

SURVIVAL RATE AND GROWTH OF JUVENILE SANDFISH (*Holothuria scabra*) IN VARIOUS REARING CONDITIONS

Lisa Fajar Indriana¹, Muhammad Firdaus¹, Supono² and Hendra Munandar¹

¹ Research and Development Division for Marine Bio Industry. Research Center for Oceanography – Indonesian Institute of Science (LIPI).

² Bitung Technical Implementation Unit for Marine Life Conservation. Research Center for Oceanography - Indonesian Institute of Science (LIPI).

*Correspondence author: lisaindriana23@gmail.com

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ABSTRACT

Techniques for producing *Holothuria scabra* or sandfish sea cucumbers from aquaculture have been developed rapidly in the recent years to address the issues of decreasing sandfish population and increasing market demand. During the process of culturing *H. scabra*, the early stages of sandfish (*i.e.* larvae and juvenile) are the most critical of the development. Therefore, the Research and Development Division of the Marine Bio Industry – LIPI conducted a study to assess the growth performance of juvenile *H. scabra* cultured in four different rearing conditions: (i) indoor concrete tank, (ii) indoor fiberglass tank, (iii) outdoor concrete tank with 1 mm hapa mesh, and (iv) outdoor concrete tank with 3 mm hapa mesh. Each of the rearing condition was set with an initial juvenile density of 15 juveniles per tank with a mean initial weight of 14 g, and fed *ad libitum* with a mixed feed consisting of *Enhalus acoroides* sea grass, *Pennisetum purpureum* napier and cow manure. The experiments were conducted for 20 weeks between April 26 - September 23, 2013. After the experimental period, juveniles reared in the outdoor concrete tank with 1 mm mesh show the best performance with respect to their survival rate ($48.89 \pm 3.85\%$), mean final weight (22.07 ± 1.01 g), final biomass (162.05 ± 18.17 g) and specific growth rate ($0.15 \pm 0.03\%$ per day). Further studies that combine rearing conditions with different bottom substrates as well as investigating the effect of light are needed to optimize the production of *H. scabra* from aquaculture.

Keywords: *Holothuria scabra*, growth, aquaculture, juvenile, tank

INTRODUCTION

Indonesia is currently increasing its sea cucumbers production, therefore several national strategic steps have been put forward including aquaculture that could supplement wild capture production. Over-exploitation has been threatening the sustainability of sea cucumbers production, more so in the face of a growing market demand. Between 2005-2014, capture-based sea cucumbers production in Indonesia reached an average increase of 0.81%, or equivalent to 5,428 tons in 2014 (DGCF, 2015). To further increase its production of sea cucumbers over the period

2015 - 2019, Indonesia has designed a 174,600 ha area for aquaculture development (DGA, 2015).

Although several techniques for increasing sea cucumbers production in hatcheries or aquaculture have been established (*e.g.* Battaglene, 1999; Pitt, 2001; Agudo, 2006), the mass production of sea cucumbers still faces issues with respect to production cost, nursery technology and survival rate of cultured juveniles. For example, the rearing of juvenile *H. scabra* in earthen seawater ponds, sea pens and sea cages takes at least two years to reach the commercial sizes of 0.7 - 1 kg (Purcell, 2004; Bell *et al.*, 2007; Purcell and Simutoga, 2008).

Moreover, the survival rate and growth performance of juvenile *H. scabra* during their early stages are relatively low. Technologies for culturing the early stages of sandfish are available, particularly rearing methods for newly settled juveniles using staked plates and sand in rearing tanks (Battaglione, 1999; Pitt, 2001). However, optimizing the growth performance of juvenile sandfishes during their grow-out stage is still a challenge. For instance, Purcell (2004) reported that the survival rates of juvenile *H. scabra* (3 - 15 g) released in sea pens or sea ranch only range between 10 - 30%.

