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The Induction of Asexual Reproduction on Holothuria scabra and Bohadschia marmorata: The Conservation Effort inTanimbar Archipelago, Maluku

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Abstract. Southeast Maluku is the leading supplier of Indonesian trepang, but lately, the production has been decreased significantly. The effort to increase the trepang population through sexual reproductive techniques still requires a long time. Therefore, another method which faster, more productive, and more accessible are needed to be applied by coastal communities, namely inducing asexual reproduction of fission using a rubber band. This research using experimental methods in the field and analyzed descriptively. Fission induction conducted by rubber binding at 1/3 of the anterior body part of trepang. This study induces asexual reproduction in two trepang species found on Matakus Island, Tanimbar Archipelago, Maluku, *Holothuria scabra*, and *Bohadschia marmorata*. The results showed that *H. scabra* (98%) and *B. marmorata* (100%) rubber binding could induce individual fission of the trepang and divide into two new individuals. The posterior part has a higher regeneration and a better survival rate than the anterior part. The survival rate of *B. Marmorata* is higher than that of *H. scabra*.

1. Introduction

The production of Indonesian sea cucumber, locally known as trepang in 2011, reached 5,800 tons [1]. Southeast Maluku is a leading supplier of Indonesian trepang [2]. The production increases along with market demand for this marine commodity [3] and leads to high exploitation of that natural resources. In the case of Maluku, it was a report that sea cucumber resources have declined significantly. A research in 2001 revealed that the density of *Holothuria* spp. in Morela was about 0.09 - 1.03 individuals/m^2, while a study conducted in 2013 at the same place was only found of 0.004-0.123 individuals/m^2 [2].

The significant decline of the trepang population has a high impact on local fishermen, especially those living in archipelago such as West Southeast Maluku Regency (MTB). According to Soselisa *et al.* [4], sea cucumber fishing in MTB is generally done artisanally by local fishermen with traditional tools, methods, and management. Some MTB regions still run the management of biological resources based on local wisdom called "Sasi". The Sasi can be defined as a moratorium on the capture of natural resources in a certain period to guarantee a good catch in the future. The 'sasi' is also applied in sea cucumber fishing in MTB.

Two sea cucumber species are well-known in MTB markets, *i.e.*, *Holothuria scabra* and *Bohadschia marmorata*. *Holothuria scabra*, or locally known as trepang gosok, has a very high economic value in the international market. Local fishers usually sell to the collectors who shipped the trepang to export destinations such as China [5]. According to Purcell *et al.* [6], the dried of *H. scabra* traded about 115 to 1668 US dollars per kg in China. This market demand has lead to over-exploitation of the natural population of *H. scabra*. Consequently, *H. scabra* has been listed as an endangered species due to populations declining by the IUCN Red List [7]. Meanwhile, *Bohadschia marmorata* is trepang that is quite dominant in the market and has important values. *B. marmorata* in Indonesia is highly exploited [8][6]. *B. marmorata* has been incorporated into Appendix II CITES, which means that the species is threatened with extinction if the trade continues without an adequate regulation [8][9]

The decline of natural resources is caused by a rapid reduction of individuals by capture, so trepang does not have the time and opportunity to breed, replacing lost members [10]. In general, trepang shows a slow growth rate with a low rate of population turnover and habitats in the intertidal zone in tropical countries [2]. This characteristic worsens the condition of trepang resources if there is overfishing, apart from the weak regulation for the capture.

The efforts to multiply trepang through sexual reproduction require a long time and inhibited by high mortality of larvae and juveniles. An alternative method that is faster, more effective, and easier to implement than the available method is urgently needed [10]. Naturally, some trepang species can breed asexually by fission, which is triggered by physical stress [11][12][13][14]. Therefore, physical stress has a high potential to be used for individual propagation. One of the physical stress is to tie the trepan body using rubber bands. It is a simple technique for the induction of asexual reproduction of trepang. This idea has been successful in several locations [15][16][17][18][19][20]. However, such a method still not applied in Indonesia, especially in Tanimbar Island, Maluku. Therefore, it is necessary to conduct a similar study in the primary trepang production center in Tanimbar Island Maluku Indonesia.

2. Methods

The study was conducted in May - July 2019, data was collected at Matakus island, Tanimbar archipelago, Maluku. Trepang Were used in this research is taken in the intertidal area*: H. scabra* as many as 50 individuals and 25 individuals of *B. marmorata*. The method to induce asexual reproduction of sea cucumber fission based on Purwati [10] with slight modification.

