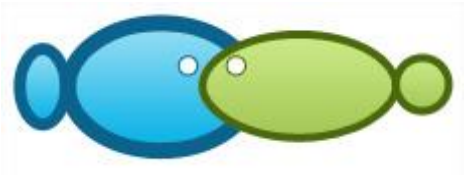

# Production performance and physiological responses of sea cucumber (*Holothuria scabra*) reared using *Penaeus vannamei* pond sediment as a source of nutrients

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**Abstract**. Shrimp pond sediment (SPS) contains a significant quantity of nutrients. The use of SPS as a source of nutrients could prevent the adverse effects of the sediment on the water's ecosystem. The objective of this study was to evaluate the performance and physiological responses of *Holothuria scabra* fed with SPS. Five substrate treatments were prepared based on the percentage of SPS. *H. scabra* held in two tanks and fed with *Sargassum* and seagrass powder mixed were used as a control group to assess the *H. scabra* physiological responses. Two months old juveniles of *H. scabra* (a density of 20 individuals $m^{-2}$) were reared for 40 days in a recirculating aquaculture system, without supplementary food. The highest production performance was observed for the *H. scabra* in 40 SPS:60 BS, with specific growth and survival rates of 4.14±0.30% and 70±10%, respectively. No significant difference in physiological parameters was observed in this treatment compared to the control group ($p>0.05$). Meanwhile, the respiratory burst and phenol-oxidase activity in 20 SPS:80 BS were significantly higher than the control ($p<0.05$). Dissolved oxygen, temperature, and salinity were in good ranges for the maintenance of *H. scabra*, except for the pH value which was fairly high in the early phase of maintenance.
**Key Words**: coelomic fluid, hemocyte count, phenoloxidase activity, respiratory burst.

**Introduction**. The primary aspect to consider in the culture of *Holothuria scabra* (sea cucumber or sandfish) is how to increase the availability of feeds in the sediment substrates to sustain their growth. Sediment substrates provide not only shelter but also a potential source of food. Robinson et al (2013) reported that the incorporation of sand into formulated diets could improve the growth of *H. scabra*.

*H. scabra* is a deposit and filter feeder animal which acquires food by filtering organic matter in sediment substrate. The higher the amount of feed utilized, the faster the growth rates of the animals. Different from shrimps or other types of fish occupying higher trophic levels in the food pyramid, *H. scabra* could utilize a formulated diet containing >25% protein to enhance growth. Juveniles of *H. scabra* could utilize a plant-based artificial diet properly (Giri et al 2017) and decomposed organic matter (Lopez & Levinton 1987). These sources of feeds generally contain low protein levels. The high-energy of artificial feeds was found to be unsuitable for juvenile of *H. scabra*, *Australostichopus mollis*. They tend to consume low-energy sources of nutrients on surface sediments. Fermentation of the carbohydrate of energy sources prior to feeding has been proven to be more effective in consumption of feed with higher energy levels, in increasing digestibility and in producing high growth levels of this species of *H. scabra* juvenile (Slater 2011).

In intensive farming, out of total feeds applied to a pond, 15% is not consumed, 20% becomes fecal material, 48% goes to energy maintenance and only 17% is converted into dry weight (Primavera 1994). Based on this reference, if 1 kg of shrimp

diet contains 4,086.3 kcal of energy, then 1,430.2 kcal from the feed will be unconsumed by shrimps, becoming metabolic waste. The unused organic matter at the bottom of the pond is known as shrimp pond sediment (SPS). The accumulation of sediment in the bottom of the pond adversely affects water quality and stimulates eutrophication when accumulated in the waters because such organic matter is rich in carbon, nitrogen, and phosphate (Sabilu et al, unpublished data). Shrimp pond sediment provides a potential source of food and organic fertilizer for important fish species. Fish occupying the second tropic level in the energy pyramid, such as Holothuroidea, and water plants that serve as a primary producer are the potential consumers of organic material.

*H. scabra*, a genus of *Holothuroidea*, is a commercial aquaculture species. Based on their feeding behavior, it is assumed that the animal could utilize shrimp pond sediment as a source of food. It is expected that the supply of food could influence the production performances of *H. scabra*. The direct contribution of organic matter from sediment waste and artificial feed to the somatic growth of *H. scabra* has been widely reported in previous studies (Slater & Jeffs 2010; Slater et al 2009; Liu et al 2009; Bell et al 2007). Although shrimp pond sediment is a potential low-cost feed source for *H. scabra* growth and physiological response, its exact composition has not been published.

Apart from providing essential nutrients, shrimp pond sediment could also release chemical substances that adversely affect the water quality of the aquaculture site. The carbonic, nitrogen, and phosphorus contents in shrimp pond sediment have the potential for raising water pH and decreasing dissolved oxygen. In fertile water, the decrease of oxygen results in the abundance of micro-organisms. This phenomenon is normally followed by an increase of $CO_2$ concentration, as a result of micro-organisms respiration. As an osmo-conformer, extreme changes in water quality are expected to influence the health status of animals that possess true coelom.

The health status of organisms is generally determined by comparing the range values of measured physiological parameters with healthy organisms. The coelomic fluid is known as the primary cellular component of the immune system in *H. scabra*, it has a homologous role with blood cells in the immune system of vertebrates. Eliseikina & Magalamov (2002) reported that in the coelomic fluid of *Holothuria armata*, the types of coelomocytes are progenitor cells, amoebocytes, vacuolated cells, morula cells, crystal cells and hemocyte cells. Amoebocytes are capable of transporting food nutrients, vacuolated cells participate in osmoregulation, morula cells participate in reproduction activity and hemocyte cells are involved in respiration. The coelomic fluid contains phenoloxidase, which is a crucial immune-related enzyme in invertebrates (Jiang et al 2014). The purpose of this study was to evaluate the production performance and physiological responses of juvenile *H. scabra* fed with shrimp pond sediment.

