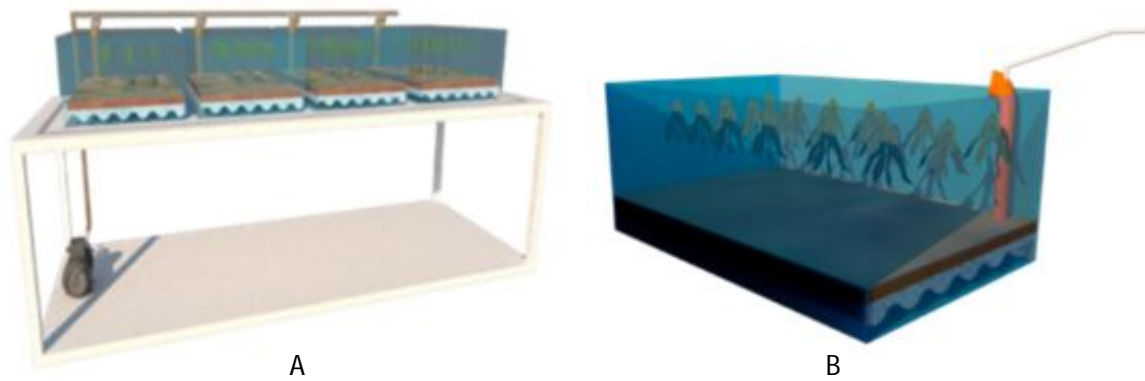


Figure 1. The design of the application of *Gracilaria* sp. in the *H. scabra* culture tank.

sea cucumbers per culture tank. Over the rearing period, the sea cucumbers were fed with 1 g of artificial pellets containing *Spirulina* once every two days (Xia *et al.*, 2012).

Water Quality Assessment

Water quality parameters, including temperature, dissolved oxygen (DO), salinity, pH, and total ammonia nitrogen (TAN), were continuously recorded three times per day during the experimental period using thermometer, DO meter, refractometer, pH meter, and spectrophotometer, respectively. Other parameters, such as ammonia, nitrite, nitrate, and total organic matter (TOM) were periodically assessed three times on day 1, 15, and 30 using phenate, sulfanilamide-NED, brucine, and Winkler method, respectively, following APHA (2005). Measurement of TOM was conducted using water samples obtained from the bottom of culture tanks.

Physiological Responses of Sea Cucumber

Measurement of the physiological responses of sea cucumbers was carried out on day 1 and 30. The physiological response parameters measured in this study were oxygen consumption rate (OCR), cholesterol level, blood glucose level, and total hemocyte count (THC) as part of the primary and secondary physiological responses of the cultured species. OCR is measured based on the amount of oxygen consumption during a certain time, following NRC (1977). Blood samples were taken from the abdominal part of the sea cucumber body. The total blood cholesterol of the sea cucumber was calculated using the cholesterol oxidase method according to Richmond (1973). The blood glucose level was determined following Barham & Trinder (1972). THC was measured using the hemocytometer method based on Chen & Wang (2006).

Growth and Survival Rate of Sea Cucumbers

The survival rate (SR) of sea cucumbers was calculated according to Goddard (1996), as the following formula:

$$SR = \frac{N_t}{N_o} \times 100\% \quad (1)$$

where: *SR* is the survival rate (%), *N_t* is the total number of surviving sea cucumbers at the observed time, and *N_o* is the initial number of sea cucumbers

Specific growth rate (SGR) was determined following Muchlisin *et al.* (2016). The formula is as follows:

$$SGR = \frac{\ln \bar{W}_t - \ln \bar{W}_o}{t} \times 100\% \quad (2)$$

where: *SGR* is specific growth rate (%), \bar{W}_t is average body weight or body length of sea cucumbers in a certain time (g), \bar{W}_o is initial average bodyweight (g) or body length (cm) of sea cucumbers, and *t* is the rearing period (days)

Statistical Analysis

Data was analyzed using the one-way analysis of variance (ANOVA) at a significance level of 0.05. When the significant effect observed, a pairwise comparison using Tukey post-hoc test was employed to identify significant differences between treatments. Statistical analysis was performed using SPSS 24.0, and was presented using Microsoft Excel 2016.

RESULTS AND DISCUSSION

Water Quality in the Rearing Water

Table 1 shows the mean range for water temperature, DO, salinity, pH, and TAN monitored daily. Generally, we found that the water temperature, DO, salinity, pH, and TAN in cultivation tanks ranged be-

Table 1. The value range of water quality parameters in the cultivation tanks of the sea cucumber *H. scabra* added with varying amounts of seaweed *Gracilaria* sp.

