Fission Reproduction of Two Stichopudidae Species (Holothuria:Echinodermata)

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Abstrak

Reproduksi Fission dari Dua Species Teripang Famili Stichopudidae (Holothuria:Echinodermata)

Terdapat dua sistem reproduksi pada teripang, yaitu seksual dan aseksual. Fission, sebagai salah satu cara reproduksi aseksual, adalah kemampuan alami teripang untuk membelah tubuhnya menjadi dua bagain dan masing-masing bagian dapat beregenerasi menjadi individu baru. Fenomena ini memberikan kemungkinan melakukan simulasi fission untuk memperbanyak benih teripang. Penelitian ini bertujuan untuk menganalisa kemampuan fission dua species Stichopudidae (Stichopus hermanii and S. chloronotus) dari Karimunjawa. Simulasi fission dilakukan dengan mengikat teripang uji dengan karet pentil dan meletakkan individu teripang yang telah diikat pada keranjang yang digantung pada permukaan laut. Pengamatan fission dilakukan pada jam ke 12, 24 dan 48. Ketika telah terjadi fission dan luka tertutup, teripang uji tetap berada di keranjang uji dan teripang tidak diberi pakan. Perlakuan berlanjut sampai penyembuhan secara morfologis terjadi dengan sempurna. Waktu terjadinya regenerasi setelah luka sembuh dicatat sebagai data pengamatan. Hasil penelitian menunjukkan bahwa simulasi fisssion dapat dilakukan pada species teripang Stichopudidae. Setelah proses fission selesai, dinding tubuh pada lokasi fission menjadi basah atau berlendir selama satu sampai dua jam. Pada pagi hari berikutnya, dinding tubuh akan nampak normal konsistensinya dan luka telah tertutup. Proses fission berlangsung selama beberapa jam sampai dua haridan tanda-tanda regenerasi timbul mulai dua sampai tiga minggu setelah fission. Waktu generasi S. hermanii terjadi lebih cepat (2 minggu setelah fission) daripada S. chloronotus.

Kata kunci : *fission, produksi benih, stichopudidae, Pulau Karimunjawa*

Abstract

There are two reproduction systems in seacucumber, i.e. sexual and asexual. Fission, as a way of asexual reproduction, is natural seacucumber ability to split their body into two part, and this natural fenomenon give possibility to conduct fission stimulation as seacucumber propagation. Present works are aimed to analyse fission capability of two Stichopudidae species of sea cucumber (Stichopus hermanii and S. chloronotus) from Karimunjawa Islands. Fission stimulation by rubber band tied was done in basket hanged on the sea cage. The fission was observed for 12, 24 and 48 hours. When fission occurred and wound recovered the sea cucumber were still kept in the basket and no food was added. This treatment continued until morphological recovery was completed. The time regeneration occurred after wounds recover was recorded. The result of present work revealed that stichopudidae species showed very good response to fission stimulation. After the entire process of fission, the bodywall at the fission site remained a liquid or mucus like consistency for at least two more hours. The following morning, the bodywall had its normal consistency and the wounds at both ends were nearly entirely closed. The fission process took several hours to two days and signs of regeneration appeared two to five weeks after fission. Fission simulation give shorter regeneration time for S. hermanii (2 week) than S. chloronatus.

Keywords: *fission, seed production, stichopudidae, Karimunjawa island*

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Introduction

Sea cucumbers (phylum Echinoderm, class Holothuroidea) can be described as worm-like organisms with elongated and soft bodies. Sea cucumber is known as teripang (Indonesia), beche de-mer (French) or teat fish. There are 23 economic important sea cucumber species in Indonesia and act as world's main beche de-mer exporter (Tuwo and Conand, 1992).

One of main sea cucumber producer is Karimunjawa waters. The Karimunjawa islands lie in semi-closed waters, located in the Java Sea (110° 07.2'-110° 37.2' BT and 5°43.2'- 5°54.6'LS), northern part of Jepara, Central of Java. There are a total of 15 species of sea cucumbers according to previous studies (Anonimous, 2008) but in more recent studies (Hartati *et al*., 2009), found 18 sea cucumber species which belong to Holothuriidae and Stichopodidae family.