The use of artificial feed to increase the growth of juveniles has been explored, yet at the cost of more expensive ingredients. Seaweed (*Sargassum* sp.) has been suggested as the best ingredient for formulated powder feed (Wu *et al.*, 2015). However, the exploitation of seaweed as a source of artificial feed raises a sustainability concern for aquaculture development. Therefore, substituting seaweed with highly available terrestrial plants has also been developed for feeding sandfishes to lower the production cost (Xia *et al.*, 2012; Wu *et al.*, 2015).

Besides nutrients from formulated feed, the surface area of nursery tank where juvenile sandfishes live in is also a major factor for their grow-out stage. *H. scabra* sandfishes are deposit feeders and require a large surface area for foraging (Bell *et al.*, 2007). Competition for space impedes the growth of holothurian juveniles, where overcrowding reduces their growth rates due to the lack of food or malnutrition, while increasing size variability (Liu *et al.*, 2004). The growth of juvenile sandfishes significantly decreases when reaching a biomass of 225 g/m² of tank floor space (Battaglione *et al.*, 1999).

Physical aspects of rearing tanks may play an important role in producing natural food sources as diatom film and bacteria (biofilm) can attach to the tank wall (*e.g.* Battaglione, 1999). Rearing tanks for juvenile sandfishes conventionally use rectangular concrete tanks, but recently fiberglass

tanks have been used as well. While the use of various rearing tanks has been massively applied, it is not well understood whether there is any difference between the growth performances of juvenile *H. scabra* reared in different types of tank.

Therefore this study aims to investigate the survival rate and growth of juvenile *Holothuria scabra* reared in various rearing conditions. In line with the effort to optimize sea cucumbers production from aquaculture, the Research and Development Division of the Marine Bio Industry – LIPI located in West Nusa Tenggara has been conducting research on the aquaculture of *H. scabra*. In 2011, the Research and Development Division has developed artificial feeds sourced from various terrestrial plants including napier grass (*Pennisetum purpureum*). The mixed of napier grass with sea grass (*Enhalus acoroides*) and cow manure can be used as a substitute feed for juvenile *H. scabra* (Dwiono, *pers. comm*). Moreover, the research unit is located within the Fisheries Management Area (FMA) 713 covering the Makassar Strait, Bone Bay, Flores and Bali Sea, which supports the highest percentage (41%) of wild captured sea cucumbers production in Indonesia in 2014 (DGCF, 2015).

MATERIALS AND METHODS

The juveniles of *H. scabra* used in the experiments were from the hatchery of the Research and Development Division of the Marine Bio Industry – LIPI. A total of 180 juveniles with an initial mean weight of 14 g was used. The juveniles were reared for 20 weeks (April 26 - September 23, 2013) under four different rearing conditions, which are: (i) indoor concrete tanks with 1 mm hapa mesh (ICT 1 mm), (ii) indoor fiberglass tanks with 1 mm hapa mesh (IFT 1 mm), (iii) outdoor concrete tanks with 1 mm hapa mesh (OCT 1 mm), and (iv) outdoor concrete tanks with 3 mm hapa mesh (OCT 3 mm). See Table 1 for details. Each treatment was conducted in triplicates and stocked with 15

juveniles.

Table 1. Set up of the four rearing conditions.

Treatment	Description
ICT-1 mm	Indoor Concrete Tank Tank dimension: 400 (L) x 50 (W) x 50 (H) cm. Floating hapa frame: 100 x 20 x 20 cm. 1 mm mesh size.
IFT-1 mm	Indoor Fiberglass Tank Tank dimension: 400 x 80 x 75 cm. Floating hapa frame: 100 x 20 x 20 cm. 1 mm mesh size.
OCT-1 mm	Outdoor Concrete Tank Tank dimension: 500 x 200 x 100 cm. Floating hapa frame: 100 x 20 x 20 cm. 1 mm mesh size.
OCT-3 mm	Outdoor Concrete Tank Tank dimension: 500 x 200 x 100 cm. Floating hapa frame: 100 x 20 x 20 cm. 3 mm mesh size.

