Application of asexual reproduction techniques and growth ability of the sandfish *Holothuria scabra*

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Abstract. Sea cucumber population in nature is reported to have experienced exploitation pressure in various places. Natural sea cucumber reproduction (sexual) takes a long time to produce a new adult individual. This is feared to be the cause of the extinction of sea cucumbers. Sandfish, *Holothuria scabra*, do not include sea cucumber species which are categorized as capable of asexual reproduction. However, several laboratory-scale studies state that sandfish can be induced for asexual reproduction. This study aims to determine the level of life graduation and the growth of sandfish produced by asexual reproduction. Individual sandfish is tied with rubber bands about 60% from the anterior to the posterior. The result showed that the sandfish were successfully induced to undergo asexual reproduction which is known as "fission". The time needed for a sandfish to break itself into 2 new individuals takes less than 2 days. The first days after dividing the body seem to be a critical period. Only 13.64% of new individuals survive the critical phase. It concluded that the growth of asexual reproduction sandfish is slow because it takes time to regenerate the scattered internal organs during the process of division.

1. Introduction

Sandfish *Holothuria scabra* Jaeger. is one of the most requested sea cucumbers on the oriental market. Sea cucumbers have long been used as food, mainly because they have a high protein content with some amino acids but have a low-fat content. Besides, sea cucumbers are also used as raw materials for medicines and cosmetics because they contain minerals, vitamins, and various bioactive ingredients. The wide use of processed sea cucumber products and the high price on the market has triggered the excessive capture of them. Sea cucumber populations in the wild are reported to have experienced exploitation pressures in various places. This is indicated by the reduction in catches in nature and the maximum size that is getting smaller as has been reported in various places in the world [1,2,3,4]. In Indonesia, local fishermen who depend on catching natural sea cucumbers are increasingly difficult to obtain large sea cucumbers so they started catching young sea cucumbers weighing around 30 g while previously sea cucumbers were caught larger than 250 g.

One of the most appropriate ways to minimize the level of exploitation of sea cucumber resources in nature is to conduct cultivation activities. Sea cucumber cultivation in Indonesia is an enlargement activity that is collecting seeds of 20-30g in size and rearing them until they reach market size. Sea cucumber hatcheries have been successfully carried out in several hatcheries such as in Gondol and Lampung BBRBL. However, the survival rate of larval reach to juvenile is still very low beside the very slow growth of them.
Sea cucumbers generally reproduce sexually in the water column. Natural sea cucumber reproduction (sexual) takes a long time to produce a new adult individual. While the population of sea cucumbers in nature has decreased every year. It is feared as a cause of extinction sea cucumbers in nature.

Several types of sea cucumbers are reported to be able to reproduce asexually through body fission, such as *H. atra* and *H. Impatiens* [5]. *H. scabra* does not include species that are categorized as capable of performing asexual reproduction. However, several laboratory-scale studies state that *H. scabra* can be induced for asexual reproduction [6,7]. Therefore, these laboratory-scale studies need to be continued to determine the survival rate of sea cucumbers are asexual reproduction results if reared in nature. The objective of studies was to know the survival rate of sea cucumbers as a result of asexual reproduction techniques that are reared in nature and absolute growth rate of them. The results of this study can provide information an appropriate asexual reproduction or fission techniques and survival rates of sea cucumbers.

2. **Material and methods**

2.1 *Research time and site location*

This research activity was carried out in November 2019 until February 2020. Rearing of sea cucumber produced by fission induction was carried out in Hitu Village, Lehiitu District, Central Maluku Regency, Maluku Province.

2.2 *Materials and equipment*

Materials and equipment used in the study are as follows: digital scales to weighing of the sandfish, plastic container to bringing of sandfish to the site location equipped with an aerator during transportation. A cage/confine of 3x3 m in the rearing site. The 2.0 mm diameter elastic band is used to tie the body of the sandfish to induce the animal to break up which is called "fission". The water quality checker consists of a refractometer and thermometer to measure salinity and water temperature. Cotton and wipes tissue for cleaning during the treatment process takes place, also alcohol to sterilize equipment other than freshwater. Meanwhile, 44 individuals of sandfish were used as treatment objects.