Parameters	Treatments				Optimal value
	Control (0 g)	Low (15 g)	Medium (30 g)	High (45 g)	
Temperature (°C)	27-32.1 ^a	27.3-30.7 ^a	27.4-30.7 ^a	27.2-33 ^a	27-32 ⁾
DO (mg/L)	6-7.8 ^a	6-7.8 ^a	6-7.6 ^a	6-7.3 ^a	> 5 ^{**)}
Salinity (‰)	30-38 ^{ab}	31-37 ^c	31-35 ^{bc}	30-35 ^a	35 ⁾
TAN (mg/L)	0.001-0.01 ^a	0.002-0.009 ^a	0.002-0.011 ^a	0.001-0.012 ^a	< 3 ^{***)}
pH	8.09-8.76 ^c	7.61-8.76 ^b	7.61-8.71 ^a	7.57-8.73 ^b	7.8 ⁾

Source: ⁾ Asha & Muthiah (2005); ^{**)} Sulardiono *et al.* (2017); ^{***)} Hastuti *et al.* (2015)

Description: The mean range followed by different upper case letters in the same parameter denotes a significant difference between treatments ($P < 0.05$) (Tukey multiple comparison tests)

tween 27°C-33°C, 6-7.8 mg/L, 30‰-38‰, 7.57-8.76, and 0.001 to 0.01 mg/L, respectively. Our study showed that the culture tank's water temperature, DO, salinity, and TAN had no significant difference among treatments ($P > 0.05$). However, pH significantly differed between treatments and control ($P < 0.05$).

In our study, the daily water temperature in all treatments lies within the optimal value (Table 1). Our study showed that DO during the experimental period ranged from 6 to 7.8 mg/L (Table 1). Fluctuating DO in the treatments was probably due to the temperature differences between culture tanks, in which higher water temperature results in a lower DO. A high and stable DO is probably due to using a double bottom system, implementing the pressure difference generated by aeration to supply oxygen in culture tanks. High oxygen concentration is able to minimize the competition between sea cucumber and seaweed in oxygen consumption, primarily at night. However, surprisingly, sea cucumbers are able to survive in hypoxia conditions (Yu *et al.*, 2012). The results showed that water salinity among all treatments ranged from 30‰-38‰ during the experiment (Table 1), which remains in the optimal salinity for *H. scabra* (Asha & Muthiah, 2005). Meanwhile, TAN varied from 0.001 to 0.01 mg/L (Table 1), remaining within acceptable levels for *H. scabra* growth (Hastuti *et al.*, 2015).

The water pH in all culture tanks varied from 7.57 to 8.76, which showed a significant difference between treatments and control ($P < 0.05$) (Table 2). Francis-Floyd *et al.* (2012) reported that low pH and temperature are able to terminate the nitrification process in the aquatic environment. According to our study, the water pH tends to be alkaline, resulting in

the decomposition process of organic matter would be more effective. The pH value is generally influenced by the amount of nitrogen and oxygen in the water environment resulted from microbial activities converting organic waste to organic acids. The increase in pH is due to protein decomposition that produces ammonium accompanied by the release of OH⁻ ions. Also, microbes can consume organic acids for their activities, causing an increase in water pH. In addition, the pH value is influenced by carbon dioxide (CO₂) utilization for photosynthesis of *Gracilaria* sp. CO₂ uptake results in water pH increases. At night, it was observed a decrease in water pH (Davies *et al.*, 2011).

An increase in the ammonia concentration was observed in the culture tanks from day 1 to 15, followed by a decrease until day 30 (Figure 2). A significant increase in ammonia on day 15 implied the biological and chemical activities due to fertilizer decomposition. Increased ammonia generated by the decomposition process was supported by optimal temperature and pH (Hastuti, 2011; Francis-Floyd *et al.*, 2012). On day 15, the highest ammonia concentration was observed in control (0.0023 mg/L), whereas the lowest ammonia concentration was detected in the low seaweed density (0.0006 mg/L) (Figure 2). On day 1 and 15, the ammonia concentrations significantly differed between treatments and control ($P < 0.05$). Ammonia concentration in all treatments was maintained at acceptable levels for the growth of *H. scabra*. The reduction of ammonia concentration in each treatment from day 15 to day 30 might be caused by biological nitrification processes by nitrifying bacteria, which converted ammonia into nitrite and nitrate (Hastuti, 2011).