A formulated feed consisted of *Enhalus acoroides* sea grass (40%), *Pennisetum purpureum* napier grass (30%) and cow manure (30%) was given *ad libitum* during the rearing period. The tanks were continuously aerated and 75% of the water was changed every 3 days. Weekly water quality was conducted on the tanks. Juveniles from each treatment were assessed for their survival rate and individual weight every 2 weeks.

At the end of the experiment, the survival rate (SR), the specific growth rate (SGR) and the final biomass (FB) of juveniles were estimated following Zhou *et al.*, (2006) and Ju *et al.*, (2015):

$$SR = (N_t/N_0) \times 100\% \quad (\text{Eq. 1})$$

where SR is the survival rate (%), N_t and N_0 are the final and initial numbers of biota, respectively.

$$SGR = 100 (\ln W_t - \ln W_0) / t \quad (\text{Eq.2})$$

where SGR is the specific growth rate (% per day), W_0 is the initial wet weight (g), W_t is the final wet weight (g), and t is the length of culturing period (days).

$$FB = W_t \times N_t \quad (\text{Eq.3})$$

where FB is the final biomass (g). W_t is the final wet weight (g) and N_t is the final number of biota.

Statistical Analysis

Data on the effect of various rearing conditions on the growth performance of juvenile *H. scabra* are analyzed with the one-way analysis of variance (ANOVA) at a significance level of 0.05 using the SPSS software (version 22). The Tukey post-hoc test is applied to investigate any significant difference between treatments.

RESULTS

Survival Rate

The survival rates of juvenile *H. scabra* in all types of tank decrease during the rearing period with less than 50% at the end of the experiments (Figure 1). It appears that the week 10 is a critical period when juveniles started to die with steady mortality rates afterwards. Juveniles reared in the OCT 1 mm experiment show the highest

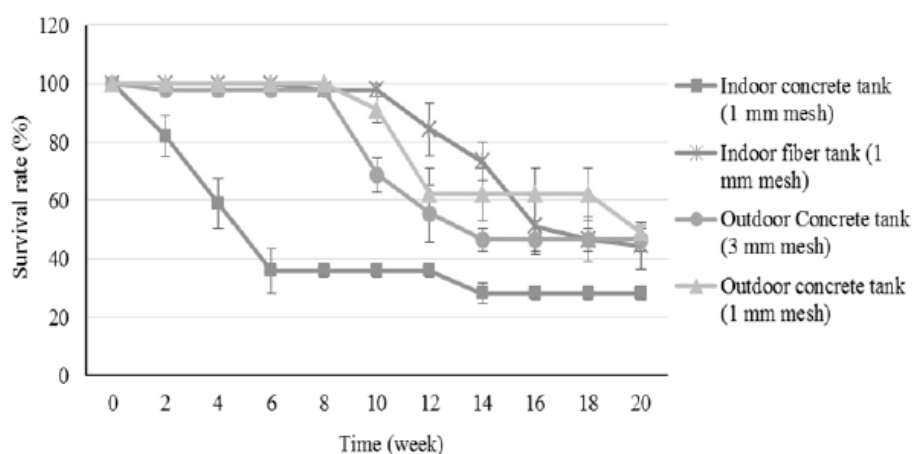


Figure 1. Survival rates (mean \pm se, in%) of juvenile *H. scabra* under the four rearing conditions.

survival rate ($48.89 \pm 2.22\%$), while those in the ICT 1 mm experiment demonstrate the lowest survival rate ($28.21 \pm 2.29\%$). However, our data analysis shows no statistically significant difference between final survival rates under the four different rearing conditions ($p > 0.05$).

Growth

Juveniles reared in the four types of tank show a general increase in growth, except for those in the IFT 1mm experiment that show more stable weights during the rearing period (Figure 2). The weights of juvenile *H. scabra* fluctuated at the beginning of the rearing period before eventually increased starting the week 12. Meanwhile, juveniles reared in the IFT 1 mm experiment show a slightly decreased mean weight during the rearing period from 13.76 ± 0.52 g to $11.81 \pm$

2.75 g.

