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Growth, Survival, and Behavior of Early Juvenile Sandfish *Holothuria scabra* (Jaeger, 1883) in Response to Feed Types and Salinity Levels under Laboratory Conditions

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Aquaculture of the tropical sea cucumber *Holothuria scabra* or sandfish is still at a developing stage, especially in the Philippines. In Mindanao, early juveniles of sandfish were successfully produced at the Mindanao State University at Naawan (MSUN) sandfish hatchery for 5 yr now. However, on-site growing of these early juveniles in ocean nurseries often suffered very low survival rates. Two separate laboratory experiments were conducted for 60 d to test for the effects of feed types (*Navicula* sp., powdered *Sargassum*, and *Sargassum* extract) and salinity levels [ambient seawater at 32–35 (as control), 20, 25, and 40 ppt] on the growth, survival, and behavior of 7-wk-old early juvenile sandfish (2–15 mm in length). Juveniles fed with *Sargassum* extract significantly produced the highest increase in width and length, followed by powdered *Sargassum* and *Navicula* sp. with a survival rate of at least 71%. The highest growth rate (GR) and survival were observed with ambient salinity, followed by 40, 25, and 20 ppt. Unusual pale coloration, sluggish movement, and destroyed integument in some parts of the body were observed in some juveniles exposed to lower salinity indication of an unhealthy individual. Overall, *Sargassum* extract is an ideal feed for sea cucumber juveniles. Low salinity is stressful to early juvenile sandfish and that growth and behavior were adversely affected.

Keywords: biological factor, environmental factor, hatchery-reared juveniles, laboratory experiments, sandfish

INTRODUCTION

Sea cucumbers are essential keystone species, bioturbators, and recyclers in many coastal ecosystems and provide vital and often overlooked ecological services in the habitats they occupy (Yuval *et al.* 2014). They are a significant component of the marine ecosystem as they help in the local buffering of ocean acidification and can be hosts for different parasites and commensals (Purcell *et al.* 2016).

In an economic sense, sea cucumbers provide substantial resources of livelihood in coastal communities because they are harvested locally as a source of food and traditional medicine (Subaldo 2011) or as export products

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(Choo 2008; González-Wangüemert 2014). Accordingly, the sea cucumber fisheries have been generating income for over 1,000 years along the Indo-Pacific region (Omar *et al.* 2013), and have now been expanding worldwide driven by the Chinese demand for dried sea cucumbers or *beche-de-mer* (Kinch *et al.* 2008). There were approximately 24–35 species that are commercially exploited (Jun 2002; Ram *et al.* 2010). Sea cucumbers with rigid body walls such as *Holothuria scabra* or sandfish were selected as high valued species and preferred by exporters (Purcell *et al.* 2012). The increasing demand for this organism leads to a depleted state of its population (Eriksson and Bryne 2013). As of now, *H. scabra* is listed as endangered species based on IUCN red list together with other high-valued sea cucumber species such as *Apostichopus japonicus*, *Holothuria lessoni*, and *Holothuria nobilis* (Conand *et al.* 2014).

To provide the demand of the Asian market, sandfish production is highly recommended, and restocking of juveniles is one solution for the depleted wild fishery (Giraspy and Ivy 2009; Giraspy and Walsalam 2010; Ghobadyan *et al.* 2012). Pond grow-out of juvenile sandfish to market size is being developed in Vietnam (Pitt and Duy 2004; Bell *et al.* 2007), and several programs and projects were made to enhance sandfish production, sea ranching, and stock enhancement – especially in the Philippines (Hair *et al.* 2011).

Several problems encountered in sea cucumber farming are often the result of drastic changes in environmental conditions and increases in parasites and predators (Lavitra *et al.* 2009). Stocking densities and periphyton abundance are also other factors for the fast growth and high survival of sandfish juveniles in floating hapas (Gorospe *et al.* 2019; Sinsona and Juinio-Meñez 2019; Altamirano and Noran-Baylon 2020). However, in Naawan, appropriate stocking densities are strictly observed yet high mortality is still experienced. The decline of the periphyton abundance might be one reason for the high mortality of sandfish juveniles during the nursery stage; thus, we are looking into the possibility of subjecting juveniles to different feed types to maximize their growth and survival. And if feeding is to be introduced, we must find alternatives to the usual feed used in the hatchery, which is the live microalgae, to minimize the cost as well as its negative impact on the environment. We looked between powdered *Sargassum* and *Sargassum* extract, as *Sargassum* species are still readily available in the coastal area of Naawan, Misamis Oriental. Depending on the formulated diet and feeding rate, these could affect the growth and survival of sea cucumbers such as in *Holothuria atra* and *H. scabra* (Seeruttun *et al.* 2008; Pattinasarany *et al.* 2014). Studies were conducted to formulate the effective diets of *H. scabra* larvae and early juveniles (Agudo 2006; Duy 2010). One of the most effective diets for *H. scabra* larvae and early juveniles is the *Chaetoceros calcitrans* (Abidin *et al.* 2019). However, during the preliminary experiment conducted last June–July 2016, it was found out that the *C. calcitrans* produced the lowest growth and survival among powdered *Sargassum* and *Sargassum* extract and the cost of production and maintenance for this microalgae is expensive, which is a bottleneck for small scale hatcheries. That is why introducing new diets that are cost-efficient yet could enhance growth performance and produce high survival is necessary for culturing this organism. One alternative for live microalgae that is easily produced in the hatchery is the *Navicula* sp. Another accessible feed is the *Sargassum* found to be a good supplemented feed for live microalgae during the early juvenile stage (Edullantes 2015). *Sargassum* species and floating mats of *Sargassum* contain a wide range of biologically active compounds and have a high ash content, which provides minerals and trace elements that are beneficial in both fertilizer and animal feed (Milledge and Harvey 2016). These also contain minerals and vitamins (Cajipe 1990) and macro and micronutrients that promote growth (Divya *et al.* 2015; Milledge and Harvey 2016).