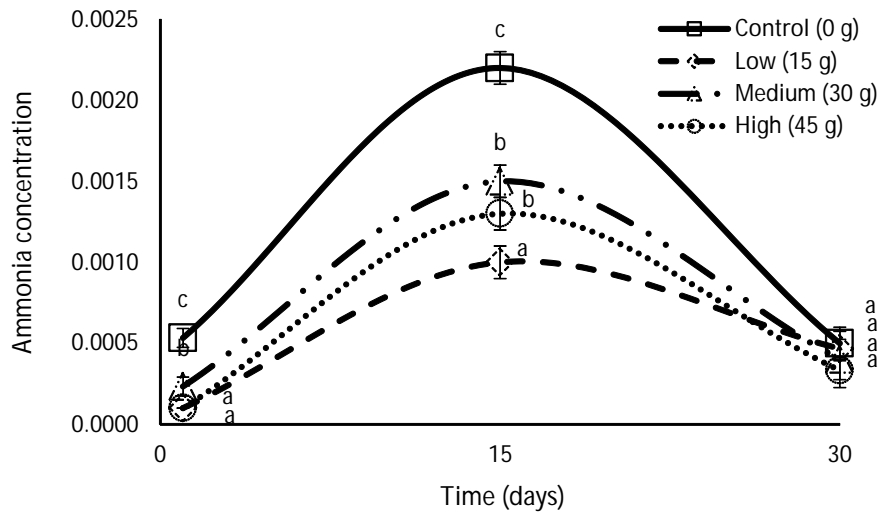


Figure 2. Ammonia concentration in the water culture of sea cucumber *H. scabra* added with seaweed *Gracilaria* sp. in varying weight treatments, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g). Different letters above the bars indicate significant differences among treatments based on Tukey’s multiple comparison tests ($P < 0.05$).

Nitrification activity produces nitrite compounds whose concentration is influenced by oxygen (Hastuti, 2011). Generally, nitrite concentration in all treatments gradually decreases from day 1 to 30, except for the medium seaweed density on day 15 (Figure 3). This result indicates that ammonia was effectively converted to nitrite, and nitrite was transformed into nitrate. The considerable difference in nitrite concentration between treatments on day 15 was probably influenced by nitrite characteristics, which tend to be less stable in water due to oxygen and micro-

bial activity. We detected the highest nitrite concentration in the medium seaweed density on day 15 (0.7 mg/L), while the lowest nitrite concentration in the high seaweed density on day 30 (0.03 mg/L) (Figure 3). Analysis of variance indicated that nitrite concentration in the high seaweed density had no significant difference compared to the control and other treatments ($P < 0.05$) (Figure 3). Generally, the nitrite concentration in this study remained in a tolerable range for sea cucumbers.

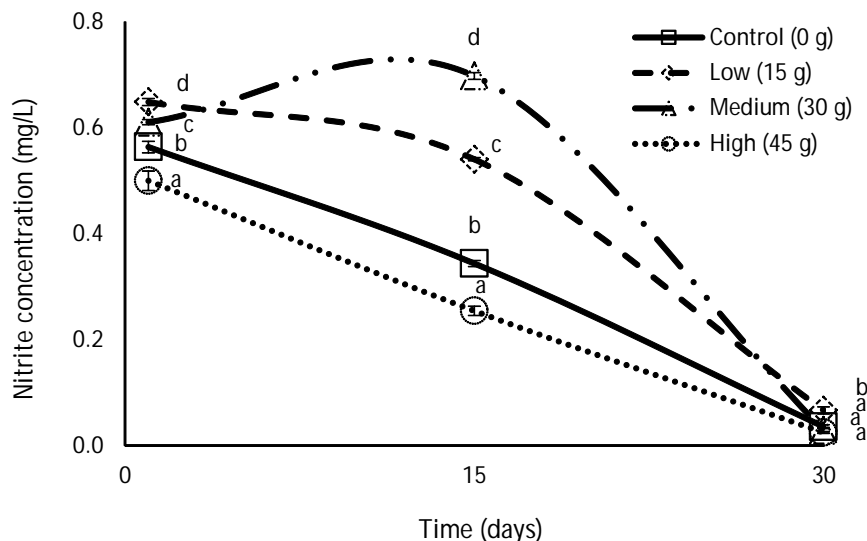


Figure 3. Nitrite concentration in the water culture of sea cucumber *H. scabra* cultured with seaweed *Gracilaria* sp. in various weight treatments, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g). Different letters indicate significant differences between treatments based on Tukey’s multiple comparison tests ($P < 0.05$).