Most final weights of juvenile *H. scabra* from different rearing conditions are significantly different ($p < 0.05$). The final weights of juveniles reared in the IFT 1 mm (11.81 ± 4.76 g) and OCT 3 mm (18.60 ± 3.48 g) tanks are significantly different from the rest. Whereas the ICT 1 mm (27.41 ± 3.98 g) and the OCT 1 mm (22.07 ± 1.01 g) tanks are not significantly different from each other.

The specific growth rate (SGR) of juveniles reared in the IFT 1 mm experiment shows a significantly ($p < 0.05$) lower value (-0.07 ± 0.09 g per day) than the rest (Figure 3). Whereas the SGRs for the ICT 1 mm (0.22 ± 0.05 g per day), the OCT 1 mm (0.15 ± 0.03 g per day) and the OCT 3 mm (0.11 ± 0.14 g per day) experiments are not statistically different from each other.

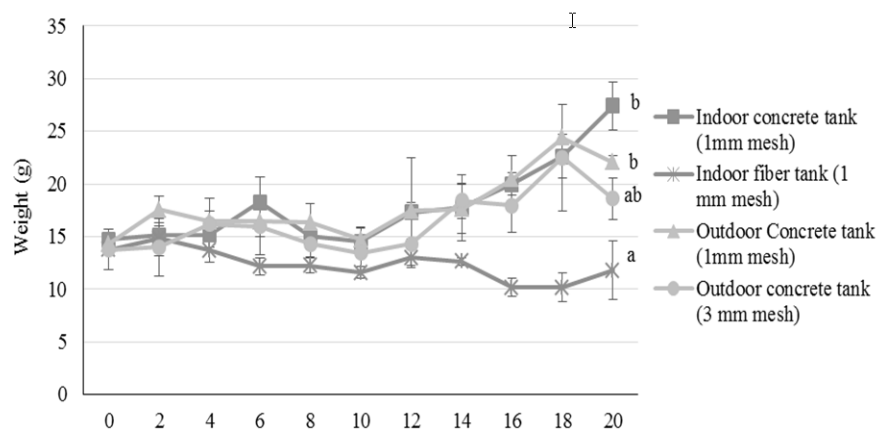


Figure 2. Weights (mean \pm SE, in gram) of juvenile *H. scabra* under the four rearing conditions. Mean values noted by different letters are significantly different from one another.

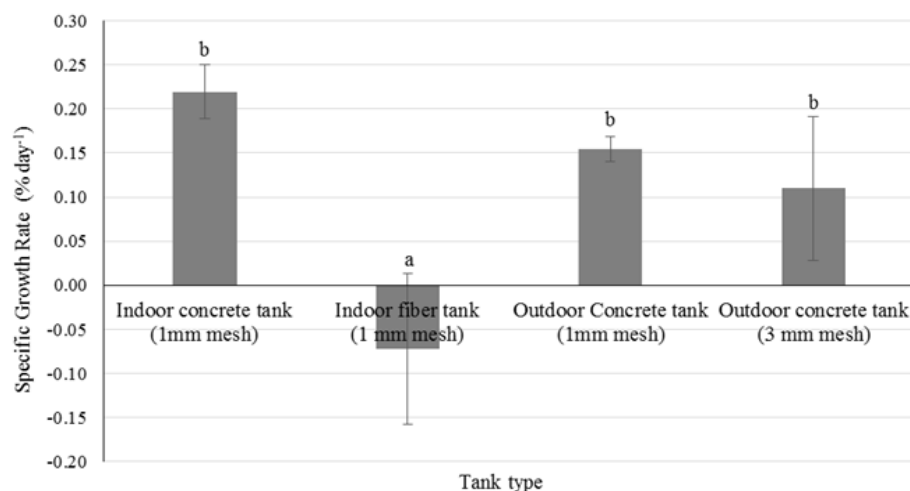


Figure 3. Specific growth rates (mean \pm SE in % per day) of juvenile *H. scabra* under the four rearing conditions. Mean values noted by different letters are significantly different from one another.