## Material and Method

***Experimental location***. The present research was conducted at the Laboratory of Marine Science Department of Bogor Agriculture University, Ancol, Indonesia. *H. scabra* was held in 15 glass aquariums (100 x 50 x 50 cm). The research was conducted from July to October 2019.

***Experimental materials***. Two months old *H. scabra* juveniles (average initial weight 2.65±0.09 g) were obtained from Hatchery Institute for Mariculture Research and Fishes Extension (IMRAFE), Gondol, Bali. The juveniles were added into polyethylene packing bags, previously loaded with filtered fresh seawater. Subsequently, the bags were provided with sand substrate, inflated with pure oxygen, and tied with rubber bands. These packaged bags were then placed in styrofoam boxes and sealed up with tape after putting ice in the boxes. Shrimp pond sediment (SPS) was obtained from the intensive pond of vannamei in Langensari Village, Subang, West Java. Beach sand (BS) used in the experiment was collected from Bali coastal area. Proximate analyses of shrimp pond sediment are presented in Table 1.

Table 1

Proximate analyses (%), nutrient quantity (g), and gross energy (kcal) of shrimp pond sediment used in the experiment (dry weight)

| *Nutrient composition* | *% in 100 g SPS[1)]* | *The quantity of nutrient in each treatment (g)* | | | | |
|---|---|---|---|---|---|---|
| | | *10 SPS[1)]: 90 BS[2)]* | *20 SPS[1)]: 80 BS[2)]* | *30 SPS[1)]: 70 BS[2)]* | *40 SPS[1)]: 60 BS[2)]* | *50 SPS[1)]: 50 BS[2)]* |
| Ash content | 45.16 | 451.58 | 903.15 | 1,354.73 | 1,806.31 | 2,257.89 |
| Protein | 9.01 | 90.09 | 180.19 | 270.28 | 360.38 | 450.47 |
| Lipid | 1.91 | 19.15 | 38.30 | 57.44 | 76.59 | 95.74 |
| Crude fiber | 9.07 | 90.70 | 181.40 | 272.10 | 362.80 | 453.50 |
| BETN[3)] | 34.85 | 348.53 | 697.07 | 1,045.60 | 1,394.13 | 1,742.67 |
| GE[4)] (kcal) | 211.90 | 2,118.97 | 4,237.93 | 6,356.90 | 8,475.86 | 10,594.83 |

[1)]SPS-Shrimp Pond sediment; [2)]BS-Beach sand; [3)]BETN-Extract matter without nitrogen; [4)]GE-Gross energy= (g protein x 5.6)+(g fat x 9.4)+(g BETN x 4.1) kcal (Watanabe 1988).

***H. scabra rearing***. A total of 20 *H. scabra* in each treatment was reared under a recirculating aquaculture system (RAS) using a double bottom method. Each treatment was carried out in glass aquariums provided with a 10 kg substrate containing beach sand and pond sediment. The experiment was conducted in five treatments (each with three replicates) based on the concentration of shrimp pond sediment in the substrate: 10% shrimp pond sediment and 90% beach sand (10 SPS:90 BS), 20% shrimp pond sediment and 80% beach sand (20 SPS:80 BS), 30% shrimp pond sediment and 70% beach sand (30 SPS:70 BS), 40% shrimp pond sediment and 60% beach sand (40 SPS:60 BS), and 50% shrimp pond sediment and 50% beach sand (50 SPS:50 BS). The control group consisted in *H. scabra* held in two juvenile tanks. The animals were reared for 40 days without providing other supplementary diets, while juveniles held in tanks were fed with seaweed and seagrass powder mixed in the same proportion. There was no water exchange during the experiment. Freshwater was added in every four days to change evaporation and maintain salinity level to 30 g $L^{-1}$.

***Sampling***. All *H. scabra* individuals were measured for weight on days 1, 10, 20, 30 to 40, respectively. Individual wet weight measurements were taken within 1 minute of removing from the aquarium and sea water was removed by drying them on a sponge (Battaglene et al 1999). Coelomic fluid was taken to evaluate physiological parameters: total hemocyte count (THC), respiratory burst (RB), phenoloxidase (PO), and concentration of glucose. The measurements of these parameters were carried out on days 20 and 40 of the experimental period. Coelemic fluid was collected using a 1 mL syringe through the posterior abdomen. The fluid was randomly obtained from three individuals, for each treatment.

***Growth analysis***. The absolute growth rate (AGR) of *H. scabra* was calculated by subtracting the mean final weight (Wt) to mean initial weight (Wo) and divided by culture period (t). AGR was calculated using the equation recommended by Effendie (1979):

$$\mathrm{AGR} = (\mathrm{Wt} - \mathrm{W0})/\mathrm{t}$$

Specific growth rate (SGR) was calculated using the equation suggested by Huisman (1987):

$$SGR = \left( \sqrt[t]{\frac{Wt}{W0}} - 1 \right) x\ 10$$

The survival rate (SR) was determined using the equation recommended by Huisman (1987):

$$SR = \left(\frac{Nt}{\mathrm{N0}}\right) \mathrm{x}\ 100$$

Where:
SR – the survival rate of *H. scabra*;
Nt, N0 – the final and initial *H. scabra* survival.

