Fission technique on sea cucumbers (*Holothuroidea*): An alternative method for seed production

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Abstract. This study aimed to determine the successive level of fission technique on several sea cucumbers (Holothuroidea) in littoral zone of Madong Bay, Tanjungpinang. The fission technique was performed by tying the middle part of the sea cucumber's body using a rubber band. Parameters observed were composed of: (a) the occurrence of autotomy on pre- and post-treatment period, (b) successive rate of fission technique (%), (c) fission duration, (d) survival rate on post-treatment period. All data were analyzed descriptively. The results showed that: (1) Only gold sea cucumber *Stichopus chloronotus* that could perform autotomy on post-treatment period, (2) The successive rate of fission technique on *Stichopus chloronotus* and *Holothuria atra* was 83.33% and 33.33%, respectively, while fission was absent on *Holothuria scabra* and *Holothuria* sp. (0%), (3) The fastest fission process was found on *Stichopus chloronotus* at 1.2 hours, after applying the fission technique at 24±2 hours and *Holothuria atra* was at 24-48 hours, (4) The survival rate on post-treatment period was 66.67% in *Stichopus chloronotus* and 41.67% in *Holothuria atra*, while *Holothuria scabra* was 66.67% and *Holothuria* sp. was 11.11%. Fission technique is more suitable for *Stichopus chloronotus* as an alternative method for seed supply in grow-out culture activities.

1 Introduction

Sea cucumbers contain various amounts of protein, fat, and amino acids, including *H. argus*, which has 16.94% (without boiling) and 11.99% (boiled for 30 minutes) [1]. There are 18 amino acids in sea cucumbers, seven of which are essential amino acids and 11 of which are non-essential amino acids. The golden sea cucumber contains higher amino acids (38.45%) [2]. This sea cucumber also contains high protein content of 95.14% and lipid of 0.20%. Dried golden sea cucumber contains amino acids, like glutamic acid (6.6049%), glycine (7.1769%), aspartic acid (3.9227%), and proline (3.4189%), while fresh golden sea cucumber contains glutamic acid (0.2281%), glycine (0.2308%), and alanine (0.2169%) [3].

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Sea cucumbers digest large amounts of sediment, allowing the upper layers oxygenation of sediment. This process prevents the accumulation of organic matter decay and may help control populations of pests and pathogens, like bacteria. The scarcity of sea cucumbers can result in seabed hardening and unsuitable habitats for other benthos and infaunal organisms [4].

Some commercial sea cucumbers have faced exploitation pressure due to being traded nationally and internationally. Sea cucumber hunting is currently not only on expensive types, but also on cheaper types in the least-concerned species groups. High exploitation pressure on these types of sea cucumbers has caused their natural populations to decline [4]. Exploitation on sea cucumbers without a proper stock management in nature will cause this benthic biota to be difficult to find in the Riau Islands, as occurred in the Southeast Maluku [5] and several areas of the Sulawesi [6]. Overexploitation on sea cucumbers can cause seawater environmental imbalance. Although a global conservation against sea cucumbers have been reported [7-8], yet this action remains unsuccessful.

Culture activities (breeding) are one way to overcome overfishing of sea cucumbers. [9] reported that sea cucumber culture is highly important to support a restocking implementation as a conservation action of sea cucumbers in nature. [10] argued that sand sea cucumbers (*Holothuria scabra*) from breeding product had high opportunity to be further reared extensively in tropical area through brackish-water pond rearing or sea ranching. [11] has successfully stimulated a gonadal maturation and a spawning activity on sand sea cucumbers (*Holothuria scabra*) through hormonal manipulation. [12] succeeded to artificially spawn sand cucumbers through temperature-shock method by elevating and lowering the temperature media at 3-5°C. Meanwhile, [13] succeeded to nurse the sand sea cucumber seeds in East Lombok. Several obstacles still suppress the sea cucumber culture's success. Low survival rate in larval and early juvenile rearing is one of the problems that often occurs in *H. scabra* breeding [10]. Critical phases in the life cycle of sea cucumbers, namely attachment and metamorphosis process, has not been fully resolved. A proper substrate type for sea cucumber attachment still needs further studies [14]. The growth rate of sea cucumbers is extremely slow and appropriate feed formulation has not been found for growth rate stimulation.

