



## BREAKTHROUGH IN INDUCED BREEDING AND REARING OF THE LARVAE AND JUVENILES OF *HOLOTHURIA (METRIATYLA) SCABRA* JAEGER AT TUTICORIN

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### ABSTRACT

*Holothuria (Metriatyla) scabra* the most valuable sea-cucumber at present from India, has been successfully induced to breed for the first time. The larvae have been reared through various stages till settlement by feeding with micro-algae. Details of various stages of larvae are given. The feeding and rearing of juveniles also are presented in this paper.

### INTRODUCTION

Export of *beche-de-mer* has earned very good foreign exchange to India and the industry was flourishing very much for sometime. Due to increasing demand for the raw materials, the sea-cucumbers were exploited indiscriminately including undersized animals resulting overexploitation and depletion of stock. It was felt very essential to take measures to preserve the holothurian resources from overexploitation, particularly the smaller size and conserve the resources for judicious exploitation. Hence Government of India imposed ban in 1982 on the export of processed holothurians less than 8 cm in size as a measure of conservation. As a result, the *beche-de-mer* industry is now facing a setback in the country. In view of this, the Central Marine Fisheries Research Institute, initiated a programme on the artificial spawning and production of young Holothurians for culture and propagation at the Institute's Molluscan Hatchery Unit, Tuticorin. The achievement in the experiment is significant which would eventually solve problem presently faced by the *beche-de-mer* industry of India.

The results of the laboratory experiments on artificial spawning and rearing of larvae, and juveniles are enumerated in the present paper.

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### MATERIALS AND METHODS

Sea-cucumbers *Holothuria scabra* form a seasonal fishery in Tuticorin. They are collected off Tuticorin in the vicinities of Kaswar and Karaichalli Islands by skin diving. The fishery starts from the late September extends till April. During this period of the year, the water clarity remains to be ideal for gathering the sea-cucumbers from the seabed at depths ranging from 6 - 10 m. Based on the availability of adult *H. scabra* from the commercial landings, experiments on the artificial spawning and production of young Holothurians were carried out.

*H. scabra* of the size ranging from 250 - 350 mm in length were collected and maintained in the laboratory as broodstock. A total of 50 *H. scabra* have been maintained under healthy conditions in the laboratory for a period of 10 months. The Holothurians were placed on a layer of mud and sand with a thickness of 10 cm in 2 x 1 x 0.5 m fibre-glass tanks. The layer of mud and sand is changed once in a week and the water in the tank is well aerated.

## OBSERVATIONS AND RESULTS

*Induced spawning*

Various methods employed to induce spawning in *H. scabra* have been attempted. Neural extract of the starfish *Pentagaster regulus* were obtained, centrifuged and the extracts was injected into holothurians at different concentrations. This did not induce the Holothurians to spawn. Chemical stimuli such as injecting isotonic potassium chloride into the body of holothurian and treatment of holothurians in water medium containing various levels of concentrations of Cystine did not provide any results. Holothurians placed in different salinity media, above and below ambient levels (35‰) for a period of 3-6 hours did not induce them to spawn.

Successful induction of spawning was achieved by subjecting the holothurians to thermal shock. Twenty numbers of holothurians measuring 300-350 mm and weighing 500 - 600 gms were conditioned for 2 days in water temperature of 25° C which is 2-5° C below the ambient temperature. Three fibre-glass tanks of 30 x 50 x 75 cm (100 l) were taken and filled with filtered seawater at temperature levels of 27° C (normal water temperature), 32° C and 37° C. In each tank, 5 numbers of holothurians were placed. The animals at water temperature of 32° C and 37° C exhibited vigorous movements than the ones in 27° C. In about 90 minutes, two males at 32° C started spawning and subsequently, one male at 37° C spawned. The spawning males were transferred to the normal seawater temperature conditions forthwith. These animals continued to spawn for 15 to 20 minutes. During spawning, the males fully extended the pharyngeal bulb with tentacles and waved back and forth releasing a thin string of spermatozoa from the gonophore. Further, the animals curved and twisted their body while spawning. The spermatozoa were very active and they get dispersed in the water evenly within minutes. On completion of spawning, the sperm suspension in the water was poured to a tank which contained ten numbers of holothurians at normal temperature conditions. Within 15 minutes of time, a single female spawned. The behaviour of the spawning female was similar to that of male, but the eggs were

released in spurts which get dispersed in the water. The eggs were visible to naked eye and initially they remained on the surface and gradually sank to the bottom. All the animals in the tank were removed and the sex cells were held in the tank for one hour, to effect the fertilization process. Total eggs spawned by the single female was estimated to be one million.