2.3 *The procedure of the study*

The sandfish research objects were obtained from natural catches originating from the villages of Suli and Hitu, Central Maluku District, Maluku Province.

![Figure 1. Asexual reproduction technique in sandfish *H. scabra*](image)

The sandfish from Suli were transported to the village of Hitu where they lived in confinement. They were transported using a styrofoam box filled with seawater and given aeration. They were then put in a 3x3 m cage and left for 1 night to be able to adapt and to recover their condition. The next day, the sandfish individual tied with an elastic band to induce fission as an asexual reproduction technique according to the method proposed by [8]. They are bound in the middle of the body about 60% toward the posterior so that the anterior portion is larger than the posterior. This was based on
previous studies where the possibility of live sea cucumbers with this technique results are higher in the posterior because in the posterior there are more internal organs [6].

2.4 Research design
This study only used one population with one treatment and 44 individuals as replications. The measured parameters were the survival and absolute growth rate. The survival rates are calculated using a formula:

$$SR(\%) = \frac{N_t}{N_0} \times 100$$

Explanation: $SR$ = survival rate (%), $N_0$ = the number of individuals at the beginning of the study, $N_t$ = the number of individuals at the end of the study.

The absolute growth rate is calculated using a formula:

$$W = W_t - W_0$$

Explanation: $W$ = absolute growth rate (g), $W_t$ = average of weight at the end of study (g), $W_0$ = average of weight at the beginning of the study (g) [10]. Sampling was done every week to estimate the growth of sea cucumbers.

2.5 Data analysis
The data obtained were analyzed using descriptive statistics and displayed with tables and figures.

3. Result and discussion
3.1 Sandfish asexual reproduction
Sandfish are not classified as fissiparous species or species that can reproduce asexually by severing their bodies naturally. However, the sandfish in this study were successfully induced to undergo asexual reproduction through binding to parts of their body or what is known as "fission". In this study as many as 44 individuals sandfish were induced by tied the body and all individuals managed to break themselves into 2 new individuals. This means that the success rate of asexual reproduction induction in sandfish is 100% (Table 1). The result obtained was 88 new individuals.

| Table 1. Sandfish asexual reproduction data |
|---------------------------------------------|
| Individual used                              | 44 individuals |
| Fission successful                          | 44 individuals |
| The number of new individuals                | 88 individuals |
| The number of new individuals which have survived | 12 individuals |
| Survival Rate                                | 13.64%         |

Moments after being tied up, the body of the sandfish will swell at the bound part. Sandfish then move forward so that the rubber would be seen pressing on the bound body part. Occasionally the sandfish appeared to move like twisting their bodies. The part of the body that was bound seems to be the point of the movement of the sandfish. Over time, the bound part of the body looks thin out and started to show division. A division that occurs causes the distance between the two parts of the body. The anterior portion keeps moving forward while the posterior portion tends to remain or move in opposite directions but with a very small velocity compared to the anterior part. The posterior part looked like it was about to be released by the anterior part. The situation continued for several hours until the body breaks into two parts. The two parts of the body are the anterior part which has the mouth to the middle of the body and the posterior part which has the anus up to the middle of the body. After separated, there were open wounds on the two severed body parts. The digestive tract also t comes out as a result of the open wound. In some sandfish, when there was swelling of the body at the beginning of the induction process, the sandfish released their gonads in the form of milky white threads and some were orange.
The time needed by the sandfish to break itself into 2 new individuals is less than 2 days. This is similar to what is obtained by several previous studies such as in [6] who tried to induce asexual reproduction of the sandfish in the laboratory. Small individu break up faster than large individu. It might be possible due to small individu have thinner skin compared to the large one [6,7]. However, the death rate of the small ones tended to be higher [6].

