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Strategies for Enhancing Grow-Out Culture Technique of Community-Based Sea Cucumber (*Holothuria scabra*): A Case Study in Malawali Island, Sabah

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ABSTRACT

Initial efforts to cultivate *Holothuria scabra* in coastal communities on Malawali faced several challenges. Most notable were the high mortality rate and slow growth, especially after several harvests. In this study, an attempt was made to improve cultivation techniques to increase the growth and survival rate of hatchery-produced *H. scabra* on Malawali Island. A comparative analysis of growth and survival rates of 50 *H. scabra* juveniles (8.68 ± 3.88 g [mean ± S.D.]) kept in 4 m² (stocking density: 12 ind. m⁻²) and 16 m² (3 ind. m⁻²) experimental pens with and without sediment enrichment with *Sargassum* spp. (enrichment ratio: 3% total biomass) for a period of 6 months was conducted. Several key biophysical parameters were recorded, including total organic matter (TOM) and chlorophyll-a (Chl-a). The results indicate that lower stocking density and sediment enrichment did not lead to a higher survival rate of juvenile *H. scabra*. However, stocking density had a noticeable effect on the growth of juvenile *H. scabra*. The average final total biomass of juveniles in enriched and non-enriched pens with low stocking density was significantly higher (1890.45 g and 1667.65 g, respectively), while juveniles in enriched and non-enriched pens with high stocking density had the lowest total biomass (889.7 g and 350.15 g, respectively). While there was no significant difference in TOM content between enriched and non-enriched pens on each observation day (one-way ANOVA; $p > 0.05$), the pooled data showed that enriched pens had significantly higher TOM content. Conversely, the enriched pens have a significantly higher Chl-a concentration than the non-enriched pens. Conclusively, the research findings indicate that a stocking density of 3 ind. m⁻² is a feasible approach to maximise the biomass of *H. scabra* in grow-out pens. On Malawali Island, it was also discovered that while a 3% sediment enrichment alters the properties of the sediment, it is insufficient to sustain *H. scabra*'s ideal development and survival. This study offers insights into sea cucumber farming in the region but highlights the need for further research. Future studies should determine optimal sediment enrichment ratios and use larger sample sizes with sufficient replicates for more conclusive results.

Keywords: Growth, grow-out sea pen, sandfish, sediment enrichment, survival

INTRODUCTION

The sea cucumber has a significant cultural value in Malaysia. About 80 out of 1200 sea cucumber species live in the marine and coastal waters of the country (Kamarudin *et al*., 2017). These sea cucumbers are mainly exported to the Chinese market (Hair *et al*., 2016; Conand, 2018) and are highly sought-after for their nutritional and medicinal properties (Conand, 2006). This has led to an increase in demand and subsequent overexploitation, resulting in some species being classified as vulnerable *(Holothuria fuscogilva*) or endangered *(H. scabra, H. nobilis, H. lessoni, H. whitmaei*) on the IUCN Red List (IUCN, 2023).

Over the past decade, Sabah has emerged as the major center of sea cucumber production in Malaysia, with the highest production in the country, particularly in Tun Mustapha Park (TMP), Sabah (Chor *et al*., 2016). Generally, the sea cucumber farms were constructed near sandy beaches, mangroves, coral reefs, and seagrass, such as Sibogo Island, Malawali Island, Tigabu Island, and Balambangan Island (Lim *et al*., 2021). For coastal communities, sea cucumber farming has become a significant livelihood option. Additionally, for many small-scale fishermen who have traditionally depended on declining wild fisheries, this sector offers a substitute source of income. These communities can lessen their economic vulnerability and improve food security through sea cucumber farming (Hair *et al*., 2019). Furthermore, the grow-out culture of sea cucumbers, like *H. scabra,* is easy and accessible for low-income households (Juinio-Meñez *et al*., 2017).

However, the initial attempts to farm *H. scabra* in the coastal regions, particularly in Malawali Island, Kudat faced numerous challenges that affected profitability. These challenges include the high mortality rate and slow growth of the farmed *H. scabra*. Farmers reported that the growth performance of their cultured *H. scabra* decreased significantly after two or three farming cycles. In addition, *H. scabra* farming practices in TMP are considered unsustainable as they rely too heavily on wild stocks, leading to the loss of marine habitats and inappropriate management practices.