Sexual sea cucumber seed production is still difficult and takes a longer time to reach the size ready for grow-out phase stocking, resulting in an insufficient production for grow-out rearing activities. Alternative solutions are necessary as an effort to provide sea cucumber seeds ready for grow-out stocking. Several types of sea cucumbers can divide themselves/fission naturally which underlies the development of artificial technology to stimulate fission, known as Fission Technique. The fission technique was chosen in this study because there are no scientific documentations, proving other natural asexual reproduction techniques in sea cucumbers. Studies related to the application of fission techniques on various types of sea cucumbers has never been performed in the Riau Islands. Therefore, the application of fission techniques on various types of Holothuroidea (sea cucumbers) needs to be carried out. This study aimed to analyze the successive rate of fission techniques on several types of sea cucumbers found in the littoral zone of Madong Bay, Tanjungpinang.

2 Methods

This study was performed on May-August, 2024 in littoral zone of Madong Bay, Kampung Bugis Village, Tanjungpinang as presented in Figure 1. Three sea cucumbers found in littoral zone were applied as study objects, namely sand sea cucumber (*Holothuria scabra*),

black sea cucumber (*Holothuria atra*), sea cucumber (*Holothuria* sp.), and one species from littoral zone of Korek Island, Batam, namely gold sea cucumber (*Stichopus chloronotus*), was also used as a study object. For rearing media, plastic baskets (48x36x17 cm^3) and speedboat were used. Fission technique was implemented in 104.47253°E and 0,97652°N, as presented in Figure 1.

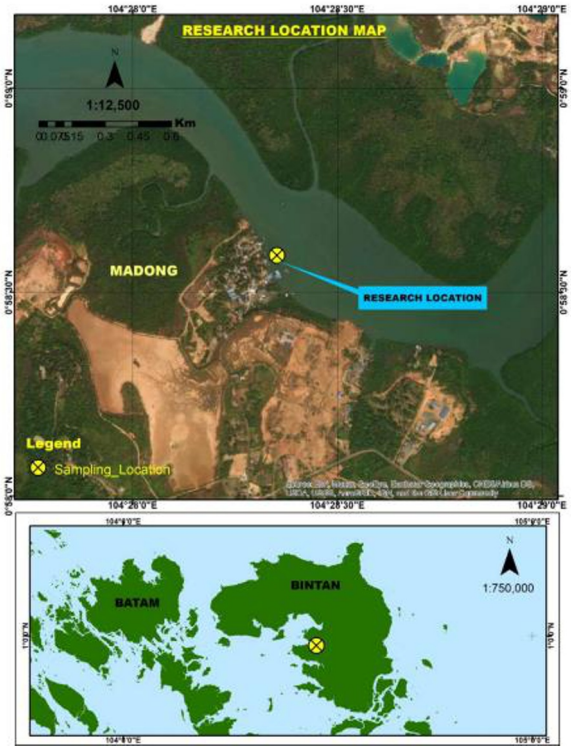

Fig.1. Study location in Madong Bay, Tanjungpinang

2.1 Experimental design

The fission technique was tested in four sea cucumbers that were divided into three size categories, namely:

(1) <60 g/sea cucumbers,
(2) 60-100 g/sea cucumbers, and
(3) >100 g/sea cucumbers

2.2 Sea cucumber preparations

Sea cucumbers divided following the size categories were obtained from the fishermen in Madong Village, Kampung Bugis District, Tanjungpinang and Korek Rapat Island, Batam. Sea cucumbers were collected until reaching the required amount. The test sea cucumbers before the Fission Technique treatment were adapted in floating net cages for 5 days.

