Asexual reproduction experiment of sandfish (Holothuria scabra) on natural condition of Morella, central maluku Indonesia

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Abstract
Some holothurian can reproduce asexually through fission. Experiment to induce fission of sandfish (Holothuria scabra) was conducted on March 2019 at Morella coastal waters, Central Maluku-Eastern Indonesia. Small (< 50g) and large (> 60g) H. scabra were induced to fission by tightening with rubber band at three different positions i.e. near to anterior, in the middle and near to posterior. Treated animals were kept at natural habitat in two separate 2x2 m² pen cultures. Observation was done every 12 hours to record duration of fission, time to heal the wound and survival of H. scabra. The results showed that small individual had shorter time to fission and to heal the wound but had lower survival compared to large animal. This result indicates that even though H. scabra is not categorized as fissiparous holothurian but it can split the body into two new animals through indincation.

Keywords: Asexual reproduction, fission, sandfish, Holothuria scabra

1. Introduction
Sea cucumber or holothurian is one of marine commodities that have high economic value in national as well as in international markets. The demand of this resource increase so its exploitation is intensive and its population tends to decline. In developing countries such as Indonesia, sea cucumber is exploited traditionally, mostly at shallow waters [1]. However, because of easy access to sea cucumber habitat makes exploitation of this resource is high and cause overexploitation in some areas [2, 3].

Maluku is one of the producers of sea cucumber in Indonesia. Exploitation of sea cucumber in Maluku has been done for long time, however, information of this resource is still lacking. Several researches had been conducted in Maluku, but mostly focused on species composition, community structure and size distribution of sea cucumber in the area [1, 3, 4, 5, 6]. One of economical holothurian and has wide distribution in Indonesia including Maluku is sandfish Holothuria scabra. High demand and good price make population of this species tend to decrease due to high exploitation [7]. Therefore, restocking of this species is needed.

Naturally, holothurian can reproduce sexually and asexually. Success of sexual reproduction depend on density of holothurian [8]. There is an effort to produce fingerling through hatchery but the result is not as expected. There are several reason for this such as difficulty to make male and female breeder to spawn simultaneously, high mortality of larvae, need modern technology and expensive [8].

Some holothurians have capability to reproduce asexually through fission in which one individual can split into two or more new individual. Experiment on fission of holothurian in Indonesian had been done for some species such as H. atra [9, 10] and H. Impatias [11] as well as species of Stichopudidae [12]. The sandfish (H. scabra) is not categorized as fissiparous species; however, this species had been induced for fission at laboratory condition [13]. Technology to induce fission is simple, cost is affordable and the duration is short [8]. This research was carried out to induce fission of H. scabra at natural habitat by using tighten method.

2. Materials and Methods
This experiment was conducted on March 2019 at Morella coastal waters, Central Maluku-
Eastern Indonesia (Figure 1). Substrate in this area is predominated by sand with mud sand, gravel, crushed coral and coral reefs are spotted in certain area. There are three species of seagrasses in the coastal waters of Morella namely Enhalus acoroides, Cymodocea rotundata and Halodule pinifolia [6].

![Fig 1: Map showing study site (red star)](image)

Sandfishes (H. scabra) used in this experiment were collected from Morella coastal waters. As many as 30 individuals (41-137g) were chosen randomly and grouped into two categories consist of 15 individuals each namely small size (<50g) and large one (>60g). Those animals were kept in two separate 2x2 m² pen cultures. Inducement of asexual reproduction through fission of sandfish (H. scabra) was done by tighten the body of holothurian with rubber band at three different positions i.e. close to anterior, in the middle and close to posterior (Figure 2). Each category consists of five animals for those three treatments.

![Fig 2: Position of tighten of sandfish (H. scabra)](image)

Observation was carried out every 12 hours for number and duration of fission holothurian. Observation also was done for number of survival animal and time to heal after fission. This experiment ended when wound of all new individuals healed.

### 3. Results and Discussion

#### 3.1 Fission Success

Some species of holothurian have been reported to reproduce asexually through fission in the field or in laboratory [15, 16] (Uthicke 2001; Conand 2004) and H. scabra is not included in those holothurians. The result in this study showed all individuals (100%) of H. scabra have ability to split their body into two parts, anterior and posterior through induction of fission in natural habitat. Similar result also had been reported in laboratory condition [13].

#### 3.2 Fission Time

Duration of fission varied, ranging from 12 - 36 hours (̄=24.78, SE=2.74) for small individuals while for large individuals ranging from 24 - 48 hours (̄=37.6, SE=2.58) (Table 1). The result of t-student test showed that size significantly affected duration of fission (t-student=3.40, df=28, P=0.002). This fission time is similar for H. scabra induced in laboratory condition [13] and for H. impatien [11]. On the contrary, duration of fission in this study is slower than fission time of H. atra (<12 hours) [11] and, H. edulis and H. leucospilota (< 24 hours) [9]. Furthermore, smaller individuals of Stichopus horrens and S. vastus had fission time less than 12 hours [17]. Duration of fission is affected by size and species of holothurian. The shorter fission time of smaller individuals might be due to their smaller diameter of the body compare to the larger ones [13].

<table>
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<tr>
<th>Size</th>
<th>≤12</th>
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<th>≤36</th>
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<td>33%</td>
<td>40%</td>
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<td>Small</td>
<td>33%</td>
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#### 3.3 Survival and healing time

Survival of animals was counted when they healed their wound. At the end of the experiment 13 new animals after fission survived which consist of seven large individuals (47%) and six small individuals (40%). Survival of H. scabra induced and kept in natural habitat is higher than the same species treated in laboratory condition [13]. For all animal
survived, survival holothurian based on position of tighten is presented in Figure 3. It can be seen in Figure 3 that percentage of survival holothurian tightened near posterior is higher than animal tightened at other positions both for small and large animals.

4. Conclusion
All individuals of Holothuria scabra used in this experiment succeeded to fission but survival rate of fission individuals is low. Duration of fission and healing time are shorter for small individuals than large ones.

5. Acknowledgement
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6. References