Original Article

Survival and growth performance of sub-adult sandfish *Holothuria scabra* (Jaeger, 1833) in tanks with different supplemental feeds

Alexis G. Sumbe[1,2,*], Jean Beth S. Jontila[1,2], Precious G. Sevidal[1], Lorelie J. Bongabong[1], Ronalyn G. Ramirez[1], and Lota A. Creencia[1]

[1]*College of Fisheries and Natural Sciences, Western Philippines University, Puerto Princesa City, Philippines*
[2]*Capacity Development on Futures Thinking and Strategic Foresight Project, College of Fisheries and Natural Sciences, Western Philippines University, Puerto Princesa City, Philippines*

[*]*Corresponding Author:* alexisgervaciosumbe@gmail.com

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ABSTRACT

Sandfish (*Holothuria scabra* Jaeger, 1833*)* is one of the most valuable sea cucumbers in local and international markets. Although listed as an endangered species, it is among the regularly harvested marine resources in Palawan, Philippines. To continue the trade of sandfish without harvesting from the wild, this study initially ventured into producing seed stocks. However, broodstocks were scarce, only sub-adults were available, and they needed to grow into broodstocks. This study evaluated the survival and growth performance of sub-adult sandfish when fed with soya bean powder (Treatment 1/T_1), rice bran (Treatment 2/T_2), and powdered seagrass leaves (Treatment 3/T_3). Each treatment and control (no supplemental feeding) had three replicates with three sandfish having 59.55 ± 10.24 g mean weight and 9.74 ± 0.98 cm mean length. The samples were cultured in aerated plastic tanks (63.5 cm × 45.72 cm × 35.56 cm) with a 10 cm layer of sandy-muddy sediment filled with 40 L seawater, which was changed twice a day. Results showed 100% survival in T_3 and control, 66% in T_1, and 0% in T_2. The mean weight and length were stable in the first two weeks of culture in all treatments and control, but the latter declined during the 3rd and 4th weeks of culture ($P < 0.05$). Such was attributed to the drop in salinity during the 3rd week. Nevertheless, seagrass powder and soya beans appeared to help maintain the growth and tolerance to stress of sub-adult sandfish while unprocessed rice bran was detrimental to the health of sub-adult sandfish and caused mortality before the end of the culture period.

Keywords: juvenile, mariculture, rice bran, seagrass, soya

INTRODUCTION

Sandfish *Holothuria scabra* (Jaeger, 1833) is considered one of the most valuable sea cucumber species that are processed and dried into "trepang" or "bêche-de-mer", and used as a main ingredient in Chinese cuisine that commands a high price in local and international markets (Akamine 2002; Brown et al. 2010; Purcell 2010; Jontila 2023). The value of a premium dried sandfish in Palawan, Philippines has steadily increased over the years, from PHP 324.00 kg^{-1} (USD 8.10) (Schoppe 2000) to PHP

6,000.00 kg^{-1} (USD 113.2) (Jontila et al. 2018). The average market price of dried sandfish in Chinese stores in Hong Kong and Guangzhou was USD 369 kg^{-1} and USD 153 kg^{-1} in 2016, respectively (Purcell et al. 2018). Due to this demand, sandfish has been heavily harvested in many areas across its distribution range. In Palawan, Philippines, it is freely collected and traded despite being IUCN-listed as an endangered species (Hamel et al. 2013; Dolorosa et al. 2017). Such has resulted in overharvesting and even depletion of its population in some near-shore areas (Jontila et al. 2018). Overharvesting has already placed the country as a hot spot area for sea cucumbers in Southeast Asia (Choo 2008). With the scarcity of sea cucumbers in near-shore areas, fishers are now venturing to farther sites to gather prime-sized and valuable sea cucumbers, particularly sandfish. In the absence of enforcement, sandfish and sea cucumber fishery will not be sustainable and will likely lead to population depletion in the long run, which would impact the livelihood of coastal communities since sea cucumber gathering is one of their substantial sources of income (Jontila et al. 2018). It would also affect the country's economy because sea cucumber is among its top 10 marine export commodities in terms of value (DA-BFAR 2021).

