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The Growth of Sea cucumber *Stichopus herrmanni* After Transverse Induced Fission in Two and Three Fission Plane

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Abstract

Transverse induced fission proven could be done in Teripang Tril, *Stichopus herrmanni*. This present works aimed to analyze wound recovery, regeneration period and growth of Teripang Trill after asexual reproduction by fission using two and three fission plane. Observations were made every day until the sea cucumber body separated into two or more (depending on treatment) and reared for 16 weeks. The results showed that there are differences in wound recovery, regeneration period and growth of *S. herrmanni* depend on their different fission plane. The wound recovery and regeneration period (days) of anterior, middle and posterior individu *S. herrmanni* resulted from two and three fission plane were varied but the two fission plane the anterior individu recover for longer period than posterior part and the wound recover process in both end for thee fission plane was same. Average growth of anterior and posterior fragment were longer for two fission plane than three fission plane. The middle fragment (M1 and M2) both fission plane was able to grow but very low. It showed that three fission plane gave very slow growth in every fragment of the body.

Keywords: growth, post-fission, fission plane, *Stichopus herrmanni*

Introduction

The ability to regenerate among Holothuroidea occur after the phenomenon of eviseration as a defend mechanism and after fission in asexual reproduction. Many marine organisms have ability to reproduce asexually (Gouyon *et al.*, 1993). Natural asexual reproduction in the sea cucumber has been observed in the order of Dendrochirotida and Aspidochirotida, and has been known to be happen in 10 species of sea cucumber from tropical waters (Conand *et al.*, 1997; Conand *et al.*, 1998; Kohtsuka *et al.*, 2005). The sea cucumber with ability to reproduce asexually by fission naturally are included as fissiparous holothuria. In asexual reproduction, sea cucumbers do divide its body or fission. After fission, anterior part of body complete with mouth and tentacles and some internal organs will regenerate and grow as well as the posterior part with anus, some intestinal and respiratory tree, become a new individual.

Fission on fissiparous species can occur naturally in the laboratory, especially when rearing is done in the period of asexual reproduction season. For example *Stichopus chloronotus* were narrowed on her wall in their habitat, immediately transferred to the laboratory to observe the process of fission (Uthicke, 2001). However the desirable is

the cleavage of sea cucumbers that can be controlled so that fission can occur in both species in the category fissiparous and non fissiparous. And it has been proven by Hartati *et al.*, (2009) in nine holothurian species from Karimunjawa Waters, Jepara.

The measurement of body weight during recovery and change in morphology of individus resulted from fission inducement has been conducted upon *H. atra* by Purwati and Dwiono (2007). Hartati *et al.* (2013) observed induced fission of two Stichopudidae species i.e. *Stichopus herrmanni* and *S. chloronotus*. They reported the species's entire process of fission, closed of the wounds and signs of short time regeneration but none has been done to determine further individual growth after induced fission of *S. herrmanni*. The objective of present work was to measure individus growth after induced fission with two and three fission plane of transversed induced fission in *S. herrmannii*.

Materials and Method

Transversed induced fission was carried out on 30 individus of *S. herrmannii* collected from Karimunjawa Waters, Jepara (average body weight 350 g.). Prior to fission inducement sea cucumber

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were kept in bottom seacage culture system sized of 5x5x3m³. Bottom of cage was sandy-mud substrate planted with seagrass of *Thalassia*, *Cymodocea*, *Enhallus* taken from surrounding cage. The seaweed of *Padina* were also put into the cage providing additional feed for sea cucumber others than microphyto-zoobenthic from the substrate.

The experiment was started in the afternoon when the day has already cool to reduce sea cucumber stress. Fission stimulation was conducted in 5 ltrs tray filled with seawater. Transverse fission was induced by placing rubber bicycle inner tubes around the body of sea cucumbers depending the treatment of experiment. The treatment was two and three fission plane, in which 15 sea cucumber were tied in two and three places respectively (Fig. 1). This treatment was modified by Hartati *et al.* (2009) from the methods of Purwati dan Dwiono (2005; 2007). After being tied for fission, the sea cucumber were placed in plastic perforated basket sized of 20x40x10cm³ covered with nets to protect the animals from predators. Stocking density of the sea cucumber was two individuus per baskets. Baskets then was placed hang in the bamboo pool in the cage just under the surface of the seawater. When fission occurred and wound recover, the sea cucumber were still kept in the basket and no food was added. This treatment continued until morphological recovery was completed. When a new anal or mouth aperture 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.