Similarly, another physicochemical factor is salinity, which is one of the principal components that affect the physiological performance (Yu *et al.* 2013) and regulate the growth and reproduction of marine animals (Purwati and Luong Van 2003). Below or above the preferred normal salinity level of the organism could result in abnormal development and deformation, which may cause larvae mortality (James *et al.* 1994; Meng *et al.* 2011) and low specific GR like in the *H. atra* (Seeruttun *et al.* 2008).

In Northern Mindanao, Philippines, an ACIAR (Australian Centre for International Agricultural Research) funded project on the "Expansion and Diversification of Production and Management System for Sea Cucumbers in the Philippines, Vietnam and northern Australia" (Project No. FIS/2010/042) – also called the Sandfish project –was implemented at MSUN. With the help of this project, sandfish juveniles have been successfully produced in the hatchery. However, the juveniles experienced high mortality rates during nursery culture in sea-based floating hapas. Before the juveniles are released to the natural environment, they have to grow up to 3 g, thus requiring at least another 2 mo of nursery rearing. During this period, their feeding efficiency and responses to eco-physiological factors are most crucial for the success and high survival of the sandfish juvenile. We have an insufficient understanding of the role of feed types and salinity during this crucial stage. Investigating these aspects can shed more light on more effective resource management. To address this knowledge gap and to generate useful information on the growth, survival, and behavioral response of *H. scabra*, a 60-d study was conducted using the hatchery-reared *H.scabra* early juveniles subjected to different feed types (*Navicula* sp., powdered *Sargassum*, and *Sargassum* extract) and exposed to various salinity levels [ambient seawater at 32–35 (as control), 20, 25, and 40 ppt] under laboratory conditions.

MATERIALS AND METHODS

Test Organism

The hatchery-reared sandfish early juveniles measuring 2−15 mm in length and weighed less than 1 g were used in the study and were provided by the MSUN sandfish project. The source broodstock of the juveniles was from Kauswagan, Lanao del Norte, which was spawned on 15 Sep 2016 at the MSUN sandfish hatchery. The 60-d experiments started on 16 Nov 2016 until 16 Jan 2017.

Experimental Design

Two experiments (feed types and salinity) were conducted indoors at the extension facility of the MSUN sandfish hatchery. Experiments were carried out simultaneously in a 50-L glass aquarium with a dimension of 59 cm x 29 cm x 30 cm (L x W x H). Nine glass aquaria were used for feed types and 12 for salinity experiments with three replicates per treatment. Glass aquaria were placed on three white tables to easily observe the juveniles. Sediments were not added to avoid other contributing factors such as organic matter, which may affect the growth of the juveniles. In each experiment, replicate aquaria were haphazardly arranged across the experimental table to discount potential spatial effects such that of light, temperature, and other factors. A total of 1,050 juveniles (length: 2–15 mm; weight: < 1 g) were selected and transferred to the aquarium. For each treatment, 50 individuals were randomly stocked per aquarium to avoid overcrowding, since the growth of *H. scabra* juvenile is being affected by high density (Battaglene *et al.* 1999). The seawater that was used was passed through an ultraviolet (UV) water filtration system. Each aquarium was filled with 40 L of UV-filtered seawater, where each set up was provided with a complete aeration system for water circulation ensuring a continuous supply of dissolved oxygen. Water management was done every 3 d by partially siphoning ~ 80% of water in the aquarium.

Feeding Experiment

Feed types experiment was conducted using three different feed types (Table 1). The amount of feed subjected to the juveniles in this experiment was based on the preliminary experiment in feed types we conducted last June–July 2016. In the first 30 d of the experiment, *H. scabra* juveniles were fed with each of the experimental feed types twice a day, *i.e.* 6:00 AM and 4:00–6:00 PM. Adjustments were done on Days 31–60 of the experiment by increasing the feeding amount and feeding only once a day at 6:00 AM. For example, F1 was fed with 500 mL of *Navicula* sp. twice a day during the first 30 d, and 2,000 mL of the former succeeding Days 31–60 of the experiment. One (1) mL of *Navicula* sp. contains ~ 20,000–40,000 cells. For F2, approximately 2 g of powdered *Sargassum* on the first 30 d and ~ 8 g on Days 31–60. Powder *Sargassum* was diluted in 50 mL of UV-filtered seawater before being administered to each treatment. For F3, 100 g of fresh chopped *Sargassum* and 100 mL of UV-filtered seawater from the tanks (1:1 ratio) were blended for 2–3 min, then strained through a 45-μm mesh size sieve. Each replicate was fed with 30 mL of the extract on the first 30 d and 120 mL on Days 31–60.

Salinity Experiment

Four salinity treatments with three replicate each treatment was conducted. Treatments that were used were 32–35 (ambient; as the control), 20, 25, and 40 ppt. Desired lower salinities were achieved by diluting UV-sterilized filtered seawater mixed with filtered freshwater. The highest salinity level (40 ppt) was obtained by adding commercial sea salt to the seawater. Juveniles were fed using powdered *Sargassum* suspended in seawater before adding, with ~ 2 g on the first 30 d in the morning and afternoon and adjusted into ~ 8 g on Days 31–60 and fed

Table 1. Different feed types/diets used in the study.

Treatment	Feed type	Description	Feeding rate per day
F1	*Navicula* sp.	Pure cultured benthic diatom	1,000 mL (Days 1–30) 2,000 mL (Days 31–60)
F2	Powdered *Sargassum*	Powdered dried brown *Sargassum* seaweed	4 g (Days 1–30) 8 g (Days 31–60)
F3	*Sargassum* extract	Blended fresh brown *Sargassum* seaweed	60 mL (Days 1–30) 120 mL (Days 31–60)

in the morning only. Powdered *Sargassum* was chosen as feed to the juveniles in the salinity experiment since it is used as an alternative feed for early juveniles cultured in tanks at MSUN hatchery.