Several developing countries, such as Fiji, Maldives, Papua New Guinea, Madagascar, Philippines, and Indonesia use the traditional sea ranching method of "place, grow and take" (Juinio-Meñez *et al*., 2012; 2013; Robinson & Pascal, 2012; Hair *et al*., 2016). Studies on sea cucumber farming have moved from monoculture to polyculture in the recent decade. According to Namukose *et al*. (2016) and Hamad *et al*. (2019), co-cultivating *H. scabra* with seaweed is one of the most effective methods for modern and successful sea cucumber farming. Additionally, Yu *et al*. (2014) and Cubillo *et al*. (2016) revealed that sea cucumbers farming in the IMTA (Integrated Multi-trophic Aquaculture) system aid in the bioturbation of sediments, improving nutrient cycling and lowering the buildup of organic matter, all of which can lessen the adverse effects of intense aquaculture system, which also indicated the ecological benefits of this farming. Small-scale fishermen in coastal communities utilise extensive sea pens to accelerate the growth of wild-caught *H. scabra* (Hair *et al*., 2016). However, the feasibility of these culture systems depends on environmental variables that promote high growth and survival of hatchery-produced juveniles after release, as emphasised by Dumalan *et al*. (2019).

Stocking density is a crucial parameter that influences the growth and survival of sea cucumbers in aquaculture. Too low a stocking density can reduce productivity and wastes space, while too high a stocking density increases competition and limits available space (Serang *et al*., 2016). A study by Lavitra *et al*. (2010) suggests that the optimal stocking density for sea cucumbers is 3 ind. m^{-2} and achieves the highest growth rate (0.64 g day^{-1}). Sea cucumbers, including *H. scabra*, feed on sediments, with organic matter being an important component of their diet (Liu *et al.*, 2010). *Sargsassum* spp. and compost fertilisers have been used to enrich the sediment in sea pen, promoting the growth of microalgae and directly supporting the growth of *H. scabra* due to increased food availability (Sinsona & Juinio-Meñez, 2018; Sabilu *et al*., 2022).

Despite the extensive sea cucumber farming, there has been no formal assessment of the area under cultivation on Malawali Island. This study aims to refine the farming technique to improve the growth and survival rate of *H. scabra,* particularly on Malawali Island, focusing on a small-scale community-based *H. scabra* farm. The research will compare growth and survival rates at different stocking densities with and without sediment enrichment with *Sargassum* spp. This study hypothesized that low stocking density with the addition of sediment enrichment with *Sargassum* spp. will result in higher growth and survival rates of *H. scabra* juveniles in sea pens.

MATERIALS AND METHODS

Access License

This project was approved by the Sabah Biodiversity Centre (SABC) with access licence reference number JKM/MBS.1000-2/2 JLD. 13 (126) and gained ethical approval for animal research studies with file number AEC 0014 / 2022.

Experimental Site

The research site chosen for this study was located on the northern side of Malawali (Figure 1). The sea pens were constructed within the existing farm owned by the community. The selected research site is characterised by extensive intertidal and shallow water areas characterised by a flat topography and sufficient sediment depth to allow for the installation of the pens. The sediment depth corresponds to the minimum requirement of at least 20 cm recommended by (Rougier *et al*., 2013).

In addition, factors such as visibility and accessibility for both the community and the monitoring team were considered when selecting the site to facilitate maintenance and monitoring of the enclosures. The rectangular sea pens of 16 m^2 and 4 m^2 were set up in a similar habitat to ensure that the habitat would not be a disturbance factor. The research sites consist of small patches of *Enhalus acoroides*, *Halodule* spp, *Cymdocea* sp. and *Thalassia hemprichii* seagrass species. However, the population density of each seagrass species was not quantified.