Observation of the sea cucumber was performed on every individual sea cucumber during and after fission. After sea cucumber underwent

fission and the wound was cured. The growth or regeneration of body part individu resulted from fission was measured for total growth to the nearest 1 mm, measured dorsally from constriction point to end of animal by means of flexible ruler. The new individuus resulted from two fission plane were anterior (A), middle 1 (M1) and posterior (P) parts of the body while three fission plane will divide the sea cucumber become four parts of body, i.e. anterior (A), middle 1 (M1), middle 2 (M2), and posterior (P). The growth of anterior part was in the posterior end of the new individu and the growth of posterior part was in the anterior end of the new individu. The growth of middle part in two fission plane (Middle 1) were in the anterior end (Anterior Middle 1, AM1) and posterior end (Posterior Middle 1, PM1) of new individu. While the growth of middle part in three fission plane (Middle 1 and Middle 2) were in the anterior end (Anterior Middle 1, AM1) and posterior end (Posterior Middle 1, PM1) of new individu of Middle 1 as well as in the anterior end (Anterior Middle 2, AM2) and posterior end (Posterior Middle 2, PM2) of new individu of Middle 2. (see schematic Figure 3). The measurement of body part of individuus growth was carried out biweekly for sixteen weeks.

Results and Discussion

Unlike sexual reproduction which is universal, asexual reproduction of fissiparous species may not occur in certain habitats. Naturally, 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.



Figure 1. Transverse induced fission with rubber bicycle inner tubes of *S. hermannii* on two and three fission planes



Figure 2. The measurement of regeneration and growth of individu after fission

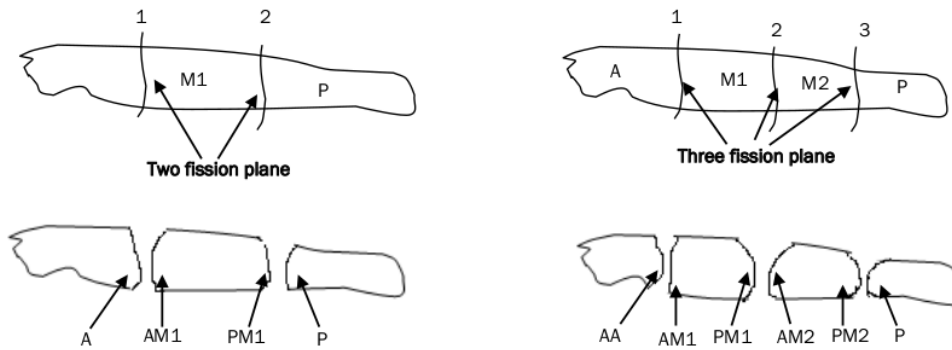


Figure 3. The schematic growth position of individu after fission of two and three fission plane
 Note : A=Anterior; M1=Middle1; AM1=Anterior Middle1; PM1=Posterior Middle1; M2=Middle2; AM2=Anterior Middle2; PM2=Posterior Middle2; P=Posterior

Fission stimulation, in which holothurians are induced to divide, has prosperous potential for seed production. This technique has proven successful for both fissiparous and non-fissiparous holothurians as has been carried out by works of Reichenbach *et al.* (1996), Laxminarayana (2006), Purwati and Dwiono (2007), Razek *et al.* (2007), Hartati *et al.* (2009; 2013; 2015a; b), Hermawan *et al.* (2012), (Nugroho *et al.* (2012), Karim *et al.* (2013); and Muttaqin *et al.* (2013).

The present work did induced transversed fission *S. herrmanni* in two and three fission plane. Observations showed that the body was more constricted at the constriction point of fission plane. The posterior part was swollen and extended. The

anterior, middle, and posterior parts was not rotated in opposite directions until that parts stretched like happened in natural fission. Naturally most holothurian species with asexual reproduction follow the twisting and stretching mode (Uthicke, 2001). Instead, in this present work, 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, as had happened in *S. chloronotus* (Uthicke, 2001). It was noted that in this experiment none individuals eviscerated at the constriction point as happened in induced fission of *H. aerenicola* (Razek *et al.*, 2007). After one day, the anterior and posterior parts were completely separated. According to Dulmatov

(2014), the difference in fission duration between various fissiparous species is most likely determined by differences in extracellular matrix properties and matrix metallo proteinases, tissue inhibitors of metalloproteinases, and other enzymatic activities. The entire process of fission lasted from one to five days.