Data Collection

During monitoring (Days 0, 30, 45, and 60), juveniles were carefully siphoned from the tank using an aeration tube to a white plastic tray, where a ruler was provided as a scale for length and width measurement using Coral Point Count with Excel Extensions (CPCe 4.1) software (Kohler and Gill 2006). The length was measured from the mouth to the anus. The widest portion of the body was measured to get the width of the juveniles. Juveniles that were curved or curled in the photo were noted in the comments and estimations of the length and width were done during photo analysis. The actual length and weight of juveniles at 60 d of culture or upon the termination of the study were determined using a celluloid ruler and a pocket weighing scale with 0.1-g accuracy, respectively.

During water management (every 3 d), survival was determined by manual counting of the number of surviving live juveniles. Dead juvenile in each tank was noted as indicated by the presence of a lesion of the integument and paleness of body color. A dead juvenile was removed but not replaced in treatments where mortality was observed. Different physical and behavioral responses of juveniles were also monitored every before and after feeding. These include skin condition, location in the tank, and activity (Table 2).

Table 2. Physical and behavior response criteria for *H. scabra* juveniles with activity response modified from Wolkenhauer and Skewes (2008).

Responses	Definition
Skin condition	
Thin	Pale/ lighter color; veins and internal organs may be seen from the skin
Medium	Medium; not too dark nor pale; gray skin coloration
Thick	Dark color; a large amount of black is mixed into the skin
Location	
On the wall	Juveniles found in the wall surface of the tanks
On the bottom	Juveniles found on the floor of the tanks
Activity	
Resting	The animal is inactive with no movement observed
Feeding	The animal is actively feeding on the floor or walls; tentacles are exposed and the head performs sweeping movements
Moving	The animal is slowly moving and not resting

Statistical Analysis

One-way analysis of variance (ANOVA) was used to test the differences in length, width, GR, survival, and FPR (feces production rate) among treatments of the two experiments at a 5% level of significance. Data were log-transformed when the assumption of homogeneity of variance was not met. When a significant difference was found, Tukey's test was performed to determine the source of variation. Average values were determined and range descriptive statistics were also employed. All analyses were performed using SPSS software (18.0).

RESULTS

Length and Width

The length and width of juveniles fed with powdered *Sargassum* and *Sargassum* extract showed a rapid increase within 30 d but not those fed with *Navicula* sp. (Figures 1A and B). Juveniles fed with *Sargassum* extract consistently showed the highest increase in both length and width across all sampling times, followed by powdered *Sargassum* and *Navicula* sp. as the lowest. All feed type treatments were significantly different in length and width (one-way ANOVA, both, $p < 0.05$). Juveniles in different salinity levels showed a linear increase in length and width and were significantly different in each day of culture (one-way ANOVA, $p < 0.05$) (Figures 1C and D). The highest length of juveniles was observed in 40 ppt while the lowest was in 20 ppt during 60 d. In contrast, juveniles under 25 ppt had the highest width while 20 ppt are still the lowest.

GR

The weights of juveniles administered with different feed types and salinity levels increased gradually until the end of the experiment. After the 60-d feed-type experiment, the GR of *H. scabra* juveniles differed significantly among treatments (one-way ANOVA, $p < 0.001$; Figure 2A). Juveniles fed with *Sargassum* extract showed the highest GR of 0.026 g d^{-1}, followed by powdered *Sargassum* and *Navicula* sp. having GR values of 0.010 and 0.003 g d^{-1}, respectively (Figure 2A). In contrast, the GR of juveniles did not differ significantly among salinity treatments (one-way ANOVA, $p > 0.05$). The highest GR of the juveniles was observed in the ambient salinity (32–35 ppt), followed by 40, 25, and 20 ppt with a range of 0.016– 0.026 g d^{-1} (Figure 2B).