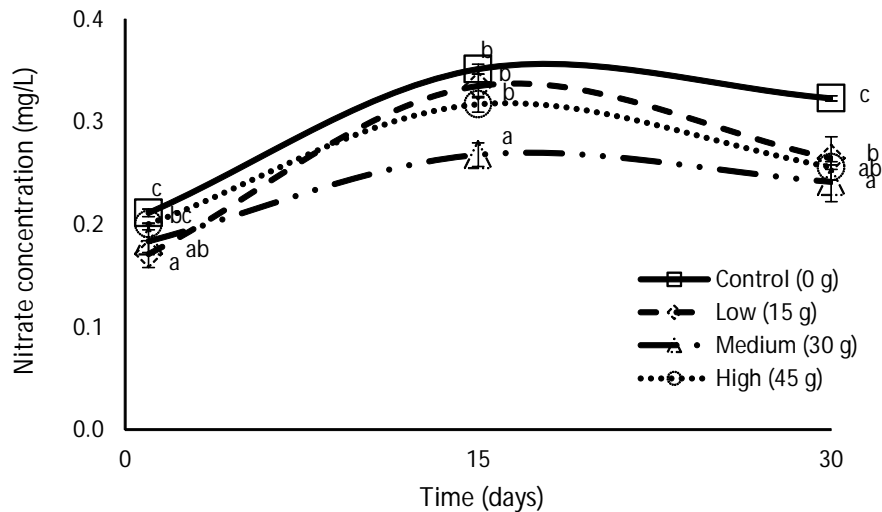


Figure 4. Nitrate concentration in the water culture of sea cucumber *H. scabra* cultured with seaweed *Gracilaria* sp. in varying weight treatments, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g). Different letters denote significant differences among treatments based on Tukey's multiple comparison tests ($P < 0.05$).

Nitrate content increased until day 15, then reduced until day 30 (Figure 4). On day 15, the maximum and minimum nitrate concentration was shown in control and the medium seaweed density reached 0.35 and 0.3 mg/L, respectively. An increase in nitrate concentration on day 15 was probably due to fertilizer decomposition on the substrate followed by nitrification process and the ineffective absorption of nitrate by *Gracilaria* sp. Subsequently, nitrate was absorbed on day 15 to 30 (Figure 4). A significant difference in nitrate concentration on day 30 was observed between treatments and control ($P < 0.05$) (Figure 4). *Gracilaria* sp. can consume nitrate as an energy source for their growth (Capillo *et al.*, 2015), which indirectly affects sea cucumbers growth. Additionally, the dynamic of ammonia, nitrite, and nitrate concentration between treatments was influenced by the abundance and activity of ammonia and nitrite-oxidizing bacteria in the culture environment (Hastuti *et al.*, 2017).

According to statistical analysis, TOM was significantly different between each treatment and control on days 1, 15, and 30 ($P < 0.05$). At the beginning of this experiment, TOM in the water environment showed a high value (Figure 5), probably caused by substrate decomposition. TOM is vital for the growth of indigenous heterotrophic microbes. Low TOM concentration in the medium seaweed density treatment might be associated with the abundance of heterotrophic microbes in the culture medium. In this present study, TOM declined gradually from day 1 until

day 30 (Figure 5). This trend might be resulted from sea cucumber consumption patterns, which tend to utilize TOM. Sea cucumber has modified their behaviour, both in foraging and digesting, in order to optimize the nutrient intake from organic compounds in sediment or substrate (Zamora & Jeffs, 2011). Organic matter can also be utilized and assimilated by seaweed as primary producers (Namukose *et al.*, 2016) and as an energy source for indigenous microbes.

Physiological Response of the Sea Cucumbers

Oxygen uptake from the water environment is associated with the oxygen demand of cultured species, indicating metabolism rate or energy production (Yu *et al.*, 2012). Results showed oxygen consumption rate (OCR) fluctuation during 60-minute observation time, either on day 1 or 30 (Figure 6). On day 1, OCR showed the fewest rate in the control, namely at minute 50 and 60 (Figure 6A). On the other hand, the highest OCR was shown by control on day 1 at minute 30 (5.94 mgO₂/g/h) (Figure 6A). On day 30, the highest OCR was observed at the low seaweed density at minute 10, reaching 7.6 mgO₂/g/h (Figure 6B). Temperature differences among culture tanks might cause fluctuating OCR. High oxygen consumption on the first day was likely due to the adaptation of sea cucumbers to the new environment. OCR observed in this present study was higher than previously reported by Kühnhold *et al.* (2019), which reached $13.2 \pm 2.7 \mu\text{gO}_2/\text{g/h}$ under ambient control (29°C).