***Total hemocyte count***. THC was determined using the method developed by Wang & Chen (2006). Hemocyte cells of coelomic fluid were counted using a hemacytometer. The quantity of hemocyte was calculated using the formula as follows:

$$THC\ = The\ counted\ number\ of\ cell\ x\ \left(\frac{1}{Vol.\ of\ the\ hemacytometer}\right)\ dilution\ factor$$

***Respiratory burst activity***. Respiratory burst activity was quantified using the reduction of nitroblue tetrazolium (NBT) to formazan, following the method suggested by Cheng et al (2004). 50 µL reaction mixture contained coelomic fluid and an anticoagulant was incubated at room temperature for 30 minutes. Then, the mixture was centrifuged at 3000 rpm for 20 minutes. The supernatant fluid was removed and the pellet was added with 100 µL NBT to be incubated for 2 hours at room temperature. The pellet was then centrifuged again at 3,000 rpm for 10 minutes. The supernatant fluid was discarded. The pellet was added with 100 µL methanol absolute and centrifuged at 3000 rpm for 10 minutes. The pellet was subsequently rinsed using 70% methanol, and then added with 120 µL KOH and 140 µL dimethyl sulfonyl oxide (DMSO). The pellet was then deposited on microplates. The optical density at 630 nm was measured using a microplate reader. The respiratory burst was expressed as an NBT reduction in 50 µL coelomic fluid.

***Phenoloxidase activity***. Phenoloxidase activity was measured using a spectrophotometer by recording the formation of dopachrome produced from L-dihydroxy phenylalanine (L-DOPA) (Liu & Chen 2004). Briefly, 1 mL coelomic fluid mixed with anticoagulant was centrifuged at 1,500 rpm for 10 minutes. The supernatant fluid was removed from the pellet and added with 1 mL solution of a cacodylate-citrate buffer. Then, the pellet was centrifuged again at 1,500 rpm for 10 minutes. The supernatant fluid was discarded from the pellet and added with 200 µL cacodylate-citrate buffer. Subsequently, 100 µL suspension was added with 50 µL trypsin as an activator and was incubated at room temperature for 10 minutes. The pellet was added with L-DOPA and 800 µL cacodylate-citrate buffer and allowed to react for 5 minutes. The optical density at 490 nm was measured using a microplate reader. The standard solution used to measure background phenoloxidase activity in all test solutions consisted of 100 µL coelomic fluid suspension, 50 µL cacodylate buffer (to replace the trypsin) and 50 µL L-DOPA. The optical density of phenoloxidase activity for all test conditions was expressed as dopachrome formation in 100 µL coelomic fluid.

***Glucose***. A sample of coelomic fluid was initially collected in a clean Eppendorf tube, and then mixed with an anticoagulant and centrifuged 1000 rpm for 10 minutes to separate plasma from the fluid. Briefly, 0.5 µL of coelomic fluid was added with 3.5 mL orthotoluidin reagent and mixed with glacial acetic acid. The mixtures were then kept in boiling water for 10 minutes and left it cool. The concentration of glucose was measured using a spectrophotometer at 635 nm. The obtained absorbance value was converted into the coelomic glucose level and calculated using the following equation suggested by Wedemeyer & Yasutake (1977):

$$GD = \frac{Abs\ Sp}{Abs\ St}\ x\ GST$$

Where:
GD - concentration of glucose in coelomic fluid (mg $L^{-1}$);
AbsSp - sample absorbance;
AbsSt - standard absorbance;
GST - standard glucose concentration (mg $L^{-1}$).

***Oxygen consumption rate***. Data on oxygen consumption rates were collected to evaluate the availability of dissolved oxygen (DO). *H. scabra* were weighted individually and placed in an external-uncontaminated oxygen respirometer. Water was bubbled to obtain constant DO (>5 mg $L^{-1}$) in the respirometer. The fluctuation of DO was recorded every minute for 1 hour. Oxygen consumption rate was calculated using the following formula as suggested by Liao & Huang (1975):

$$TKO = \{(\Delta DO)/W \times t\} \times V$$

Where:
TKO - oxygen consumption rate (mg $O_2$ $g^{-1}$ $h^{-1}$);
ΔDO - the amount of consumed oxygen for 60 minutes (mg $L^{-1}$);
W - weight (g);
T - observation period (hr);
V - water volume in respirometer (L).

***Statistical analysis***. Data on water quality parameters were analyzed descriptively, while growth and health status of *H. scabra* were analyzed using one-way ANOVA with Tukey post hoc tests at the significance of $p<0.05$.

**Results and Discussion**

***Survival rate***. The survival of juvenile *H. scabra* attained 100% in five different treatments over 20 days. At the end of the experiment, we found that the *H. scabra* survival rate fluctuated according to the SPS concentration in the total weight of substrate: 83.33±15.27% at 10% SPS, 76.67±5.77% at 30% SPS, 70.0±10.0% at 40% SPS, 70.0±10.0% at 50% SPS and 26.67±5.77 at 20% SPS (Figure 1).