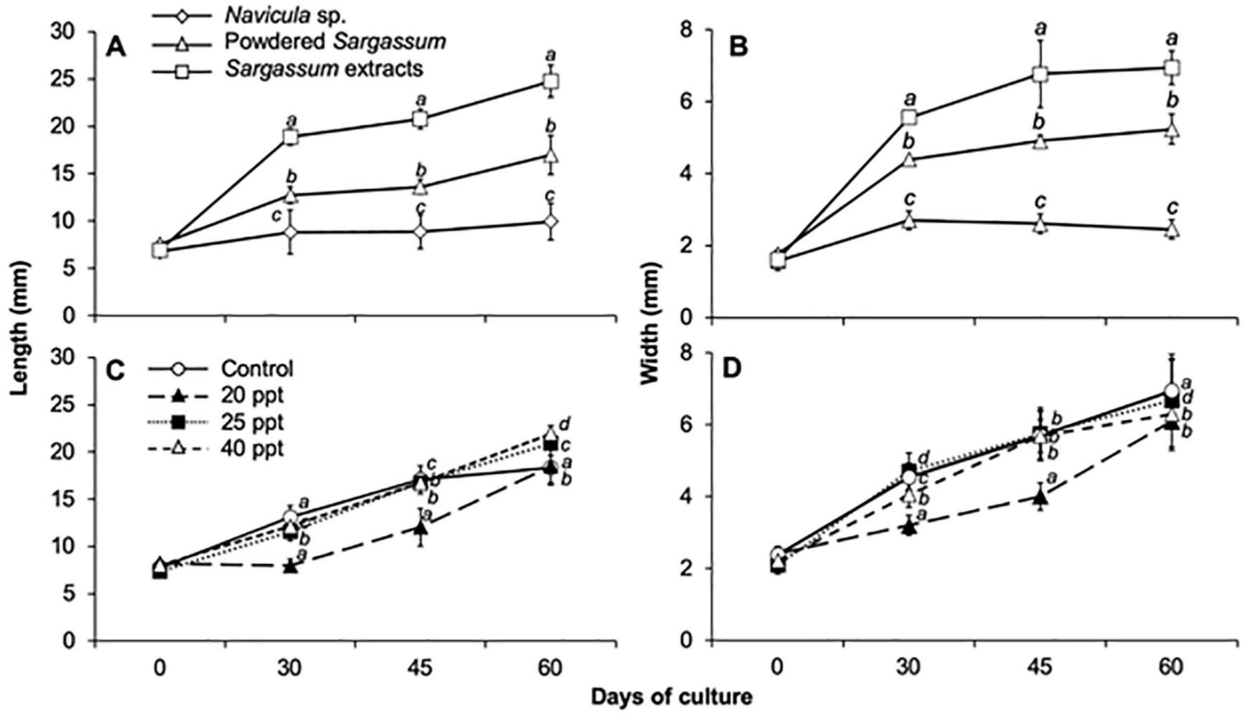

Figure 1. Growth of *Holothuria scabra* juveniles in terms of length and width in different feed types (A, B) and salinity (C, D) experiments.

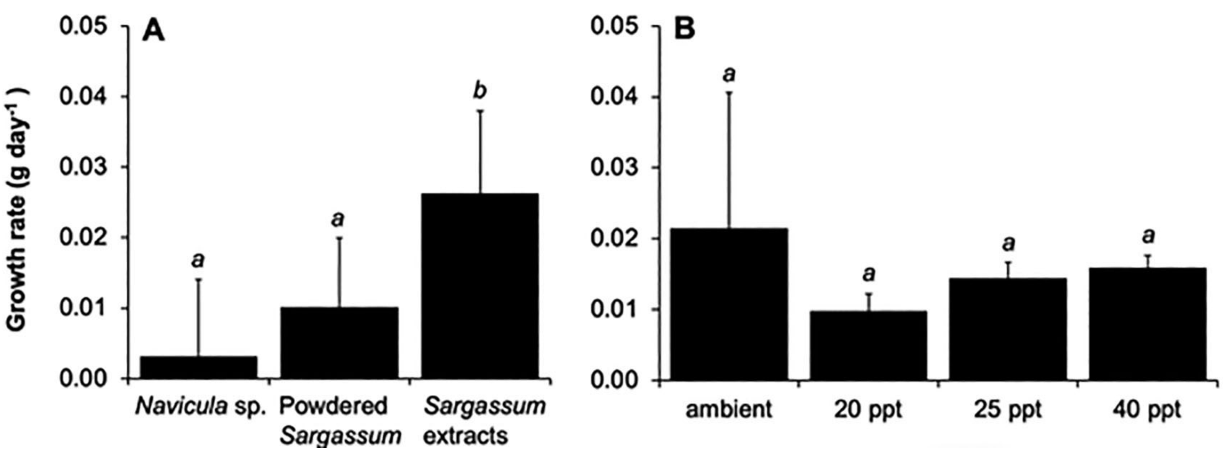

Figure 2. The GR of *Holothuria scabra* juveniles in different feed types (A) and salinity (B) experiments. Error bar represents the standard deviation of the experiments in three replications.

Size Distribution

Size ranges of juveniles administered in different feed types were approximately the same at the start of the experiment. All treatment size ranges were within the range of 2–12 mm. At the end of the experiment, the length of juveniles in all treatments greatly varied and diversified (Figure 3A). Juveniles fed with *Navicula* sp., powdered *Sargassum*, and *Sargassum* extract obtained wide size ranges of 0–28, 2–38, and 2–54 mm, respectively. Moreover, all treatments showed a different higher frequency of size ranges. Juveniles fed with *Navicula* sp. had a higher frequency in the range of 2–10 mm while in powdered *Sargassum*, the length-frequency was higher at 10–22 mm and in *Sargassum* extract at 20–34 mm. The size ranges of juveniles grown in different salinity levels were also approximately the same at 6–15 mm at the start of the experiment. The length of juveniles cultured in the ambient reached 3–39 mm and juveniles in 20, 25, and 40 ppt reached 6–45 mm at the end of the experiment (Figure 3B). The highest frequency of juveniles was found at 24

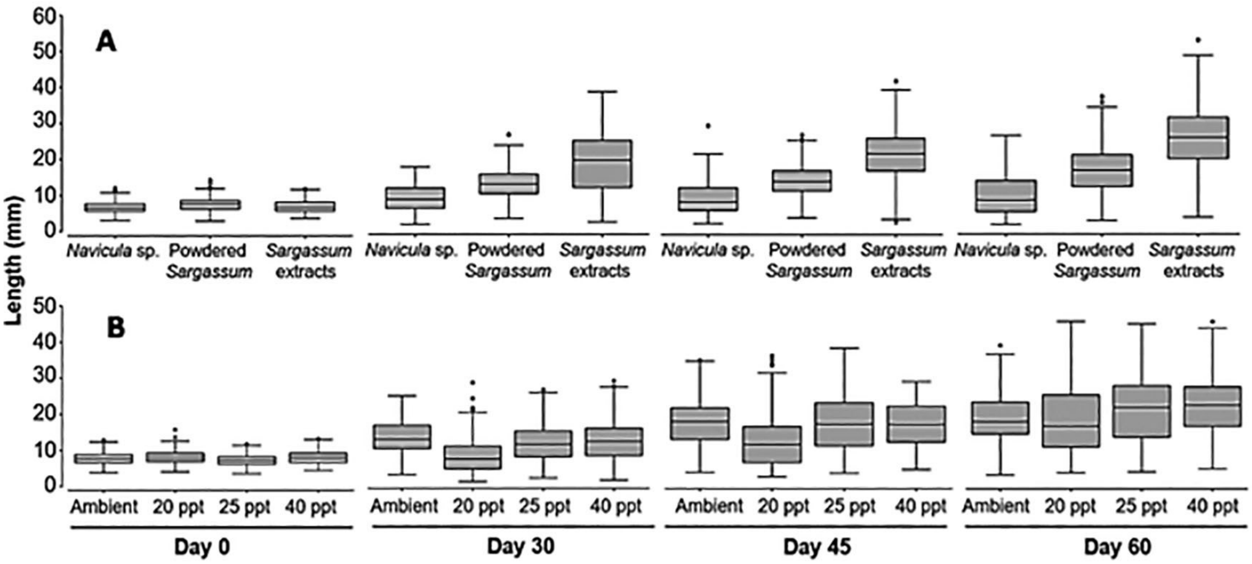

Figure 3. Size distribution of *Holothuria scabra* juveniles in different feed types (A) and salinity (B) experiments. The number of individuals indicated on each bar.