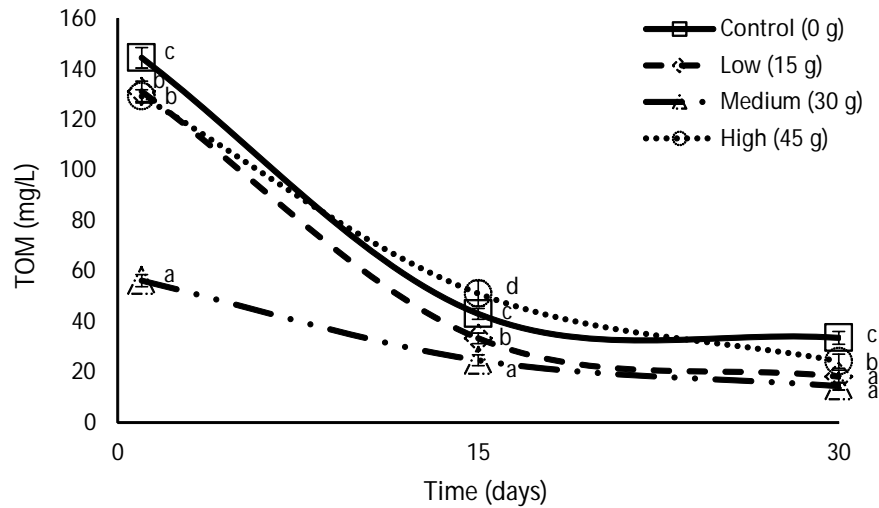


Figure 5. The concentration of TOM in the water culture of the sea cucumber *H. scabra* added with seaweed *Gracilaria* sp. in various weight treatments, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g). Different letters denote significant differences between treatments based on Tukey's multiple comparison tests ($P < 0.05$).

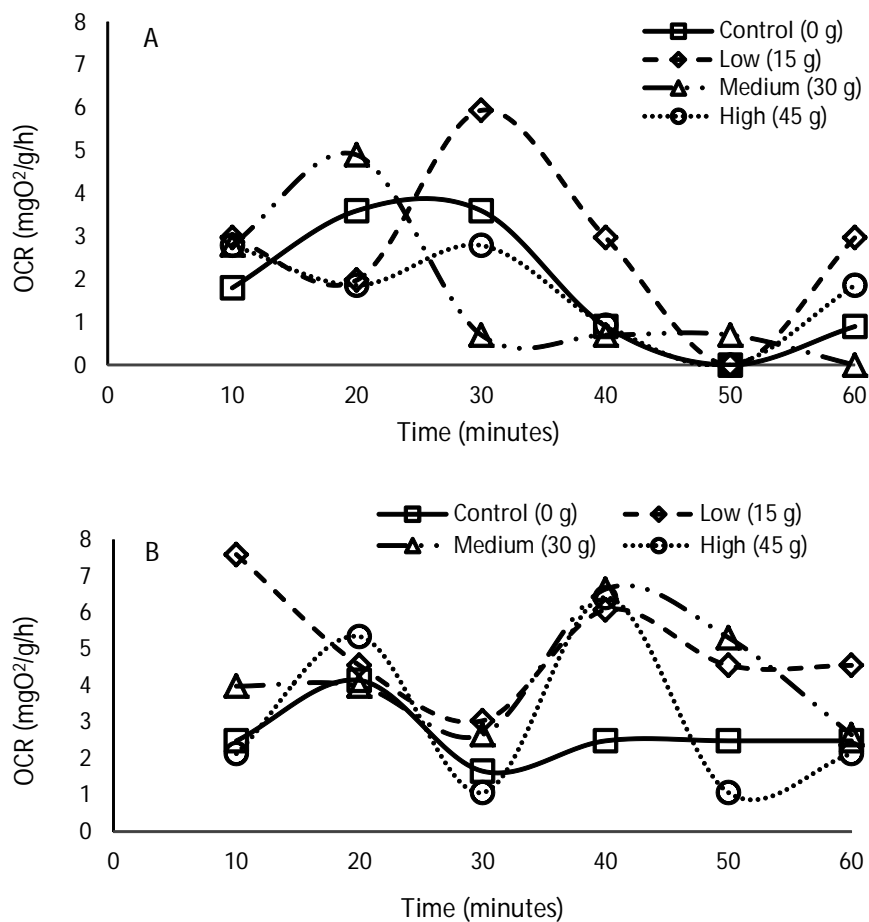


Figure 6. Oxygen consumption rate (OCR) of the sea cucumber *H. scabra* cultured with seaweed *Gracilaria* sp. with varying weight, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g) on day 1 (A) and day 30 (B).