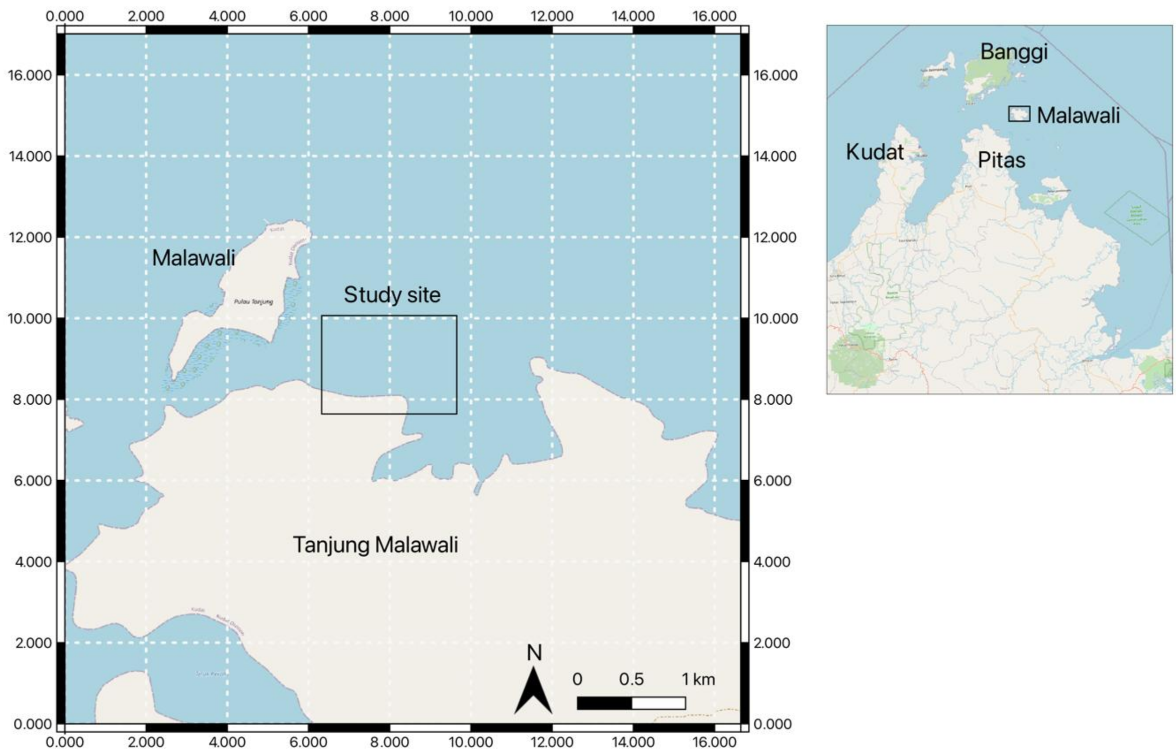

Figure 1. Location of the study site (Malawali)

Experimental Juveniles

The experimental animals used in this study were produced at the Borneo Scabra Hatchery in Tuaran, Sabah. A total of 600 *H. scabra* juveniles (8.68 ± 3.88 [S.D.] g body weight) from a single spawning were transported from the hatchery to the experimental sites in plastic bags filled with water (70%) and oxygen (30%) at a cool temperature.

Grow-Out Pen Trial and Sediment Enrichment

Sea pens measuring 2×2×1 m and 4×4×1 m (W×L×H) were constructed to maintain the natural habitat of the *H. scabra* juvenile while allowing a regular water supply and water exchange. These sea pens were built with an 8 mm thick HDPE mesh net and stakes made from a rust-prone PVC pipe. The PVC pipes were reinforced by filling them with cement to create robust stakes. These stakes were buried 20–30 cm deep into the sediment together with the bottom net of the pen to prevent the juveniles from escaping by burrowing, as recommended by Purcell & Simutoga (2008) and Robinson & Pascal (2012).

In this experiment, a comparison of growth and survival rates was conducted with 50 *H. scabra* juveniles placed over a period of 188 days (6 months 4 days) in experimental pens of 4 m^2 (stocking density: 12 ind. m^{-2}) and 16 m^2 (3 ind. m^{-2}) with or without sediment enrichment with *Sargassum* spp. Four treatment pens were used (16U (control) = 3 ind. m^{-2} stocking density without enrichment; 16E = 3 ind. m^{-2} stocking density with enrichment; 4U = 12 ind. m^{-2} stocking density without enrichment; 4E = 12 ind. m^{-2} stocking density with enrichment). The initial mean stocking weight of *H. scabra* juveniles in each treatment pens showed no significant difference (one-way ANOVA, p = 0.488).

The preparation and method of enrichment followed the approach described by Sinsona & Juinio-Meñez (2018). *Sargassum* spp. were collected, rinsed with freshwater to remove debris, and then sun-dried. After drying under natural sunlight, the *Sargassum* spp. was ground with an electronic grinder for about 25 seconds. Prior to enrichment, the *Sargassum* spp. was soaked overnight to increase its specific gravity and prevent it from floating during enrichment to ensure that it remained submerged in the sediment. The soaked *Sargassum* spp. was mixed directly into the top 2 cm layer of the sediment layer in pens 4E and 16E on day 1 and re-enriched every 30 days (Sinsona & Juinio-Meñez, 2018). During the 6-month experiment, the monthly enrichment amount was determined based on the body weight of the *H. scabra* juveniles in each treatment pen, using 3% of the body weight and adjusted every 30 days according to the weight of the juveniles. Ahmed *et al*. (2018) used an enrichment ratio of 3% total biomass for daily enrichment. However, since the enrichment in this study was only performed once a month during the monthly site visit, the 3% total biomass was multiplied by 30 days.

Data Collection and Calculation for Growth and Survival of Juveniles in Grow-out Pens

Before transporting and stocking juveniles into each pen, their weight was measured using digital scales with 1 decimal point, individually (Dumalan *et al*., 2019). During the measurement, the *H. scabra* juveniles were manually removed from the pens and placed in a basin. To facilitate the expulsion of excess water from the body, the animals were allowed to remain out of the water for 2 minutes before weighing. Following the method described by Dumalan *et al*. (2019), the juveniles were carefully dried with a towel before being weighed using a digital scale accurate to one decimal place. Only the weight of *H. scabra* was used as their growth measurement because their body length is inconsistent since it is influenced by stress (Lavitra *et al*., 2010).

Monitoring of growth and survival was conducted monthly during the spring low tide to facilitate access to the experimental sites: day 0, 33, 63, 90, 119, 157, 188. The monitoring of the growth and survival of *H. scabra* juveniles involved the active participation of Malawali community members, who formed the monitoring team and supervisor of this community-based project. The field supervisor took responsibility for daily or weekly surveillance of the experimental pens to prevent poaching.

