

Spawning induction and larval rearing of the sea cucumber *Holothuria scabra* in Malaysia

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Abstract

The sea cucumber *Holothuria scabra* was induced to spawn by the methods of thermal and algal stimulation. Thermal stimulation proved to be the better method. The larvae were given a mix of microalgal diet, the concentration of which was based on the larval growth. Doliolaria larvae appeared 11 days after fertilisation, and then became pentactula 18 days after fertilisation. A survival rate of 4.2% was recorded from three successful spawning.

Keywords: *Holothuria scabra*, spawning method, survival rate, thermal induction.

Introduction

The processed beche-de-mer of *Holothuria scabra* is a source of income and food for the coastal communities of eastern Malaysia. However, severe overfishing has led to a significant decrease in the natural sea cucumber population. This continuous over-exploitation is likely to have a huge impact on the ecosystem and marine environment as a whole (Conand 2004). Research into the feasibility of restocking and stock enhancement of tropical sea cucumber is being conducted in many tropical countries (Purcell et al. 2011, 2014; Eriksson et al. 2014; Watanabe et al. 2014). There is also an urgent need for restocking and stock enhancement in Malaysia. This paper focuses on breeding and cultivation of *H. scabra*, a high-value, widely distributed species and currently the only tropical species that can be mass produced in hatcheries. This is the first report of breeding by induced spawning of *H. scabra* and larval rearing in Malaysia.

Materials and methods

Broodstock collection

Healthy *H. scabra* weighing between 450 and 750 g were collected from the wild by divers in April 2012. Broodstock collected from the nearby coastal area were carefully transported to the hatchery in tubs of fresh seawater, while broodstock collected from far away were packed in polythene bags supplied

with oxygen and fresh seawater. At the hatchery, the broodstock were placed in 10 m³ flow-through tanks filled with sandy substratum. There were about 30 to 40 animals per tank. They were fed with algae, seaweed and mud and the substratum was changed every two weeks.

Induced spawning

We used two methods to induce spawning: thermal stimulation and algal stimulation. Thermal stimulation was found to be the better method and was used thereafter.

For thermal stimulation, 30 to 40 sea cucumbers were gently washed, cleansed and prepared for spawning. The temperature of the spawning tank was raised by 3–5°C from the ambient temperature of 27–28°C to induce spawning. The males released their sperm 30 minutes after spawning induction for two hours and then, after one hour, the females released their eggs.

For algal stimulation, 100 g of dried *Spirulina* was mixed with seawater and dissolved in a one-litre spawning tank. Additional dried *Spirulina* was added if the concentration was still insufficient to induce spawning. Thirty to 40 sea cucumbers were introduced into the tank and left for an hour to spawn. Once the spawning was over, the broodstock were put back in the broodstock tanks.

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Three spawning trials were conducted using each of the spawning induction methods. After each successful spawning, the eggs were collected, washed in 75- μm sieves, and counted using a hemacytometer.

Larval rearing

The larvae were stocked in a 1,000 L larval rearing tank with a stocking density of 200 to 250 larvae per litre. The tank was filled with 1 μm UV sterilised seawater at a temperature between 26 and 30°C. Salinity was maintained between 32 and 36 ppt, and pH between 8.0 and 8.2. The larvae were checked every day for changes in shape, size and stage, as well for the presence of bacteria and predators.

The auricularia larvae were fed with microalgae, and the algal concentration in the rearing tanks was maintained at 20,000–35,000 cells mL^{-1} , depending on the growth stages. A microalgae diet consists of *Isochrysis galbana*, *Pavlova lutheri*, *Chaetoceros mueleri*, *Nitzschia acicularis* and *Navicula* sp.

Continuous aeration was provided to the tanks and the water was changed every day by flow through. A 75- μm sieve was placed inside the tank to prevent the larvae from flowing out. The bottom of the tanks was siphoned out every day to clear any sediment. The doliolaria stage began 11 days after larval stocking. The larvae were transferred into juvenile rearing tanks filled with settlement plates covered with *Spirulina* sheets. After seven days, the doliolaria transformed into pentactula and actively fed on benthic diatoms, dead algae, seagrass, seaweed powder and *Spirulina*. They were harvested once they reached an average length of 15 mm.

Results

Results from Table 1 and Figure 1 show that thermal induction was the more effective method, producing a total of 1,073,000 eggs from three spawning trials, while induced spawning via the algal induction method was successful only in one trial out of the three, producing 120,000 eggs.

Table 1. Number of eggs produced from different types of spawning method of *Holothuria scabra*.