The Stichopodidae consists of two genera *Stichopus* and *Thelenota*. Their characteristics are a body square-shaped or trapezoidal in cross section, cuvierian organs always absent, gonad in two tufts appended on each side of the dorsal mesentery and dominant spicules in branched rods and C- and S-shaped rods. The genus *Stichopus* has a bivium covered by tubercules and papillae at least on its sides; the spicules develop as tables, branched rods and C and S rods (Hartati *et al*. , 2014). *Stichopus chloronotus* is a rather small species with a firm body of quadrangular shape; its green tegument gives it the name of greenfish the ventral mouth is surrounded by a row of 20 stout tentacles. It lives in shallow areas of the coral environment. Whilst *Stichopus hermanni (*former *S. variegatus*) is a medium-sized species (mean about 35 cm) with a firm body wall which disintegrates easily when collected as the other *Stichopus*. Its color yellow to greenish gives it its name of curryfish. The bivium is covered by irregular conical warts arranged in 8 longitudinal rows (Conand, 2006), with smaller papillae in between. It occurs in reefs and lagoons, in sea-grass beds, rubbles and muddy-sand bottoms.

Good economic prospect lead to increase catching of sea cucumber. The sign of depleting population are shown by Hartati *et al*. (1996), Hartati (1998), Pringgenies *et al*. (2007; 2008). Therefore, it is urgently need an effort to recover the population through sea cucumber culture and restocking. Culture of sea cucumber (ongrowing) has already practiced by fishermen of Karimunjawa but the seed are only supplied from natural population. Hence, for culture and restocking purposes, the continuation seed supply immediately has to be done.

Sea cucumbers can reproduce through sexual reproduction as well as asexual reproduction (Conand, 1996; Darsono, 1999). Asexual reproduction through fission of fissiparous holothurian species does not always undergo fission at all geographic locations and the reasons for this variability among populations are unknown (Purwati and Dwiono, 2007). It has been suggested that fissiparous populations undergo fission when the habitats are euthrophicated (Conand, 1996) or the sexual reproduction failed (Purwati, 2001b; 2004).

Induced fission as asexual reproduction may be an option for effective population recoveries (Nugroho *et al.,* 2012). Purwati (2002) and Purwati and Dwiono (2007) has discussed the possibilities of using this asexual reproduction capacity on stock restoration of fissiparous holothurians and also did stimulation of asexual reproduction (Purwati, 2001a; 2002; Purwati and Dwiono, 2008) on low economic value sea cucumber species, *Holothuria atra*. But none has been done on Stichopus species. This paper is aimed to show the fission capability of *Stichopus hermanii* and *S. chloronotus* from Karimunjawa waters.

Material and Methods

Twenty live adult specimens of sea cucumbers (average body weight 250 g) were collected from local fishermen of Karimunjawa waters. Fission inducement method applied in this experiment was modification of Purwati and Dwiono (2005; 2008) who did the experiment in controlled environment. Prior to fission inducement, ten individuals of *Stichopus hermanii* and *S. chloronotus* were kept in bottom seacage located in Tajung Gelam waters, northern side of Karimunjawa Island. The experiment was started in the afternoon when the day has already cool to reduce sea cucumber's stress.

Rubber bicycle inner tubes were used to tighten sea cucumber up which was proven most suitable to do fission stimulation by Purwati and Dwiono (2005; 2008). The rubber was placed tightly around each specimen at the middle of their body. Every three tied sea cucumbers were then placed in a small basket hang just under the surface of seawater. The basket was tied loosely onto bamboo pool on the cage to allow it moving according tide. The fission was observed for 12, 24 and 48 hours.

When fission occurred and wound recovered, the sea cucumber were still kept in the basket and no food was added. This treatment continued until morphological recovery was completed. The time regeneration occurred after wounds recover was recorded. When a new anal aperture or mouth started to appear, the animals were moved in the cage. There is no treatment in the cage as food has already available in the substrate in the cage bottom.

Results and Discussion

Unlike sexual reproduction which is universal, asexual reproduction of fissiparous species may not occur in certain habitats. Fission area tends to be specific. Each species may need particular requirement of triggers, including failure of sexual recruitment. Active fission effects individual size of the populations while the population density remains stable.

The results show that the fission process in most of seacucumber (70-80%) less than 12 hours (Table 1). The common phenomenon when inducement injured the animals, such as evisceration, was not happened in this experiment. The result of this experiment was higher than Hermawan *et al.* (2012), and confirmed that fission stimulation was more effective for fissiparous species, like *S. hermanii, S. chloronotus* and S. *horrens* than non-fissiparous one (*S. vastus*).