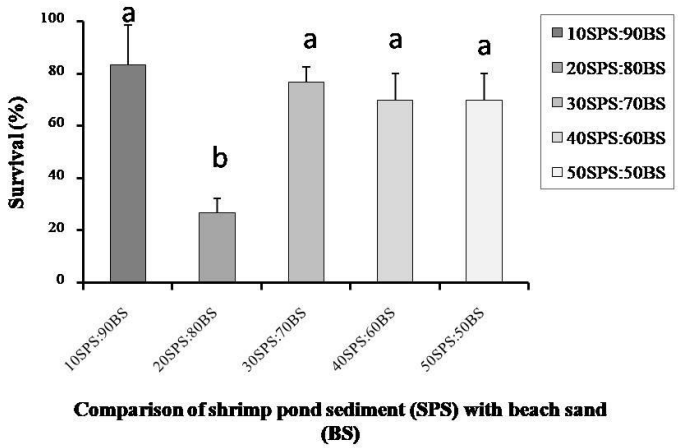


Figure 1. Survival of juvenile *Holothuria scabra* fed with different concentrations of shrimp pond sediment. 10% SPS concentration from the total weight of substrate (10 SPS:90 BS), 20% (20 SPS:80 BS), 30% (30 SPS:70 BS), 40% (40 SPS:60 BS) and 50% (50 SPS:50 BS). Significant differences from different treatments are given as $P<0.05$. Bar represents standard deviation.

Different concentrations of SPS significantly influenced the survival of the *H. scabra* ($p<0.05$). The lowest survival was found in juveniles reared on 20 SPS:80 BS and was significantly different from the other four treatments ($p<0.05$). However, there was no significant difference among treatments containing 10, 30, 40, and 50% SPS ($p>0.05$).

***Growth rate***. The growth of juvenile *H. scabra* reared for 40 days is provided in Table 2. Final body weight, daily growth, and specific growth rates were significantly higher in juveniles reared on 40 SPS:60 BS and 50 SPS:50 BS compared to the other treatments.

Table 2

Growth of juvenile *Holothuria scabra* fed with different concentrations of shrimp pond sediment

| *Parameter* | *Concentrations of shrimp pond sediment and beach sand* | | | | |
|---|---|---|---|---|---|
| | *10 SPS: 90 BS*[4] | *20 SPS: 80 BS*[5] | *30 SPS: 70 BS*[6] | *40 SPS: 60 BS*[7] | *50 SPS: 50 BS*[8] |
| Initial body weight (g) | 2.60±0.10 | 2.63±0.06 | 2.60±0.10 | 2.70±0.10 | 2.73±0.06 |
| Final body weight (g) | 8.71±1.34$^{a}$ | 8.24±0.39$^{a}$ | 11.32±1.67$^{a}$ | 19.11±2.02$^{b}$ | 19.06±1,53$^{b}$ |
| ΔW[1] | 6.11±1.34$^{a}$ | 5.61±0.37$^{a}$ | 8.72±1.34$^{a}$ | 16.41±1.97$^{b}$ | 16.33±1.54$^{b}$ |
| GR[2] (g day$^{-1}$) | 0.15±0.03$^{a}$ | 0.14±0.01$^{a}$ | 0.22±0.04$^{a}$ | 0.41±0.05$^{b}$ | 0.41±0.04$^{b}$ |
| SGR[3] (%) | 2.07±0.35$^{a}$ | 1.92±0.10$^{a}$ | 2.71±0.46$^{a}$ | 4.14±0.30$^{b}$ | 4.10±0.29$^{b}$ |

[1)] ΔW-increase in final weight;[2)] GR-daily growth rate ;[3)] SGR-Specific Growth rate;[4)] 10 SPS:90 BS (10% shrimp pond sediment and 90 % beach sand);[5)] 20 SPS:80 BS (20% shrimp pond sediment and 80% beach sand);[6)] 30 SPS:70 BS (30% shrimp pond sediment and 70% beach sand);[7)] 40 SPS:60 BS (40% shrimp pond sediment and 60% beach sand);[8)] 50 SPS:50 BS (50% shrimp pond sediment and 50% beach sand);[*)] Different notation letters in the same line are significantly different (P<0.05) among treatments.

***Physiological response***. Total hemocyte count in juveniles reared on 10SPS:90BS and 20 SPS:80 BS on day 20 was lower, reaching 4.37±2.60 and 5.43±0.84 cell mm$^{-3}$, respectively, compared to the control group (6.93±0.78 cell mm$^{-3}$). Whereas, juveniles reared on 30 SPS:70 BS, 40 SPS:60 BS, and 50 SPS:50 BS showed higher THC attaining 7.50±1.21, 8.13±1.00 and 9.67±2.08 cell mm$^{-3}$, respectively (Figure 2). Differences between different treatments were determined against a significance level of P<0.05.

