

# EXPERIMENT ON FISSION STIMULATION OF *HOLOTHURIA ARTA* (HOLOTHUROIDEA, ECHINODERMATA): CHANGING IN BODY WEIGHT AND MORPHOLOGY

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## ABSTRACT

Experiment on *Holothuria atra* provided evidence that asexual reproduction by fission was possible to be stimulated at laboratory. The success of the experiment was determined based on the high survival of stimulated individuals that grew into intact. After being tightened, induced individuals divided into anterior- and posterior-end individuals. During regenerating into intact individuals, the body weight decreased to relatively minimum weight. Developing of new tentacles in posterior-end individuals and anal aperture in anterior-end individuals required 7-8 weeks after fission. These indicators may lead to anatomical recovery which may be a sign of feeding necessity.

**Keywords:** *Holothuria atra*, Induced fission, Fresh weight, Morphology

## INTRODUCTION

Ten holothurian species reproduce asexually by fission (self division), in which the body divides into anterior-end (A individual) and posterior-end (P individual). Among those, four species are common throughout Indonesian waters: *Holothuria atra*, *H.leucospilota*, *H.edulis* and *Stichopus chloronotus* (Purwati, 2001a). Yet, natural fissiparity has been poorly observed and discussed in Indonesia.

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. In *H.atra*, fission is present at Wanlitung, Taiwan (Chao *et al.* 1993; Chen *et al.* 1993) and Reunion Island (Conand, 1996), but fission does not occur at Nanwan, Taiwan (Chao *et al.*, 1993) and Fanning Island (Townesley and Townesley, 1973). Triggers working on natural fission have been in debate for almost two decades (Harriott, 1982; Conand, 1996; Uthicke, 1997). It has been suggested that fissiparous populations undergo

fission when the habitats are eutrophicated (Conand, 1996) or the sexual reproduction failed (Purwati, 2001b; 2004). Regardless the possibly absence of natural fission in Indonesian waters, Research Center for Oceanography-LIPI allocated budget for experiment on induced fission in 2004. The main target was to assure that fission of certain holothurian species could be induced at the laboratory. Specimens used were local species *Holothuria atra* collected from waters of Desa Kombal, west Lombok. This experiment will promote asexual propagation through fission in holothurians, from which efforts on population recoveries can be encouraged.

Fission may be defined as a transversely division separating the body into mouth (anterior) end and anal end (posterior) end. Each part regenerates into a new intact individual (Crozier, 1917; Deichmann, 1922; Doty, 1977; Emson and Wilkie, 1980; Chao *et al.*, 1993; Conand, 1996; Conand *et al.*, 1997). Therefore, fission accomplishment is determined based on the high survival of fissioning individuals to reach intact (Purwati and Dwiono, 2005). During the process, the individuals change in body weight. This paper

presents results of weekly measurement during recovery and change in morphology of the individuals resulted from fission inducement.

## METHODS

### Species and measurement procedure

The experiment on induced fission used *H. atra* collected from sea grass beds of Teluk Kombal, west Lombok. Ten individuals mostly weighed 200-250 g. in fresh were induced in 22 June 2004. Experiment proceeded until 17 August 2004 when all individuals showed regenerating in terms of morphology.

It is essentially important to standardize the measurement procedure because the holothurian body changes easily as a result of freely water-passing through the skin and body cavity. Body weight was measured as follow: relax specimen on the water was held with bare hand, with one finger plugged up the anal pore. Rapidly, the specimen was raised up and put into a plastic bag. Accordingly, weight measurement included the free water in the body. By this way, stress to the animals would also be minimized.

Fresh weight of anterior (A) and posterior individuals (P) resulted from fission inducement were measured every week, and ended when new

tentacles and anal pore developed. Weight lost during subsequent week was estimated in percentage, as

$$\frac{n \text{ weight} - (n-1 \text{ weight})}{n \text{ weight}}$$

in which  $n$  was the weight of particular week.

Morphology of individuals resulted from fission were monitored, mainly focused on area where division occurred (former fission plane). Observation on *H.leucospilota* was additionally conducted which was resulted from similar experiment.

### Inducing technique

The use of vent pipeline for bicycle facilitated easy operation when it was tightened up strongly around the body of *H. atra* at the point of 40-50% from posterior end. Fission commonly occurs in few hours. Fissioning individuals were kept individually in small buckets with continuous flowing water. No food added because these individuals were in the state of non-feeding behavior. The technique has been presented thoroughly in Purwati and Dwiono (2005).