With this method (tied with rubber band), fission occur with closed wound in fission plane in both species, just like in *S. horrens and S. vastus* on previous experiment (Hermawan *et al.,* 2012). This differ from the result of fission *H. atra* with open wound (Purwati and Dwiono, 2005; 2008). Induced individuals did not show twisting as normally occurs in specimens undergoing natural fission as described for species of the genus Holothuria (Emson and Wilkie, 1980). Instead, the mechanical properties of the body wall allow this organ to become semi-fluid, and the sections may separate rapidly, apparently with minor effort simply by forward movement of the anterior section.

Table 1. Time needed for seacucumber to fission as percentage test individuals

Species	Time to fission		
	<12 hours	24 hours	2 days
S. hermanii	70	20	10
S. chloronatus	80	10	10
*S. horrens**	27	41	32
*S. vastus**	14	29	52

Note : *) Hermawan *et al.* (2012)

The mechanical properties of the bodywall of many holothurians are well described for *S. chloronotus* (Motokawa, 1982; 1984). Connective tissue in holothurians (and other echinoderms) is named "catch-connective-tissue" (Motokawa, 1984) or "mutable collagenous tissue" (Wilkie, 1984). These tissues may contract or expand nearly instantaneously without the action of muscles, probably under control of the nervous system (Wilkie, 1984).

After the entire process of fission, the bodywall at the fission site remained a liquid or mucus like consistency for at least two more hours. The following morning, the bodywall had its normal consistency and the wounds at both ends were nearly entirely closed. It appears from the result of this experiment that, in Sticholudidae family, such as *Stichopus hermanii* and *S. chloronotus*, another important function of the catch connective tissue is to aide in asexual reproduction by transverse fission and to warrant rapid wound healing.

The fission process took several hours to two days (Tabel 1), and signs of regeneration appeared two to five weeks after fission (Table 2). The process of fission and regeneration are presented in Figure 1-6.

Table 2. Time needed for seacucumber for regeneration

Species	Week	% Regeneration	
		Anterior	Posterior
S. hermanii	2-5	60	30
S. chloronatus	4-5	60	70

Figure 1. Fission stimulation in *S. herrmanni* : Tying with rubberband

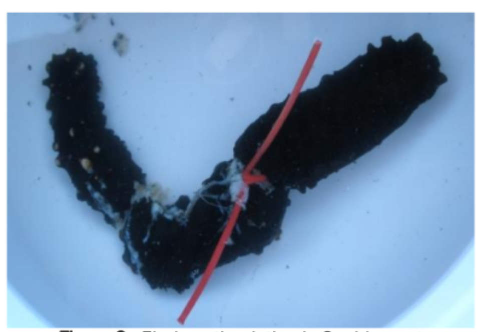

Figure 2. Fission stimulation in *S. chloronatus*

Figure 3. Hanging the basket on the surface of the cage

Figure 4. Result of fission of *S. hermanii*

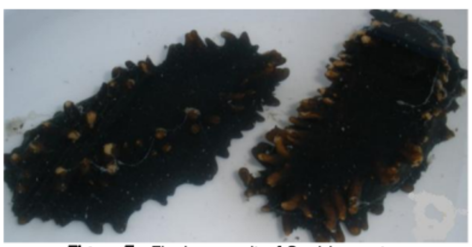

Figure 5. Fission result of *S. chloronatus*

Figure 6. Regeneration of posterior part of *S. hermanii*

The previous work done by Hermawan *et al*. (2012) which stimulated and observed fission ability and regeneration *S. horrens* and *S. vastus* showed that in both species, smaller group showed easier and quicker fission, but large group has higher regeneration rate.

The inducement of two species of Stichopus produced varied regeneration of anterior and posterior parts (Table 2). Regeneration of anterior part of *S. hermanii* specimens were higher than posterior part but otherwise happened in *S. chloronatus*. The result of Reichenbach and Holloway (1995) showed that *S.chloronotus* regenerated anterior and posterior parts into whole animals within 3 months. In addition, the posterior parts of *S. horrens* and *S. vastus* the succesfully formed a new mouth complex and posterior parts have higher survival rate than anterior Hermawan *et al.* (2012).

