

Fission inducement in Indonesian holothurians

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Introduction

Fissiparous holothurians are capable of reproducing asexually through binary fission. Inducing fission in order to double individual numbers would be a promising alternative for holothurian re-population, as suggested by Lokani et al. (1996). Fission is described as asexual reproduction in which an adult splits into two individuals, A anterior and P posterior. Induced fission is only successful if both A and P survive and grow into intact individuals.

Only 10 species of holothurians are known to be fissiparous. Among those, four are common in tropical Indonesian waters: *Holothuria atra*, *H. leucospilota*, *H. edulis* and *Stichopus chloronotus*.

Research on fission of sea cucumbers in their natural habitat has indicated that the fission plane is specific for each species. The body of *H. atra* splits at 44% (Chao et al. 1993) and *H. leucospilota* at 20–33% of its body length when measured from the anterior end (Townsend and Townsend 1973; Conand et al. 1997; Purwati 2004).

Fission has been observed in natural stocks but has rarely been observed under specific conditions (i.e. laboratory). Uthicke (2001) published his observations when he brought a fissioning *S. chloronotus* individual to the laboratory. Reichenbach and Holloway (1995) induced fission in eight holothurians species. Their results showed a higher survival rate when the rubber bands were placed on the animal's natural fission plane. Inspired by this information, we conducted induced fission experiments that we present here.

Methods

The target species *H. atra* and *H. leucospilota* were collected from the village Teluk Kombal, Mataram, in west Lombok. Prior to fission inducement, 20 individuals of *H. atra* and 20 individuals of *H. leucospilota* were kept overnight in a tank with aerated and filtered fresh seawater in order for them to empty their gut contents.

The experiment took place from June–August 2004, at the Marine Bioindustry Technical Implementation Unit Mataram, RC Oceanography, LIPI, in Indonesia.

Initially, four different items — nylon string, cable tie, rubber band and rubber bicycle inner tube — were used to tighten up the animal's body. Results showed that the bicycle inner tube was the most suitable, as it was flexible and harmless to the animal's skin. Nylon string and cable ties created open wounds on the skin, triggering evisceration. Rubber bands were difficult to put on, and scratched and injured the skin when placed too tightly.

In the following experiment, we used rubber from bicycle inner tubes. The rubber was placed tightly around each animal, and animals were then placed in a small basket with a small amount of seawater to relax. Each band was then tightened around the sea cucumber, at 40–50% posteriorly for *H. atra* and 25–30% anteriorly for *H. leucospilota*.

Constricted individuals were reared in small buckets filled with fresh, filtered seawater and slowly aerated. Ten individuals of each species were reared separately, while the rest were kept together. When fission occurred, sand-filtered seawater was flowed over the animals (temperature and salinity similar to natural seawater). No food was added. This treatment continued until morphological recovery was completed. When a new anal aperture or mouth started to appear, the animals were moved outdoors and reared in concrete tanks with 10 cm of sand substrate and water flowing for four to six hours a day. Water was fully changed each week.

Results and discussion

Figure 1 shows the inducing techniques used in this experiment. When inducement injured the animals, evisceration was the usual response. Even so, fission could still occur and commonly resulted in an open wound on the fission plane. When the inducement position was 25–30% posteriorly on *H. atra* specimens, A and P specimens

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with open wounds were common. Inducement at 40–50% posteriorly produced at least 90% survival of A and P specimens.

Induced individuals did not show twisting as normally occurs in specimens undergoing natural fission. Occasionally, viscera appeared on the fission plane of either A or P after splitting (Fig. 2). But the wound healed not long afterwards. The fission process took several hours to three days, and signs of regeneration appeared two to three weeks after fission. Figures 3 and 4 show obvious signs of re-growth.

These preliminary results support previous studies that indicate fission can be induced. Even though fission only allows doubling of a sea cucumber stock, its advantages are: inducement can be done on any number of individuals; survival rates can be very high, as the produced individuals are already adapted to the habitat and begin growing from a relatively large size; threats (predation, etc.), which normally occur during the larval and juvenile stages, are reduced; and costs and technology are low.

Lokani et al. (1996) mentioned that fission can be induced in non-fissiparous *H. scabra*. During our experiment, we induced *Bohadschia marmorata* to divide using the same technique; nearly all induced individuals survived and recovered. For future work, we will focus on the regenerating efforts of fission products.