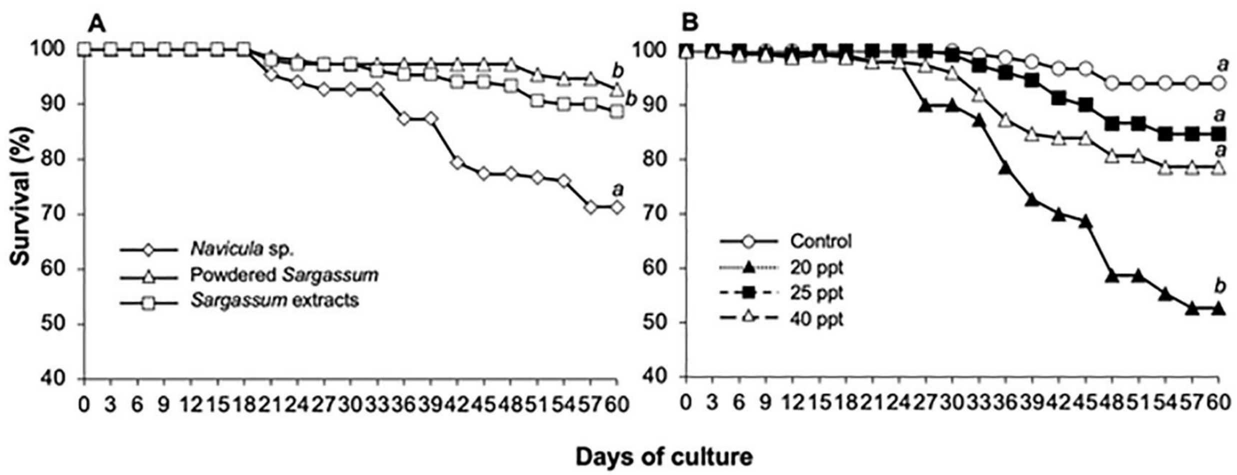

Figure 4. Survival of *Holothuria scabra* juveniles in different feed types (A) and salinity (B) experiments.

mm exposed at 25 and 40 ppt, followed by ambient and 20 ppt at 18 and 12 mm, respectively.

Survival

Percent survival of juveniles in feed types and salinity experiments differed significantly among treatments (one-way ANOVA, $p < 0.001$, both experiments; Figures 4A and B). The survival of juvenile was highest on powdered *Sargassum* at 92.67%, followed by *Sargassum* extract at 88.67%, and lastly, those fed with *Navicula* sp. at 71.33% (Figure 4A). The start of mortality occurred at 21 d of rearing and continuously decreasing until 60 d of the experiment. In contrast, the mortality of juveniles in different salinity levels began on Day 6 in 40 ppt, Day 15 in 20 ppt, Day 30 in 25 ppt, and Day 33 in ambient salinity. The trend showed the highest mean number of live juveniles was in the ambient salinity and lowest in 20 ppt at the range of 52.87–94.00% survival (Figure 4B

Physical and Morphological Behavior

The change of skin condition of the juveniles was observed inside the aquaria in both experiments, in which the number of the thin and medium juveniles decreases while the thick skin condition increases until Day 60 (Figures 5A and B). Aside from the change in skin condition, some of the *H. scabra* juveniles exposed to the lower salinity treatments (*i.e.* 20 ppt) had a sluggish movement and a disintegration of some body parts during the middle