Survival rates in each pen were calculated monthly as a percentage of the remaining *H. scabra* juveniles. The absolute growth rate (AGR) was calculated as difference between the juvenile's average final weight and average initial weight over the number of rearing days (Yussuf & Yahya, 2021):

$$AGR\left(\frac{g}{day}\right) = \frac{final\ weight - initial\ weight}{rearing\ days}. \quad \textbf{Eq.(1)}$$

The total biomass was calculated by adding the individual weights per pen and expressing the result in grammes. Biomass increment was calculated to determine how much the total biomass in each experimental pens increased throughout the experimental period:

$$Biomass\ increment\ (\%) = [\frac{(final\ biomass - initial\ biomass)}{initial\ biomass}]\ x\ 100. \quad \textbf{Eq.(2)}$$

Sediment Enrichment

To assess relative food abundance, sediment samples were collected from all treatment pens to analyse chlorophyll-a (Chl-a) and total organic matter (TOM). The sediment samples were collected prior to the next sediment enrichment. Analyses were performed on day 0, 33, 63, 90, 119, 157 and 188. Samples were collected from the top 2-3 cm of the surface at five random points within the pens using a 20 ml cut-off syringe as described in the method of Dumalan *et al*. (2019).

To ensure representative data, the samples from a single pen were combined in a zip-lock bag. As direct on-site analysis was impractical, all samples were stored in a cool box with ice to prevent oxidation and transported to the Borneo

Marine Research Institute. Upon arrival at the facility, the samples were frozen prior to analysis. To determine the TOM of the sediments, the loss on ignition method was used according to the procedure described by (Heiri *et al*., 2001). For the determination of Chl-a in the sediment, the spectrophotometric method explained by Slater & Carton (2009) was used.

Water Quality

The water parameters such as temperature (˚C), salinity (ppt), pH, and dissolve oxygen (mg L^{-1}) of each site were measured in situ using a YSI multiparameter at every monitoring time (monthly).

Statistical Analysis

Normality and homogeneity of variance were tested using the Shapiro–Wilk test or Levene test, with the significance level set at $p < 0.05$. Logarithmic and square root transformation were conducted to normalize the data. A one-way ANOVA was used to assess significant differences in mean survival, total biomass, mean weight, and biomass increment in *H. scabra* juveniles between treatments on the same observation day. Post-hoc comparisons were performed using Duncan's multiple range test to identify specific differences between treatment groups. Similarly, a one-way ANOVA with Duncan's multiple range test was used to assess the difference in TOM and Chl-a content in the same monitoring day. Meanwhile, A Kruskal-wallis was used to assess significant differences of TOM and Chl-a, with pairwise comparison to identify the specific difference between treatment groups for the pooled data (combined from day 0 to day 188). The combined effect of stocking density and additional sediment enrichment on the survival rate, total biomass, mean weight, and biomass increment of *H. scabra* was analysed using a two-way ANOVA. A significance level of $p < 0.05$ was used for all statistical analyses, and SPSS Statistics version 28 was used for data processing.

RESULTS

Growth and Survival of Juveniles in Grow-Out Pen

Although there were no statistically significant differences in survival rates related to the addition of enrichment, stocking density, or the interaction between these two factors (Two-way ANOVA; p>0.05; Table 1), the pen with low stocking density and enrichment (16E) consistently showed higher survival throughout the experiment. Monthly data on survival percentage, total biomass, and mean weight during the grow-out trials are illustrated in Figure 2. The highest mortality occurred within the first two months after stocking across all pens. Notably, the pen with high stocking density and no enrichment (4U) experienced a significant decline in juvenile numbers from June to July, ending the experiment with the lowest survival rate (7 ± 5.66%) and total biomass (350.15 ± 213.6 g pen^{-1}).

Additionally, total biomass and mean weight of juveniles were not significantly different with respect to additional of enrichment and interaction between two main factors ($p > 0.05$; Table 1) but both total biomass and mean weight were significantly different with respect to stocking density (Two-way ANOVA; $p = 0.022$; Table 1). Maximum biomass (g pen^{-1}) was reached after 4 months of rearing at low stocking density (16U; 2422.25 ± 169.25 g pen^{-1} and 16E; 2727.25 ± 444.25 g pen^{-1}) and after 3 months of rearing at high stocking density (4U; 709.4 ± 97.8 g pen^{-1} and 4E; 1172.5 ± 74.05 g pen^{-1}). AGR is not significantly different with respect to additional of enrichment, stocking density and interaction between two main factors (Two-way ANOVA; $p > 0.05$; Table 1). AGR of juveniles reared in 16U was higher (0.63 ± 0.18 g day^{-1}) but not significantly different with 16E (0.52 ± 0.03 g day^{-1}), 4U (0.49 ± 0.08 g day^{-1}), and 4E (0.41 ± 0.11 g day^{-1}).

