

## REPRODUCTION AND LARVAL REARING OF SANDFISH (*Holothuria scabra*)

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### ABSTRACT

The natural stock of sea cucumber *Holothuria scabra* (known as sandfish) has been declining in both population and size, making it difficult to collect broodstock and juvenile sandfishes for aquaculture. This research aims to evaluate the reproduction of domesticated broodstock (F-1) and performance of the juveniles (F-2). Broodstock were reared in two rectangular concrete tanks with a dimension of 190 x 290 x 70 cm<sup>3</sup>. There were 12 individuals (7 males and 5 females) of broodstock (F-1) with total length and mean body weight of 12.0 ± 1.21 cm and 122.6 ± 32.37 g, respectively. Six broodstock were put in each tank and fed with compressed benthos at 4% biomass daily in the afternoon. During our experiment, domesticated sandfish broodstock successfully spawned twice. The first spawning (occurred in January 2017) had one female spawned that released 1,350,000 eggs with a hatching rate of 57.4%. Another broodstock spawned in April 2017 and produced 3,280,000 eggs with a hatching rate of 78.66%. The growth performance of 170 days-old juveniles (F-2) shows a total length of 5.66 ± 0.90 cm and a mean body weight of 10.08 ± 2.07 g. The survival rates are 5.19% and 8.68% for juveniles spawned in January and April, respectively. We conclude by showing that sandfish could be domesticated to produce seeds for further aquaculture development.

**Keywords:** Broodstock, domesticated, juvenile, reproduction, sandfish.

### INTRODUCTION

Sandfish (*Holothuria scabra*) is a high economically valued sea cucumber (Rustam, 2006), whose population has been declining in Indonesia. Sea cucumbers as an Asian delicacy are packed as fresh, frozen or dried products (Wen *et al.*, 2010). Besides being consumed as foods, sea cucumbers are also used in traditional medicines (Zhang *et al.*, 1995; Akamine, 2004) due to the presence of bioactive compounds (Beauregard *et al.*, 2001). Wild-captured sea cucumbers have supplied high demand for sandfish export in Indonesia. Consequently, the natural population of sandfish in Indonesian waters has been

declining significantly every year. It underlines the importance of sea cucumber aquaculture to support the increasing demand while declining natural population.

As the availability of wild broodstock depletes in nature, domesticated broodstock could help to sustain aquaculture production. The Institute for Mariculture Research and Fisheries Extension (IMRAFE) develops the breeding technology of sandfish to support aquaculture production. In Indonesia, IMRAFE has developed hatchery technology for mass production using wild broodstock from Bali, South Sulawesi and Southeast Maluku (Sembiring *et al.*, 2016).

This research aims to examine the reproduction of domesticated broodstock (F-1) and performance of the juveniles (F-2) produced from domesticated broodstock. Currently, there is a limited understanding the reproduction of domesticated sandfish broodstock and the performance of the juveniles. To evaluate these, several parameters such as minimum size of an individual with sexual maturity, age, spawning frequency, number of eggs produced and hatching rates, as well as growth and growth rates of the juveniles (F-2) need to be observed.

## MATERIALS AND METHODS

### The Culture of Sandfish Broodstock

Twenty sandfish broodstock collected from Southeast Maluku were treated at IMRAFE Laboratory in Bali to produce F-1 broodstock. The broodstock were acclimated in two fiberglass tanks of 100 L in volume. Only 18 healthy broodstocks (11 males and 7 females) were stimulated to spawn by raising water temperatures from 28 to 30°C. Among the 18 broodstock, three of them spawned, but only one produced healthy hatched larvae for further larval rearing. The larvae were reared up to a juvenile size, and their performance was determined based on their growth parameters when they reached 6 cm in total length. The best 12 juveniles were selected and reared in two concrete tanks. Each tank (200 x 150 x 70 cm<sup>3</sup>) was stocked with six selected juveniles (density of 2 individual/m<sup>2</sup>) and reared for 22 months to develop into domesticated broodstock (F-1). The broodstocks were fed benthos at 4% of biomass daily in the afternoon (Sembiring *et al.*, 2017).