Final Biomass

Data on the final biomass (FB) of juveniles reared in the four types of tank (Figure 4) reveal that the OCT 1 mm experiment yields the highest FB (162.05 ± 10.49 g) compared to the ICT 1 mm (101.84 ± 16.44 g), OCT 3 mm (112.37 ± 17.58 g) and IFT 1 mm (61.84 ± 6.40 g) tanks. And, the estimated FBs of the ICT 1 mm and OCT 3 mm experiments are not significantly different.

DISCUSSION

Cost-effective methods for mass rearing of juvenile sea cucumbers have been recently developed. These include applying co-culture with other species such as tilapia (Sithisak *et al.*, 2013), *Kappaphycus striatum* red seaweed (Beltran-Gutierrez *et al.*, 2014), *Litopenaeus stylirostris* shrimp (Bell *et al.*, 2007) and *Penaeus monodon* black tiger shrimp (Lebata-Ramos *et al.*, 2012), as well as substituting natural feeds with terrestrial plants (Wu *et al.*, 2015) and applying different grow-out rearing techniques (Purcell, 2004; Lavitra *et al.*, 2010; Purcell and Agudo, 2013). Currently, there are two reported common methods for commercial mass-rearing of juvenile holothurians, which are plate-culture (for the early larval stage, up to 13 mm juveniles) and bottom-culture (for >20 mm juveniles (Battaglione, 1999).

In general, the survival rates of juvenile *H. scabra* in this study are relatively high compared to previous works. Battaglione *et al.* (1999) reported that the survival rate of juvenile *H. scabra* reared in concrete square tank with different types of substrate range between 4.5 - 34.0%. Meanwhile, juvenile *H. scabra* reared in fiber tank and fed with Algamac demonstrate a 34% of survival rate (Pitt, 2001). In all, previous works showed that variation in the survivorship and growth rate of sea cucumbers are associated not only with rearing techniques, but also the initial stocking density, bottom substrates, commercial feed and environmental conditions during rearing period (Qiu *et al.*, 2014; Tolon *et al.*, 2015; Giraspy and Ivy, 2008; Lavitra, 2010).

In this study, water quality during the rearing period was maintained at the optimum level for the growth of juvenile *H. scabra* (25.35 ± 0.01 to $29.35 \pm 1.11^\circ\text{C}$ for temperature, 28.50

± 0.78 to 34.81 ± 0.51 ppt for salinity, 7 ± 0.16 to 8.15 ± 0.28 for pH). These ranges of water quality parameters are similar to the optimum environmental conditions reported in existing studies (Battaglione *et al.*, 1999; Mercier *et al.*, 1999; Utchike, 2001; Pitt and Duy, 2004).

In this study, the use of different mesh sizes in the outdoor concrete tanks demonstrates no significant effect on the growth performance of juvenile *H. scabra*, while the different tank types (fiber glass versus concrete) show significant differences in the SGR and growth of juvenile *H. scabra*. The SGR and growth of juveniles reared in the concrete tanks (both indoor and outdoor ones) are higher than of those reared in the fiberglass tanks. Limited information is available for studies that compare the effect of different tanks (fiberglass and concrete) on the SGR and growth of juvenile *H. scabra* as most of previous studies focused on the application of different techniques (i.e. pond system, plastic tank) and/or using different feed formulas. For instance, Sithisak *et al.* (2013) showed higher SGR for juvenile *H. scabra* reared in round plastic ponds and fed by shrimp feed (0.27 ± 0.28 g).

Looking at the effects of indoor versus outdoor rearing tanks, there are noticeable differences in the SGRs and growths under these different set-ups. The lowest growth performance (weekly weight gain, SGR and final biomass) is demonstrated by juveniles reared in the fiberglass tank placed indoor. As the indoor tank was not equipped with a light source, the indoor tanks likely had lower light intensity (data on light intensity was not recorded). Dong *et al.* (2011) reported the effects of photoperiod on holothurians of other species (*Apostichopus japonicus*). Low light availability during rearing period significantly affected the movement and reduced feeding time of this sea cucumber compared to those reared under natural light cycle. In our study, juvenile *H. scabra* were exposed to continuous low light intensity. Thus, we speculate that the low survival rate and growth of juvenile *H. scabra* reared in the indoor fiber experiment is also possibly due to low feeding rate.