Table 1 shows time needed for sea cucumber to recover the wound in the constriction site or fission plane. It shows that the two fission plane the anterior individu recover for longer period than posterior part but the wound recover process in both end for three fission plane was same (average of 68 days). All of middle wound part (M1 and M2) recover for longer period of time than anterior (A) and posterior (P). Razek *et al.* (2007) found that temperature had a considerable effect on the fission process. The dividing rate was greater at high temperatures (30 °C) than at low temperatures (25 °C). On the contrary, the survival rate of divided parts increased as water temperature decreased. The wound-healing period lasted longer at high temperatures than at low temperatures, so they concluded that the dividing rate increased with an increasing water temperature, while the survival rate of divided parts and the wound-healing period increased when the temperature decreased. This phenomenon might also happened in this experiment, since the temperature of seawater of Karimunjawa waters were 25-32 °C.

Regeneration period of different part of the body varied with fission plane (Table 1). The two fission plane give regeneration period (52-86 days) faster than three fission plane (68-125 days). Regeneration is in fact a distinct type of developmental process typically occurring in echinoderm: it can involve limited processes of cell turnover and tissue repair, replacement of lost parts or organs, cast off following self-induced or traumatic mutilations, and even complete regrowth of whole individuals from body fragments (thus contributing to typical asexual reproductive processes). Carnevali (2006) showed that in Holothuroids, regeneration mechanisms consists of epimorphic-like regeneration with presumptive contribution of undifferentiated cells, pseudo-blastema formation and nerve-dependent regeneration. He also proposed that In holothurians the regenerative potential can differ a lot between different groups (Dendrochirota, Aspidochirota, Apoda) and also vary with the age of the individuals.

Following fission process, there are two regeneration of sea cucumber, i.e. regeneration of internal organs and body growth. Although in present work, the observation only carried out for body growth, the internal organ development was

discussed based on Dolmatov (2014). The set of organs remaining in the fragments after fission, in two and three fission plane, the anterior fragment retains the aquapharyngeal complex (AC), gonads, and one or two segments of the gut (Conand *et al.*, 1998; Tipper *et al.*, 2002; Dolmatov *et al.*, 2012; Dolmatov, 2014). The posterior fragment of these animals contains the cloaca, a larger portion of the gut, the respiratory trees, and, if any, cuvierian tubules and gonad. In the middle fragments (Middle 1 in two fission plane and Middle 1 and Middle 2 in three fission plane) may retains small portion of intestine, gut and respiratory trees. According to Dolmatov (2014), in the anterior fragment, regeneration after fission begins with partial atrophy of the damaged first descending portion of the intestine, simultaneously the wound at the posterior end is repaired. The intestine then begins to grow backward, down the mesentery, simultaneously, the cloaca develops at the posterior end of the anterior fragment in which intestine grows joint into the cloaca, and the integrity of the digestive system is restored. At this point, sea cucumber restore the terminal regions of the longitudinal muscle bands (LMB), it's ends become thinner and grow toward the cloaca. After the intestine and cloaca merge, the primordia of the respiratory trees appear on the dorsal side of the cloaca (Dolmatov, 2014). According to Garcia-Ararras *et al.* (1998; 2001), Garcia-Ararras and Greenberg (2001) and Dolmatov (1992; 1996) the new digestive tube develops from the mesenterial lamina, which anchored the original gut to the body wall. On the basis of indirect evidence, the overall process is considered to be a nerve-dependent regeneration. At the posterior end of anterior fragment, a small outgrowth emerges and subsequently becomes longer, thus forming the posterior region of the animal. Respiratory trees develop with the growth of the posterior end.

The main event in the posterior fragment following fission is the regeneration of aquapharyngeal complex (AC) (Dolmatov, 2014) include radial nerve cords, radial water-vascular canals, nerve ring and circular water-vascular canal as well as anterior region of the intestine. The intestine becomes thinner and begins growing forward up the mesentery, into the AC and the integrity of the digestive system is restored. Then the animal begins to grow. At the anterior end, a small outgrowth emerges and gradually grows longer, thus forming the anterior region of the animal. integrity of the digestive system is restored. Respiratory trees in the anterior fragment develop through the transformation of the dorsal wall of the anterior region of the cloaca (Dolmatov and Ginanova 2009; Dolmatov *et al.*, 2011). According to Dolmatov and Ginanova (2001) muscle regeneration process appears to be due to the coelomic epithelial cells

which apparently dedifferentiate, migrate and invade the muscle bands where they differentiate into muscle bundle rudiments, finally giving rise to new muscle cells.