Spawning method followed a method by Sembiring *et al.* (2004). Two transparent 200 L volume fiberglass containers were used to induce spawning of F-1 broodstock. Each container was filled with 150 L of filtered seawater. One container was equipped with a heater to maintain a water temperature of 30°C, while another container was filled with ice blocks placed in a plastic bag to decrease water temperature by 3-5°C below the standard (30°C). All the 12

broodstock from the initial tanks with a water temperature of about 28°C (2°C from the normal) initially were put into the tank with higher water temperature (30°C). If they did not spawn within 1-2 hours, they were transferred into the tank with lower water temperature (3-5°C from the normal). The spawned eggs were then collected and put into an incubated tank. Their embryonic development was monitored under a binocular microscope. Viable eggs were incubated in a transparent 200 L fiberglass tank, and the water temperature was set at 30°C using a heater. Within 24 hours, the eggs hatched out to an auricularia larval stage.

### Larval Rearing

Sandfish larvae at auricularia stage were transferred to a rectangular 190 x 290 x 70 cm<sup>3</sup> concrete tank with a density of 100 larvae per liter. The tank was filled with filtered seawater using a sand filter and a 5 µm cartridge filter. During larval rearing from auricularia up to the juvenile stage (36 days), the larvae were fed with phytoplankton *Chaetoceros sp.* at a density of 20,000 cells/mL (Notowinarto and Putro, 1992; Sembiring *et al.*, 1996).

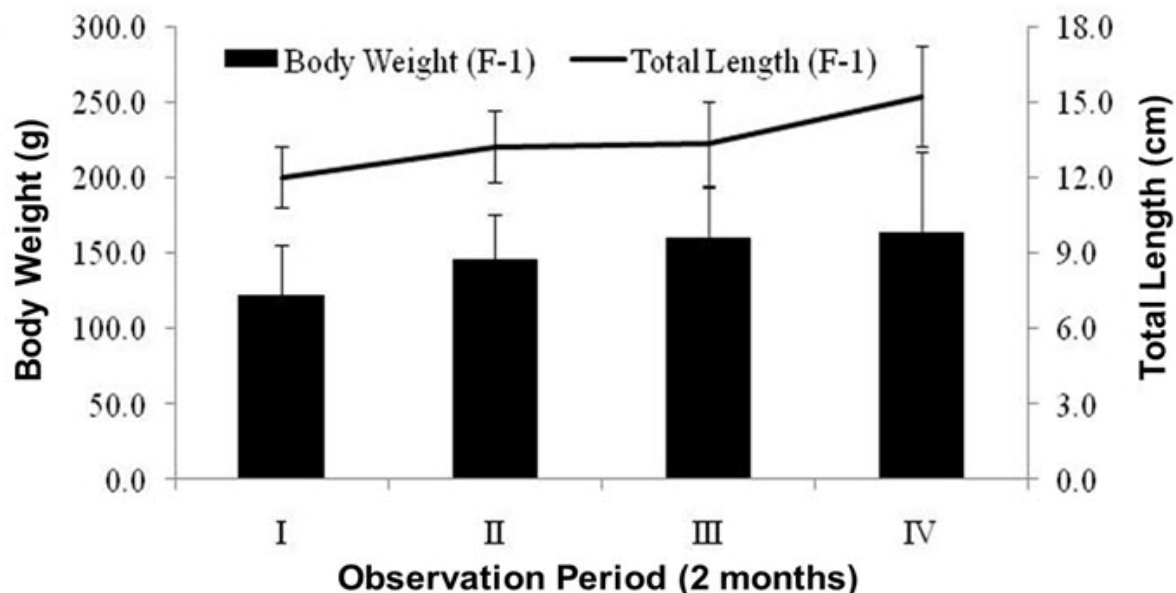
Juveniles of 36 days-old were then harvested and transferred into hapas (100 x 70 x 50 cm<sup>3</sup>) in nursery tanks (200 x 150 x 70 cm<sup>3</sup>) and reared up to the fry stage. Six hapa nets were set in each nursery tank. The density of juvenile in each hapa was 500 individuals. During the nursery period, the juveniles were fed with natural fresh benthos at 2% of biomass given in the late afternoon. The waste at the bottom of the nursery tank was siphoned daily using a flow-through system at a rate of 1 L/minute (Sembiring *et al.*, 2015).