Based on the monthly survival rate (Figure 2) in grow-out sea pens, there was a dramatic decline of juvenile sandfish in almost all treatments after one month of stocking. Throughout the experimental period, 4U pens exhibit lowest survival although not significantly different from the other pens (One-way ANOVA; $p > 0.05$) Meanwhile, 16E consistently has slightly higher survival compared to the other treatments. At the end of the experiment, no treatment showed higher than 45% survival rate, with the highest is 35.00 ± 12.73%, followed by 29.00 ± 21.21%, 20.00 ± 5.66% and the lowest is 7.00 ± 1.41% for 16E, 16U, 4E and 4U respectively. On month 3 and 4 of the experiment, some of the juveniles in site A spotted with skin ulceration disease (SKUDs).

Despite the lack of proof, there is always a possibility that the disease contributed to the deaths of the juveniles. Monthly patterns indicate that continuous decline of juveniles occurred in all treatments. The inconsistent pattern of survival especially in 4U and 4E pens could be due to the difficulty in retrieving the juveniles during monitoring.

Sediment Characteristics: Total Organic Matter and Chlorophyll-A

The TOM and Chl-a content for each therapy are displayed monthly in Table 2. During the observation day, the TOM content in all treatments did not differ statistically significantly (p>0.05), even though TOM was consistently slightly higher in the enriched pens (4E and 16E). However, the pooled data of TOM throughout the study showed that enriched pens (4E) and (16E) are significantly higher than those unenriched pens (4U) and (16U). (Kruskal-wallis with pairwise comparison; p=0.10) (see Table 2). At the start of the experiment, chlorophyll-a content was significantly higher in the high stocking density and enrichment pens (4E) but was not significantly different from 16E. The Chl-a content in the pens with enrichment (4E and 16E) remained consistently higher than in the pens without enrichment (p< 0.05). Similarly, the pooled data of Chl-a throughout the experiment showed that enriched pens are significantly higher than those unenriched pens (p< 0.01). From these results, it can be concluded that *Sargassum* spp. has altered the sediment characteristics of the sea pens.

Water Quality

The monthly seawater temperatures in the experimental sea pens were relatively high during the study (28.3 – 32.9 °C) but still within the optimal range. The lowest temperature was recorded in June with a range of 28.8 – 29.1 ˚C and in November (28.3 – 29.5 ˚C) as it was cloudy during the monitoring. The highest temperature was in September (32.8 – 32.9 ˚C). Salinity ranged from 30.5 – 33.41 ppt, with the lowest salinity measured in November (28.2 – 31.2 ppt) which is still in optimal range for sea cucumber farming. Meanwhile, dissolved oxygen ranged from 3.65 to 6.08 mg L^{-1}.

Table 1. Two-way ANOVA (significant at p<0.05) of survival (%), total biomass (g), mean weight (g) and AGR (g day^{-1}) of juveniles between each treatment for 7 months.

		DF	MS	F	p-value
Survival (%)	Enrichment	1	180.500	1.118	0.350
	Stocking density	1	684.500	4.238	0.109
	Enrichment x stocking density	1	24.500	0.152	0.717
	Error	4	161.500		
Total biomass (g)	Enrichment	1	0.098	2.689	0.176
	Stocking density	1	0.485	13.378	0.022*
	Enrichment x stocking density	1	0.048	1.325	0.314
	Error	4	0.036		
Mean weight (g)	Enrichment	1	1274.163	1.534	0.219
	Stocking density	1	4523.357	5.447	0.022*
	Enrichment x stocking density	1	65.310	.079	0.780
	Error	87	830.486		
AGR (g day^{-1})	Enrichment	1	0.018	1.452	0.295
	Stocking density	1	.031	2.509	0.188
	Enrichment x stocking density	1	.001	0.040	0.851
	Error	4	0.012		

Significant difference at p<0.05. Asterisk (*) indicates significant difference.