Regeneration is defined as a process of secondary (postembryonic) development of an injured or autotomized organ or structure. Therefore, since the same structure is created as an outcome of both regeneration and embryogenesis, regeneration is often stated to involve a reactivation of developmental mechanisms (Mashanov and Garci'a-Arrara', 2011). The regeneration could not be an exact reproduction of developmental programs, since regeneration always involves unique processes, such as wound healing and dedifferentiation. The degree to which regeneration recapitulates embryonic development seems to be different in each particular case. As to the digestive tube, there are interesting parallels between normal development and regeneration of this organ in sea cucumbers. Mashanov and Garci'a-Arrara', 2011 also mentioned that the extraordinary regenerative potential of holothurian visceral organs is mostly due to the ability of specialized cells to dedifferentiate and rebuild the lost structures through proliferation and migration.

An attempt to fission stimulation of Holothuroidea family done by Muttaqin *et al* (2013) for *H. atra* and Nugroho *et al.* (2012) for *H. impatiens* showed that the posterior parts grew faster than other parts. In their study, Thorne and Byrne (2013) found that in natural the fissiparous tropical sea cucumber *Holothuria atra* population densities remain stable indicating that asexual propagation through fission may balance mortality and that a portion of fission products suffer post-split mortality. The reason why this clonal species severs its body with the potential death of one half is not known. Fission in *H. atra* results in production of anterior and posterior fission products that differ in anatomy with the anterior portion containing the buccal complex and gonads, while the posterior product has the cloaca and respiratory trees. They also investigated post-split survivorship of the two fission products at three sites to address the possibility that there is a 'sacrificial half'. Due to possession of the feeding complex and gonads, the anterior portion might be the more physiologically valuable body portion to maintain, and we hypothesised that the anterior section would exhibit differential survival. Over the 6 year study however, Thorne and Byrne (2013) found that there was no difference in survival success of anterior and posterior segments across all sites and months sampled, with ca. 1000 individuals examined.

In the work on induced transverse fission of *H. atra* and *B. marmorata* (Laxminarayana, 2006) revealed that all the cut pieces survived in first species whereas the second survival was 92.5–95%. The mortality that occurred was for the anterior parts during the first two days after duplication. There was initial weight loss immediately after the induction of transverse fission in the sea cucumbers, but thereafter, there was a gradual increase in weight once the sea cucumbers fully regenerated. The growth rate was slightly better for *H. atra* (0.62–0.64 g day-1) compared with *B. marmorata* (0.52–0.54g day-1).

Asexual reproduction in nature is a seasonal event mainly occurring in winter in natural populations (Laxminarayana, 2006). Most holothurian species with asexual reproduction follow the "twisting and stretching" mode (Emson and Wickie 1980; Uthicke 2001a, b); the anterior and posterior sections rotate in opposite directions, resulting in a constriction in the holothurian. In the second step, the two halves slowly move in opposite directions until the body wall tears at the constriction and the two halves become completely separated.

Natural fission reproduction has been observed in ten species of sea cucumber of Dendrochirota and Aspidochirota, such as *Cucumaria lactea*, *C. planci* Brandt (Smiley, *et al.*, 1991), *H. parvula* (Emson and Mladenov, 1987), *H. atra* Jager (Harriott, 1982; (Conand, 1993; Conand, 1996), *H. edulis* (Uthicke, 1997), *H. leucospilota* (Conand, *et al.*, 1997), *S. chloronatus* (Uthicke, 1997; Conand *et al.*, 1998), *S. horrens* (Harriott, 1982; Kohtsuka *et al.* (2005), *A. difficilis* Semper and *A. mauritiana* (Kandan, 1994). So that species are grouped as fissiparous holothurians. This process is believed to be an important mechanism in maintaining population size (Uthicke, 2001a).

These present results support previous studies (Purwati and Dwiono, 2005; 2008) that also indicate fission can be induced, but the first attempt of fission stimulation in *S. hermanii*. Disadvantage of fission reproduction is only produce two individus. But there are several advantages, such as applicable to fishermen, only need simple and cheap technology, and could be done for any number of seacucumber.

Conclusions

It could be concluded that confirming previous result that seacucumber stichopudidae species showed very good response to fission stimulation. Asexual reproduction through fission stimulation could be applied for seacucumber seed production.

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