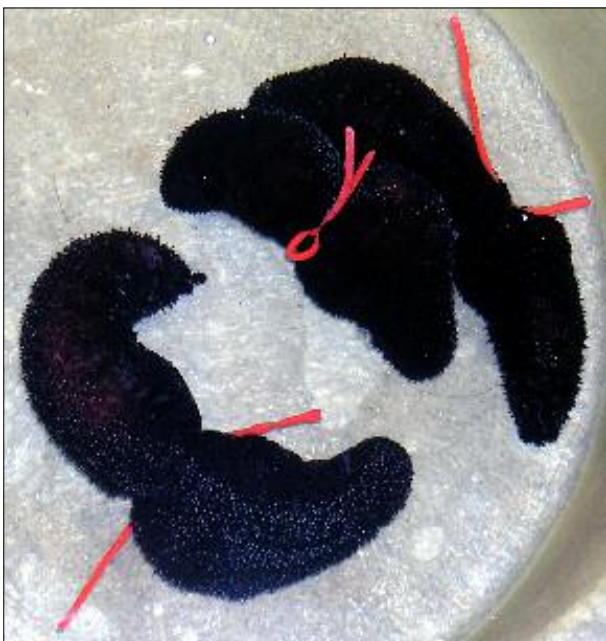


Figure 1. Induced fission of *Holothuria atra*.

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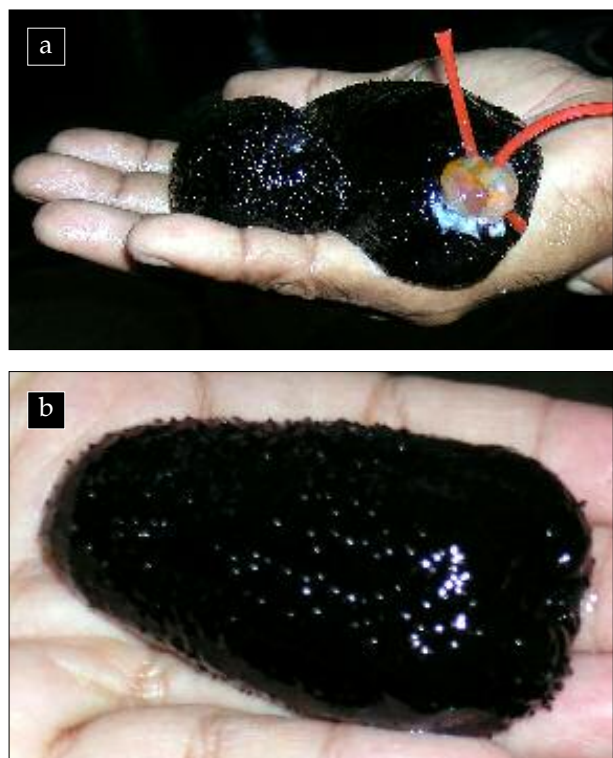


Figure 2. Result of inducement (a), and P individuals (b) of *Holothuria atra*.

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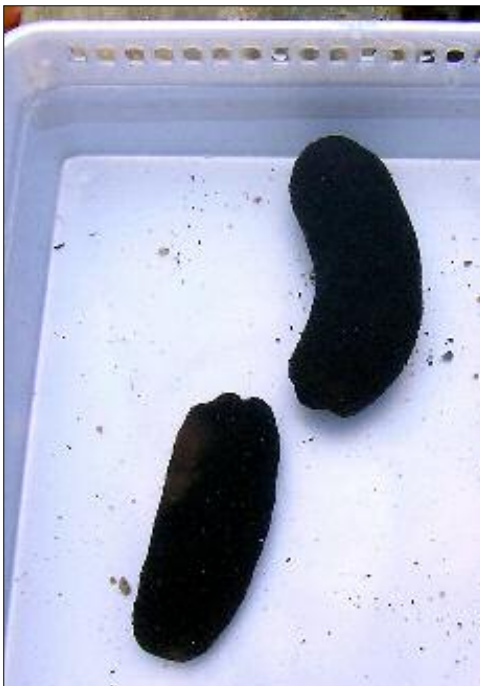


Figure 3. Regenerating individuals of *Holothuria atra*: 4 weeks after fission (left) and 9 weeks after fission (right).



Figure 4. Regenerating individuals of *H. leucospilota*. New mouth part (left) and anal aperture (right), 9 weeks after fission.