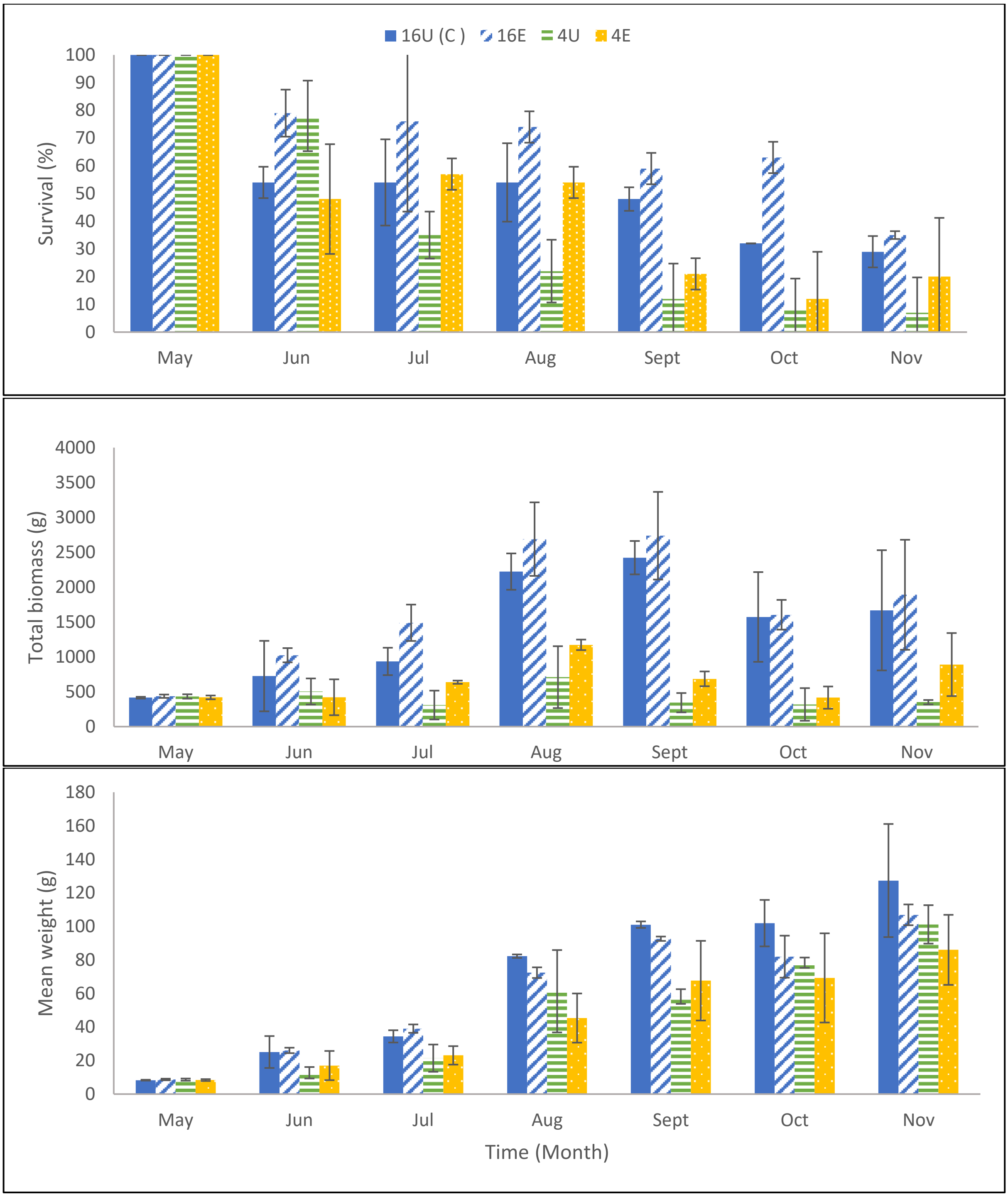

Figure 2. Monthly survival rate, total biomass and mean weight of juveniles. Error bars indicate standard deviation. 16U (3 ind.m^{-2}, non-enrich), 16E (3 ind.m^{-2}, enriched), 4U (12 ind.m^{-2}, non-enrich) and 4E (12 ind.m^{-2}, enriched).

Table 2. Monthly TOM and Chl-a ± S.D in enriched and unenriched pens.

Day/Parameter/ Treatment		16U (Control)	4U	4E	16E
0	TOM (%)	$3.10±0.53^{a}$	$3.33±0.75^{a}$	$3.07±0.11^{a}$	$3.27±0.31^{a}$
	Chl-a (ug/g)	$1.04±0.13^{b}$	$0.88±0.35^{b}$	$2.60±0.27^{a}$	$1.74±0.29^{ab}$
33	TOM (%)	$3.06±0.33^{a}$	$3.05±0.21^{a}$	$3.21±0.23^{a}$	$3.64±0.74^{a}$
	Chl-a (ug/g)	$1.08±0.29^{ab}$	$1.14±0.42^{b}$	$2.32±0.8^{ab}$	$2.61±0.79^{a}$
63	TOM (%)	$3.24±0.12^{a}$	$3.07±0.24^{a}$	$3.07±0.26^{a}$	$3.31±0.29^{a}$
	Chl-a (ug/g)	$1.23±0.42^{ab}$	$0.71±0.08^{b}$	$1.34±0.31^{a}$	$1.78±0.31^{a}$
90	TOM (%)	$2.89±0.69^{a}$	$2.94±0.61^{a}$	$3.56±0.35^{a}$	$3.33±0.82^{a}$
	Chl-a (ug/g)	$1.29±0.42^{a}$	$0.71±0.25^{a}$	$1.82±1.30^{a}$	$1.42±0.48^{a}$
119	TOM (%)	$3.14±0.23^{a}$	$2.91±0.29^{a}$	$3.16±0.28^{a}$	$3.24±0.19^{a}$
	Chl-a (ug/g)	$0.87±0.29^{a}$	$0.74±0.12^{a}$	$1.05±0.25^{a}$	$1.13±0.19^{a}$
157	TOM (%)	$3.16±0.20^{a}$	$2.95±0.32^{a}$	$3.09±0.22^{a}$	$3.51±0.55^{a}$
	Chl-a (ug/g)	$1.34±0.81^{a}$	$1.36±0.65^{a}$	$2.45±0.20^{a}$	$2.42±0.72^{a}$
188	TOM (%)	$3.39±0.20^{a}$	$3.1±0.10^{a}$	$3.61±0.36^{a}$	$3.66±0.31^{a}$
	Chl-a (ug/g)	$0.79±0.42^{b}$	$1.05±0.15^{b}$	$2.08±0.52^{a}$	$2.18±0.90^{a}$