Sandfish culture is a way to increase the supply to capture the benefits of the high prices and market demand for sea cucumber products (Juinio-Meñez et al. 2016). Given the huge market for sandfish, full-cycle production of this species has been developed but production in the Philippines has not yet scaled up to commercial level, mainly because rearing of sandfish in the hatchery until maturity is not economically viable. To shorten the rearing time in a hatchery, juvenile sandfish can be grown in ocean nurseries using low-cost technology (Juinio-Meñez et al. 2012). In an attempt to demonstrate this, the team sourced out broodstocks from fishers for seed stock production. However, there were not enough broodstocks coming from the same area, which is important in maintaining genetic diversity. The fishers' catch were mainly late juveniles or sub-adults with very few adults. With such available resources, the team thought of growing the sub-adults and seeing if supplemental feeding would improve their growth performance while acclimating them in tanks before rearing them in their natural habitat (Dumalan et al. 2019). The addition of feeds to improve the growth and condition of sandfish in tanks yielded positive outcomes for broodstocks (Agudo 2006) but not for the sub-adults. Also, most of the studies in the Philippines were on diversity, abundance, and population (Schoppe 2000; Olavides et al. 2010; Dolorosa and Jontila 2012; Cabansag and Romero 2014; Jontila et al. 2014; Dolorosa 2015; Jontila et al. 2018) including substrate preference, feeding, and growth both in tanks and natural habitat of sandfish (Juinio-Meñez et al. 2014; Altamirano et al. 2017; Altamirano and Baylon 2020). Considering the different preferences of sandfish at each stage, this study compared the survival and growth of sub-adult sandfish in tanks with natural sediment supplemented with soya bean powder, rice bran, and powdered seagrass leaves and compared with the control (no supplemental feeding). Specifically, this study monitored the weekly survival, length (cm) and weight (g) of sub-adult sandfish. Information on this would be useful in improving the culture techniques for sandfish.

METHODS

Sub-adult Sandfish

The study used sub-adult sandfish bought from fishers of Sitio Silangan, Barangay Tagburos, Puerto Princesa City. A total of 36 sub-adults with a mean weight of 59.55 ± 10.24 g and a mean length of 9.74 ± 0.98 cm was used. The culture was conducted in the Inland Sea Ranching Station (ISRS) of the Department of Agriculture - Bureau of Fisheries and Aquatic Resources (DA-BFAR) situated in Barangay Sta. Lucia, Puerto Princesa City from 06 April 2022 to 12 May 2022.

Supplemental Foods

Three types of supplemental foods were prepared: soya bean powder, rice bran, and pulverized seagrass (*Enhalus acoroides)* leaves. The soya bean was used as a food supplement in sandfish broodstocks (Battaglene 1999) while the rice bran and seagrass powder were used as feed components of sandfish (Agudo 2006). The soya beans were toasted over a medium heat pan, cooled down, and grounded until powdery. The rice bran was purchased from the local market and was no longer processed. As for the seagrass, the leaves were washed, cleaned, cut into small pieces, and roasted in a frying pan until they became dry. It was then pounded until a powdery texture was attained.

Experimental Setup

This study used 40-L plastic crates (63.5 cm × 45.72 cm × 35.56 cm) filled with around 10 cm layer of natural sandy-muddy sediment taken from the natural habitat of sandfish. Three treatments were prepared: soya bean powder (Treatment 1/T_1), rice bran (Treatment 2/T_2), and powdered seagrass leaves (Treatment 3/T_3) including the control with no supplemental food and only sandy-muddy substrate. Treatments and control were aerated continuously for 24 hours and the water was changed twice a day, every seven o'clock in the morning and five o'clock in the afternoon to ensure the continuous supply of dissolved oxygen (DO) and eliminate unwanted chemicals from the waste of sandfish (Tuwo et al. 2019). The amount of food given ranged from 3.5 to 5.0 g based on the body weight of sandfish (Agudo 2006). The supplemental foods were added every morning after changing the water by thoroughly mixing it with salt water in a dipper before pouring it into each tank. They were allowed to settle by turning off the aeration until the water became clear. Table 1 shows the initial mean weight (± sd) and length (± sd) of 36 sub-adult sandfish with a total stocking biomass of 238.19 g in weight and 38.94 cm in length with three replicates containing three individuals each.

Table 1. Initial mean weight (± sd) and length (± sd) of sub-adult sandfish. T- treatment.