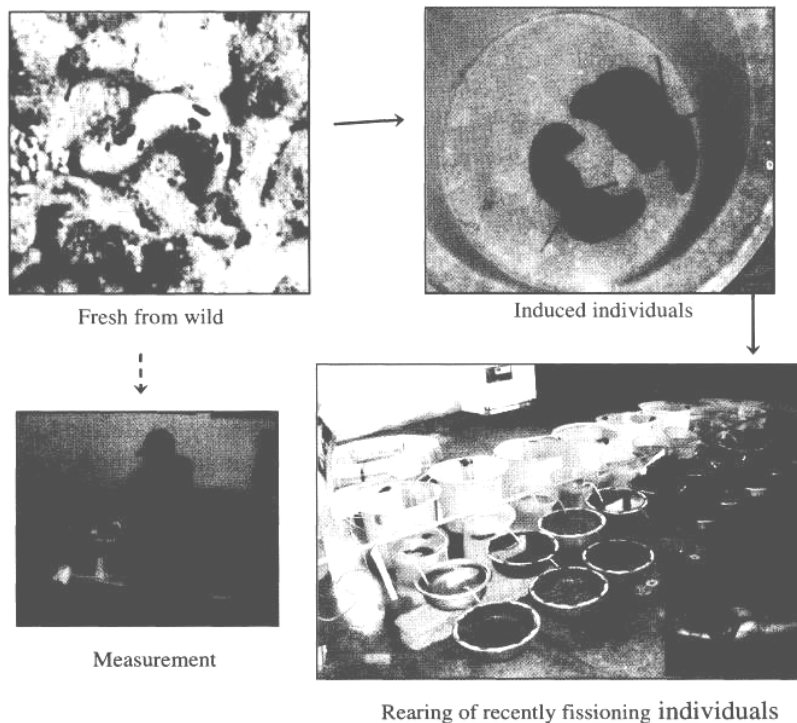


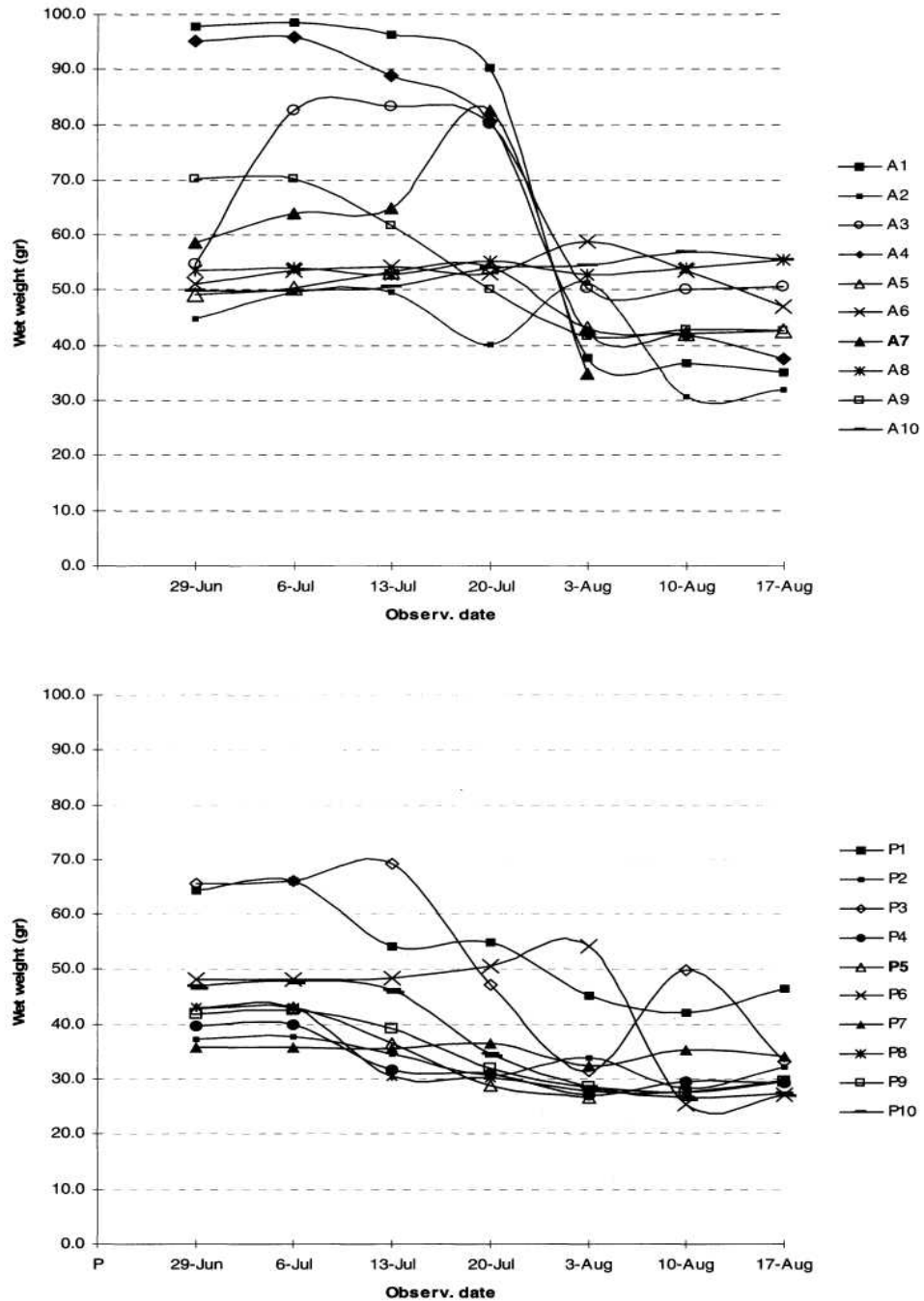
Figure1. Procedures of fission inducement on *Holothuria atra*

