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Short Communication

Survival Rate and Growth Performance of *Holothuria scabra* Towards Different Stocking Densities and Feeding with *Spirulina*

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ABSTRACT

Holothuria scabra is widely used in traditional medicine or consumed as a healthy or exotic food, causing wild stocks to decrease. Therefore, aquaculture is the best solution to address this issue and support the growing market demand. However, up to now, *H. scabra* production is facing problems related to the nursery phase, survival rate of larvae and juveniles, production cost, and broodstock for mass production. In this study, the juveniles of *H. scabra* were divided into three different stocking densities and fed with 1 g of dissolved *Spirulina* powder once on alternate days. Their length was recorded every two weeks. After six weeks, the juveniles reared with 100 individual densities showed the highest survival rate at 80% compared to 200 and 400 stocking densities. Meanwhile, every tank showed a positive growth rate, indicating that *Spirulina* powder could potentially promote the growth of juvenile *H. scabra*. The specific growth rate for stocking density at 100, 200, and 400 were 1.2, 0.86, and 1.37%/day, respectively. In conclusion, the optimum initial stocking density is between 100 and 200 individuals for a 1-ton fibreglass tank with a 500 L water capacity. The *Spirulina* can be used as the main protein source as compared to other diets for juvenile *H. scabra*.

Keywords: *Holothuria scabra*, sandfish, sea cucumber, *Spirulina*, stocking density

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INTRODUCTION

Holothuria scabra Jaeger, also known as sandfish and locally known as 'trepang' and 'gamat pasir' in Malaysia, is a species of sea cucumber from the family Holothuriidae (Kamarudin et al., 2019). Holothurians are invertebrate species without a backbone and inhabit coastal areas with sandy bottoms. People believe this sea cucumber is highly medicinal and widely used in traditional medicine or consumed as a healthy or exotic food. However, the demand for it has increased over time, and until today, the current stock of sandfish cannot support the market demand, neither locally nor internationally. Uncontrolled harvesting of wild sandfish has led to overexploitation, resulting in this species being listed as endangered (EN) under the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Barclay et al., 2017; Battaglene & Bell, 2004; Han et al., 2016).

Aquaculture is considered the best solution to address sandfish's declining wild stock issue and support market demand. The technique for producing *H. scabra* in hatcheries was developed in early 2000 and has now become one of the new target species in aquaculture (Battaglene & Bell, 2004). However, up to date, *H. scabra* production has faced problems related to the nursery phase, the survival rate of larvae and juveniles, production cost, and broodstock for mass production. A study on the biological life cycle of *H. scabra* has been done, and new technology has been developed to cope with the life cycle stage in aquaculture. The early stages of culturing *H. scabra* (larvae and juveniles) are the most critical because of the low survival rate (Indriana et al., 2017). When the larvae of *H. scabra* develop into the second stage (aurikularia to doliolaria), they need more space for attachment or settlement on substrates like tank walls. The technology for site attachment was developed by using stacked plates and sand in rearing tanks (Altamirano et al., 2017; Sitoresmi & Pursetyo, 2020). However, the survival rate of juvenile *H. scabra* at the grow-out phase (3–15 g) is still low, with only 10–30% after being released in sea pens (Purcell, 2004).

Stocking density is the most important factor for the survival rate and growth performance of *H. scabra* at all rearing levels, from larval, juvenile, and grown-up levels. The survival rates are inversely related to the stocking density. A study showed that the environment or nature of the hatchery did not affect its growth, but the stocking density influenced its growth (Lavitra et al., 2010). At the larval level, many studies suggest the stocking density of the larvae is between 1 and 1.5 per ml (Abidin et al., 2019; Asha & Diwakar, 2013). At the juvenile level, studies showed significantly faster growth and higher survival in low densities compared to high densities (Altamirano & Noran-Baylon, 2020; Cantero et al., 2016; Gorospe et al., 2017). Besides, the growth rate of the juveniles was stunted after being held at higher densities (Battaglene et al., 1999).