Spawning method	Date	No. of broodstock	No. of eggs
Thermal induction	28-05-2012	30	154,000
Thermal induction	01-06-2012	30	385,000
Thermal induction	25-07-2012	30	534,000
Algal induction	28-05-2012	30	0
Algal induction	01-06-2012	30	0
Algal induction	25-07-2012	30	120,000

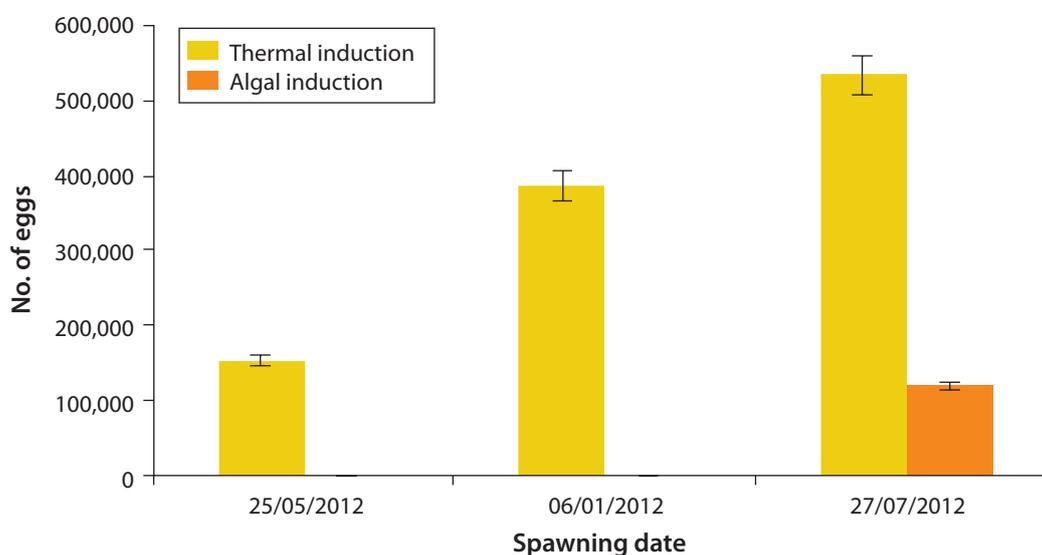


Figure 1. Number of eggs produced from different spawning method of *Holothuria scabra*.

The percentage of larval hatch was calculated on the appearance of auricularia larvae, approximately 48 hours after fertilisation. From the three spawning trials, $92\% \pm 2.9$ of auricularia larvae hatched. Auricularia is the start of the feeding stage, and during this stage the larvae were fed with $20,000 \text{ cells mL}^{-1}$ of microalgae. The feed was subsequently increased by the larval demand. Auricularia sizes range from about 425 to $450 \mu\text{m}$. Middle auricularia were bigger, with a size range between 750 and $950 \mu\text{m}$ (Fig. 2).



Figure 2. Auricularia larva of *Holothuria scabra*.

After 11 days, about $38 \pm 12.9\%$ auricularia larvae metamorphosed into the non-feeding stage, doliolaria — A very active non-feeding, fast moving larva between 450 and $650 \mu\text{m}$ (Fig. 3).



Figure 3. Doliolaria larva of *Holothuria scabra*.

Doliolaria transformed into creeping stage pentactula on the 18th day. They can be seen on the settlement plates, on the bottom and on the wall of the tanks as they search for suitable substratum to settle on. The pentactula larvae size ranges from 350 to $750 \mu\text{m}$. The survival rate of *H. scabra* larvae was $4.2\% \pm 0.5$ (Fig. 4).

Discussion

Restocking, reseeded or stock enhancement is seen as a way of rebuilding after the depletion of the wild stock of sea cucumbers. Many countries have started their own stock enhancement programme by doing research into the feasibility of tropical sea cucumber breeding (Purcell et al. 2011, 2014; Eriksson et al. 2014; Watanabe et al. 2014). Successful