T	Feedings	No. of stock per replicate	No. of stock per treatment	Mean ± sd initial weight (g)	Mean ± sd initial length (cm)	Duration of culture (d)
T_1	Soya bean powder	3	9	59.38 ± 5.76	10.5 ± 1.36	30
T_2	Rice bran	3	9	73.91 ± 6.52	10.61 ± 0.59	21
T_3	Powdered seagrass leaves	3	9	54.39 ±16.64	9.22 ± 1.35	30
Control	No supplemental feedings	3	9	50.51 ± 6.64	8.61 ± 0.75	30

Monitoring of Survival and Growth

The survival of sub-adult sandfish cultured in tanks with natural sediment was determined by counting the number of live individuals every week. As for growth, the weight (g) and length (cm) of each individual were recorded every week for 30 days using a digital weighing scale and transparent plastic ruler, respectively. To minimize the error in measuring the body size, the samples were taken out of the tank by hand and were given 30 seconds to relax and drain the excess water in their bodies before taking the measurements. At the end of the culture period, all sub-adult sandfish were measured for their final weight and length. Water parameters such as salinity (ppt), pH, and temperature (ºC) were also monitored daily at seven o'clock in the morning, noon, and five o'clock in the afternoon using a refractometer (Atago brand), colorimetric method, and mercury thermometer, respectively. Observations on the burrowing behavior of sandfish and the appearance of the natural sediment were also noted.

Data Analysis

One-way analysis of variance (ANOVA) was used to test the differences in length and weight among the treatments at a 5% level of significance.

RESULTS

Survival

Among treatments, T_3 (powdered seagrass leaves) and the control (no supplemental food) had 100% survival after 30 days of culture (Figure 1). Treatment 1 (soya bean powder) had mortalities during the second week but retained a 66% survival for the rest of the culture period. Treatment 2 (rice bran) had higher mortality during the 2nd week with only 33% survival which eventually went down to 11% until all samples died at the end of the culture period.

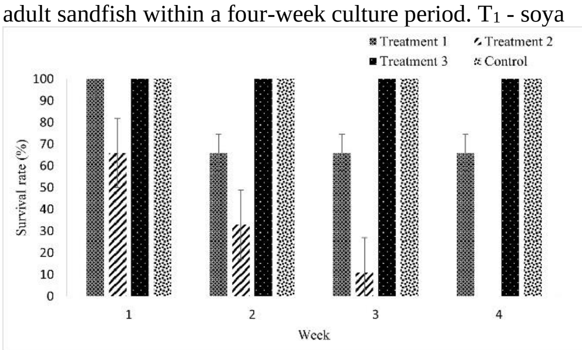

Figure 1. Average percentage (± sd) of survival of sub-adult sandfish within a four-week culture period. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.

Growth Performance in Weight

Sandfish displayed a stable mean (± sd) body weight in the first two weeks of culture (Figure 2). Among the treatments, only individuals in T_3 (powdered seagrass leaves) displayed a slight increase in weight while T_2 (rice bran) slightly decreased, though both changes were not significant ($P > 0.05$).

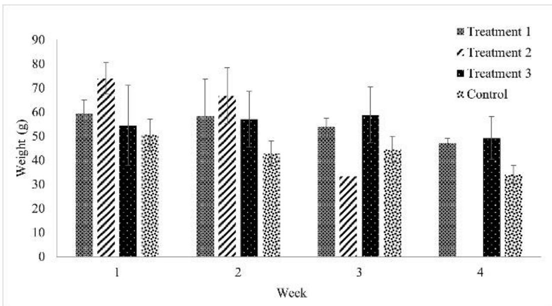

Figure 2. Mean (± sd) body weight of sub-adult sandfish within a four-week culture period. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.

On the third week of culture, the weight of sandfish in all treatments remained almost the same, except for T_2 (rice bran), wherein the mean (± sd) weight decreased significantly from 73.91 ± 6.52 g to a remaining individual weighing 33.27 g ($P < 0.05$). The final mean weight (± sd) of sandfish decreased in all treatments including the control (Table 2), but overall, the decrease was not significant ($P > 0.05$).

Growth Performance in Length

The mean length of sandfish in all treatments was almost the same in the first and second weeks of culture. It was in the third week that a significant decrease in mean (± sd) lengths of T_1 (soya bean powder) and T_2 (rice bran) was observed, from 10.5 ± 1.36 cm to 7.11 ± 6.16 cm and 10.61 ± 0.15 cm to a remaining individual measuring 12.5 cm, respectively (Figure 3). On the fourth week of culture, the length of sandfish in all treatments decreased significantly ($P < 0.05$). The final mean length (± sd) in all treatments including the control showed decrease as shown in Table 2.

Absolute Growth Rate (AGR)

After 30 days of culture, all samples displayed a negative absolute growth rate (AGR) (Figures 4 and 5). All treatments including the control almost had the same AGR (-0.16 cm to -0.36 cm), except for Treatment 2 and the control which had -0.17 g and -2.46 g in weight, respectively.