## RESULTS

The body weight just after division was not recorded, and first weighing was done one week after fission (Fig. 2). Evisceration did not occur in this experiment. Increase in body weight took place in the second measurement for all A and P individuals. After since, increase was in occasion.

During experiment, survival rate attained 90% when one P and one A individual failed in week-6 after fission.

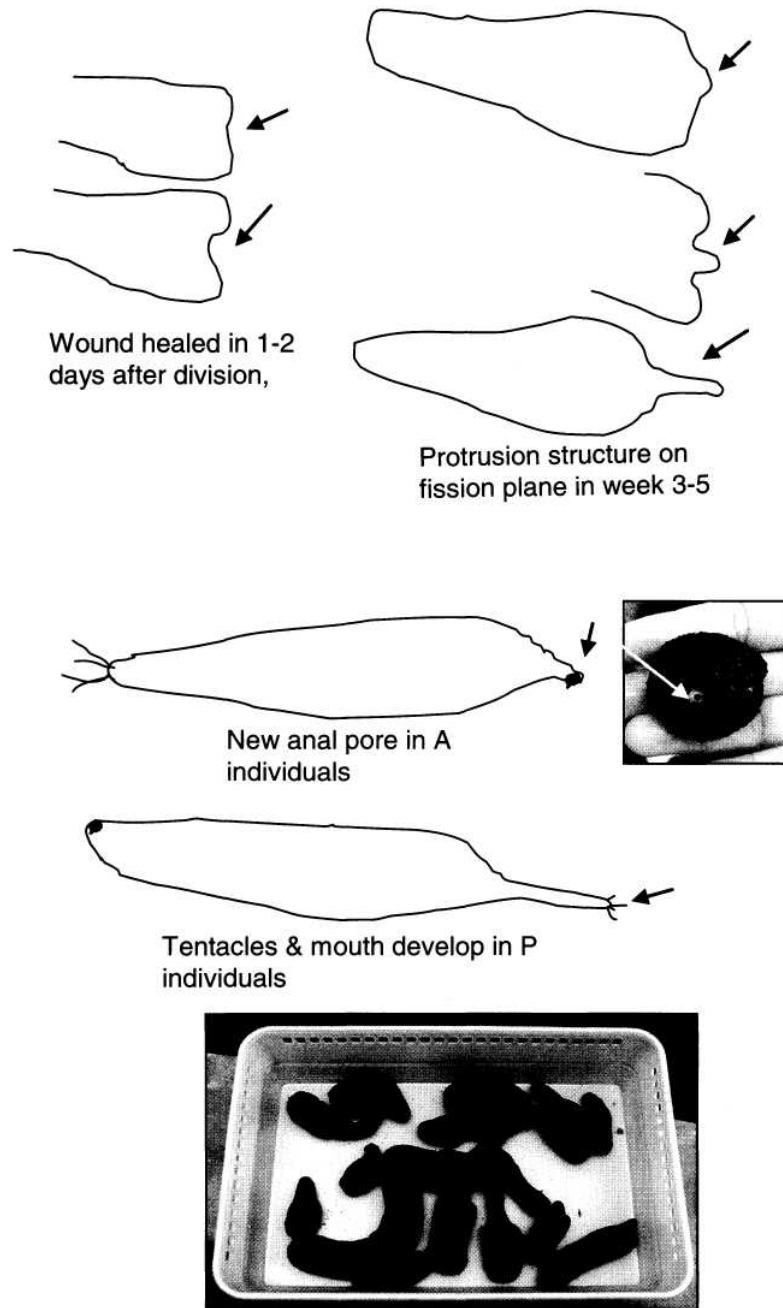
Weight lost ranged between 12.50% and 0.66% in A, 10.81%-0.61% in P individuals. While average lost amongst individuals was varied between 1.02 % and 12.50 %. During 5-7 weeks of weight monitoring, the animals seemed to manage the body weight, to some extent, stable.