### Biological Parameters

Reproduction performance of domesticated broodstock (F-1) was observed as growth (total length and mean body weight), spawning frequency, number of eggs produced and egg diameter. In the nursery stage, the performance of juvenile (F-2) was observed as growth (total length and mean body weight) measured every 30 days started as a 50 days-old juvenile.

**Table 1.** Number, diameter and hatching rate of sandfish *H. scabra* eggs.

Spawning Period	Number of Eggs (pieces)	Egg Diameter ( $\mu\text{m}$ ) (N=20)	Hatching Rate (%)
I	1,350,000	181.26 $\pm$ 4.78	57.40
II	3,280,000	181.05 $\pm$ 5.16	78.66



**Figure 1.** Mean body weight and the total length of domesticated sandfish, *H. scabra* (F-1) during the experiment.

## RESULTS

### Reproduction of Domesticated Broodstock (F-1)

During the research, spawning of domesticated sandfish broodstock (F-1) occurred twice (January and April 2017). Spawning of sandfish stimulated by heat occurred in three days after a full moon. The numbers of spawned broodstock occurred from one female and one male (out of the 12 broodstock) and eggs produced are shown in Table 1. The total length and mean body weight of domesticated broodstock (F-1) are 12.0  $\pm$  1.21 cm and 122.6  $\pm$  32.37 g, respectively (Figure 1).

Domesticated broodstock (F-1) at the age of 22 months were able to spawn and produce 2,315,000  $\pm$  1,364,716 eggs, or equal to 18,975  $\pm$  11,186 eggs per a gram of body weight. Domesticated broodstock (F-1) at the age of 22 months have a total length of 12 cm and a mean body weight of 122 g, with a male-to-female ratio

of 1.4. The average diameter of eggs produced by domesticated broodstock (F-1) is 181.05  $\pm$  4.78  $\mu\text{m}$  (with a range of 172.34 to 188.51  $\mu\text{m}$ ).

### Performance of Sandfish Juvenile (F-2)

Growth performance of sandfish juvenile (F-2) as expressed in total length and mean body weight are shown in Figures 2 and 3. A sharp increase in the total length of larvae occurred between 36 days (0.200  $\pm$  0.005 cm) and 70 days (3.90  $\pm$  0.52 cm) that may be due to a change in food preference from phytoplankton to the benthos. During this 34 days-period, sandfish juvenile (F-2) grew by 1.1 mm per day. The total length of sandfish juveniles (F-2) at 170 days-old is 5.66  $\pm$  0.90 cm, while the mean body weight is 10.08  $\pm$  2.07 g.

The survival rates of sandfish juveniles (F-2) that grew from 0.2 to 5.66 cm are 8.68% (spawned in January 2017) and 5.19% (spawned in April 2017).

## DISCUSSION

### Reproduction of Domesticated Broodstock (F-1)

Previous works suggest that wild broodstock may be more productive than domesticated broodstock, however, our research shows that domesticated broodstock may have high productivity as well. Wild broodstock from Southeast Maluku with total length and mean body weight of  $14.64 \pm 1.80$  cm and  $186.61 \pm 74.68$  g, respectively, produced 620,000 eggs or equal to 3,333 eggs per gram of broodstock body weight (Sembiring *et al.*, 2016). In comparison, results from the current work suggests that domesticated broodstock (F-1) could produce 3-6 times more eggs (1,350,000 to 3,280,000 eggs)