Water Parameters

The water pH throughout the culture period in all treatments and control was alkaline ranging from 7.8-8.2. The temperature ranged from 26°C to 30°C with the highest mean value at 30.5°C at five o'clock in the afternoon. As for salinity, the value ranged from 31 ppt to 33 ppt, except when there was a Low-Pressure Area (LPA) that brought heavy rain on the 17th day of culture, dropping the salinity in the reservoir to 27 ppt.

Table 2. Final mean weight (± sd) and length (± sd) of sub-adult sandfish.

Treatment	Feedings	Final no. of stock	Mean ± sd final weight (g)	Mean ± sd final length (cm)
T_1	Soya bean powder	7	47.15 ± 1.93	2.77 ± 2.42
T_2	Rice bran	0	-	-
T_3	Powdered seagrass leaves	9	49.17 ± 8.88	4.31 ± 1.49
Control	No supplemental feedings	9	34.1 ± 3.79	2.99 ± 1.81

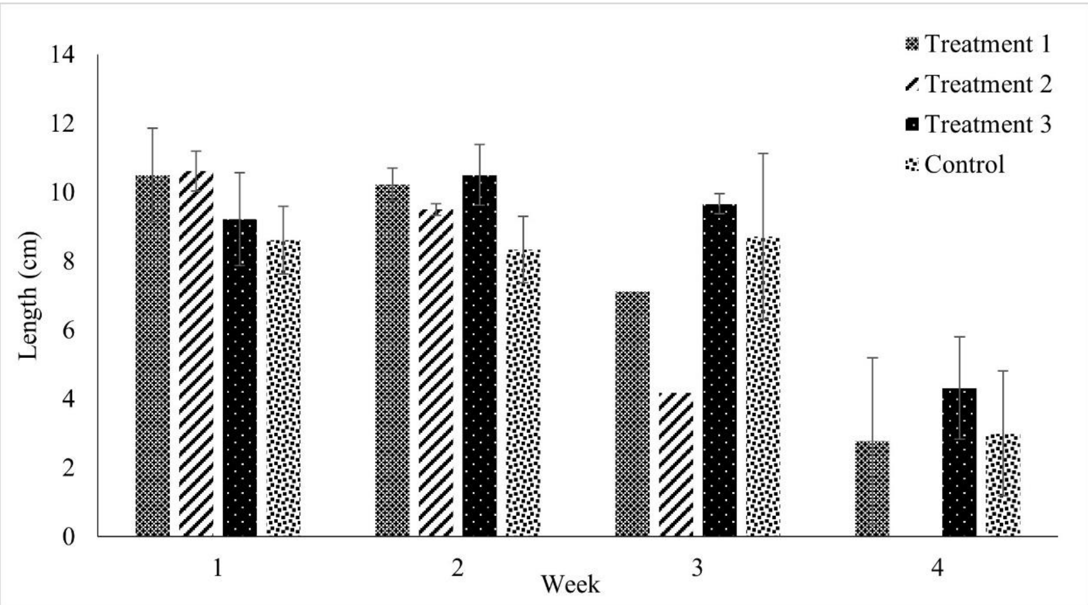

Figure 3. Mean (± sd) body length of sub-adult sandfish within a four-week culture period. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.