Based on discussion of Dolmatov (2014) the formation of internal organs of *S. herrmanni* before the growth of an external region of two fission plane occurs for approximately 40 days (Table 1). Consequently, the sea cucumber have a small outgrowth at the anterior and posterior end after fission occurred over almost two months. This results were shorter than other holothurians which may range from 1.5 to 3 months (Reichenbach and Holloway, 1995; Purwati et al., 2009; Dolmatov et al., 2012). In *C. schmeltzii*, the formation of internal organs without the growth of an external region occurs for approximately 30 days (Dolmatov et al. 2012). For three fission plane, the posterior and anterior end of anterior and posterior fragment was recover in almost similar period of time, but regeration time is shorter in the posterior end of anterior fragment. The regeration period of middle fragment showed very slow, i.e. more than 75 days.

Growth of the body begins when the internal organs are formed. Initial signs of regeneration at the fission site, the dermis is depigmented and a protuberance forms. As carried out in present work, assessments of fission and regeneration rates are typically based on the external morphology of the animals, particularly the presence of the growing anterior or posterior end of the body. Table 2 and Table 3 showed that average growth of anterior and posterior fragment were 24,5 and 29 mm for two fission plane and 11,5 and 2,4 mm for three fission plane. The middle fragment (M1 and M2) both fission plane was able to grow but very low, i.e range of 1,85-13,1 mm. In anterior end of the middle 1 of two fission plane was longer than posterior end. In the contrary, anterior and posterior end of M1 is

longer than M2 of three fission plane. The growth duration of the body varies broadly and apparently depends on the species (Dolmatov, 2014). The same related experiment still scarce. In *C. schmeltzii* individuals showed 2-3 mm long outgrowth (10–15% of body length) formed within 25 days when the animals were maintained under artificial conditions after fission (Dolmatov, 2014). In *S. chloronotus*, growth of the external region to normal size required up to one month (Conand et al., 2002). According to Jaquemet et al. (1999) the regeneration of *H. atra* after fission took six months.

The survival rate of individu after fission with two fission plane were generally greater (70-80%) than those fission with three fission plane (30-60%) (Tabel 2). Sea cucumber survivorship also indicate that the posterior part (Table 2 and Table 3) have a higher percentage than the other part (middle and anterior). These results are similar to work done by Reichenbach et al., (1996) where *S. variegatus* (now *S. herrmanni*) on the posterior part has a higher value than the anterior part of the regeneration stage. The same result also showed the fission stimulation of 10 species of sea cucumber by Hartati et al. (2009), *S. horrens* dan *S. vastus* (Hermawan et al., 2012), *H. impatiens* (Nugroho et al., 2012), *S. herrmanni* (Hartati et al., 2013; 2015a; b), *H. edulis* and *H. leucospilota* (Karim et al. (2013); and *H. atra* (Muttaqin et al., 2013).

The weight of sea cucumber may affect the survival rate of fission result. The average weight of sea cucumber tested in present work was 350 g, the two fission plane gave better result than three fission plane. . The studies of Lambeth (2000) showed that small (median weight 600 g) and medium size (median weight 1,300 g) *S. variegatus* (now called *S. herrmanni*) were able to regenerate both anterior and posterior parts (with 100 per cent survival) into whole animals in around 40-80 days.

Tabel 1. Wound recovery and regeneration period (days) of anterior, middle and posterior individu *S. herrmanni* resulted from two and three fission plane

Treatment	Individu	Wound location	Wound recovery period day - (average)	Regeneration Period started at day - (average)
2 Fission plane	Anterior	Anterior	43-57 (50)	52-67 (59,5)
	Middle 1	Anterior Middle1	57-79 (68)	67-86 (76,5)
		Posterior Middle1	54-67 (60,5)	64-74 (69)
	Posterior	Posterior	42-54 (48)	58-72 (65)
3 Fission plane	Anterior	Anterior	58-78 (68)	68-89 (78,5)
	Middle 1	Anterior Middle1	62-88 (75)	76-95 (85,5)
		Posterior Middle1	67-92 (79,5)	75-108 (91,5)
	Middle 2	Anterior Middle2	73-94 (83,5)	85-120 (102,5)
		Posterior Middle2	74-94 (84)	90-125 (107,5)
	Posterior	Posterior	60-76 (68)	78-90 (84)

Tabel 2. The growth (length range and average, mm) of body part *S. herrmanni* after fussion with two fission plane and their survival rate (%) in 16th week.