than those of wild broodstock (F-0). Our results show a higher number than reported by Al Rashdi *et al.*, (2012), where female wild broodstock with a mean body weight of 500 g produced at least one million eggs or equal to 2,000-6,000 eggs per gram of body weight. Ivy and Giraspy (2006) reported that seven female broodstock with sizes of 350-500 g produced a total of 14,230,000 eggs (2,032,000 eggs per broodstock or 4,064-5,805 eggs per gram of body weight). Whereas our study shows nine female broodstock with the same size produced a total of 32,760,000 eggs (3,640,000 eggs per broodstock or 7,280-10,400 eggs per gram of body weight). We suggest that the relatively high reproduction performance of domesticated broodstock in our experiment may be due to better feeding management.

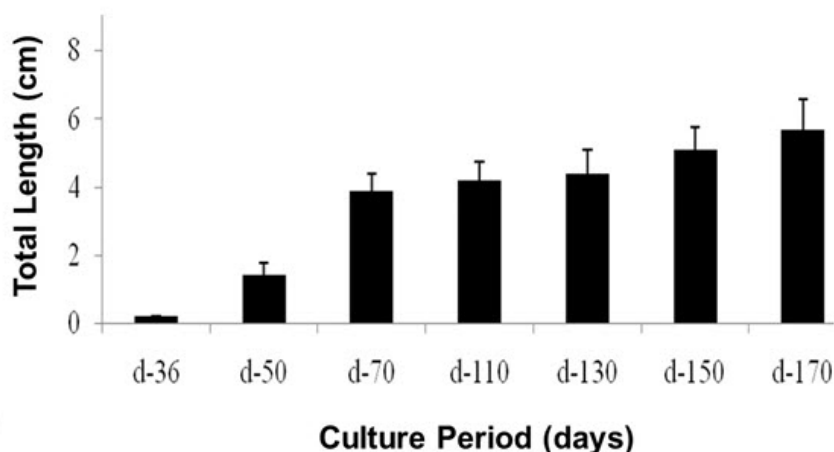


Figure 2. The total length of sandfish *H. scabra* juvenile (F-2) produced from domesticated broodstock (F-1).

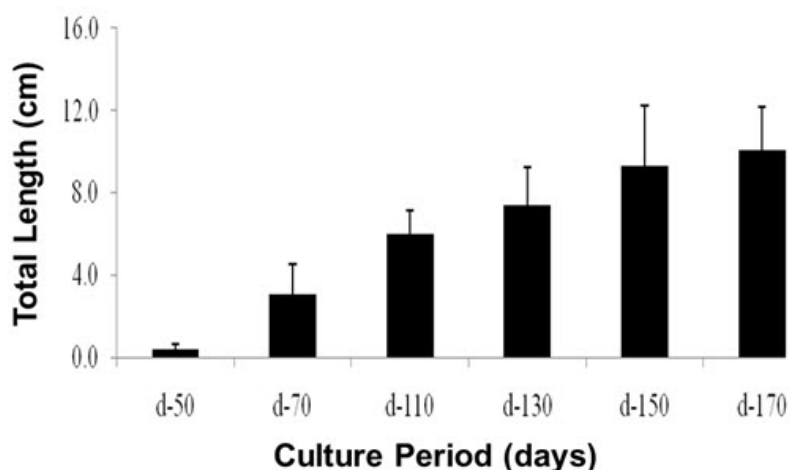


Figure 3. Mean body weight of sandfish *H. scabra* juvenile (F-2) produced from domesticated broodstock (F-1).

In the current research, average values for egg diameter of domesticated broodstock (F-1), *i.e.*,  $181.26 \pm 4.78 \mu\text{m}$  and  $181.05 \pm 5.16 \mu\text{m}$ , are larger than egg diameter of wild broodstock (F-0), *i.e.*,  $172.86 \pm 6.82 \mu\text{m}$  (Sembiring *et al.*, 2016). However, egg diameter in the current study is smaller than the result reported by Ivy and Giraspy (2006), which was  $205.36 \pm 17.54 \mu\text{m}$ . The size of the egg may depend on the geography with lower values for the tropical region compared to the temperate region (*e.g.*, Australia).