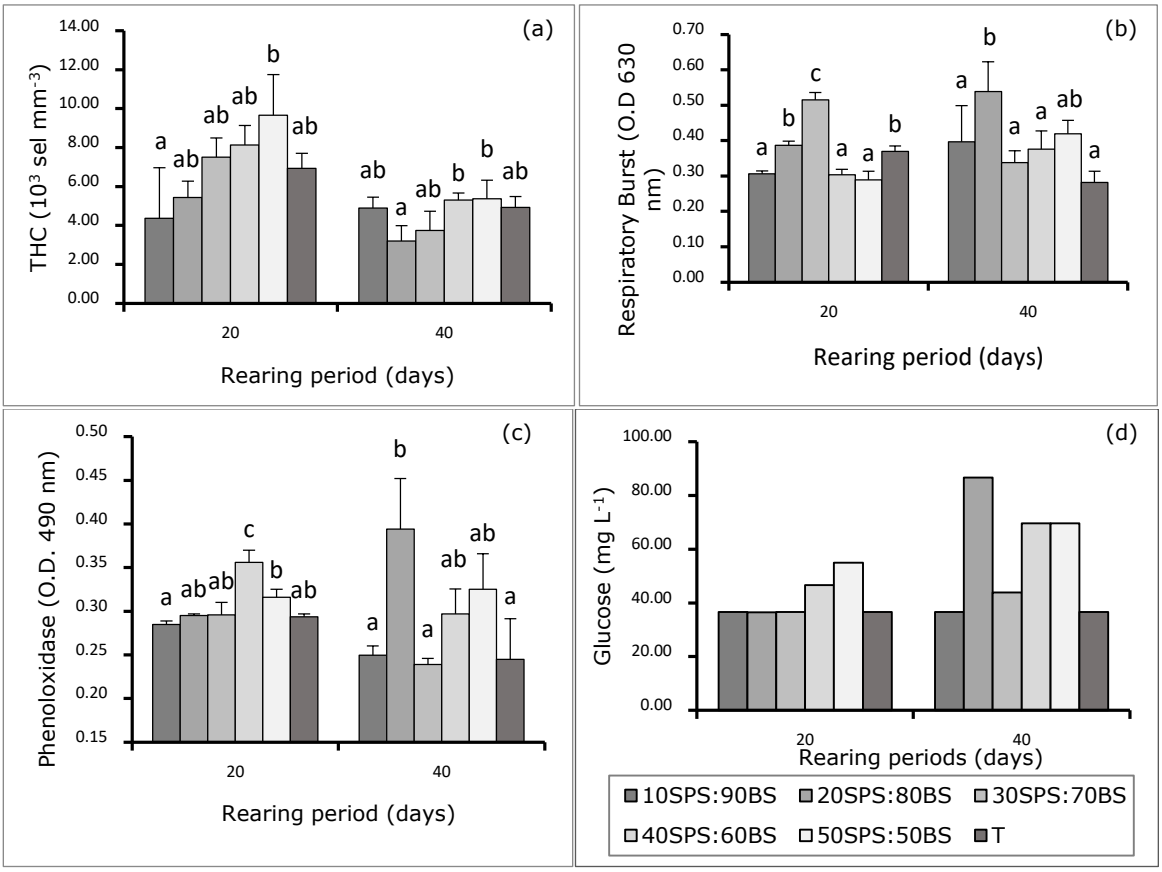


Figure 2. Physiological conditions of coelomic fluid in *Holothuria scabra* fed with different concentrations of SPS on days 20 and 40. (a) Total hemocyte count (THC), (b) phenoloxidase activity, (c) respiratory burst activity, (d) glucose concentration. 10% SPS concentration from the total weight of substrate (10 SPS:90 BS), 20% (20 SPS:80 BS), 30% (30 SPS:70 BS), 40% (40 SPS:60 BS), 50% (50 SPS:50 BS) and control group (T). Bar represents standard deviation.

THC on day 20 and 40 in 10 SPS:90 BS was 4.37±2.6 cells $mm^{-3}$ and 4.9±0.56 cells $mm^{-3}$, in 20 SPS:80 BS was 5.43±0.84 cells $mm^{-3}$ and 3.72±0.79 cells $mm^{-3}$, in 30 SPS:70 BS was 7.50±1.21 cells $mm^{-3}$ and 3.73±0.60 cells $mm^{-3}$, in 40 SPS:60 BS was 8.13±1.0 cells $mm^{-3}$ and 5.3±0.36 cells $mm^{-3}$, in 50 SPS:50 BS was 9.67±2.08 cells $mm^{-3}$ and 5.37±0.96 cells $mm^{-3}$, and control group was 6.93±0.78 cells $mm^{-3}$ and 4.93±0.55 cells $mm^{-3}$. There was a reduction in THC on day 40. The THC was significantly lower for *H. scabra* for 20 SPS:80 BS than for 40 SPS:60 BS and 50 SPS:50 BS ($p<0.05$). No significant difference in THC was observed between the control group and five treatments ($p>0.05$).

The highest phenoloxidase activity (OD: 0.356±0.014) in *H. scabra* was observed at 40% SPS on day 20 and was significantly different from the other treatments and control group. On day 40, phenoloxidase activity was significantly higher for 20 SPS:80 BS than in the control group ($p<0.05$). The activity was reduced to 0.297±0.041 mg $L^{-1}$ at 40% SPS and was not significantly different from the control group ($p>0.05$).

The highest respiratory burst (OD: 0.52±0.02) occurred for the *H. scabra* at 30 SPS:70 BS on day 20 and was significantly different from the control group (OD: 0.37±0.02) and the other 4 treatments. On day 40, the observed respiratory burst was higher for 20 SPS:80 BS and was significantly different from 30 SPS:70 BS, 40 SPS:60 BS, 10 SPS:90 BS and control group ($p<0.05$).

The highest concentration of glucose on day 20 was obtained for 50 SPS:50 BS (54.94 mg $L^{-1}$) and 40 SPS:60 BS (46.50 mg $L^{-1}$), while the other treatments and control group was 36.63 mg $L^{-1}$. On day 40, the highest concentration of glucose was found in 20 SPS:80 BS (86.63 mg $L^{-1}$), followed by 40 SPS:60 BS and 50 SPS:50 BS (69.59 mg $L^{-1}$), 30 SPS:70 BS (43.95 mg $L^{-1}$), and the lowest was 10 SPS:90 BS and control group (36.63 mg $L^{-1}$).

***Oxygen consumption rate***. The oxygen consumption in *H. scabra* was 0.26±0.05 mg $O_2$ $g^{-1}$ $h^{-1}$ (Figure 3). Dissolved oxygen in the respirometer increased between 0.1 and 0.4 mg $L^{-1}$ when it reached 2.4 mg $L^{-1}$.