Week	Anterior	Anterior Middle 1	Posterior Middle 1	Posterior
0	0	0	0	0
2	0	0	0	0
4	0	0	0	0
6	0-3 (1,5)	0-2 (2)	0	0-2 (1)
8	3-8 (5,5)	1-4 (2,1)	0-3(1,5)	3-7 (5)
10	5-13 (9)	3-7 (4,4)	0,7-3,5 (2,1)	6,5-16 (11,3)
12	8-19 (13,5)	5-8 (6,7)	1,1-3,6 (2,35)	9-25 (17)
14	11-26 (18,5)	7-12 (9,75)	1,3-3,7 (2,5)	14-34 (24)
16	16-33 (24,5)	9-18 (13,1)	1,6-3,8 (2,7)	19-39 (29)
Survival Rate (%)	70	70	80	

Tabel 3. The growth (length range and average, mm) of body part *S. herrmanni* after fussion with three fission plane and their survival rate (%) in 16th week.

Week	Anterior	Anterior Middle 1	Posterior Middle 1	Anterior Middle 2	Posterior Middle 2	Posterior
0	0	0	0	0	0	0
2	0	0	0	0	0	0
4	0	0	0	0	0	0
6	0	0	0	0	0	0
8	0-2 (1)	0	0	0	0	0-0,4 (0,2)
10	3-5,5 (4,3)	0-2 (1)	0-1 (0,5)	0-0,1 (0,05)	0	0,2-1,4 (0,8)
12	7-9 (8)	1-3 (2)	1-2 (1,5)	0,6-1,4 (1)	1-1,6 (1,3)	0,8-2,4 (1,6)
14	8-13 (10,5)	2-3,3 (2,65)	1-3,2 (2,1)	1,1-2,7 (1,9)	1,1-2,1 (1,6)	1,1-2,7 (1,9)
16	9-14 (11,5)	2-3,5 (2,75)	2-3,4 (2,7)	1,4-2,9 (2,15)	1,4-2,3 (1,85)	1,4-3,4 (2,4)
Survival Rate (%)	50	40		30		60

The shortest regeneration time was for the posterior parts of the smallest weight class of *S. variegatus*. In the contrary, adult *S. variegatus* with a median wet weight of 3,650 g were able to regenerate only the

Transverse cutting experiments have shown that not all holothurians can restore both body fragments (Reichenbach and Holloway, 1995; Reichenbach et al., 1996; Dolmatov and Mashanov, 2007; 2626, Dolmatov et al. 2012). For example, both anterior and posterior fragments of *Actinopyga mauritiana* die after constricting of the body with a rubber band (Reichenbach and Holloway, 1995). In *Holothuria fuscogilva*, *A. miliaris*, and *Stichopus variegatus* only the posterior parts can regenerate into whole animals (Reichenbach and Holloway, 1995). On the other hand smaller (younger) individuals of *A. mauritiana*, *H. fuscogilva*, and *S. variegatus* have higher survivorship and shorter regeneration time relative to adults (Reichenbach et

posterior part into a whole animal, in around 100 days, with zero per cent survival of the anterior parts and 80 per cent survival of the posterior parts.

al., 1996). After transverse cutting both fragments of *Holothuria pervicax*, *H. impatiens*, and *Massinium magnum* die (Dolmatov et al. 2012; Dolmatov, 2014). Only posterior parts of *Apostichopus japonicus*, *Holothuria scabra*, *Ohshimella ehrenbergi*, and *Colochirus quadrangularis* can regenerate lost anterior structures after such operation (Dolmatov and Mashanov, 2007; Dolmatov et al. 2012; Dolmatov, 2014)

Conclusion

The Wound recovery and regeneration period (days) of anterior, middle and posterior individu *S. herrmanni* resulted from two and three fission plane

were varied but the two fission plane the anterior individu recover for longer period than posterior part and the wound recover process in both end for three fission plane was same. Average growth of anterior and posterior fragment were longer for two fission plane than three fission plane. The middle fragment (M1 and M2) both fission plane was able to grow but very low.

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