Hatching rate of eggs for domesticated broodstock (F-1), *i.e.*, 78.66% is not significantly different compared to wild broodstock (F-0), *i.e.*, 76.0% (Sembiring *et al.*, 2016). Water temperature is very important for sea cucumber biological activities such as spawning, eggs incubation and also for growth (Mercier *et al.*, 1999; Purcell and Kirby, 2005; Wolkenhauer, 2008). Studies have suggested that the main factor that affects hatching rate is water temperature with an optimum range of 29-30°C (Battaglione *et al.*, 1999; Mercier *et al.*, 1999).

### Performance of Sandfish Juvenile (F-2)

This research yields a relatively higher growth of juveniles (F-2) compared to previous works. Ivy and Giraspy (2006) reported that the total length of juvenile *H. scabra* var *versicolor* reached a final size of 20-25 mm (56 days-old), although they reported a larger egg diameter compared to our work. The growth of the juveniles in this research is higher compared to the results of Battaglione *et al.* (1999) where the total length of juveniles increased by 0.5 mm per day for *H. scabra*. The better result on growth could be due to better feeding and environmental controls. In all, the total length of juveniles in this study indicates that juvenile produced from domesticated broodstock (F-1) are suitable for aquaculture.

The difference in mean body weight between F-2 and F-1 may be due to grading frequency. When juveniles reached 3 g in weight, it was suitable to do grading and grouping by size. Grading for F-2 juveniles in this study did not occur as frequently as for F-1 juveniles. This

condition leads to the F-2 juveniles to compete for space and food. The density of juvenile F-2 with a body weight of 3-10 g in this study (0.07 individual/cm<sup>2</sup>) was lower than the result of Battaglione *et al.* (1999) that reported an optimum density of 1.7 juvenile/cm<sup>2</sup> when reached 21 days-old. Much bigger juveniles may cause the difference in this research. On the other hand, the juvenile density of F-2 in this study is higher compared to the density of sea cucumber reported in its natural habitat of 0.0002 individual/cm<sup>2</sup> (Madang, 2011).

The survival rate of sandfish is relatively low in our study with high mortality occurred at the beginning of the experiment when juveniles were about 0.2 cm in size. The high mortality may be due to the transition phase of feed from phytoplankton *Chaetoceros* *sp.* to diatom benthos, as well as competition for food and space with animals that entered the nursery tanks through seawater supply flow. Indeed, the survival rate of *H. scabra* juvenile could be increased by preventing the competitor animals from entering the nursery tank (James *et al.*, 1994).

### CONCLUSION

This research aims to evaluate the reproduction of domesticated broodstock (F-1) and performance of the juveniles (F-2). During our experiment, domesticated sandfish broodstock successfully spawned twice. Our experiment shows that sandfish could be domesticated to produce seeds for further aquaculture development.