The cholesterol level of the sea cucumbers on day-1 varied from 3.76 to 8.15 mg/dL. On day-1, the highest and lowest cholesterol level was consecutively exhibited by the high seaweed density and the control. Subsequently, cholesterol level on day-30 declined ranging between 0.8 ± 0.2 mg/dL and 2.06 ± 0.3 mg/dL. On day-30, low and high seaweed density showed the largest and the smallest cholesterol level, respectively ($P < 0.05$) (Figure 7). Cholesterol enhancement will increase catecholamines and steroids. An increase in catecholamine concentrations will affect secondary and tertiary physiological responses (Schreck & Tort, 2016). Catecholamine enhancements also influence the performance of cardiorespiratory system, thereby increasing oxygen uptake to tissues (Rodnick & Planas, 2016).

Blood glucose level can be used as a biomarker for stress responses of organisms, which is a secondary physiological response due to metabolic disruption. We detected that sea cucumbers reared in the low seaweed density on day-30 demonstrated the highest glucose concentration, while those in high seaweed density showed the lowest glucose level (Figure 8). Statistical analysis showed that the high seaweed density on day-30 significantly differed from the control ($P < 0.05$).

Environmental parameters are directly related to the physiological response of culture species. Not only temperature, ammonia and nitrite also affect the stress level of culture biota (Eddy, 2005). It is suspected that the concentrations of ammonia and nitrite indirectly affect the stress levels of sea cucumbers reared in low and medium seaweed densities treatments, so the cholesterol and glucose levels were

higher than those of the control on day-30. Also, it can be observed that the cholesterol and blood glucose level of sea cucumber responds to nitrite concentration in a water environment, indicated by similar patterns (Figure 3; 7; 8).

THC as a biomarker of secondary physiological responses ranged between 1.32×10^6 and 2.84×10^6 cells/mL on day-1, then increased up to 4.52×10^6 - 5.74×10^6 cells/mL on day-30 (Figure 9). A rise in THC leads to the production of phagocytic cells to fight against pathogens, including viral or bacteria (Hastuti *et al.*, 2015). Stress is able to enhance sea cucumbers' immune system through antibody synthesis by lymphocytes (hemocytes). Hemocyte formation is influenced by cortisol (Schreck & Tort, 2016). On day-30, the highest THC was in the high seaweed density treatment compared to other treatments (Figure 9). Statistical analysis exhibited that the THC in the high seaweed density treatment on day-30 was significantly different from other treatments ($P < 0.05$) (Figure 9). The highest THC on day-30 reached by high seaweed density treatment suggests high antibody production. A high amount of THC in sea cucumbers exhibited a high immune response, phenoloxidase activity, and phagocytic activity (Verghese *et al.*, 2007).

Growth Performance of Sea Cucumbers

Our study showed that the survival rate as one of the sea cucumbers' production responses implied the success of sea cucumber cultivation in a controlled technology system. The survival rate of sea cucumber in all treatments was 100% over 30-day rearing period ($P > 0.05$) (Figure 10). The results proved that the cultivation system in this study did not negatively affect the growth of sea cucumber. Sea cucumbers

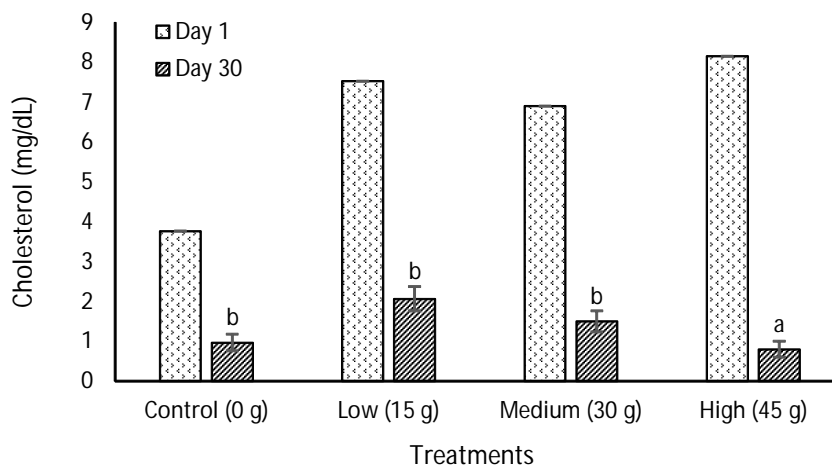


Figure 7. Cholesterol level of the sea cucumbers *H. scabra* cultured with seaweed *Gracilaria* sp. with varying weight, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g). Different letters denote significant differences between treatments based on Tukey's multiple comparison tests ($P < 0.05$).

