

Induced spawning and larval rearing of the sea cucumbers, *Bohadschia marmorata* and *Holothuria atra* in Mauritius

A. Laxminarayana¹

Abstract

Two species of sea cucumbers, *Bohadschia marmorata* and *Holothuria atra* have been induced to spawn by thermal stimulation. The larval stages of these sea cucumbers were reared on diet of unicellular algae, artificial feed and seaweed paste. The pentactula stage was reached in 20 days. Survival rates obtained up to the pentactula stage were 12.5% and 6.4% for *B. marmorata* and *H. atra*, respectively.

Introduction

In Mauritius, sea cucumbers are regularly collected by fishers, mainly for domestic consumption. The loss of critical stocks of sea cucumber is likely to have a significant impact on the ecosystem condition and the adjacent marine environment, as a whole. Therefore, there is an urgent need for developing a technology for seed production and culture of sea cucumbers in Mauritius. Although about 1400 species have been identified worldwide, there is little information about holothurian species in Mauritius. Conand (1998) mentioned 11 edible species in the southwestern Indian Ocean region, including Mauritius. Luchman et al. (2001) carried out a study to assess the distribution and abundance of the holothurians in the lagoon at Preneuse (west coast) and in Baie du Cap (south coast) in Mauritius. The dominant species of sea cucumbers found in Mauritian waters are the chalkfish, *Bohadschia marmorata* and the lollifish, *Holothuria atra*. Among these two species, *B. marmorata* has a better commercial value and grows to a larger size (2.0 kg) than *H. atra* (1.5 kg).

Hence, the present work was undertaken in order to develop appropriate technologies for the breeding, seed production and culture of the two species of sea cucumbers, *B. marmorata* and *H. atra*. The results obtained are presented and discussed in this paper.

Materials and methods

The work was carried out at the Albion Fisheries Research Centre, Mauritius. Sea cucumber broodstock were collected from the wild at low tide, and stocked in 1-tonne tanks filled with a sandy substratum of six inches thickness to enable the sea cucumbers to bury in the sand. The water in the tanks

was changed everyday and sand was changed every fortnight. Fresh seaweed was ground into a paste in a mixer and put in water at least twice a week. The sea cucumbers thrived on the organic matter present in the mud. The algal paste settled to the bottom and was consumed by the sea cucumbers along with the mud. Fifteen to twenty adults were kept in each tank. The methods used for inducing spawning in these two species of sea cucumbers are described below.

i) *Thermal stimulation*: The temperature of the seawater was reduced by 3–5°C by the addition of ice, and the sea cucumbers were then introduced into this tank. After 5 minutes, the sea cucumbers were introduced into another tank filled with filtered seawater at normal temperature (3–5°C higher than the first tank temperature). A rise of 3 to 5°C was enough to induce spawning. The males spawned first, which induced the females to release their eggs.

ii) *Stimulation through drying and powerful jet of seawater*: This method was used with breeders that were conditioned for more than one week in the hatchery. The sea cucumbers were dried in the shade for 30 minutes. Then, the specimens were subjected to a powerful jet of seawater for 30 minutes. After 1–1.5 hours, the specimens began to move up the tank wall and began to show swaying movements. The males released the sperms first and then, 30 minutes later, the females started reacting. The anterior region of the female became bulged due to the inside pressure. The eggs were released in powerful jets intermittently.

The success achieved for *B. marmorata* and *H. atra* in inducing spawning and larval rearing are described separately.

1. Ministry of Fisheries, Albion Fisheries Research Centre, Petite Rivière, Albion, Mauritius. Email: drlaxminar@yahoo.co.uk

Bohadschia marmorata**Induced spawning**

This species was induced to spawn by both of the methods described above, although successful egg fertilization was only obtained with the thermal stimulation method. The water temperature was reduced to 25°C and after 5 minutes the sea cucumbers were transferred to a tank where the seawater was at 30°C. The males released their sperm after 10 minutes, which induced the females to spawn after one hour.

Fertilization took place in the water. After the eggs and sperm were released, the breeders were removed from the tank. The eggs were washed several times in order to remove excess sperm. Two of the females released 624,000 eggs. The eggs are spherical, white, and visible to the naked eye. After fertilization, the first polar body appeared within 20–25 minutes. The first cleavage took place after 20 minutes. Early gastrula was formed after 45 minutes. In 4 hours, the blastula — which is oval and motile — was fully formed. After 50 hours, early auricularia larvae were formed. The total number of early auricularia larvae obtained was 450,000. The hatching rate was 72.1%.