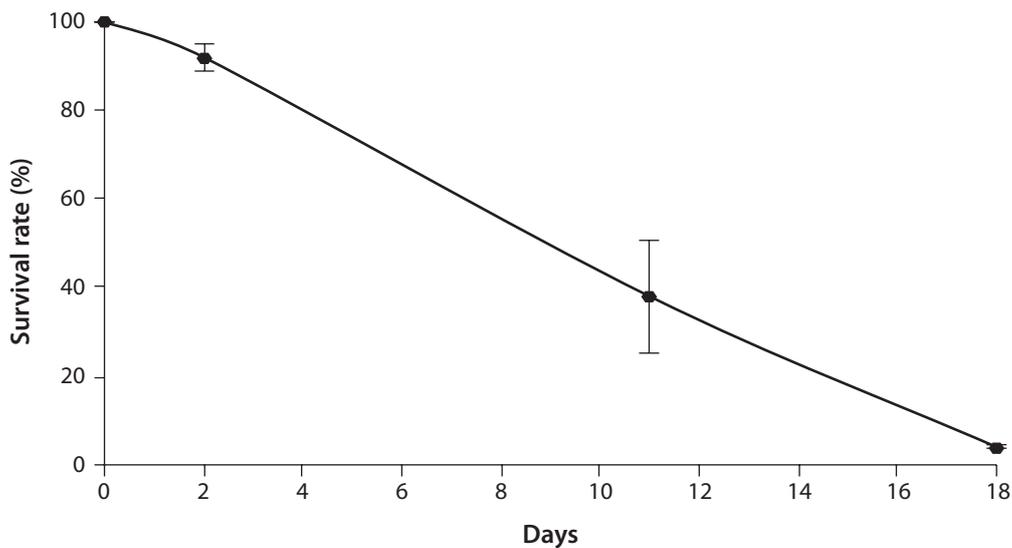


Figure 4. Survival rate of *Holothuria scabra* larvae.

breeding by induced spawning has been widely reported and most of the authors claim that thermal induction is the best method for sea cucumber spawning (Battaglione 1999; Battaglione et al. 2002; Laxminarayana 2005; Ivy and Giraspy 2006; Eeckhaut et al. 2012). In this study, two methods were compared and thermal induction gave a better result than algal stimulation. While thermal induction was effective on most sea cucumber species, it is less effective on *Holothuria fuscogilva* (Battaglione et al. 2002).

There are other methods for induced spawning, such as powerful water jets on drying individuals (James 1996; Liu et al. 2004; Wang and Yuan 2004) and blended gonad as stimulant (Battaglione 1999). *Stichopus* sp. (curry fish) was induced to spawn by open-air drying followed by flow-through seawater stimulation.

Successful spawning and larval rearing of several species of sea cucumber have been reported in various studies (Battaglione et al. 2002; Chen 2003; Laxminarayana 2005; Giraspy and Ivy 2005; Ivy and Giraspy 2006; Dabbagh et al. 2011). However, the only tropical species that can be mass-produced in hatcheries is *H. scabra* (Battaglione and Bell 1999). *H. scabra* culture started in 1988 in India for farming purposes, although the possibility for stock enhancement was suggested (James et al. 1988, 1994; James 1996). This species appears capable of spawning all year long without the influence of lunar periodicity (Ong Che and Gomez 1985; Conand 1993). They are dioecious, highly fecund and are broadcast spawners. The life cycle of cultured *H. scabra* starts with the appearance of early auricularia 48 hours after fertilisation of the eggs, followed by mid and late auricularia. Eleven days after fertilisation, the larvae morph into the non-feeding state of doliolaria. Then the first appearance of pentactula larvae is observed 18 days after fertilisation. The larval growth characteristics vary from species to species. For example, *Holothuria atra* and *Bohadschia marmorata* take 20 days to reach pentactula stage (Laxminarayana 2005) while *H. lessoni* take 19 days (Ivy and Giraspy 2006).

The survival rate of *H. scabra* from this study was 4.2% ±0.5. The poor survival rate of tropical sea cucumber species has been reported by Purcell et al. (2012). Ivy and Giraspy (2006) reported that the survival rate for *H. scabra versicolor* was 1.12% in 2004 and 4.53% in 2005. The survival rates for *B. marmorata* and *H. atra* were 12.5% and 6.4% respectively (Laxminarayana 2005). A few studies suggest that survival rate and algal concentration are related. A good survival rate of *H. lessoni* was obtained with algal concentration 4×10^4 cells mL⁻¹ (Ivy and Giraspy 2006) while *Holothuria spinifera* had the highest survival rate at algal concentration

of between 2 and 3×10^4 cells mL⁻¹ (Asha and Muthiah 2002).

Decline in the percentage of the survival rate occurs during the metamorphosis stage in our study. From 92% hatched larvae, only 38% of auricularia larvae morphed into doliolaria. The rate further declined to 4.2% when they transformed into pentactula larvae. In this experiment, the mortality rate was up to 96%, starting from when the eggs were fertilised to the formation of the pentactula larvae. Battaglione (1999) reported that the highest mortality occurred at the first feeding and settlement. This also may be due to the food demands and the induction of the larval metamorphosis. The presence of bacteria and predators in the tank also affects the mortality and survival rate. Furthermore, it may also relate to the anatomy and physiology of the larvae of the sea cucumber species.

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