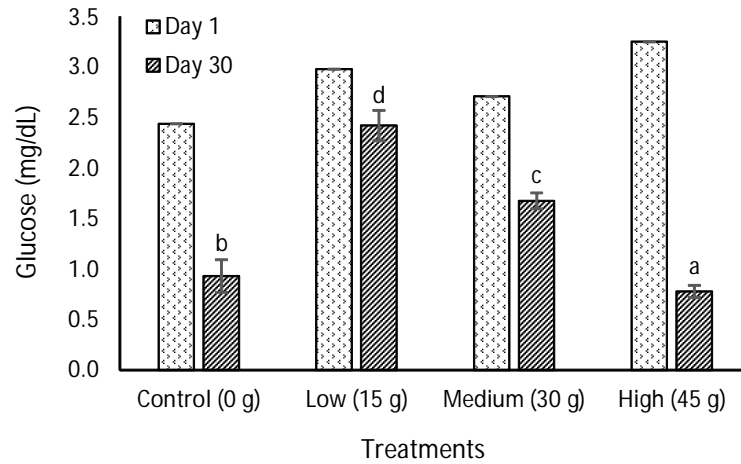


Figure 8. Blood glucose level of the sea cucumber *H. scabra* cultured with seaweed *Gracilaria* sp. with varying weight, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g). Different letters denote significant differences between treatments based on Tukey's multiple comparison tests ($P < 0.05$).

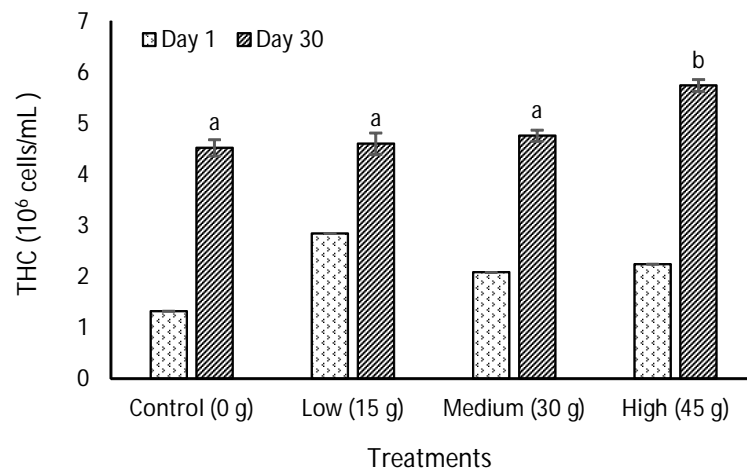


Figure 9. Total hemocyte count of the sea cucumber *H. scabra* cultured with seaweed *Gracilaria* sp. with varying weight, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g). Different letters denote significant differences between treatments based on Tukey's multiple comparison tests ($P < 0.05$).

were relatively able to survive in a controlled environment without changing the water for 30 days.

Based on the result, the highest weight-SGR was possessed by sea cucumbers reared in the high seaweed density treatment ($0.59 \pm 0.2\%$), while the lowest weight-SGR was in the control ($0.32 \pm 0.08\%$) (Figure 11A). The highest length-SGR was observed in the high seaweed density treatment, whereas the lowest length-SGR was detected in the control reached $1.16 \pm 0.09\%$ and $0.97 \pm 0.06\%$, respectively (Figure 11B). On the contrary, the length-SGR of sea cucumber in medium and high seaweed density treat-

ments significantly different from the control ($P < 0.05$) (Figure 11B). Previously, Indriana *et al.* (2017) reported that the SGR in weight of sea cucumber cultivated in a fiber tub without *Gracilaria* sp. throughout 30 days was $0.15 \pm 0.03\%$ per day.