2.3 Fission Technique Application

The fission technique applied in this study referred to [15] and [16] with slight modifications. The stages of Fission Technique were: (1) Before the Fission Technique, sea cucumbers were drained for a minute and weighed using a digital scale, (2) The middle part of the sea cucumber body was tied with a rubber band to stimulate the sea cucumber to divide its body into two parts, and (3) Sea cucumbers were placed in a plastic basket covered with a net on the bottom and sidelines of the basket. The plastic basket containing sea cucumbers after fission treatment was then placed in a floating net cage as shown in Figure 2.

Fig. 2. Plastic baskets as sea cucumbers rearing media on post-treatment period

2.4 Fission Technique Result Observation

The post-treatment condition on sea cucumbers was observed in plastic baskets at 1, 2, 3 and 4 hours, then at 24 and 48 hours. The successive rate of the Fission Technique was marked by the separation of the post-treatment sea cucumber body into two new individuals. The survival rate of post-treatment sea cucumbers was observed every day until the 5th day.

2.5 Parameters

Parameters were composed of: (1) duration (hours) of first fission occurrence to 48-th hour of fission period, (2) number of sea cucumbers that successfully performed fission, and (3) survival rate of sea cucumbers. All formula to calculate the parameters were:

Fission successive rate (%) = number of sea cucumbers that performed fission/total number of sea cucumbers × 100 (1)

Survival rate = N_t/N_0 x 100% (2)

N_t=Number of sea cucumbers at final observation period
N_0= Number of sea cucumbers at initial observation period

3. Data analysis

All data, containing the number of sea cucumbers which performed fission, fission duration, and survival rate, were analyzed descriptively.

4. Results and discussion

4.1 Sea cucumbers in Littoral Zone of Madong Bay as Fission Technique Samples

There are three sea cucumber species found in the littoral zone of Madong Bay, Tanjungpinang, namely (a) sand sea cucumber *Holuthuria scabra* (sandfish), (b) black sea cucumber *Holothuria atra* and (c) sea cucumber *Holothuria* sp., while another type comes from Korek Rapat Island, Batam namely gold sea cucumber *Stichopus chloronotus*, as presented in Figure 3. These species were taken as samples for Fission Technique.

Fig. 3. Three sea cucumber species found in littoral zone of Madong Bay, Tanjungpinang (a, b and c), while another type comes from Korek Rapat Island, Batam (d)

These sea cucumbers were taken alive from muddy sand substrate near the seagrass bed, except for *Stichopus chloronotus* which lives on sandy substrates. When taken, sea cucumbers were found burrowing in their substrate. Therefore, searching for sea cucumbers in the littoral zone was performed when the sea water receded at night with the help of a flashlight.

4.2 Fission Technique Results

The fission technique results in four sea cucumber species, based on body weight, number of sea cucumbers performed fission, survival rate, and autotomy capability, are presented in Table 1.

Tabel 1. Fission technique results in four sea cucumber species

No	Sea cucumber spesies	Successful fission (%)	Survival rate (%)	Autotomy (Yes/No)
1	*Holothuria scabra*	0	91.67	No
2	*Holothuria atra*	33.33	41.60	No
3	*Holothuria sp*	0	11.11	No
4	*Stichopus chloronotus*	83.33	66.67	Yes

Fission was absent on sand sea cucumber *Holothuria scabra* from the first to the last day of observation. The rubber bands binding the sea cucumbers were all released, but sea cucumbers were still alive on post-treatment period. This condition was in line with [17], that *Holothuria scabra* was unable to undergo fission.

In the first day, fission was absent on black sea cucumber *Holothuria atra*. This sea cucumber underwent fission after Fission Technique treatment on day 3, which separated two new sea cucumber individuals. One of the new individuals that survived was the posterior part of the sea cucumber as shown in Figure 4.

Fig. 4. Anterior and posterior part of black sea cucumber *Holothuria atra* after fission technique treatment.