The results obtained in this study indicate that sea cucumbers can be induced to reproduce asexually. Even so, the survival rate of the life of these two parts of the sea cucumber's body is not very encouraging. Only 12 individuals from 88 individuals survived until the 3rd day after successfully severing their bodies. All individuals who succeed in living are the posterior part. The success of individual posterior survival is also reported in H. edulis and H. leucospilota [10,11] and H. scabra [6,7]. It is suspected that the success of the posterior individual survives because the posterior part has more organs than the anterior part. This is reinforced after the anterior and posterior body parts were opened. In the posterior part, there was a respiratory tree while in the anterior part most were empty except organs attached to the mouth i.e tentacles.

The first 3 days after successfully breaking down the body seems to be a critical period for sea cucumbers. This happens because of open wounds and energy spent to break up the body [6]. Besides, a suspected bacterial attack is observed through the presence of white spots that cover the surface of the injured skin. Another thing that is thought to trigger the death of new individuals due to asexual reproduction is stress. This research was conducted in nature where individuals have recently experienced environmental changes due to tides such as rising water temperatures at low tide and changes in salinity due to runoff from the land. Thus, in this study, only 12 individuals were used to look at survival rates and growth rates.

3.2 The survival rate of the sandfish from asexual reproduction
The 12 individuals that were used as tested animals turned out to survive until the end of this study which took 6 weeks. After passing through the first 3 days which seems to be a critical period, sandfish tend to survive. The early days that were thought to be critical were also reported [7] where the greatest death rates occurred in the first few days after the induction of this division.

3.3 Growth of the sandfish from asexual reproduction
The average absolute weight gain of sandfish from asexual reproduction obtained in this study can be seen in Figure 1. It can be seen that sandfish gain body weight every week. The average absolute weight gain obtained for 42 days or 6 weeks is 1.34 g with a growth rate of 0.03 g / day.

![Figure 2. Average weight gain of the sandfish produced by asexual reproduction for 6 weeks.](image)

The average weight gain of sandfish as a result of asexual reproduction is quite low compared to the average weight gain of sandfish produced by sexual reproduction reported by several researchers. Some sandfish had growth about 400 g during 12 months of maintenance [12]. Other reported that sandfish weight gain was 1.8 g per day [13] as well as the absolute growth of sandfish in a controlled
container of 4.1-22.25 g obtained during 6 weeks of maintenance in the laboratory [14]. On the contrary, there are also results from a fairly slow growth rate of sandfish (0.04-0.23 g.day\(^{-1}\)) even though the sandfish were placed in the rich nutrients area [13,15]. These facts suggested a wide range of growth rate of the sandfish.

The slow growth of sandfish from asexual reproduction is understandable given the sandfish when splitting out most of the body organs, especially organs or digestive tract. Thus to be able to digest food, the sandfish asexual reproduction needs to regenerate the organs in the body to digest the food. After the division, sandfish seems to stop eating because there is no digestive tract, but sandfish still need the energy to maintain their basal body needs. It can thus be understood why the growth of sandfish after asexual reproduction is low. This is in line with the opinion which states that after regeneration the weight of sandfish that divide will be low because, in addition to stopping eating, sandfish also have to spend more energy which hurts their growth [16].

Figure 1 shows the increase in body weight of sandfish asexual reproduction starting from week 1 after induction. This means growth occurs. Generally, to grow, organisms need to consume food, which is then digested and absorbed by the intestine and used for basal and growth purposes. Allegedly regeneration of digestive organs has occurred rapidly as a result of asexual reproduction sea cucumbers. Sandfish are known to be organisms that can regenerate organs. But it is not yet known how fast the regeneration ability is. Another allegation is that sandfish can grow not only through digested nutrients, but it is suspected that other mechanisms allow sea cucumbers to obtain food to grow.

4. Conclusion

It can be concluded that sandfish can be induced to divide their bodies to form 2 new individuals but only a few individuals can survive through the “critical phase”. After passing the critical phase of the individual sandfish from asexual reproduction are generally successful in living and growing. The growth of the sandfish from asexual reproduction is slow because it needs time to regenerate the scattered internal organs during the process of division.

References

[1] Lawrence A J, Hanafy A, Gabr M, Ibrahim H, Gab-Alla A 2004 Status of the sea cucumber fishery in the Red Sea-the Egyptian experience. Advances in Sea Cucumber Aquaculture and Management. Eds. Lovatelli, A., C. Conand, S. Purcell, S. Uthicke, J-F. Hamel, and A. Mercier 2004. Food and agriculture organization of The United nations. FAO Fisheries and Agriculture Technical Paper 463.