*Early development*

The eggs measured 180 to 200  $\mu$  and the average diameter was 192  $\mu$ . The sequential stages of development of the fertilized eggs have been observed in the following pattern for the first three hours.

12.00 hrs	- Fertilization
12.15	- First cleavage
12.20	- Four celled stage (Radial cleavage)
12.25	- Eight celled stage
12.30	- Sixteen celled stage
12.35	- Thirty two celled stage
12.40	- Early blastula
13.00	- Blastula (partly developed)
15.00	- Blastula fully developed (Pl. I A)

The egg of *H. scabra* has a jelly coating. After fertilization, the cleavage starts and the embryo further develops inside the embryo sac (jelly coating). The blastula stage occurs within an hour after fertilization. It soon develops cilia and begin to rotate inside the sac. The embryo hatches out after 26 hours of fertilization. The larvae assumes typical gastrula stage after 27 hours. The body becomes slightly elongated and moderately curved at the oral end of the larvae. At the end of 48 hours, the larvae assumed Dipleurula stage. The larvae floats and exhibits slight movements with the formation of a single band of cilia running along the sides of the larval body and loops over the pre-oral and aboral aspects of the larvae.

*Auricularia stage (Pl. I B)*

Dipleurula stage larvae transforms to early Auricularian stage just after 50 hours. The

total number of larvae at early Auricularian stage have been estimated to 0.9 million. The larvae become a transparent and more elongated. The ciliary band assumes more sinuous curves along the body of the larvae which increases the motility of the larvae. The interior of the larvae shows the appearance of a well defined digestive track comprising of foregut, midgut and a narrow hindgut, leading to the larval anus. The larvae start feeding from this stage onwards. The early auricularian stage larvae measures on an average 563  $\mu$ . The auricularia grow and on the 13th day, measure on an average 1.1 mm. At this stage, the auricularians metamorphose to Doliolarian larvae or the 'pupa' stage.

#### ***Doliolaria* stage (Pl. I C)**

The larvae reach this stage on the 14th day. The size of the doliolarian larvae is much reduced, almost half the length of Auricularia, and assume the barrel-shape. The sinuous ciliary band re-organised into five rings around the body similar to that of the muscular strand of the ascidian *Doliolum*. Rapid changes occur inside the body and all the adult features of the holothurian gradually set in. The larvae measure at this stage 460-620  $\mu$ . This stage is a short phase extends to 2-3 days and subsequently transform to a swimming and creeping stage known as Pentactula.

#### ***Pentactula* stage (Pl. I D)**

The young sea-cucumbers with five primary tentacles and one or two podia are termed as Pentactula. The adult characters particularly tube-feet developed all over the body. The pentactula creep over the sides and bottom of the tanks. On the 22nd day, the colour of the pentactula become dark showing greenish-grey tinge. They actively move on the bottom and feed on the benthic algae and other detritus matter. The pentactula measured 600-700  $\mu$  and on the 28th day, they become the typical sea-cucumbers, measuring 1.5 to 2 mm.

#### ***Larval rearing***

The auricularians are reared in one tonne fibre-glass tanks with filtered seawater under

proper aeration. The larval density was maintained at 1 larva per 2 ml.

Initially a mixture of live micro-algal feed such as *Isochrysis galbana* (Haptophyceae), *Tetraselmis gracillis* and *Chlorella salina* (Chlorophyceae) were provided. Of these, the larvae preferred the flagellate *I. galbana*. Hence from the 4th day onward till it reaches the Doliolarian stage, pure culture of this flagellate has been provided as feed.

The larvae were fed with *I. galbana*, harvested during the growing phase in the mass culture system. Initially the larvae were provided with *I. galbana* at the rate of 10,000 cells/larva/day. The feeding rate gradually increased to 25,000 to 30,000 cells as the larvae approaches the pentactular stage.

The filtered seawater in the larval rearing tanks are completely changed once in alternate days on receiving the larvae in filter-heads. On penultimate days, the water is partially changed through filter-heads. The water changes were always effected just before feeding.