In the sea pen and earthen pond, the food for *H. scabra* depends on the naturally occurring microorganisms and algae. However, in the hatcheries, artificial feed is provided for *H. scabra*. The study on the effect of *Sargassum* sp. on juvenile growth performance has been explored. The result showed positive growth performance, but other issues related to the exploitation of seaweed as a source of artificial feed will affect sustainable aquaculture development (Magcanta et al., 2021). To face this issue, *Sargassum* sp. was replaced with a mixture of seagrass (*Enhalus acoroides*), Napier (*Pennisetum purpureum*), and cow manure as artificial feed for juveniles, which showed a positive result in growth performance (Indriana et al., 2017).

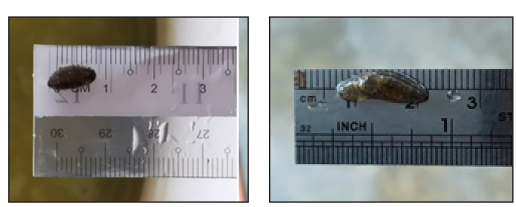
Another study using corn leaf in the diet of *H. scabra* as a substitute for seaweed also showed positive effects on the specific growth rate without any negative effects on weight gain or body composition (Wu et al., 2015). *Spirulina* has been recognised as one of the commercial diets for sea cucumbers. Some successful large-scale nurseries used *Spirulina* to feed the juveniles and broodstock of *H. scabra* (Militz et al., 2018; Simoes & Knauer, 2012). However, some claimed that no significant difference was observed in the growth in length and weight of the juveniles fed with varying proportions of *Spirulina* (Asha et al., 2004). Therefore, this study aims to obtain the best survival rate of juvenile *H. scabra* reared at three different stocking densities and to determine the potential of *Spirulina*'s effect on the growth of juvenile sandfish.

MATERIALS AND METHODS

Survival Rate at Different Stocking Densities

The origin of the *H. scabra* brood stocks was Johor, Malaysia. These mature stocks were induced using the algae bath method (Abdelaty et al., 2021). The juveniles from the same batch with an initial mean length of 1 cm (± 0.19 cm) aged 42 days were used as in Figure 1(a). One-ton fibreglass tanks were used to rear the juveniles, and 500 L of salt water were filled into each tank. Then, 1 g of *Spirulina* powder (Ocean Star International, USA) was dissolved into each tank and allowed to settle completely at the bottom. After that, the juveniles were reared for six weeks under three different stocking densities, namely 100, 200, and 400 individuals per tank. Each treatment was conducted in triplicate. The total number and initial length of juvenile sandfish were recorded every 2 weeks from the first week until the sixth week. The survival rate (SR) of juvenile *H. scabra* was measured after 42 days of culture based on the equation below:

$$\mathrm{SR} = \left(\frac{\mathrm{Nt}}{\mathrm{No}}\right) \times 100\%$$



(a) (b)

Figure 1. The size of juvenile *Holothuria scabra* : (a) Before; and (b) After 42 days rearing in the tank

where SR calculated the percentage survival of juvenile *H. scabra* (Nt), which is the final number of individuals left in the experiment tank after 42 days, and 'No' represents the initial number of individuals stocked at the beginning of the experiment. Statistical analysis was calculated using a one-way analysis of variance (ANOVA, SPSS 27.0). The results were considered significant if the p-value < 0.05.

Specific Growth Rate with *Spirulina* Feeding

The cultured juveniles in each tank were fed 1 g of *Spirulina* powder (Ocean Star International, USA) dissolved in 1 L of seawater once every two days. A 50% (250 L) seawater exchange was conducted before the feeding sessions. For control, the juveniles were not fed any algae. The specific growth rate (SGR) of juvenile *H. scabra* was estimated after 42 days of culture based on the equation:

$$\mathrm{SGR} = \frac{100(\mathrm{In\ Lt} - \mathrm{In\ Lo})}{\mathrm{t}}$$

where SGR calculated the percent growth of juvenile *H. scabra* per day, 'Lt' and 'Lo' represent the final length (cm) and initial length (cm), and t is the rearing period throughout the experiment period.