Different letter superscripts indicate significance differences (One-way ANOVA; $p < 0.05$). 16U = Low stocking density with no enrichment, 4U = high stocking density with no enrichment, 16E = low stocking density with enrichment, 4E = high stocking density with enrichment.

DISCUSSION

The aim of this study was to evaluate the effects of stocking density and sediment enrichment with *Sargassum* spp. on the growth and survival rate of pen cultured *H. scabra*, commonly known as sandfish. Sea cucumber aquaculture is of significant interest due to its economic importance and ecological value. The main results of this study show that stocking density influences the total biomass and mean weight of farmed *H. scabra* (initial size at stocking: 8.68 ± 3.88 [S.D.] g body weight). Although the stocking density of *H. scabra* has been studied in a number of studies, similar research is essential to verify results across a range of environmental conditions and methods of farming. Although no statistically significant difference ($p > 0.05$) was found between the growth and survival rate of *H. scabra* juveniles in enriched and unenriched sediment, it is still necessary to consider possible trends or patterns that could have practical implications for future studies. Statistically, stocking density had no significant effect on *H. scabra* survival in this study ($p > 0.05$), although survival was slightly higher at low stocking densities (3 ind. m^{-2}). Similar results were found in a previous study that suggested a stocking density of 3 ind. m^{-2} to give sea cucumbers a better chance of finding sufficient food sources in an environment with limited supply and available area (*Serang et al*., 2016).

Results of previous studies have shown that the growth of sea cucumbers is positively influenced by the enrichment of sediments with *Sargassum* spp. (Sinsona & Juinio-Meñez, 2018). Although the study found no significant differences in the survival and growth performance (total biomass and AGR) of *H. scabra* between the pens with enriched sediment and those without, it is important to note that the pens with lower stocking density with the sediment enriched with *Sargassum* spp. consistently showed better survival rates and growth performance. The statistical insignificance of this result could be caused by the small sample size. However, this result is consistent with other studies by Sinsona & Juinio-Meñez (2018) which show that sediment enrichment has no significant effect on the survival of *H. scabra* juveniles in pen cultures.

In addition to TOM, Chl-a found in the sediment is an indicator of benthic microalgae, which are a food source for deposit feeders such as sea cucumbers (Hartati *et al*., 2017). A previous study reported that Chl-a levels of ~ 4 µg g^{-1} can increase by more than 17 g in just 30 days (Sinsona & Juinio-Meñez, 2018) and that an enrichment ratio of 0.9 kg m^{-2} increases Chl-a by ~2.4 µg g^{-2}. In the same study, the organic matter content in the enriched sediments was high (14.4 – 15.5%) compared to this study (~3%). Of note, Chl-a and TOM content is low at these sites, which was probably consumed by the cultured *H. scabra* in the existing farms owned by the community. Additionally, enriched pens consistently have a slightly higher TOM and Chl-a content than non-enriched pens, suggesting that powdered *Sargassum* spp. in sea pens support the development of microalgae in the sediment. Enrichment of the sediment provided more food resources. However, the enrichment ratio (3% of total biomass) is not sufficient to support the ideal development and survival of juvenile *H. scabra* at a high stocking density, suggesting that a higher enrichment ratio needs to be used in future studies.

In this study, juveniles of *H. scabra* raised in pen cultures showed poor survival rates (7 – 35% with initial weight: 8.68 ± 3.88 g), similar to the reports of Dumalan *et al*. (2019). In a previous study, only 12% of the juveniles (initial weight: 6.7 ± 0.7 g) survived after 8 months of grow out in seagrass bed areas while the mortality rate in the fish farm was 100%. In the same study, however, a survival rate of 65% was recorded for larger juvenile fish (initial weight: 57.4 ± 4.3 g) after 8 months of rearing in the seagrass area and 49% in the fish farm. Since neither stocking density nor sediment enrichment had a significant effect on the survival rate of *H. scabra* ($p>0.05$), escape was more likely the main cause of survival in this study. In addition, the highest mortality rate in all enclosures occurred in the 1st and 2nd month after stocking, suggesting that transport stress could be one of the main reasons for the low survival rate. The transport of the juveniles from the hatchery to the experimental sea pen took about 4 hours by car and 1.5 hours by boat. According to Purcell *et al*. (2006), the issue in transporting live sea cucumber in water medium is the declining pH level when the water temperature is not maintained properly. The declines in pH at ambient water temperature would be stressful for the juveniles, as high-water temperature could lead to higher metabolic activity and ammonia excretion, thus increasing stress (Estudillo & Duray, 2003).