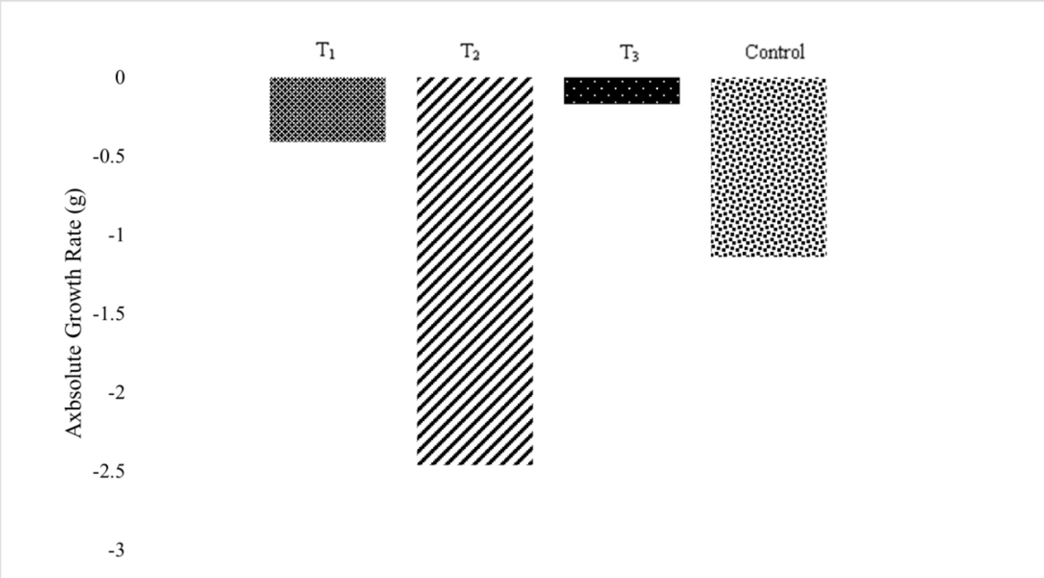

Figure 4. Estimated absolute growth rates (g) of sub-adult sandfish. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.

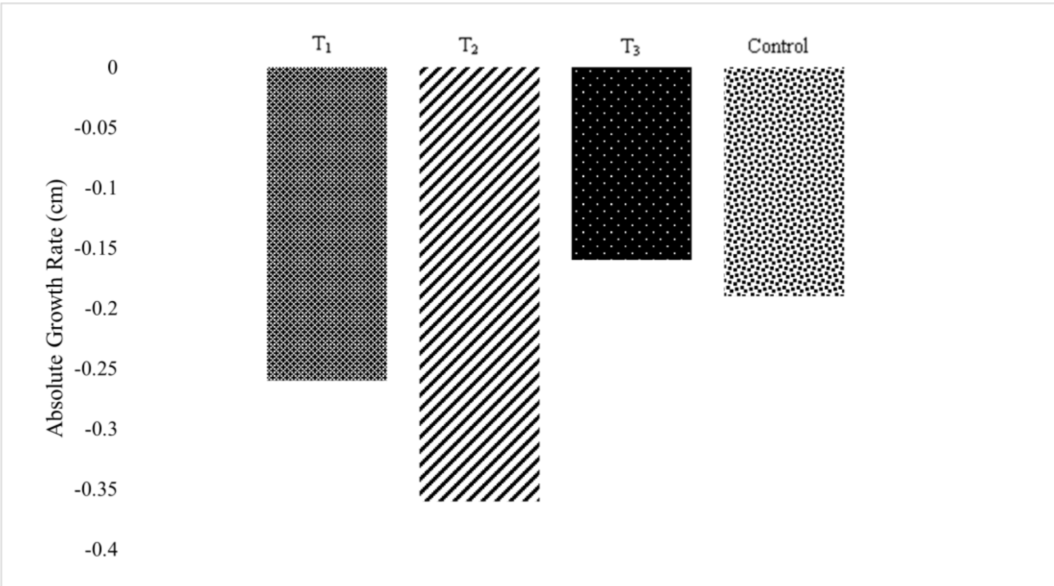

Figure 5. Estimated absolute growth rates (cm) of sub-adult sandfish. T_1 - soya bean powder, T_2 - rice bran, T_3 - powdered seagrass leaves, and control - no supplemental food.

DISCUSSION

Survival

The survival of sandfish varied among treatments. The highest was noted in T_3 (powdered seagrass leaves) and control (no supplemental food), both with 100% survival after 30 days of culture. This was expected because aside from the natural composition of the substrate taken from the habitat of sandfish in the wild, the addition of powdered seagrass leaves as a supplemental feed in T_3 (powdered seagrass leaves) could have helped achieve good results in survival rate since it naturally occurs on the sandfish's environment and rich in organic matter. Sandfish are generally found in seagrass beds (Purcell et al. 2012, Floren et al. 2021) and seagrasses are known to enrich the organic material in sediments. Seagrasses were also used as an ingredient in feeds of broodstocks (Agudo 2006). Floren et al. (2021) also reported that seagrass contributed 32.0% of the organic matter in the sediment and as much as 70% of the sea cucumber diet was derived from seagrasses. Earlier studies have shown as well that sandfish prefer organically rich sediments (Mercier et al. 1999;

Altamirano and Baylon 2020). Additionally, the distribution of sandfish in the tropical Indo-Pacific region coincides with the distribution of the seagrasses particularly *E. acoroides* and *Thalassia hemprichii* (Short et al. 2007).