Each juvenile was reared under ambient conditions, and four aeration points were set up for every tank. Water quality monitoring was conducted weekly. Statistical analysis was calculated using one-way ANOVA (SPSS 27.0). The results were considered significant if the p-value < 0.05.

RESULTS AND DISCUSSION

Survival Rates

The survival rates of juvenile *H. scabra* for all the stocking densities at 100, 200, and 400 individuals per tank showed decreased per cent survival during the rearing period. There was a more than 50% survival rate of juvenile *H. scabra* in all the treatment tanks starting from the first day of the experiment until the end (Figure 2). Treatment A (100 individuals) showed the best survival rate after the 42-day rearing period, with 80 (± 1.15%) survival rate and 80 individuals surviving on average. As for Treatment B (200 individuals), 143 individuals, on average, survived up to the end of the rearing period with 71.5 (± 0.87%) survival. Treatment C (400 individuals) showed a 53.75 (± 8.22%) survival rate, with an average of 215 individuals left from the initial stocking density. A comparison of these three treatments showed that

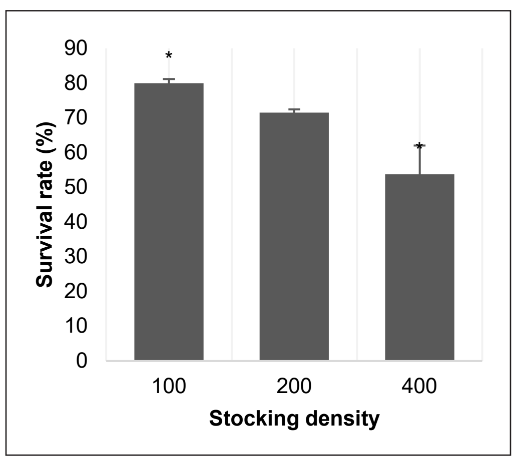


Figure 2. Survival rates ± standard deviation for the different stocking densities of juvenile *Holothuria scabra* after 42 days of culture
Note. * = The results were considered significant if the p-value < 0.05

juvenile *H. scabra* had the highest survival rates at a stocking density of 100. In contrast, a stocking density of 400 showed the lowest survival rate. The survival rate for Treatment A was significantly higher than that of Treatment C, with a *p*-value of 0.006.

The juvenile stage of *H. scabra* can attach to the walls of the tank, which act as substrate. They fed on the food attached to the tank wall and bottom (Ridwanudin et al., 2018). Competition for space and food occurred, resulting in decreased juvenile survival rates due to increased juvenile *H. scabra* in the nursing tanks (Cantero et al., 2016). Previous studies on the stocking density of juvenile *H. scabra* showed that when three different stocking densities (100, 200, and 400) were used for rearing in the ocean-based floating hapa, the lowest stocking density exhibited the highest survival rate (Yussuf & Yahya, 2021). The total area of the tank covered with water was 2.5 m^2. For Treatment A (100 individuals), every juvenile had 0.025 m^2 space, while Treatment B (200 individuals) was 0.0125 m^2/individual, and Treatment C was 0.00625 m^2/individual. The movement of *H. scabra* is quite slow, but there is high competition for space and food. The monitored water quality showed normal levels for salinity, pH, temperature, ammonia, nitrate, and dissolved oxygen without drastic changes for the duration of the experiment, as recorded in Table 1.

Growth Performance

Generally, juveniles reared at all three different stocking densities showed positive growth in all the treatment tanks. According to Table 2, the specific growth rate for Treatment A was 1.19% growth/day, while Treatments B and C showed SGR of 0.86 and 1.36%/day, respectively. The initial mean length of juvenile *H. scabra* for Treatment A was 1.1 (± 0.27) cm, and at the

Table 1
Average water parameter levels for all the treatment tanks

Parameter	Range
Salinity (ppt)	28.00 ± 0.51
pH	8.05 ± 0.09
Temperature (°C)	29.0 ± 1.1
Ammonia (ppm)	0.05 ± 0.01
Nitrate (ppm)	0.01 ± 0.01
Dissolve oxygen (ppm)	4.82 ± 0.20