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## REFERENCES

- Akamine, J. (2004). The status of the sea cucumber fisheries and trade in Japan: past and present. In *Advances in sea cucumber Aquaculture and management. FAO Fisheries Technical Paper No. 463*. Food and Agriculture Organization of the United Nations. Rome. Italy. (pp. 39-48).
- Al Rashdi, K. M., Eeckhaut, I., and Claereboudt, M. R. (2012). *A manual on hatchery of sea cucumber *Holothuria scabra* in the Sultanate of Oman*. Ministry of Agriculture and Fisheries Wealth, Aquaculture Centre, Muscat, Sultanate of Oman. (pp. 27).
- Battaglione, S. C. (1999). Culture of tropical sea cucumbers for stock restoration and enhancement. *The ICLARM Quarterly*, 2(4), 4-1.
- Beauregard, K. A., Truong, N. T., Zhang, H. Y., and Beck, W. Y. (2001). The detection and isolation of a novel antimicrobial peptide from the echinoderm, *Cucumaria frondosa*. *Adv. Exp. Med. Biol.*, 484, 55-62. doi: 10.1007/978-1-4615-1291-2\_5.
- Ivy, G, and Giraspy, D. A. (2006). Development of large-scale hatchery production techniques for the commercially important sea cucumber *Holothuria scabra* var. *versicolor* (Conand, 1986) in Queensland, Australia. *SPC Beche-de-mer Information Bulletin*, 24, 28–34.
- James, D. B., Gandhi, A. D., Palaniswamy, N., and Rodrigo, J. X. (1994). Hatchery Techniques and Culture of Sea Cucumber *Holothuria scabra*. *CMFRI Special Publication No. 57*. India. (pp. 41).
- Madang, H. M. (2011). *Ekobiologic and dynamics stocks of sandfish (*Holothuria scabra* Jaeger), black sea cucumber (*Holothuria edulis*) and relation with exploitation at Lalin village, North Maluku Province*. Thesis. Magister. Bogor Agriculture University. (pp. 126).
- Mercier A., Battaglione, S. C., and Hamel, J. F. (1999). Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. *J. Exp. Mar. Bio. Ecol.*, 239(1), 125–156 doi:10.1016/s0022-0981(99)00034-9.
- Notowinarto and Putro, D. H. (1992). Preliminary study on larval development of White sea cucumber *Holothuria scabra*. *Bull. Mariculture*, 5, 15-25.
- Purcell, S. W., and Kirby, D. S. (2005). Restocking the sea cucumber *Holothuria scabra*: Sizing no-take zones through individual-based movement modelling. *Fish. Res.*, 80, 53–61. doi: 10.1016/j.fishres.2006.03.020.
- Rustam. (2006). Sea cucumber culture. In *Training on Mariculture COREMAP Phase II. Selayar Residence*. Mattirotasi Makassar Foundation. (pp. 11).
- Sembiring, S. B. M., Darmansyah, Arfah, R., and Sugama, K. (1996). The effect of feeding frequencies on the growth and survival rates of larvae of sea cucumber *Holothuria scabra*. *Indonesian Fisheries Research Journal*, 2(2), 42-47.
- Sembiring, S. B. M., Sugama, K., Suastika, I. M., Makatutu, D., and Jufri. (2004). *Technical guidelines of sea cucumber, *Holothuria scabra* breeding Technology*. Central Research Institute for Aquaculture. Marine and Fisheries Research Agency. Department of Marine and Fisheries. Jakarta. (pp. 23).
- Sembiring, S. B. M., Wardana, I. K., and Haryanti. (2015). Sea cucumber, *Holothuria scabra* breeding technology. In *Recommendation technology of marine and fisheries*. Marine and Fisheries Research and Development Agency. Ministry of Marine Affair and Fisheries. Jakarta. (pp. 187-200).
- Sembiring, S. B. M., Wardana, I. K., and Haryanti. (2016). Performance of sea cucumber, *Holothuria scabra* juvenile from different sources of broodstock. *Aquac. Res. J.*, 11(2), 147-155. doi: 10.15578/jra.11.2.2016.147-154.
- Sembiring, S. B. M., Wardana, I. K., Giri, N. A., and Haryanti. (2017). Performance of gonad

- re-maturation of sea cucumber, *Holothuria scabra* broodstock fed with different feed ingredients. *Aquac. Res. J.*, 12(2), 147-159. doi: 10.15578/jra.12.2.2017.147-159.
- Wen, J., Hu, C., and Fan, S. (2010). Chemical composition and nutritional quality of sea cucumbers. *J. Sci. Food. Agric.*, 90, 2469–2474. doi: 10.1002/jsfa.4108.
- Wolkenhauer, S. M. (2008). Burying and feeding activity of adult *Holothuria scabra* (Echinodermata: Holothuroidea) in a controlled environment. *SPC Beche-de-mer Information Bulletin*, 27, 25–28.
- Zhang, B., Sun, D., and Wu, Y. (1995). Preliminary analysis on the feeding habit of *Apostichopus japonicus* in the rocky coast waters off Lingshan Island. *Mar. Sci.*, 3, 11–13.