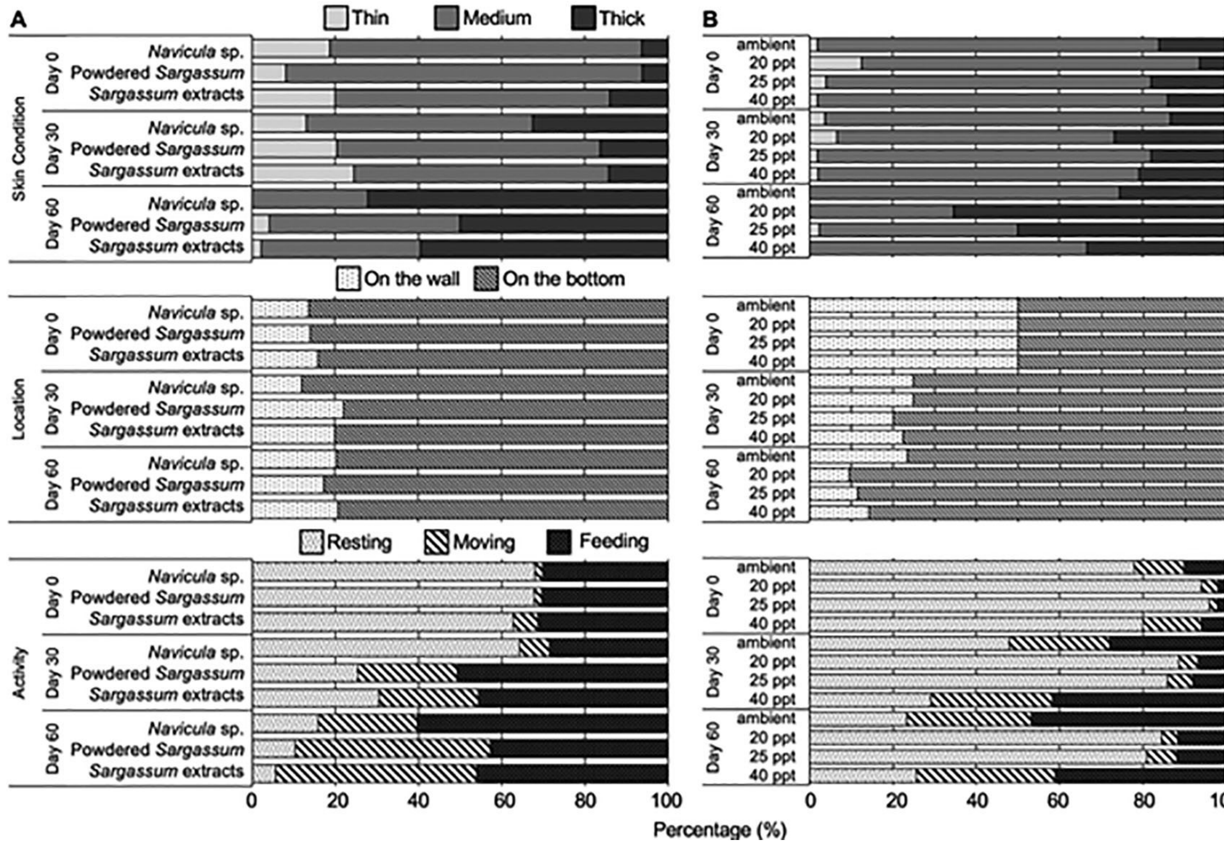

Figure 5. Percentage of skin coloration, location, and activity responses of *Holothuria scabra* juveniles in different (A) feed types and (B) salinity levels during Days 0, 30, and 60.

until the end of the salinity experiment. In the feed types experiment, most of the juveniles in *Sargassum* extract were observed to be abundant on the walls of the aquarium tank during feeding. In contrast, juveniles in the *Navicula* sp. and powdered *Sargassum* treatments were abundant at the bottom of the tank. *H. scabra* juveniles exposed to different salinity levels were mostly observed at the bottom rather than on the walls of the tank during feeding. Juveniles activity in both experiments were found to spend more time resting on the first 30 d and actively feeding thereafter; however, less movement on lower salinity of 20 ppt was observed (Figures 5A and B).

FPR

The FPR in the feeding experiment did not differ significantly among treatments (one-way ANOVA, $p > 0.05$). The highest FPR was found in powdered *Sargassum* (1.38 g g^{-1} d^{-1}), followed by *Navicula* sp. (1.01 g g^{-1} d^{-1}), and lastly, *Sargassum* extract (0.72–1.38 g g^{-1} d^{-1}). *Sargassum* extract has the highest total weight of animal casting at the end of the experiment, yet they exhibited the lowest FPR of 0.72%.

DISCUSSION

Length and Width

Here, we systematically demonstrated the effects of feed types and salinity on the growth and survival of *H. scabra* juveniles. The feed types significantly affect the increase in length and width of the juveniles. When juveniles fed with *Sargassum* extract, it showed the highest increase in length and width, not only because *Sargassum* contains bioactive compounds plus micro and macronutrients that are beneficial as animal feed (Milledge and Harvey 2016) but also because brown algae such as *S. thunbergii* and *S. polycystum* are widely used as a source of alginate, a compound with high viscosity (Wu *et al.* 2002). This greater viscosity enables sea cucumbers to handle more easily and feed more efficiently on it (Xia *et al.* 2012). The low viscosity makes it hard for sea cucumbers to handle such as the powdered *Sargassum*, which may cause the sea cucumber to reject the feed or reduce its consumption. In the case of the live feed like *Navicula* sp., Watanabe *et al.* (2011) found that *Navicula ramosissima* resulted in the lowest growth than shrimp tank detritus and shrimp feces. They are only favorable as supplemental feed if mixed with *Sargassum* extract for better results in early juveniles. Tuwo *et al.* (2012) mentioned that salinity is one of the important factors affecting the growth of *H. scabra* juveniles. In this study, the length and width of *H. scabra* juveniles were significantly affected when exposed to different salinity levels. The highest lengths were observed at the highest salinity (40 ppt) compared to the lower salinity treatment (20 ppt). Similar results were also reported by Yuan *et al.* (2010), which concluded that sea cucumber grew better in higher salinity rather than low salinity at its optimum temperature, although this result is species-specific. Possibly, the effect of lower salinity causes stress to juveniles, resulting in slower growth. The findings of the study may also suggest that juveniles exposed to ambient salinity may limit their energy in increasing its length and width and spend their energy more in growing other aspects such as their weight since salinity is an indirect factor that modifies numerous physiological responses such as the growth of marine organisms (Hu *et al.* 2010).

GR

Juveniles fed with *Sargassum* extract showed the highest GR followed by powdered *Sargassum* and *Navicula* sp., which concurred in the study of Battaglene (1999), in which juveniles fed with *Sargassum* resulted in a GR of 0.2 ± 0.02 g d^{-1}. In this study, juveniles fed with *Sargassum* extract reached larger sizes. Nutrients extracted from *Sargassum* such as *S. latifolium* gave the best growth and high survival rates on early juveniles of *H. scabra* (Lavitra *et al.* 2009; Dabbagh and Sedaghat 2012). Many biologically active compounds like terpenoids, flavonoids, sterols, sulfated polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, and pheophytine can be isolated from different *Sargassum* species (Yende *et al.* 2014). This could be the reason for the higher growth of the juveniles produced when fed with *Sargassum*. Also, some macro- and microorganisms are still present in the *Sargassum* extract than the powdered *Sargassum*, which has undergone drying and pulverizing. The results of the study also showed that ambient seawater around 32–35 ppt was best for the rearing of the *H. scabra* juveniles, which is consistent with the findings of Mills *et al.* (2012) and Seeruttun *et al.* (2008). Asha *et al.* (2011) observed the highest GR in early-stage juvenile *H. scabra* at 30 ppt with 0.008 g d^{-1} and lowest at 0.0032 g d^{-1} at 20 ppt while juveniles did not grow in 40 ppt. Lower GRs were observed in the lower salinity treatment. This finding corroborates with other studies which reported that lower salinities reduced the GRs and increased mortality in sea cucumbers (Meng *et al.* 2011; Mills *et al.* 2012). Furthermore, reduced salinity would be a difficult environment for the juveniles to adapt and grow, which causes stress that eventually affects their burrowing behavior and feeding cycles (Mercier *et al.* 1999, 2000).