The observation has been done on sea cucumber's time of fission, successful rate of fission, the regeneration, and their survival rate. Fission is marked by the separation of individuals into two parts, namely the A (anterior) and P (posterior). The success rate of trepang fission after induced was calculated by Boyer *et al.* [21], while the rate of regeneration is expressed in R(X) and calculated by Purwati *et al.* [17]. The survival rate of sea cucumbers was calculated using Tatalede & Salindeho [22]. The parameters were calculated using the following formulas:

Rate of fission = $\frac{A+P}{2T}\ X\ 100\%$ ...... [21]

R(X) = $\frac{Total\ number\ of\ individuals\ that\ have\ regeneration}{total\ number\ of\ individuals}$ x 100% ...... [17]

$Survival\ rate\ total = \frac{At+Pt}{2T}\ x\ 100\%$ ...... [22]

$The\ survival\ rate\ of\ an\ anterior\ body\ part\ \frac{was\ calculated\ based\ on\ a\ formula\ S= At}{Aa}\ x\ 100\%$ ...... [22]

$The\ survival\ rate\ of\ the\ posterior\ body\ part\ \frac{was\ estimated\ according\ to\ the\ formula\ S= Pt}{Pa}\ x\ 100\%$ ..... [22]

Note:
Aa = Number of initial anterior individuals
At = Number of final anterior individuals
Pa = Number of initial posterior individuals
Pt = Number of final posterior individuals
T = Total number of individuals

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3. Results

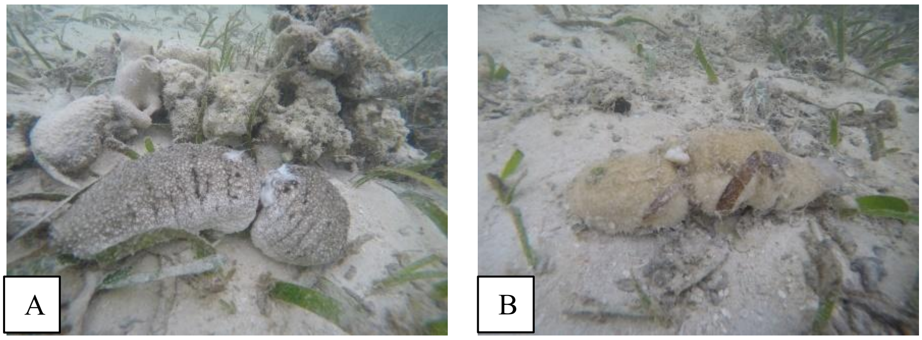

Figure 3.1. Trepang after induced by a rubber band (A: *H. Scabra*, B: *B. Marmorata*)

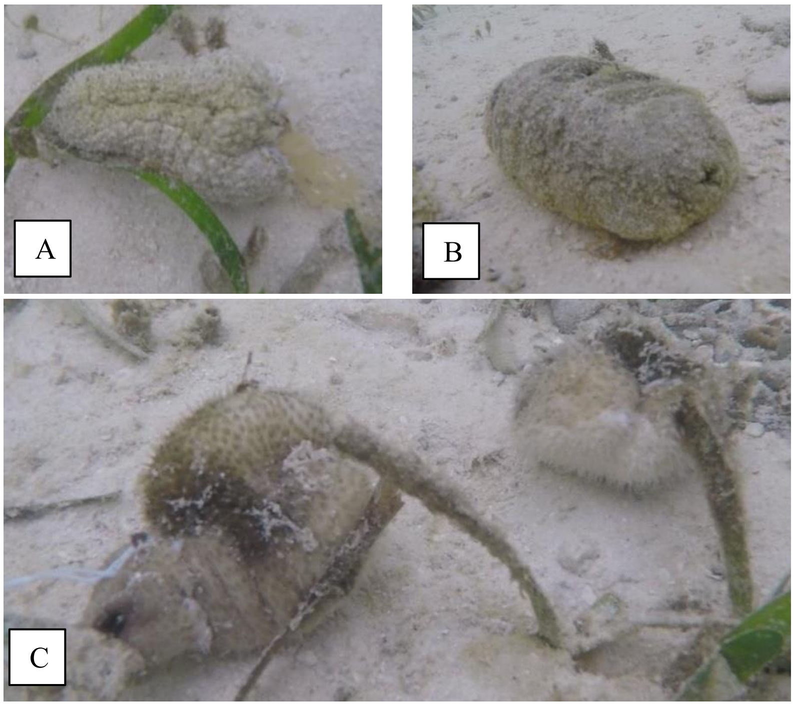

Figure 3.2 Body part of trepang after fission(A: Anterior body part of *H. scabra*; B:Posterior body part of *H. scabra*; C: Anterior and Posterior body parts of *B. Marmorata*)

Before being fissioned, both trepangs did contraction in the area tied by rubber (Figure 3.1). The results showed that *H. scabra* (98%) and *B. marmorata* (100%) could induce fission and divide into two parts. The time needed for the two species of sea cucumbers to split is 24-72 hours after induction of rubber bands (Table 3.1). *H. scabra* needs 36 hours to split, while *B. marmorata* requires 13 hours. The highest production of survival individuals recorded on 72 hours for *H. scabra* and 24 hours for *B. marmorata*. *H. scabra* has a lower regeneration rate compared to *B. marmorata* (Table 3.2). Many individuals from the anterior part of *H. scabra* died during the recovery process.

Table 3.1. The time requirement and the success rate of fission.

Species	The average of individuals length	The number of individuals that is fissioned at time						Successful rate of fission
		12 hours	24 hours	36 hours	48 hours	50 hours	72 hours	
H. scabra (n=50)	16 cm	0	0	3	13	5	28	98%
B. marmorata (n=25)	16,5 cm	0	13	4	8	0	0	100%

Table 3.2. The regeneration rate of *H. scabra* and *B. marmorata*.