We observe a positive relationship between light availability and the growth performance of juvenile *H. scabra* that may be associated with their juvenile density and total biomass. Low density and biomass minimize competition for

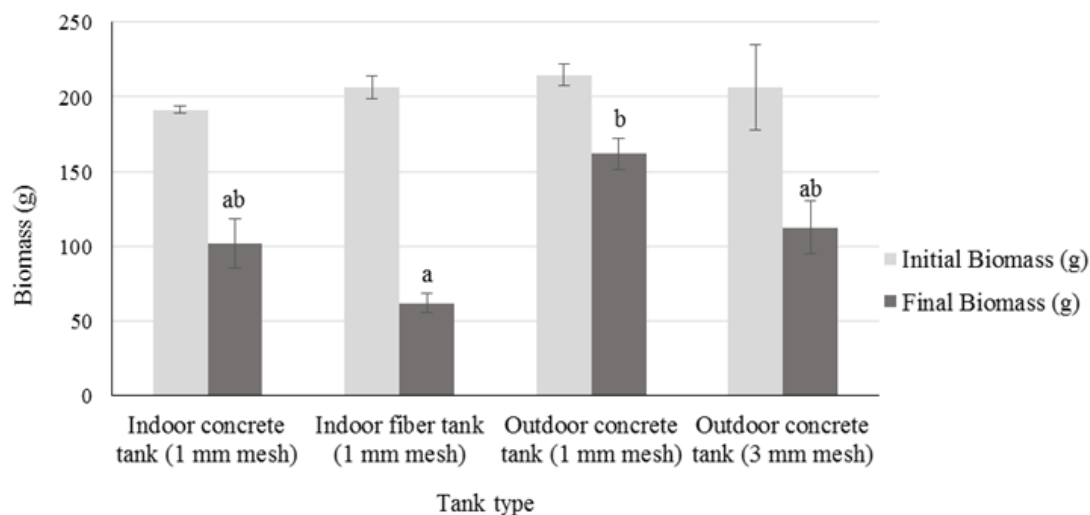


Figure 4. Mean \pm SE biomass of juveniles *H. scabra* reared in various types of tanks. Means with different letters are significantly different from one another.

space and food between biota. The adverse effects of density have been widely documented during the grow-out of juvenile holothurians (Battaglione and Bell, 1999; Liu *et al.*, 2004; Lavitra, 2010; Ramofafia *et al.*, 1997). The study of Asha and Diwakar (2013) showed that the growth rate of juveniles *H. scabra* decreased gradually with increasing stocking density. The recommended stocking density to achieve maximum growth rates varies depending on the size of juveniles, habitat condition, natural food availability and water changes. A range of 2 to 20 juveniles per m² is recommended, with 10 juveniles per m² being a commonly used density (Asha and Diwakar, 2013).

Light intensity also has been associated with the growth of diatom or epiphytic microalgae as natural food sources for juvenile *H. scabra* in aquaculture. Reduced light intensity have been shown to lower the growth of diatoms and microalgae (Battaglione, 1999; Lopes *et al.*, 2009). In our study, the biomass of diatom or epiphytic microalgae between indoor and outdoor tank was not recorded during the experiments.

CONCLUSION

Our findings suggest that concrete tank and outdoor rearing condition provide higher growth performance of juvenile *H. scabra* for aquaculture as assessed from their higher survival and specific

growth rate, average final body weight and final biomass. Whereas the use of different sizes of hapa mesh does not any significant effect the growth of juveniles.

Further studies are needed to validate the growth performance of juvenile *H. scabra* reared in different tank types and conditions to understand their optimum growth rate. Future works could include investigating the effect of different photoperiod cycles, tank material that may stimulate the growth of microalga as natural food sources for the juveniles, as well as the effect of tank colors in relation to the feeding behavior of juveniles.

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