A lower survival rate was noted in T_1 (soya bean powder), which was supplemented with soya beans. All individuals in one of its replicates died after one week, yielding a 66% survival that was maintained until the end of the culture period. It is possible that the mortality in T_1 (soya bean powder) during the first week was caused by stress during transport and handling and not really due to the nature of the substrate or the addition of powdered soya beans since all the remaining individuals survived the 30-day culture period. Soya beans are organic in nature and were also added as a food component of sandfish broodstocks (Agudo 2006). However, its use must be studied further since pinkish coloration in the bottom of the tanks was observed during the third week of culture, which is an indication of bacterial build-up that is unfavorable and may cause disease and infection to sandfish.

In contrast, samples fed with rice bran (T_2) displayed a gradual decrease in survival until all were extirpated after 30 days of culture. This shows that unprocessed rice bran is not a suitable supplement for sandfish. As the rice bran was locally sourced and bought straight from the market, it did not undergo fermentation to help improve digestibility which may have also negatively affected the sub-adult sandfish. Nevertheless, rice bran can be used as one of the components of feeds for broodstocks of sandfish (Agudo 2006), but adding it directly to the substrate may have detrimental effects on sub-adult sandfish.

Growth Performance

The growth of sandfish across all treatments including the control (no supplemental food) had minimal variations and was almost stable after two weeks of culture, with a slight increase in the length of individuals in T_3 (powdered seagrass leaves). The same performance was observed during the 3rd week except for T_2 (rice bran), wherein individuals significantly decreased in weight and length from 47.68 g and 6.39 cm down to 11.09 g and 4.17 cm ($P < 0.05$), respectively. Individuals in T_1 (soya bean powder) also decreased in length ($P < 0.05$) but not in weight ($P > 0.05$). However, in the 4th week, there was a significant decrease in length in all treatments including the control (no supplemental food) ($P < 0.05$) with 100% mortality in T_2 (rice bran) ($P < 0.05$). Such could likely be attributed to the sudden drop in salinity (27 ppt) during the 3rd week when heavy rains due to LPA diluted the seawater in the reservoir. It has to be noted that sea cucumbers are stenohaline and osmoconformers with a low level of tolerance to salinity change (Sembiring et al. 2019), thus the abrupt salinity change of 3-5 units at 27 ppt is already detrimental to them. Agudo (2006) also reported that heavy rains resulted in mass mortality of sandfish cultured in ponds while strong typhoons resulted in negative growth increments of sandfish in pens (Juinio-Meñez et al. 2014). In addition, all samples in T_2 (rice bran) were observed eviscerating, which is a sign of stress due to fluctuating salinity but could also be a defense mechanism, to the unfavorable condition before their eventual death. The viscera were removed the moment they were noted, but they already contaminated the water, which could aggravate the condition and lead to mortality (Tuwo et al. 2019). Further, the increasing temperature towards the afternoon reaching up to 30.5°C could have also been stressful to sandfish (Mackey and Hentched 2001), but this does not extend for long as the water is changed constantly in the afternoon and morning.

Overall, supplementing the diet of sandfish with powdered seagrass leaves appeared to have positive results because, among the treatments, individuals supplemented in this treatment (T_3) showed a slight increase in growth after one week of culture and were able to withstand the harsh condition without significant weight change. The same is true for sandfish fed with soya beans, in which only the length decreased considerably but not the weight. Individuals in the control did not show any significant changes in length and weight, only after 3rd week when salinity dropped to 27 ppt. The mean weight and length of individuals decreased significantly at the end of the culture period. In addition, their weight was significantly lower than the individuals in T_1 and T_3, which were fed with soya beans and seagrass leaves, respectively.

It appeared that individuals fed with soya bean and powdered seagrass leaves were able to withstand the stress brought by reduced salinity and fluctuating temperature than those fed with rice bran and in the control. Therefore, the use of seagrass powder is recommended to enhance the growth performance of sub-adult sandfish. The soya beans can also be added but must be studied further as the suspected bacterial build-up in the sediment was noted in this study. Although reported as a feed component of sandfish (Agudo 2006), the use of unprocessed rice bran is not recommended due to potential traces of chemicals and digestibility issues, which were detrimental to sub-adult sandfish. This study also highlights the importance of maintaining the water quality (salinity, pH, and temperature) at the optimum level during the acclimation and culture of sandfish in tanks, even for a short period, as they could be more detrimental to sandfish than the quality of the sediment.

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ETHICAL CONSIDERATIONS

The sandfish used in this study were handled with utmost care particularly during transport to avoid stress and mortality. The surviving individuals after culture were not disposed of but were cultured in pens.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests.

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