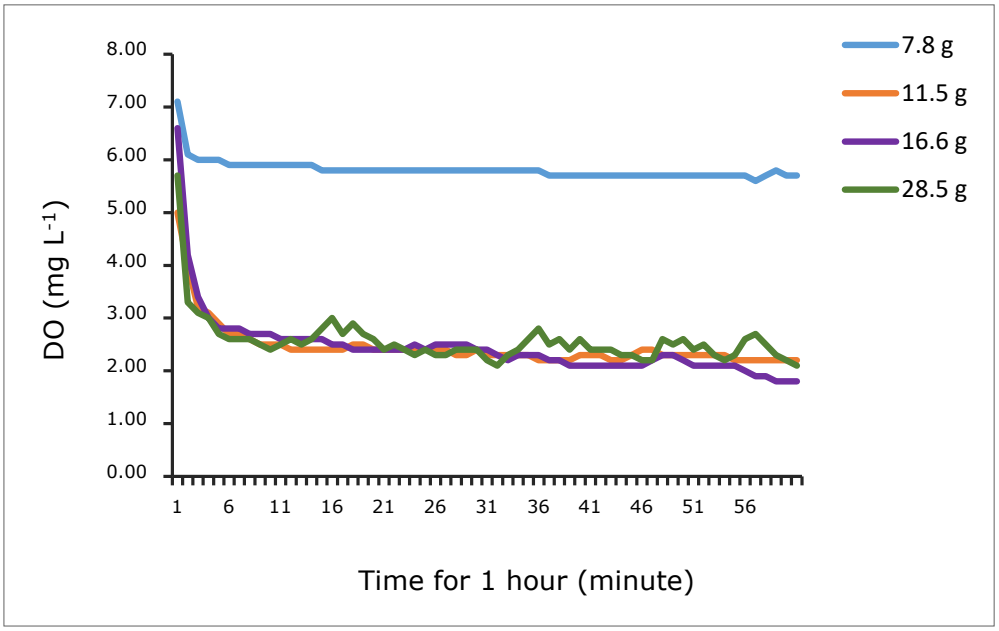


Figure 3. Oxygen consumption rates of *Holothuria scabra* were measured for 1 hour.

***Water quality***. The measurements of water quality parameters (temperature, pH, DO, and salinity) during the experimental period were presented in Table 3.

Table 3

Temperature, pH, DO, and salinity of rearing media during the experimental period

| *Variable* | *The concentration of shrimp pond sediment and beach sand* | | | | |
|---|---|---|---|---|---|
| | *10 SPS: 90 BS[1)]* | *20 SPS: 80 BS[2)]* | *30 SPS: 70 BS[3)]* | *40 SPS: 60 BS[4)]* | *50 SPS: 50 BS[5)]* |
| | Temperature ($^0$C) | | | | |
| In the morning | 26.7±0.6 | 26.9±0.5 | 26.6±0.6 | 26.7±0.6 | 26.9±0.6 |
| In the afternoon | 31.3±0.8 | 31.6±0.7 | 31.3±0.7 | 31.0±0.5 | 31.3±0.5 |
| Range | 26.0-32.3 | 26.2-32.2 | 25.9-32.1 | 25.8-31.7 | 26.1-32.1 |
| | | pH | | | |
| In the morning | 7.63-9.01 | 7.76-8.85 | 8.06-8.99 | 7.83-8.88 | 8.01-8.96 |
| In the afternoon | 8.34-9.39 | 8.19-9.31 | 8.39-9.40 | 8.34-9.29 | 8.31-9.23 |
| DO (mg L$^{-1}$) | 4.1-6.5 | 3.5-6.7 | 4.3-6.8 | 4.1-5.9 | 4.3-7.1 |
| Salinity (g L$^{-1}$) | 30-34 | 30-34 | 30-34 | 30-34 | 30-34 |

[1)]10SPS:90BS (10% shrimp pond sediment and 90% beach sand);[2)]20SPS:80BS (20% shrimp pond sediment and 80% beach sand);[3)] 30SPS:70BS (30% shrimp pond sediment and 70% beach sand);[4)]40SPS:60BS (40% shrimp pond sediment and 60% beach sand);[5)]50SPS:50BS (50% shrimp pond sediment and 50% beach sand).

In the early periods of the experiment, the level of water pH ranged from 9.18 to 9.35. Then, the level gradually reduced to 7.6-8.5 from day 5 to the end of the experiment. Different patterns, however, were seen in 20% SPS where pH level on days 20 and 40 increased to 8.62±0.17 and 8.85±0.17, respectively.