Size Distribution

Food preference experiments are widespread in the ecological literature (Roa 1992). However, algal diets in several studies were not selected based on nutritional value (Xia *et al.* 2012), as well as in this study. The peak of the length sizes of the juveniles in response to different feed types was observed at 8, 16, and 32 cm in *Navicula* sp, powdered *Sargassum,* and *Sargassum* extract, respectively. As observed, the peak in sizes in different feed types doubled and quadrupled from the lowest peak because most animals have evolved senses that enable them to discriminate against foodstuffs, resulting in dietary preferences (Xia *et al.* 2012). The length frequencies of juveniles exposed to different salinity levels do not greatly vary between treatments in this study, which may suggest that juveniles spent more energy on survival rather than growth. Similarly, Yuan *et al.* (2010) found *Apostichopus japonicus* subjected to lower salinity have more energy allocation spent on consumed food on feces, respiration, and excretion but less energy on growth. Findings of Liu *et al.* (2004) indicated that different sizes of *A. japonicus* juveniles thrive to different salinity levels, indicating that salinity tolerance of juvenile holothurians is size-specific. In this study, juveniles exposed to 40 ppt and ambient salinity exhibited unimodal patterns while 20 ppt and 25 ppt showed polymodal patterns, although these were not clearly shown on Day 60. These patterns showed that juveniles under 40 ppt and ambient were not as a variable in size compared to lower salinities treatments. The juveniles under lower salinity treatments have different adaptive capability probably results in their varied sizes, leading to a polymodal pattern. Aside from the ambient salinity, the other treatments had a wide representation of small and large individuals on Day 60. But still, all treatments are normally distributed.

Survival

In this study, a clear difference was observed in the survival of all feed-type experiments. Mortality substantially started to occur during the 3rd week of the experiment. Although Lavitra *et al.* (2009) reported that the highest mortality could occur during the first 2 wk for < 5 mm juveniles (Lavitra *et al.* 2009), both food availability and feed types affect the survival of early juveniles of *H. scabra*. The results of the salinity experiments showed that juveniles could tolerate higher salinity around 40 ppt and reduced salinity up to 20 ppt for about 24 d. However, after 24 d, rapid mortality in the juveniles occurred. The mean percentage survival of *H. scabra* juveniles varied in all treatments, indicating the influence of salinity on the survival of juveniles. The study of Tuwo *et al.* (2020) revealed that the mortality of H. *scabra* exposed to lower salinity is evident regardless of whether the drop is due to a sudden or gradual change. It was highly unfavorable to culture the sea cucumber in low salinity (Seeruttun *et al.* 2008), especially below the body salinity of saltwater bony fish, which is 18 ppt (Tuwo *et al.* 2020). *Holothuria scabra* could tolerate reduced salinity (20 ppt) for a short period (Pitt *et al.* 2001; Agudo 2006), but it does not show preferences for brackish waters (Mercier *et al.* 2000). These observations may substantiate the findings of the present study, wherein ambient water has shown to be the preferred salinity level for the rearing of juveniles. Several sandfish nursery systems were successfully developed in marine ponds (Duy 2012) and successful grow-out trials for monoculture in marine earthen ponds (Bell *et al.* 2007). In this study, sandfish juveniles were found to have no potential for brackishwater pond nursery due to their limited tolerance to lower salinity of lesser than 20 ppt. Moreover, there are other physicochemical factors aside from salinity that need to be considered in putting a brackish-water pond nursery production of sandfish in the Philippines such as silty muddy sediment that seems to be unsuitable for sandfish cultures (Altamirano *et al.* 2017).

Physical and Behavioral Responses

Juveniles showed and exhibited no marked abnormal physical and behavioral responses on both experiments except for some instances in the low salinity treatments. The change in the skin condition of juveniles observed in both experiments was due to the increase in the body length of the juvenile in which such changes were observed when organisms were growing and performing adaptation in the environment. However, there are some juveniles in low salinity treatments that have a sluggish movement with the integument destroyed in some parts of the body and unusually thin (pale coloration), which could be an indication for their inability to adapt to the lower salinity levels. These observations concurred with the study of Purcell and Eeckhaut (2005) that juveniles become unhealthy when they become sluggish and have an unusual pale body coloration. The motility of juveniles was only a normal behavior/adaptation in searching for food where they stayed on the bottom or on the walls depending on the availability of food. This is also the same normal behavior for the activity of the juveniles, in which they spend more time resting at the start of the experiment as they are still adjusting to the environment. Aside from the 20 ppt treatment, all juveniles in the remaining treatments in the two experiments were adapted to the environment, resulting in their active feeding and movement. Sea cucumbers exhibit general plasticity of behavioral and feeding strategies in response to variations in resource availability (Roberts *et al.* 2000) and to animals that spent time feeding and spent periods on burying in sediments (Robinson *et al.* 2015).