This condition was in line with [18], that the monthly regeneration rate of individual sea cucumbers originating from the anterior (3.7%) and posterior (5.1%) sections showed a higher survival rate in the posterior section. Small black sea Cucumbers (<150g) have a high division rate (20% of the population), while large sea cucumbers (up to 300 g) rarely experience fission [18]. The third sea cucumber type, *Holothuria* sp. (9 individuals), had tube feet that were generally long, but the body size was small at only between 5-29 g and this species was unable to undergo fission naturally. Only one individual that did not die after treatment and did not undergo fission, as the rubber band binding the sea cucumber's body came off by itself.

Gold sea cucumber *Stichopus chloronotus* obtained the highest successive rate of fission (83.33%) after Fission Technique treatment, compared to other three sea cucumber species. Fission Technique stimulated the sea cucumber to divide itself transversely into two separate new individuals, as shown in Figure 5.

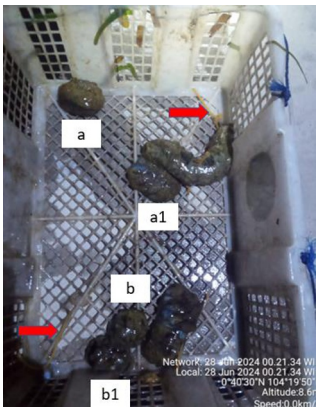

Fig. 5. a, a1, b, b1: Gold cucumber *Stichopus chloronotus* on post-fission technique. Red arrow: rubber bands binding the sea cucumber

Difference in survival rate and the successive application of Fission Technique on sea cucumbers is strongly suspected to the assosiaction of health and size of the sea cucumber. Healthier and larger sea cucumbers tend to have a higher successive rate, because they have more energy reserves to support the regeneration process.

Stichopus chloronotus is one of sea cucumber species from *Stichopus* that can undergo self-division[19-20]. Fission stages of *Stichopus* sp. that were described by according to [21, 19], and [20], are:

(1) Initiation, marked by longitudinal contractions in the middle of the sea cucumber body which can be triggered by many factors, including environmental changes, stress, and physical damage
(2) Contraction and Separation, the sea cucumber body begins to show more intense contractions in certain areas, commonly around the middle. The longitudinal and transverse muscles work together to separate the body into two parts. This process usually takes several hours to several days,
(3) Tissue Regeneration, each fragment of the body after fission begins to regenerate. This includes the re-formation of internal organs, such as the digestive tract, respiratory system, and other tissues for body function,
(4) Wound closure, the wound from fission is immediately closed by new epithelial tissue. Epithelial cells move to close the wound and prevent infection.
(5) Organ regeneration, the regeneration process involves the formation of new organs from the existing tissue. Pluripotent cells in the wound area begin to differentiate into the various types of cells to form new organs, and
(6) Growth and Recovery, after the new organs are formed, the body fragment begins to grow and develop into a complete sea cucumber individual. This process can take several weeks to several months, depending on environmental conditions and the species of sea cucumber.

Although the survival rate of gold sea cucumbers *Stichopus chloronotus* in this study was quite good (66.6%), the risk of failure and a decrease in the survival rate of sea cucumbers remained high. This condition occurs because : (1) In this artificial fission technique, open wounds emerge in the sea cucumbers' samples, that are highly susceptible to infection by bacteria, fungi or viruses, (2) Physiological stress occurs in sea cucumbers after division because some of their organs are trimmed unnaturally and have yet to regenerate normally. If environmental conditions are in imbalance condition,the survival rate of the sea cucumbers will be extremely low.

Stichopus chloronotus can perform autotomy. This species slowly "sheds" its ventral skin, but the ventral part is unlikely shown in Figure 6. This process lasts for a short time at about 2-3 minutes.

Fig. 6. Autotomy in the ventral part of gold sea cucumber *Stichopus chloronotus* (a), (b) the shed ventral body part after autotomy

5 Conclusion

Fission technique is more suitable for gold sea cucumber *Stichopus chloronotus* as an alternative way to supply sea cucumber seeds that are ready for grow-out phase culture activities. Authors suggest to perform a further study related to the ideal environmental condition to improve the sea cucumber's regeneration success on post-fission period.

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