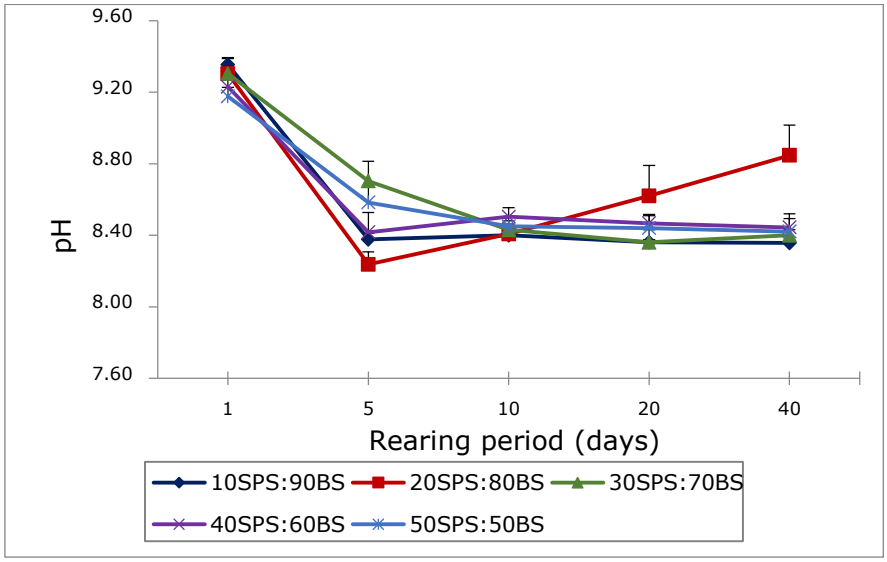


Figure 4. Water pH over the experimental period. Bar represents standard deviation.

**Discussion**. Shrimp pond sediment can be used for *H. scabra* diets due to the fairly high quantity of nutrients. Protein concentration levels in organic matter resulted from microbial decomposition (3-12%) were lower than in the formulated diet for *P. vannamei* culture (>30%; SNI 2006) and higher than in the sand sediments (0.21±0.01%). Rearing *H. scabra* using shrimp pond sediment as a source of diet showed a significantly high survival rate, ranging from 70 to 83.3%, except for data collected for 20% SPS, which is considered anomalous. Survival was significantly lower ($p<0.05$) in *H. scabra* held in 20 SPS:80 BS (26.67%) due to a high mortality level on day 35, only occurred in this treatment. In total, the average mortality rate of *H. scabra* was 56.67%, where 40% in the first range of essays, 50% in the first replication, and 80% in the last replication three, which may be due to the long exposure to high water pH. *H. scabra* is an osmoconformer, sensitive to changes in water pH (Collard et al 2014).

An increase in body weight, ranging from 5.61±0.37–16.41±1.97 g, during 40 days experimental period showed that the use of SPS as a diet can enhance the growth of *H. scabra*. The highest body weight was recorded in 40 SPS:40 BS and 50 SPS:50 BS that attained 16.41±1.97 g and 16.33±1.54 g, respectively. *H. scabra* can grow rapidly with food sources in the form of organic fraction of ingested sediment. The SGRs of *H. scabra* were different among the treatments. On day 10, the highest SGR was recorded in *H. scabra* fed with 10% SPS followed by 30, 20, 40 and 50%. On day 20, the animals fed with 40% SPS showed a higher SGR than the other treatments. However, there was no significant difference in all treatments between days 10 and 20 ($p>0.05$). These results mean that the ability of SPS in all treatments to provide nutrients was relatively the same until day 20. An increase in weight of juvenile *H. scabra* during the early rearing periods is the consequence of their transfer from rearing tanks to an aquarium containing very good substrates. Such a phenomenon is also observed at stunted juvenile of *H. scabra* reared on high densities, then transferred to low densities, where they grow normally (Battaglene et al 1999). This suggested that *H. scabra* may grow faster when they are cultured in favorable conditions, where nutrients are available at sufficient quantity. SGRs on day 40 showed the same pattern as those on day 30, where juvenile *H. scabra* reared on 40 and 50% SPS: SGR was significantly higher ($p<0.05$) than in juveniles reared on 30, 20, and 10% of SPS. At the end of the experiment, although juveniles reared on 40% SPS had better performances in weight, daily growth, and specific growth rate compared to 50% SPS, the two treatments were not significantly different ($p>0.05$). This proved that SPS-enriched substrates affected the concentration of nutrients, and subsequently the body weight. One kilogram of SPS contains 63.7-176.0 g organic carbon, 13.4-19.4 g nitrogen and 2.7-4.3 g phosphorus (Sabilu et al, unpublished data). Deposit-feeding animals use carbon to gain energy for maintenance and nitrogen for growth (Lopez & Levinton 1987).

The bodyweight decrease in *H. scabra* held in 10 SPS:90 BS on day 30 and 20 SPS:80 BS, as well as 30 SPS:70 BS on day 40. This suggested that energy for basal metabolism and normal activities were derived from protein deposited in the body, possibly due to: 1) low number of nutrients contained in the substrates, 2) a poor water quality status and 3) a poor *H. scabra* condition. On day 35, the death of *H. scabra* held in 20 SPS:80 BS indicated a severe deterioration of the physiological condition. The function of the coelomic fluid is the first line in immune defense in responding to infection or extreme changes in external conditions (Prompoon et al 2015). Matranga et al (2005) reported that coelomic fluid plays a role in defense mechanisms such as phagocytosis, encapsulation, bacterial clearance, or other foreign agents and oxygen transportation, activated by adverse external conditions.

The examination of coelomic fluid on day 40 revealed that: 1) the least total hemocyte count was found in *H. scabra* held in 20% SPS, 2) the highest levels of phenoloxidase, respiratory burst, and glucose were obtained at *H. scabra* held in 20% SPS. Total hemocyte count is an important parameter for respiration and the immune system of *H. scabra*, and the quantity may vary due to infections and exposure to extreme environmental factors. A high quantity of hemocyte during the early phase of experimental period was a response to an increase in pH value, while a low quantity of hemocyte for *H. scabra* held in 20% SPS occurred in day 40 may have been due to insufficient nutrient supply. This can happen because of prolonged stress leading to low feed intake and/or stress response caused by a wound in the posterior part making the animals unable to collect food from substrates (Figure 5).

