

Asexual reproduction by induced transverse fission in the sea cucumbers *Bohadschia marmorata* and *Holothuria atra*

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Introduction

Although holothurians are known for their ability to reproduce asexually by fission, specific data on this subject is scarce. Approximately 10 species of sea cucumbers have been reported from field and laboratory observations to reproduce asexually (Emson and Wilckie 1980; Conand 1989, 2004; Uthicke 2001a,b). Some of the species that reproduce asexually in the field are: *Holothuria atra*, *H. parvula*, *H. edulis*, *H. leucospilota*, *Actinopyga mauritiana* and *Stichopus chloronotus*. Other studies present experimental induction by constriction (Reichenbach and Hollway 1995). In the present study, the sea cucumbers *Bohadschia marmorata* and *H. atra* were induced to reproduce by transverse fission by cutting them slightly anteriorly to the middle portion (45%) of the body.

Materials and methods

This study was carried out at the Albion Fisheries Research Centre in Mauritius. The sea cucumbers *B. marmorata* and *H. atra* were collected from the wild during low tide or by free diving from August to December 2004. The sea cucumbers were maintained in one-tonne tanks containing about 15 cm of sand. The tanks were aerated with an air-blower. Water in the tanks was changed every day and the sand was changed once every two weeks. The sea cucumbers were fed with seaweed powder.

Two experiments were conducted. In the first experiment, 6 *H. atra* and 4 *B. marmorata* were studied, and in the second experiment, 10 *H. atra* and 20 *B. marmorata* were examined. The total initial weights of *H. atra* were 1387.0 g in the first experiment, and 961.0 g in the second experiment. The total initial weights of *B. marmorata* used for the first and second experiments were 440.0 g and 2609.9 g, respectively. The mean weight of *H. atra* for the first and second experiments were 216.7 g and 96.1 g, and the mean weights of *B. marmorata* were 110.0 g and 130.5 g in the first and second experiments.

The sea cucumbers were cut into two after making a constriction slightly anterior (45%) to the middle portion of the body. The total weights of the anterior and posterior parts were recorded immediately after induced transverse fission. The mean

weights of the anterior parts of *H. atra* were 86.7 g and 39.5 g in the first and second experiments. The mean weights of posterior parts of *H. atra* in the first and second experiments were 120.2 g and 52.5 g. The mean weights of anterior parts of *B. marmorata* were 42.8 g and 50.5 g in the first and second experiments, respectively. The mean weights of posterior parts of *B. marmorata* in the first and second experiments were 61.7 g and 72.8 g. All anterior and posterior halves were introduced into one-tonne fiberglass tanks provided with about 15 cm of sand. The sea cucumbers were fed daily with seaweed paste/powder.

Figure 1 shows the area of constriction before cutting *H. atra* into two, slightly anteriorly (45%) to the middle portion of the body. Figure 2 shows the process of cutting *H. atra* into two halves; Figure 3 shows the two cut portions after they regenerated.

The survival and weight of the sea cucumbers were monitored up to 373 days in the first experiment, and up to 288 days in the second experiment. The mean weights of sea cucumbers were calculated from the total final weights of the sea cucumbers at the end of the experiments. The results of the growth of asexually reproduced sea cucumbers are summarised in Tables 1 and 2.

The experiments were conducted from October 2004 to October, 2005. The water temperature variation during the period of study was 24.5–28 °C. Salinity varied from 34–36 ppt and pH varied from 8.1–8.4.

Results

In the first experiment, all the posterior and anterior halves survived in both species. The mean weights for *H. atra* were 447.6 g and 280.4 g in the first and second experiments, respectively. The mean weights of *B. marmorata* were 304.0 g and 286.0 g in the first and second experiments. The growth per day was 0.62–0.64 g for *H. atra* in the first and second experiments, and growth per day for *B. marmorata* was 0.52 g–0.54 g in the first and second experiments. In the second experiment, survival rates after 288 days were 95% and 92.5% for *H. atra* and *B. marmorata*. In the second experiment, initial mortality was noted during the first two days after duplication of the sea

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Figure 1. Area of constriction before cutting *Holothuria atra*



Figure 2. Process of cutting *H. atra* into two halves



Figure 3. The two cut portions.

cucumbers. All of the four pieces that died were the anterior portions of the cut ends of the body. After two days of duplication, the body wall had its normal consistency and wounds at both the ends were healed and entirely closed.

Discussion

Asexual reproduction in sea cucumbers by fission has been studied by several researchers (Emson and Wilkie 1980; Emson and Maldenov 1987; Conand 1989, 1993, 1996; Conand and Ridder 1990; Chao et al. 1993; Reichenbach and Hollway 1995; Boyer et al. 1995; Reichenbach et al. 1996; Uthicke 1997, 1998, 2001a, b; Conand and Uthicke 2001; Howaida et al. 2004). Asexual reproduction in nature is a seasonal event mainly occurring in winter in natural populations. 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. This process of asexual reproduction by transverse fission was noted in natural populations of *H. atra* by Chao et al. (1993), Conand and Ridder (1990), Boyer et al (1995), Conand (1996) and Uthicke (1997, 1998, 2001a, b). Reichenbach and Hollway (1995) described the asexual propagation potential of several commercially important species of sea cucumbers. Asexual reproduction was observed in *H. edulis* and *Stichopus chloronotus* (Uthicke, 1997, 1998, 2001a, b). Howaida et al. (2004) conducted experiments on the asexual reproduction of *Actinophyga mauritiana* by placing rubber bands in the midbody of *A. mauritiana*. Immediately after this, the animals started to constrict slightly in the middle and showed some swelling in

Table 1. Asexual reproduction in *H. atra*

Expt. no.	No. used	Initial total wt. (g)	Initial mean wt. (g)	Mean wt. (g) anterior part	Mean wt. (g) posterior part	Final total wt. (g)	Final mean wt. (g)	Growth / day (g)	Survival (%)
1	6	1387.0	216.7	86.7	120.2	2687.8	447.6	0.62	100.0
2	10	961.0	96.1	39.5	52.5	2701.8	280.4	0.64	95.5

Table 2. Asexual reproduction in *B. marmorata*

Expt. no.	No. used	Initial total wt. (g)	Initial mean wt. (g)	Mean wt. (g) anterior part	Mean wt. (g) posterior part	Final total wt. (g)	Final mean wt. (g)	Growth / day (g)	Survival (%)
1	4	440.0	110.0	42.8	61.7	1215.8	304.0	0.52	100.0
2	20	2609.9	130.5	50.5	72.8	5291.0	286.0	0.54	92.5

the posterior section. After one hour, the constriction became slightly more distinct, giving a heart shape to the posterior half. The anterior and posterior ends slowly rotated in opposite directions resulting in a more distinct constriction. The entire process of fission lasted for an entire day. After two days, the body wall had its normal consistency and wounds at both ends were healed and nearly closed. Howaida et al. (2004) also observed a survival rate of 65% for anterior parts and 85% for posterior parts.

In the present work, all the cut pieces survived in the first experiment whereas in the second experiment, 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). So far, there are no experimental studies on the growth rates of sea cucumbers after asexual reproduction. Therefore, the present study provides some useful information on this, although more studies are needed in this important area of sea cucumber research.

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