FPR

The findings of the feed experiment showed that *Sargassum* extract had the highest total feces weight followed by powdered *Sargassum* and *Navicula* sp. However, the result showed FPR was greatly affected by the initial and final wet weight of the juveniles. *Sargassum* extract had the highest average wet weight of juveniles; thus, the FPR was being affected by the wet weight of the juveniles, resulting in *Sargassum* extract having the smallest FPR value. Results also showed that diets affected feces production, as well as the growth and energy budget. Compared with other echinoderms, in holothurians, the energy deposited in growth is lower and the energy loss in feces accounts for the majority of the ingested energy (Yuan *et al.* 2006). The inorganic component of the feces is primarily undigested dietary elements that also depend on its dietary supply (Rose *et al.* 2015). This claim explains the reason why lower FPR resulted in high growth because more dietary elements were digested rather than excreted.

CONCLUSION

This study tested two factors, *i.e* the effect of different feed types and salinity levels on the growth and survival of the hatchery-reared sandfish juveniles towards the improvement of its nursery stage production. Results of the feed types experiment showed that *H. scabra* juveniles fed with *Sargassum* extract yielded the highest length, width, GR, and survival, as well as no abnormal behavioral responses. Thus, *Sargassum* extract is the most suitable feed for achieving higher growth and survival of *H. scabra* juveniles. This brown alga is readily available from the wild as drifters in the local coastal area of Naawan, Misamis Oriental, and has never been used or exploited by local communities. It should be noted, however, that in small-scale aquaculture operations, this dependence on *Sargassum* is potentially viable but not at an industrial level because of the potential risk of overexploitation.

Ambient seawater salinity (32–35 ppt) resulted in a higher GR and the highest survival, although it could only reach a length of 39 mm after 60 d of the experiment. Nevertheless, *H. scabra* juveniles can still grow and survive at higher salinity of 40 ppt and lower salinity of 20 ppt but only for a short duration, *i.e.* 24 d. However, some of the *H. scabra* juveniles exposed to lower salinity were observed to have a sluggish movement, destroyed integument in some parts of the body, and unusual thin pale body coloration, which can be concluded to be unhealthy. Hence, it will be challenging to culture sandfish juveniles in brackish-water systems.

RECOMMENDATION

Further research needs to be conducted to identify an appropriate formulated feed diet for *H. scabra* juveniles. In addition, salinity experiments still need to be done to determine the level of tolerance and exposure limit of juveniles to lower salinity that may address the viability of sandfish production in brackishwater ponds. These studies will enhance the state of knowledge in making this activity economically and environmentally sustainable for the improvement of the rearing conditions suitable for mass production of the sandfish juveniles for stock restoration.

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NOTES ON APPENDICES

The complete appendices section of the study is accessible at https://philjournalsci.dost.gov.ph/

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APPENDIX

Data Collection

The feces production rate was analyzed in the feed types experiment only to determine the digestibility of the feed to know the best feed type that could result in higher growth of the sandfish juveniles. Water management was also done every three days to ensure the physico-chemical conditions of the water such as salinity maintained throughout the experiment. Animal fecal matter was collected every 3 d during water management by siphoning using an aeration tube and placed in a filtration paper. The collected feces was air-dried for 2–7 d and then oven-dried at 60° C to constant weight for each replicate. After drying, casts were weighed using an analytical weighing scale. Upon termination, all replicated feces were pooled for each treatment.

The water parameters monitored during the experiments were salinity, water temperature, light intensity, dissolved oxygen, and ammonia. Salinity was monitored every 3 d using a portable hand refractometer before and after water management. Water temperature and light intensity were monitored every day using a data logger (HOBO® Pendant Temperature/Light 64K Data Logger). Dissolved oxygen and ammonia in water were analyzed using the Winkler's method and the Phenate method, respectively (APHA 1995).

Data Analysis

The average lengths of *H. scabra* juveniles were converted to average weight using the formula of Purcell and Agudo (2013):

$$W = 0.000614 \times L^{2.407} \quad (1)$$

$$L = 22.826 \times w^{0.370} \quad (2)$$

where L is the length in mm and W is weight in g. The values obtained were also used to determine the length-frequency of juveniles in each day of the culture of the two experiments. The length and width of the juveniles in the two treatments were presented in linear graphs while the length-frequency in box plots.

The growth rate indicates the gain in biomass in different experiments. Absolute growth rate (GR) was calculated using the formula:

$$GR(gd^{-1}) = \frac{(W2 - W1)}{T} \quad (3)$$

where *W1* and *W2* are the mean initial and final wet weight of sea cucumbers in each aquarium, and T is the duration of the experiment in days. The growth rate of the juveniles was presented using bar graphs.

Survival was calculated using the formula:

$$S = \frac{final}{initial} \times 100\% \quad (4)$$

wherein S is the survival (%), "final" is the number of live juvenile sandfish, and "initial" is the number of all juvenile stock in the aquarium. The survival of the juveniles in the two experiments was represented using linear graphs.

The FPR was calculated as follows (Yuan *et al.* 2006):

$$FPR\ (g\ g^{-1}\ d^{-1}) = \frac{F}{\left[\frac{T(W2 + W1)}{2}\right]} \quad (5)$$

where W1 and W2 are the initial and final body weight of sea cucumbers in each aquarium, T is the duration of the experiment (60 d), and F is the dry weight of feces.

Physicochemical Parameters

The physico-chemical water parameters during the experiments were in a normal range in both experiments throughout the 60-d experimental period (Table I).

Table I. The physico-chemical parameters of the feed types and salinity levels experiments in 60 d (NA = not applicable).

Physico-chemical parameters	Feed types	Salinity levels
Salinity	32–35 ppt	NA
Water temperature	25.58–29.75 °C	25.5–29.7 °C
Light intensity	0.14–186.68 lum/ft²	0.07–310.80 lum/ft²
Dissolved oxygen	5.18–9.36 ppm (30 d) 6.13–9.14 ppm (60 d)	6.44–7.36 ppm (30 d) 6.25–7.24 ppm (60 d)
Ammonia	0.07–0.08 ppm (30 d) 0.09–0.23 ppm (60 d)	NA