[2] Tuwo A 2004 Status of sea cucumber fisheries and farming in Indonesia. In: Lovatelli, A., C. Conand, S.W. Purcell, S. Uthicke, J.-F. Hammel and A. Mercier, 2004. Eds. Advances in Sea Cucumber Aquaculture and Management. FAO Fisheries Technical Paper 463. FAO, Rome. 49-55.

[3] Uthicke S 2004 Overfishing of holothurians: lessons from the Great Barrier Reef. Advances In Sea Cucumber Aquaculture And Management. Eds. Lovatelli, A., C. Conand, S. Purcell, S. Uthicke, J-F. Hamel, and A. Mercier, 2004. Food and Agriculture Organization Of The United Nations. FAO Fisheries and Agriculture Technical Paper 463.

[4] Al-Rashdi K M and Claereboudt M R 2010 Evidence of rapid overfishing of sea cucumbers in the Sultanate of Oman SPC Beche-de-mer Information Bulletin 30 10-13.

[5] Nugroho D, Hartati R, Suprijanto J 2012 Stimulasi fission reproduksi aseksual teripang Holothuria atra dan teripang Holothuria impatiens. Jurnal of Marine Research, 1(2) 161-166.

[6] Pattinasarany M M and Jamal E 2016 Study on asexual reproduction of Holothuria scabra induced by the tie method Triton (In Indonesian) 12(1) 10-17.

[7] Ongkers O T S, Pattinasarany M, Mamesah J A B, Uneputty Pr A, Pattikawa, J A, Natan Y, Tetelepta J M S 2020 Asexual reproduction experiment of sandfish (Holothuria scabra) on the
natural condition of Morella, central Maluku Indonesia *International Journal of Fisheries and Aquatic Studies* 8(1) 279-281.

[8] Purwati P and Dwiono S A P 2008  Asexual reproduction as an alternative for restocking sea cucumber. *Ilmu Kelautan* 13(1):37-42.

[9] Solanki Y, Jetani K L, Khan S I, Kotiya A S, Makawana N P, Rather M A 2012  Effect of Stocking Density on Growth and Survival Rate of Spiny Lobster *Panulirus Polyphagus* in Cage Culture System. *International Journal of Aquatic Science* 3 4-14

[10] Conand C 1990  The fishery resources of Pacific Island countries. Part 2: Holothurians. FAO Fisheries Technical Paper No. 272.2. Rome. 143 pp.

[11] Karim R A, Hartati R, Widianingsih 2013  Fission ability of sea cucumber *Holothuria edulis* and *Holothuria leucospilota* (Holothuridae) at different body sizes in the islands of Karimunjawa. *Journal of Marine Research* 2(1):154-160.

[12] Bell J D, Agudo N N, Purcell S W, Blazer P, Simutoga M, Pham D, Patrona L D 2007  Grow-out of sandfish *Holothuria scabra* in ponds show that co-culture with shrimp *Litopenaeus stylirostris* is not viable. *Aquaculture* 273 (4) 509-519.

[13] Tsiresy G, Pascal B, Plotieau T 2011  An assessment of *Holothuria scabra* growth in marine micro-farms in southwestern Madagascar. *SPC Beche-de-mer Information Bulletin* 31 17-22.

[14] Pattinasarany M M 2015  Profil protein dan evaluasi insulin-like growth factor-1 teripang pasir *Holothuria scabra* yang diberi formula pakan berbahan tepung cacing sutera *Tubifex sp* Dissertation University of Brawijaya Malang.

[15] Purcell S W and Simutoga M 2008  Spatio-temporal and size-dependent variation in the success of releasing cultured sea cucumber in the wild. *Reviews in Fisheries Science* 16: 201-214.

[16] Boyer C, Caillason S, Mairesse K 1995  Asexual reproduction in *Holothuria atra* on a reef of Reunion Island in The Indian Ocean. *SPC Beche-de-mer Information Bulletin* 7 7-9.

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