Larval rearing

The early auricularia larvae (Fig. 1) were stocked in 1-t FRP tanks, filled with 750 litres (L) of filtered sea water. Stocking density was 200 larvae L⁻¹. Larvae were fed with unicellular algae, and the algal concentration in the rearing tanks was maintained at 20,000–25,000 ml⁻¹. The quantity of phytoplankton was increased or decreased depending on the quantity of food in the stomach of the larvae. This was checked every-day before feeding.

Water was changed every day by keeping a sieve inside the tank with a mesh size of 80 µm. Sediments at the bottom of the tank were siphoned out completely every three or four days.

Seawater in the larval rearing tanks ranged from 27–30°C, pH ranged from 8.1–8.3, and salinity between 34 and 36 ppt. Continuous aeration was provided.

Seven to eight days after stocking, late auricularia (Fig. 2) were formed. The auricularia is slipper-shaped, transparent and pelagic. It has a preoral loop anteriorly, and an anal loop posteriorly. The



Figure 1. Early *B. marmorata* auricularia larva.

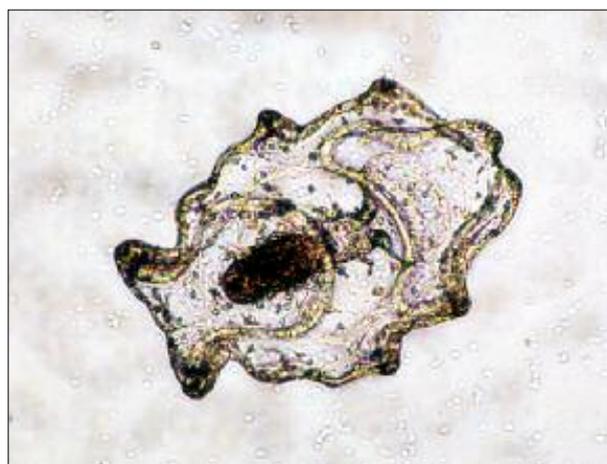


Figure 2. Late *B. marmorata* auricularia larva.



Figure 3. Late *B. marmorata* doliolaria larva.

digestive tract consists of a mouth, an elongated pharynx and a sacciform stomach. The early auricularia measures on average 480 µm. The late auricularia measures on average 420 µm. On the day 12, the auricularia larvae metamorphosed to doliolaria larvae (Fig. 3).

The doliolaria is barrel-shaped, with five bands around the body. It measures on average 370 µm. This stage is short, lasting three to four days after which the larvae transformed into a creeping stage known as pentactula.

The pentactula is tubular with five tentacles at the anterior end and a singular tube foot at the posterior end. The pentactula measures on average 560 µm and feeds actively on benthic algae and other detritus matter.

When the larvae reached the doliolaria stage, "settling plates" were put in the tank. The settling plates consisted of polythene sheets that were kept in advance in a tank filled with seawater. Seaweed extract was added, and this eventually stuck to the polythene sheets. Every day, seawater was changed and fresh seaweed extract was added. After a week, the polythene sheets were covered with a fine coat of algal extract and this served as a good settling base for the larvae.

Holothuria atra

Methods for broodstock collection, maintenance, spawning induction and larval rearing of *Holothuria atra* are similar to those used for *B. marmorata*.

Spawning induction

H. atra was induced to spawn by thermal stimulation and also by drying and using a powerful jet of seawater. Fertilized eggs were obtained by using the thermal stimulation method.

Breeders were first introduced into the seawater with a temperature of 27°C. After 10 minutes, the sea cucumbers were introduced into a tank containing seawater with a water temperature of 30°C. Within 5 minutes, the male released sperm which induced one of the females to release eggs after 30 minutes. This female spawned 800,000 eggs, which hatched into 750,000 early auricularia after 48 hours. The hatching rate was 93.8%.

Larval rearing

The early auricularia larvae were stocked in 1-t FRP tanks at the stocking density of 330 L⁻¹. The procedures used for larval rearing were similar to those followed for *B. marmorata*.

Seawater in the larval rearing tanks ranged between 26 and 28°C, and had a pH of 8.2–8.4, and a salinity of 34–35 ppt.

After 10 days of stocking, the late auricularia larvae were formed. At this stage, the shape is similar to *B. marmorata*'s. The early auricularia measured an average of 440 µm. The late auricularia measured an average of 404 µm. On the day 15 day, the auricularia larvae metamorphosed into doliolaria larvae.