Although the top of the pens was covered with mesh net, the juveniles were able to slip through the gaps at the bottom of the pens more easily. A similar problem had previously been documented by Namukose *et al*. (2016). In addition, these openings attracted other marine animals, including potential predators such as crabs, which could enter the pens and prey on the sea cucumbers (Pitt *et al*., 2004; Namukose *et al*., 2016). In this study, crabs and fish were observed in the sea pens, which could pose a direct threat to the survival of *H. scabra*. Lavitra *et al*. (2009) and Altamirano *et al*. (2017) considered crabs such as *Thalamita crenata* as the most severe predator of juvenile sea cucumber.

The monthly water quality in this study is in the optimal range for temperature and salinity. Optimal temperature of *H. scabra* varies from 26-33˚C (Agudo, 2006; Lavitra *et al*., 2010; Kühnhold, 2017). Optimal salinity for *H. scabra* cultivation ranging from 27-35 ppt (Agudo, 2006) and 28-32 ppt (Serang *et al*., 2016). However, the DO level in this study may be low (<5.0 mg L^{-1}), while the optimal DO level for sea cucumber culture according to Oh *et al*. (2015) is above 5.0 mg L^{-1}. Towards the end of the experiment (November), frequent rainfall occurred due to the onset of the northeast monsoon, which brings heavy rainfall to Peninsular Malaysia, west of Sarawak and east of Sabah (Malaysia Meteorological Department: https://www.met.gov.my/). The sharp decline in the survival rate of *H. scabra* in sea pens might coincides with this rainy season. Juinio-Meñez *et al*. (2013) previously found a sharp decrease in biomass in sea pens after a thypoon, which can be attributed to a change in sediment quality in the sea pen.

Based on this observation, the northeast monsoon in Malawali can occur from October to January, suggesting that the ideal grow-out period in Malawali is between February and September (8 months) to avoid high mortality or negative growth due to harsh weather. However, to reach a harvestable size in 8 months, which is typically > 300 g (Dumalan *et al*., 2019), the growth rate should be at least 1.2 g day^{-1}. Comparing this growth rate with the current average absolute growth rate in Malawali, which is between 0.413 and 0.633 g day^{-1}, it becomes clear that reaching a harvestable size within 6 months is a challenge. However, the use of larger juveniles at release may be considered in a future study.

While sea pens are effective in many ways, they come with their own challenges in open environments. These challenges include high costs of construction, cleaning and maintenance (Robinson & Pascal, 2012). Sea pens are also susceptible to unfavourable weather conditions such as storms and rough seas, which can shorten their lifespan if they are not designed and maintained appropriately. In our case, heavy rain and storms led to the collapse of our experimental sea pens after 5 to 7 months of operation. This unfortunate event led to the escape of juvenile sea cucumbers and the interruption of data collection, so the experiment had to be cancelled prematurely and shortened from the originally planned 10 months to 6 months. For future studies, an improved construction of the enclosures that can withstand strong waves and heavy rain should be considered.

The presence of a clear trend in survival and growth of cultured *H. scabra* despite a lack of statistical significance is an intriguing aspect of our results. There are a number of possible causes for this scenario, all of which should be carefully considered. The sample size is limited due to a limited source of hatchery-produced *H. scabra* juveniles, replication is insufficient due to limited sea pens, and the variability within the data is large, so the statistical test is unable to detect a significant difference.

CONCLUSION

As a conclusion, lower stocking density led to a higher biomass, but did not significantly affect the survival rate. In pens with enriched sediment, the results were slightly better, although not statistically significantly different. This study suggests that low stocking density (3 $ind.m^{-2}$) with sediment enrichment by *Sargassum* spp. may promote higher biomass, but the optimal enrichment ratio remains to be determined. Several problems such as escape, and predation were observed. The study has also shown the importance of considering seasonal weather effects. This study provides valuable insights into sea cucumber farming in this region, but also acknowledges limitations and emphasises the need for future research and consideration of environmental aspects when optimising practises. Future studies ought to concentrate on figuring out the best ratios of sediment enrichment for large-scale, sustainable sea cucumber farming to increase biomass while preserving high survival rates. Increasing the length of the study might facilitate the capturing of seasonal fluctuations and long-term impacts. Additionally, further research with a larger sample size, and sufficient replicates of sea pen are required for conclusive findings.

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