#### ***Rearing of young holothurians***

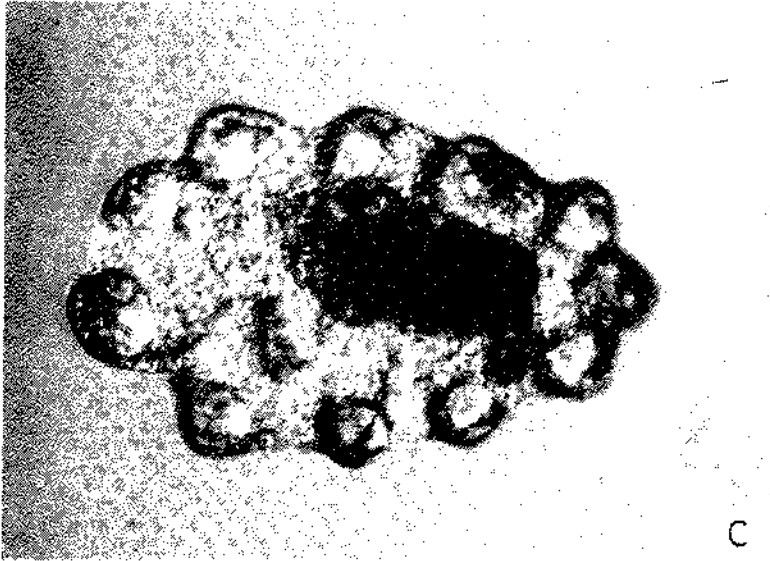
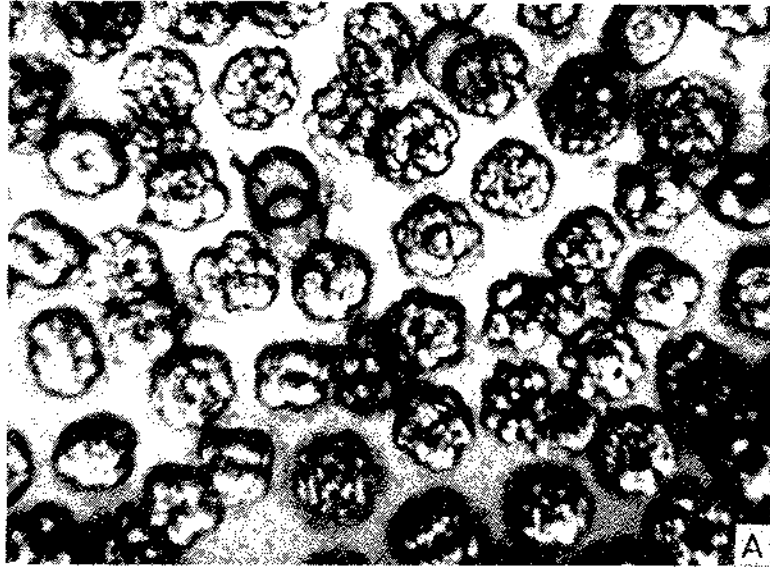
Young holothurians are reared in tanks on a layer of oven-dried silt and fine sand particles, collected from the intertidal area of Tuticorin Bay. Dried and powdered leafy alga *Ulva lactula* was dusted on the water which gradually settled at the bottom to form the food of the young holothurians. The bottom materials are changed and the operation is repeated once in 2 days.

#### ***Brood survival and the growth of young holothurians***

A total of 32,559 young holothurians was produced during the rearing process. This worked out to be 3.6% of the initial larval strength. The young holothurians reared in the laboratory registered a growth of 10.28 mm (in total length), initially during end of first month and subsequently 7 mm per month.

#### **DISCUSSION**

Mortensen (1937) made observations on the spawning and early larval development of



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PLATE I A. Fully developed blastula. B. Auricularia. C. Doliolaria and D. Pentactula.

*H. scabra* along the Egyptian Coast of the Red Sea. However, pioneering works have been done by the Chinese and Japanese in breeding and culture of holothurians, particularly on *Stichopus japonicus*. Recently, the Chinese have

commercialised the hatchery production of sea-cucumbers and their farming. The present work on the artificial spawning and production of young *H. scabra* is the first attempt in India.

#### REFERENCES

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