**Figure 2.** Change in body weight of individuals resulting from fission inducement; A individuals (above), P individuals (below).

Change in morphology of fissioning individuals were showed in Figure 3. Fission caused wound at the fission plane, which occasionally open. The wound recovered in less than one week, remaining curve-form appearance. In the week 3 and 5, protrusion appeared which gradually became more obvious. In advanced development, new anterior

of Pa individuals (P regenerating anterior complex) grew longer than new posterior part of Ap (A regenerating posterior complex). During these weeks, both A and P were not actively moved. In the week 8 and 9, tiny tentacles turned up in the anterior part of Pa, and anal aperture appeared in Ap individuals.



**Figure 3.** Diagram illustrating morphological changing during regenerating the lost part of the body after fission

## DISCUSSION

Triggers of natural fission are still in debate, and it is suggested that each fissiparous species (or population) may need specific stimulation (Purwati, 2001b). The abundance of food, role of tidal and extreme solar radiation which may be important on natural fission (Conand, 1996; Uthicke 1997, 2001), in fact, can be replaced with artificial constriction.

Division which took several hours after stimulation may be considered short, compared to *H. surinamensis* or *S. chloronotus* observed by Crozier (1917) and Uthicke (1997). When stimulated individuals divided, visceral organs remained inside of each part of the bodies. The artificial constriction may have similar function as twisting and constriction prior to natural fission on *H. leucospilota*, which are suggested both to prevent open wound and the lost of the internal organs (Purwati, 2001b).

Reduce in weight was a consequence of non-feeding behavior, which is common on fissiparity (Emson and Mladenov, 1987; Conand et.al., 1997). During regeneration, tentacles of Pa were inactive, so do the podia. This may lead to a minimum metabolic behaviour. From this point of view, old visceral organs may be self-digested and the energy was allocated for recovery and regeneration. As in *Parastichopus californicus*, seasonal visceral lost have been recognized. Instead of being expelled out, the visceral organs are absorbed, in which coelomocytes are involved in the phagocytosis (Frankboner and Cameron, 1985). Other possibility was that coelomocytes play the role in energy providing by catching diluted diets that passed through the skin. Some research indicates that tegument of holothurians may be involved in feeding and respiration (Hyman, 1955). In the case of fission stimulation, certain agents were very possible to replace the function of digestive and respiratory systems in order to maintain metabolism or physiological stability. Such case may be of interest in the future studies.

Stimulated *S. chloronotus* and *Thelenotia ananas* (non-fissiparous) take 3 and 5-7 months (Reichenbach and Holloway, 1995). In nature, *H. parvula* needs three weeks to two months to

start feeding (Kille, 1942; Emson and Mladenov, 1987). In most observed individuals of the current experiment, weight lost maximized in week 3-5, coinciding with the development of protrusion which indicated morphological regeneration. During week 5-7, the weight lost was reduced, prior to the appearance of new tentacles on Pa and anal pore on Ap individuals. The weight of P and A did not less than 25 g. and 30 g. respectively, regardless how different the initial weight and fluctuated the early body weight was. This point may be determined as the start of feeding which may refer to the complete regeneration of digestive organs and the need of energy for normal metabolism.

Only recently, Laxminarayana (2006) reported similar experiment conducted in Mauritius. He measured body weight of individuals A and P in total of 6-10 individuals, rather than individual weight. This may cause difficulties to observe whether the individuals showed similar pattern, and also makes the comparison to the current results impossible. Morphological change in A or P individuals did not described but he mentioned that the wounds healed in two days and mortality occurred during this state. As his results also showed more than 90% survival rate, this may lead to the conclusion than *H. atra* is easy to be induced to split up.

Information on the regeneration of digestive organs is important for aquaculturists who produce individuals through fission, as the knowledge will drive to a decision when the individuals resulting from fission inducement should be fed or released to their natural habitats. The advance of induced fission is that critical point facing by larvae and juveniles is not necessary to be experienced by individuals resulting from fission. In addition, the individuals may have well adapted to the habitat. This experiment gives other alternative to the efforts on population recoveries, anticipating issues on hothurian extinction due to heavy harvests.

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