CONCLUSIONS

Application of seaweed *Gracilaria* sp. in a controlled cultivation system maintained water quality parameter within the acceptable levels for sea cucumber *H. scabra*. High seaweed density (45 g or 0.005 g cm^{-3} of *Gracilaria* sp.) was the best treatment for the

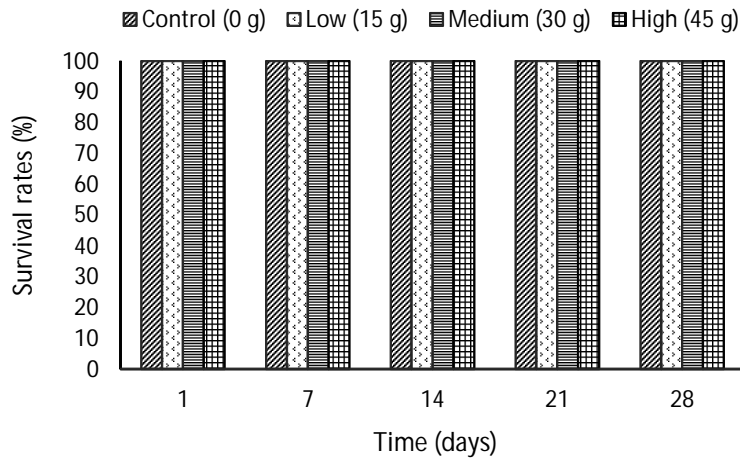


Figure 10. Survival rate of the sea cucumber *H. scabra* cultured with seaweed *Gracilaria* sp. with varying weight, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g).

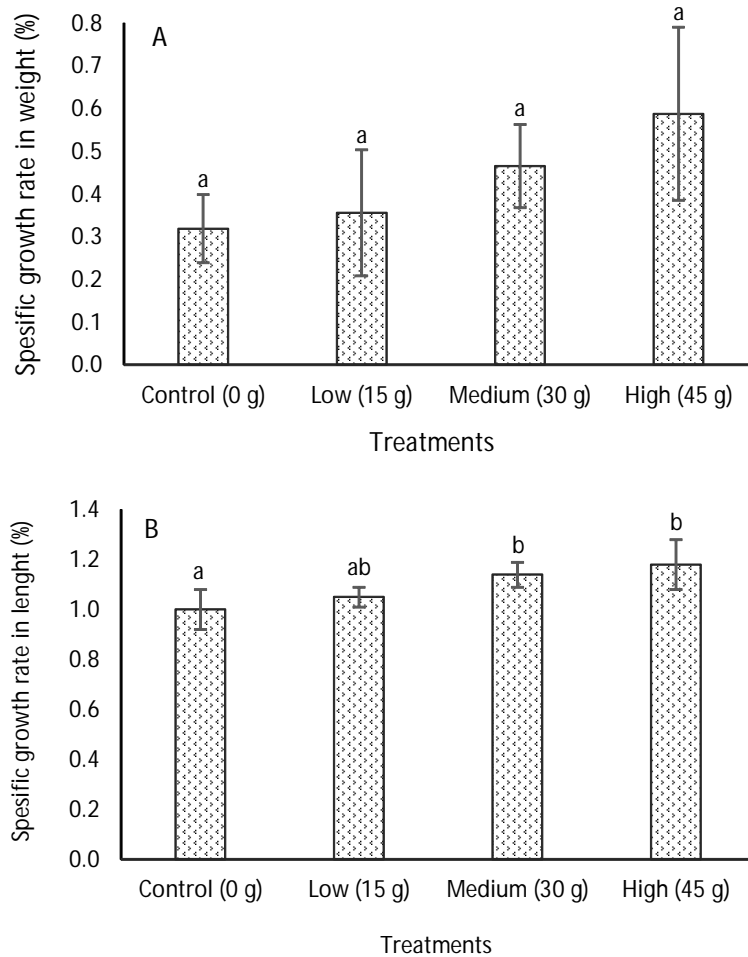


Figure 11. Specific growth rate in weight (A) and specific growth rate in length of the sea cucumber *H. scabra* (B) cultured with seaweed *Gracilaria* sp. with varying weight, i.e., control (0 g), low (15 g), medium (30 g), and high (45 g). Different letters denote significant differences between treatments based on Tukey's multiple comparison tests ($P < 0.05$).

stability of water quality in culture tanks. This treatment controls the physiological response of primary (cholesterol level) and secondary level (blood glucose level and total hemocyte count) of sea cucumber and supports the survival of sea cucumber *H. scabra*. Application of seaweed *Gracilaria* sp. under a controlled cultivation system without water exchanges for 30-days rearing period also enhances the specific growth rate of sea cucumbers.

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