Species	Anterior	Posterior	Total	Regeneration rate
H. scabra	5	45	50	51%
B. marmorata	20	24	44	88%

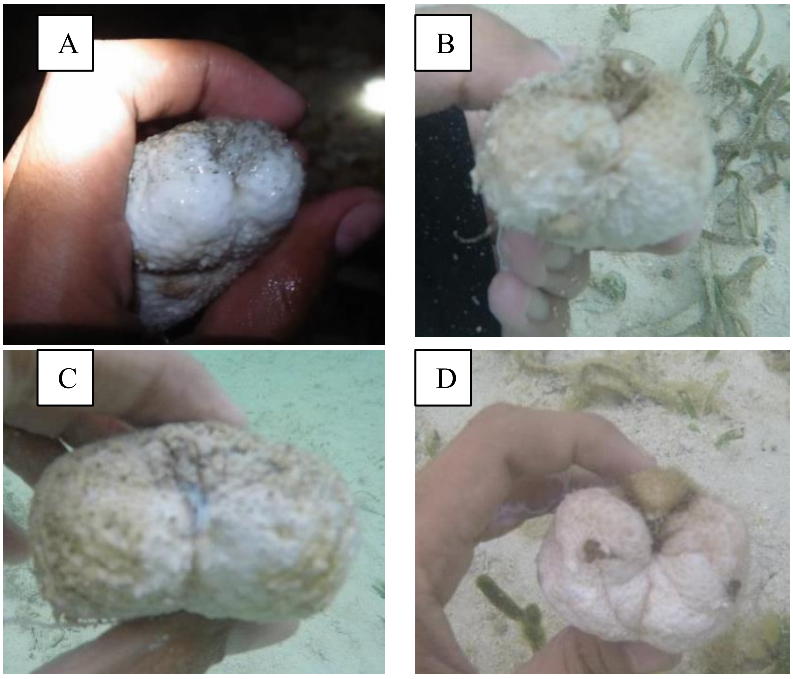

Figure 3.3. Individuals who have a close wound (A: Anterior body part of *H. scabra*; B: Anterior body part of *B. marmorata*; C: Posterior body part of *H. scabra*; D: Posterior body part of *B. marmorata*).

On the 19th day of rubber bands induction, all the H. scabra died (Table 3.3). The posterior body part of both species has a better survival rate compared to the anterior parts. Both body parts of *B. marmorata* have a total survival of 88%. The survival rate of the posterior body part on *B. marmorata* reaches 96%.

Tabel 3.3. The survival rate of fission.

Species	Survival rate		
	Anterior	Posterior	Total
H. scabra	0	92 %	46 %
B. marmorata	80 %	96 %	88 %

4. Discussion

The time of the fission after induced rubber induction are varied between sea cucumber species [10] and can be influenced by the thickness of the trepang body wall [19]. The amount of time required for *B. marmorata* to be divided is relatively short compared to *H. scabra* since *B. marmorata* has a thinner body wall. The body fission time of trepang in this study is faster than *H. tubulosa* and *H. polii* [20]. It is longer than *Stichopushorrens*, which only needs 6 hours after induction, due to a fragile body wall[18].

Naturally, *H. scabra* and *B. marmorata* had not been detected as having the ability to perform asexual reproduction by fission [12]. However, both species can still be divide after being induced by rubber bands. The success of body division is influenced by the presence of mutable connective tissue in the body of sea cucumber, responsible for the curvature and rigidity of the sea cucumber's body [23]. Both species have a higher success rate of fission than *S. horrens* and *S. vastus* [18].

Regeneration is marked by wound healing in the anterior and posterior parts of the body that are severed (Figure 3.3). Regeneration is influenced by the presence of binding tissue that functions as a tissue restorer, connecting, and developing body walls with nerve-muscle control [24]. This tissue plays a role in wound healing during the regeneration of parts of the body after fission [25]. The posterior part has a high regeneration rate because it has more binding levels [26].

Previous researchers state that the survival rate of the anterior part is higher than in the posterior [27] [15]. In contra, *H. scabra* and *B. marmorata* showed the difference result due to the posterior portion has a larger size and a higher ability to hold on the seagrass substrate compared to the anterior portion. Therefore, the posterior part has a better ability to withstand strong currents [26] [28].

In general, comparing the two trepang species, *B. marmorata* exhibits the high intensity of regeneration, less sensitivity to induction, which is shown in the absence of evisceration, and a high survival rate. The result showed that *B. marmorata* has a low level of resistance to fission induction treatment. The binding point on the 1/3 anterior part of the body [10] is useful in the process of fission on *H. scabra* and *B. marmorata*, but not for the regeneration process of *H. scabra* because it caused the anterior part to have a smaller body and fewer internal organs) [27].

5. Conclusion

H. scabra and *B. marmorata* were successfully fissioned after induced by rubber binding treatment. The posterior part has a better regeneration and survival rate than the anterior part. The survival rate of *B.marmorata* is higher than that of *H.scabra* at the end of the observation.

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