Phenoloxidase and respiratory burst activity were significantly higher than in the control group on day 40. This strengthens our assumption that *H. scabra* placed in 20 SPS:80 BS was under stress conditions. Thomas (2017) reported that when *H. scabra* is infected or exposed to extreme environmental factors, oxygen absorbance level increased in coelomic fluid, including lymphocyte, phagocyte, spherocytes and giant cells. It was associated with the release of a high amount of reactive oxygen species (ROS), anion superoxide ($O_2^-$), and anion peroxide ($H_2O_2$) as immune responses. In this situation, oxygen is not only used for respiration but it is also a microbiocidal production agent. The oxidation of glucose was involved in the regeneration of the consumed NADPH through

oxygen reduction for producing a microbiocidal agent. Prolonging stress leads to energy loss, and death may eventually occur because the animals are unable to acquire necessary energy to maintain the physiological function of their body.


Figure 5. a) Juvenile of *Holothuria scabra* in health condition, b) *Holothuria scabra* infected with lesion observed on day 35 at 20 SPS:80 BS (original).

Different physiological responses exhibited in the five treatments may be affected by water pH. The level of pH for all treatments during the early phase of the experimental period increased following the addition of SPS in rearing media. The level then gradually decreased and returned to normal. Ecologically, such conditions may be closely related to the association of microbe with sediment and water. Prokopenko et al (2006) reported that microbe plays an important role in reducing ammonium ($NH_4$-N) and oxidizing sulfide. Mudatsir (2007) reported that factors affecting the association of microbe with sediment as well as water are temperature, conductivity, salinity, and total organic matter. The high level of pH during the early phase of experimental period is affected by the following factors: 1) High use of oxygen by respiratory burst activity of cells in a coelomic fluid to produce reactive oxygen species (ROS), anion superoxide ($O_2$), and anion peroxide ($H_2O_2$) caused by extreme changes in water parameters following the addition of SPS (Thomas 2017); 2) The activity of aerobic bacteria using the organic material of sediment is leading to a high concentration of $CO_2$ in water; 3). The role of bio-turbator played by *H. scabra* (Mc-Call & Tevesz 1982), which increases calcium carbonate ($CaCO_3$) concentration at the bottom. Carbonate and hydroxide ion reacts to hydrogen, leading to a decrease of acidity and an increase in pH values.

An anomaly of water pH obtained in treatment 20 SPS:80 BS may result from the difference in chemical composition of substrate sediment. In our experiment, analysis of the chemical composition of beach sand and microbial characteristics were not undertaken. Nevertheless, the addition of beach sand from Bangka island waters as a substitution of beach sand from the Bali island waters (BS), unavailable in 20SPS:80BS, containing a high concentration of silica may affect water pH anomaly in this treatment. Malau (2014) reported that Bangka sand is rich in silica (77.54%). High silica content in the sand indicates a high quantity of active groups of silica anion, leading to the high binding cations of silica. Moreover, sand sediment also influences water conductivity. A decrease in cation and an increase in silica anion results in higher pH values (Ikhsan et al 2015). Water conductivity is a limiting factor to microbial population in the water (Abdel et al 2004; Estella et al 2004), while the microbial decomposition in sediment and water may cause the anomaly of water pH in *H. scabra* held in 20 SPS:80 BS.

Daily water temperature has fluctuated in all treatments to ±5$^o$C. The lowest water temperature was observed in the morning ranging from 26-27.8$^o$C, and the highest was found in the afternoon in the range of 30-32.3$^o$C. The temperature may affect the growth of *H.scabra* in their feeding behavior. In the present study, the presence of *H. scabra* in the surface area of substrates occurred at 02.30 p.m., suggesting that the animals were stimulated to begin foraging activity when water temperature was ±31$^o$C. In contrast, burrowing behavior occurred at 03.00 a.m., indicating that the animals

tended to hibernate at low temperatures (±27°C). Nevertheless, metabolism continuity was indicated by the presence of feces. Kuhnhold et al (2017) reported that juvenile *H. scabra* are capable of sustaining their energy balance and oxygen consumption within the homeostatic range, even at 33°C. Lavitra et al (2010) reported that juvenile *H. scabra* can survive at 39°C water temperature, above which they become weak, and die at 41°C. This strengthens our argument that the fluctuation of daily water temperature was not the cause of the death of cultured *H. scabra*.

The requirement for oxygen consumption was 0.23±0.08 mg $O_2$ $g^{-1}$ $hr^{-1}$. The increase of oxygen ranged from 0.1 to 0.4 mg $L^{-1}$ in the respirometer when DO reached 2.4 mg $L^{-1}$ indicated that the specimen released oxygen to maintain homeostatic conditions. We assume that *H. scabra* was under a critical condition when DO reached 2.4 mg $L^{-1}$, particularly concerning the capacity to use shrimp pond sediments as food. However, in the present study DO levels were in the range of 3.5-7.1 mg $L^{-1}$ in all five treatments, which are appropriate for the maintenance of *H. scabra*.

**Conclusions**. The highest production performance was observed for the *H. scabra* fed with 40% shrimp pond sediment (40 SPS:60 BS), with the specific growth rate and survival of 4.14±0.30% and 70±10%, respectively. Moreover, the health status of *H. scabra* placed in 40 SPS:60 BS was not significantly different from *H. scabra* fed with the mixture of seaweed and seagrass powder, which had THC, phenoloxidase, respiratory burst and glucose levels of 5.30±0.36 cells $mm^{-3}$, OD: 0.30±0.04, OD: 0.38±0.05 and 69.9 mg $L^{-1}$, respectively.

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