Table 2
Specific growth rates calculated for each treatment

Treatment (Individual)	Mean length (cm)				Specific growth rate (%/day)
Days	0	14	28	42	
A (100)	1.1 ± 0.27	1.34 ± 0.17	1.75 ± 0.43	1.82 ± 0.38	1.19*
B (200)	0.96 ± 0.54	1.18 ± 0.53	1.31 ± 0.51	1.38 ± 0.38	0.86
C (400)	0.71 ± 0.26	1.06 ± 0.61	1.22 ± 0.50	1.26 ± 0.51	1.36*
Control	1.43 ± 0.46	1.18 ± 0.65	1.09 ± 0.77	0.84 ± 0.56	-1.26

Note. The experimental data represented as mean length ± standard deviation; * = The results were considered significant if the *p*-value < 0.05

end of the experiment, it was 1.82 (± 0.38) cm, respectively. While the initial mean lengths of Treatments B and C were 0.96 (± 0.54) and 0.71 (± 0.26) cm, respectively. After the cultured day, 1.38 (± 0.38) and 1.26 (± 0.51) cm were recorded at the end of the experiment for B and C, respectively. It means *Spirulina* powder showed positive results in feeding the juvenile *H. scabra* in all treatments. The SGR for Treatments A and C were significantly higher than the control, with *p*-values of 0.049 and 0.040, respectively.

Several types of food were used as alternative foods to feed the post-metamorphic juvenile *H. scabra*, such as *Nannochloropsis* sp., *Chaetoceros calcitrans*, *Chaetoceros gracilis*, *Isochrysis galbana*, and *Tetraselmis chuii* (Abdelaty et al., 2021; Abidin et al., 2019). *Sargassum latifolium* gave the best growth when the juveniles attained 73 mm in 8 weeks from an initial size below 10 mm (Magcanta et al., 2021). However, the issue of using *Sargassum* sp. is the exploitation of seaweed sources as food for sea cucumbers, and artificial feed is a good substitute for the sustainability of aquaculture development at a commercial level (Wu et al., 2015). Besides, Dabbagh (2012) has compared the growth performance of *H. scabra* treated with four commercially available feeds (such as Algamac 2000, Algamac protein plus, *Spirulina*, and *Dunaliella* gold). Although the growth rate of *H. scabra* treated with *Spirulina* powder was slower compared to others, a lower mortality rate was recorded.

The dry *Spirulina* powder on the market has a high protein content, with 60% crude protein present, and is the best source to complement the main diet for sandfish (Abdelaty et al., 2021). In addition, 1 g of *Spirulina* contains 130 mg of phycocyanin, 9 mg of chlorophyll, 8 mg of phosphorus, and 7 mg of calcium, which are beneficial for the growth of *H. scabra*. Phycocyanin was reported to have potential medicinal benefits for animals (Yuniati & Sulardiono, 2020). Besides protein, *H. scabra* also consumes calcium to produce a thin protective layer as they grow up. Calcium and phosphorus were two of the main mineral compositions in *H. scabra* (Ardiansyah et al., 2020). In addition, *Spirulina* crude formed the settlement faster and with a higher settlement compared with live *Navicula* sp. and *C. calcitrans* (Sibonga et al., 2022). Moreover, *Spirulina* provided a higher volume of epibenthic or seabed biomass that indirectly increased the optimum rearing density, survival, and growth rate of juveniles of *H. scabra* (Lavitra et al., 2009).

CONCLUSION

Based on the results, a high stocking rate resulted in high mortality in juvenile *H. scabra*. Competition for space and food was a major problem, causing high mortality or disappearance on day 42. The optimum initial stocking density is between 100 and 200 individuals for a 1-ton fibreglass tank with a 500 L water capacity. Grading activities must be conducted every 2 weeks, depending on the growth performance of juvenile *H. scabra*, to reduce competition.

Due to the positive growth rates, *Spirulina* can be used as the main protein source compared to other diets for juvenile *H. scabra*. The results of this study can be used as a preliminary reference for future studies to improve the production of *H. scabra* for the aquaculture industry.

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