The barrel-shaped doliolaria larvae measured on average 360 µm. This stage was short and lasted 4–5 days. Larvae then transformed into pentactula, a tubular shape with five tentacles at the anterior end and a tubefoot at the posterior end. The pentactula measured on average 550 µm and fed on benthic algae and other detritus.

As in the case of *B. marmorata*, "settling plates" were put in the tank. The details of induced spawning and larval rearing are summarized in Table 1.

The survival rate of larvae up to the pentactula stage was 13.2% for *B. marmorata* and 6.4% for *H. atra*.

Discussion

Successful spawning and larval rearing has been achieved only in some species of sea cucumbers. *Apostichopus japonicus* juveniles were produced in Japan more than 60 years ago (Inaba 1933) and were successfully reared (Imai et al. 1950). Subsequently, Shuxu and Gengeheo (1981) and Li (1983) reported on the breeding and culture of this species in China. James et al. (1988) produced sandfish, *Holothuria scabra* juveniles. Chen and Chan

Table 1. Details of spawning events and larval rearing of *B. marmorata* and *H. atra*.

Species	No. of broodstock used	Date of spawning	No. of eggs	No. of early auricularia larvae	No. of pentactula larvae
<i>Bohadschia marmorata</i>	8	04-02-05	624,000	450,000	59,400
<i>Holothuria atra</i>	12	26-02-05	800,000	750,000	48,000

(1990) reported on the larval development of *Actinopyga echinites* and James et al. (1993) on the spawning of *A. mauritiana*. Hamel et al. (1993) studied the gametogenesis and spawning of the sea cucumber *Psolus fabricii*. Artificial induction of oocyte maturation and development in *H. leucospilota* and *H. paradis* was reported by Mayurama (1980). Ramofafia et al. (1995) achieved the spawning and early larval rearing of *H. atra*. Breeding of *H. scabra* was reported by Pitt and Duy (2004) and Morgan (2000). Breeding of *Isostichopus fuscus* in Ecuador was reported by Mercier et al. (2004). Hamel and Mercier (2004) investigated the role of perivisceral coelomic fluid (PCF) in the spawning induction of holothurians. Hamel and Mercier (1996) induced gamete release in *Cucumaria fondosa* by manipulating temperature and light. The use of a powerful jet of water on drying sea cucumbers also triggered spawning in *H. scabra* (James 1994) and *Parastichopus japonicus* (Liu et al. 2004; Wang and Yuan 2004) and also in the present study on *B. marmorata* and *H. atra*. In the present study, fertilized eggs of both species were only obtained when the thermal stimulation method was used. Battaglione et al. (2002) were able to trigger spawning in 10% of mature *H. fucogilva* females by adding a solution of dried algae, *Schizochrytrium* sp. (Algamac). As there has so far been no published work on the induction of spawning and larval rearing of the sea cucumber *B. marmorata*, this is the first report on induced spawning and larval rearing of this species. *B. marmorata* larvae were reared on a diet of unicellular algae, *Chaetoceros calcitrans*. After settling, the late larval stages were fed on the algal extract present on the "settler plates" and in the artificial feed. The species of algae that have been fed to the larvae of sea cucumbers *A. echinites*, *P. californicus* and *S. californicus* include: *Isochrysis galbana*, *Phaeodactylum tricornerutum*, *Dunaliella salina*, *D. tertiolecta* and *Pavlova lutheri*. Battaglione et al. (1999) fed *H. scabra* larvae with the microalgae, *C. mulleri*, *C. calcitrans*, *P. salina*, and *Tetraselmis chuii*. James (2004) fed *H. scabra* larvae with cultures of *I. galbana* and mixed cultures of *Chaetoceros* sp. Morgan (2001) observed that at a concentration of 1 and 2×10^4 cells ml⁻¹ of the alga *I. galbana*, growth and development of larvae increased substantially. At higher algal concentrations, larval survival was less and growth and development were inhibited. Ramofafia et al. (1995) fed *H. atra* larvae with *Tetraselmis* sp. and artificial feed, and reared the larvae to the doliolaria stage. In the present study, pure culture of unicellular algae, algal extract and artificial feeds were used. The concentration of unicellular algae was maintained at 20,000 to 25,000 cells ml⁻¹ in the rearing tanks.

Future experiments on larval rearing of *B. marmorata* and *H. atra* are planned using a variety of microal-

gae such as *I. galbana*, *C. calcitrans* and other species of microalgae to obtain better survival rates.

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