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Spawning and larval rearing of sea cucumber *Holothuria* (*Theelothuria*) *spinifera* Theel

P.S. Asha[1] and P. Muthiah[1]

Introduction

In India, the beche-de-mer industry mainly depends on *Holothuria scabra*, commonly called sandfish, a highly valued and widely distributed species. In addition to this, another species, *H. spinifera*, commonly known as brown sandfish, is also fished in large quantities and widely processed along the Gulf of Mannar and the Palk Bay, on the south-east coast of India. The animal is brown on the upper surface and lighter on the lower surface, with sharp projections all over the body (Fig. 1a). Being a highly burrowing species, it is found on clean sand in slightly deeper waters (James 2001). This species, locally called *Cheena attai* (or *Raja attai*), was once rated high in the market and was in good demand in China. At present, the market value is moderate, the freshly caught specimens are priced at Rs. 10–15/piece and the processed ones (Fig.1b) fetch Rs. 500–1000/kg depending on the size.

H. spinifera is fished throughout the year, usually by trawlers that form the major part of the sea cucumber fishery. It is also caught as a by-catch of *thallumadi*, a local fishing gear, and by skin diving during peak seasons. James et al. (1997) reported an estimated landing of 460 tonnes by a trawl net, modified to collect chanks locally known as *chanku madi*, during 1994–95 along the Rameswaram coast of the Palk Bay area. Sea cucumber caught in trawlers command a lesser price than those collected by skin diving, due to quality difference. Moreover, *H. spinifera* is very sensitive in nature and even a slight disturbance leads the animal to eviscerate, usually the gut along with the right respiratory tree and sometimes the gonad also. Hence, the specimens collected through skin diving were used as broodstock. Considering their commercial value, attempts were initiated for the hatchery production of seed. The hatchery technology for *H. scabra* has been developed by James et al. (1988). This paper presents the results of the

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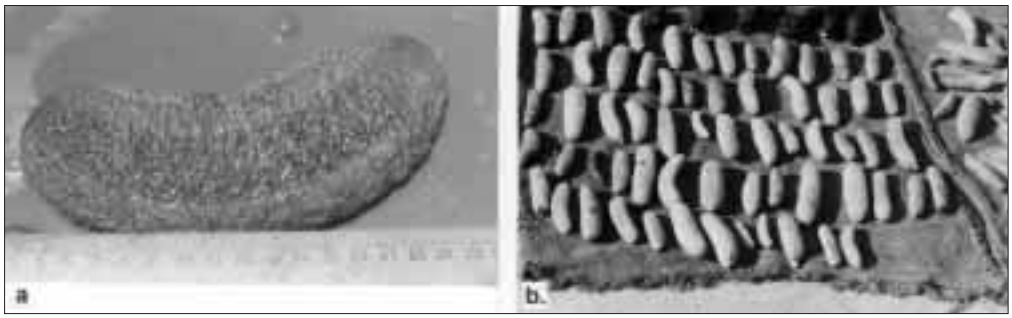

Figure 1. a. *Holothuria spinifera*, b. Processed product.

spawning and the subsequent larval rearing of *H. spinifera* in the laboratory conditions.

Material and methods

Broodstock

Eight *H. spinifera* (average length and weight 245 mm and 275 g) were collected from the wild, and maintained in the hatchery in a 1-tonne FRP tank containing six inches of coral sand. The water in the broodstock tank was changed daily and the sand every week. Animals were given an artificial feed made of four parts of rice bran, two of soya bean meal, and one of seaweed powder, at the rate of 5 g/day.

Spawning and larval rearing

Spawning was spontaneous, without any stimulation. After fertilisation, the eggs were washed to remove the excess sperm and the numbers estimated. The fertilised eggs were maintained at a rate of 0.5 larvae/ml in a 100-l tank with seawater filtered through a 40-µm sieve. The water in the larval rearing tank was changed completely and the larvae were taken out to find out the survival rate by counting the average numbers in three 1-ml samples on alternate days. Water was then changed at a rate of 50% per day, keeping the sieve (80 µm) inside the tank. This was followed up to 10 days and thereafter, a flow-through system was maintained. During the larval rearing period, the water temperature ranged between 29 and 31°C, salinity was 34.8–36.0 ppt, pH was 8.1–8.2, and the dissolved oxygen varied from 4.1–5.2 ml/l.

Feeding of the larvae

Feeding the auricularia larvae started from the second day onwards. A mixture of three microalgae, *Isochrysis galbana, Chaetoceros calcitrans* and *Nanochlorosis salina* (1:1:1), at the rate of 20,000 cells/ml, was given as initial feed, and slowly raised to 40,000 cells/ml in the later stages. After 10 days, once the larvae had reached the non-feeding doliolaria stage, the effectiveness of different settlement cues — like *Sargassum* powder, Algamac, *Spirulina* powder (0.05 g/l/day) and diatom, and dead algae (2 ml/l) — was tested using 2-l plastic bowls containing 5 doliolaria in filtered seawater.

Results

Spawning

On 2 March 2001, one of the males, after exhibiting the typical swaying movement, liberated sperm, as white threads, from the gonopore situated anteriorly. After the other animals were introduced to this sperm suspension, one of the females liberated eggs in a sudden spurt.

The eggs were spherical and visible to the naked eye, and their mean size was 143.59 ± 22.83 µm (Fig. 2a). The embryonic development was similar to that of *H. scabra*. Times after fertilisation for the different development stages are given in Table 1.

Table 1. Time after fertilisation for the different development stages of *Holothuria spinifera*.

Development stage	Time after fertilisation
Blastula	3 hours
Gastrula	24 hours
Auricularia (early)	2 days
Auricularia (late)	10 days
Doliolaria	10–12 days
Pentactula	13–15 days

The number of fertilised eggs was estimated as 60,000. The first polar body was released after 40 minutes and cleavage started in the next 20 minutes. Blastula, with a single blastopore, were observed after three hours. Motile gastrula (Fig. 2b) with ciliated and oval shaped bodies were developed in 24 hours with an average size of 265.40 ± 14.86 µm.

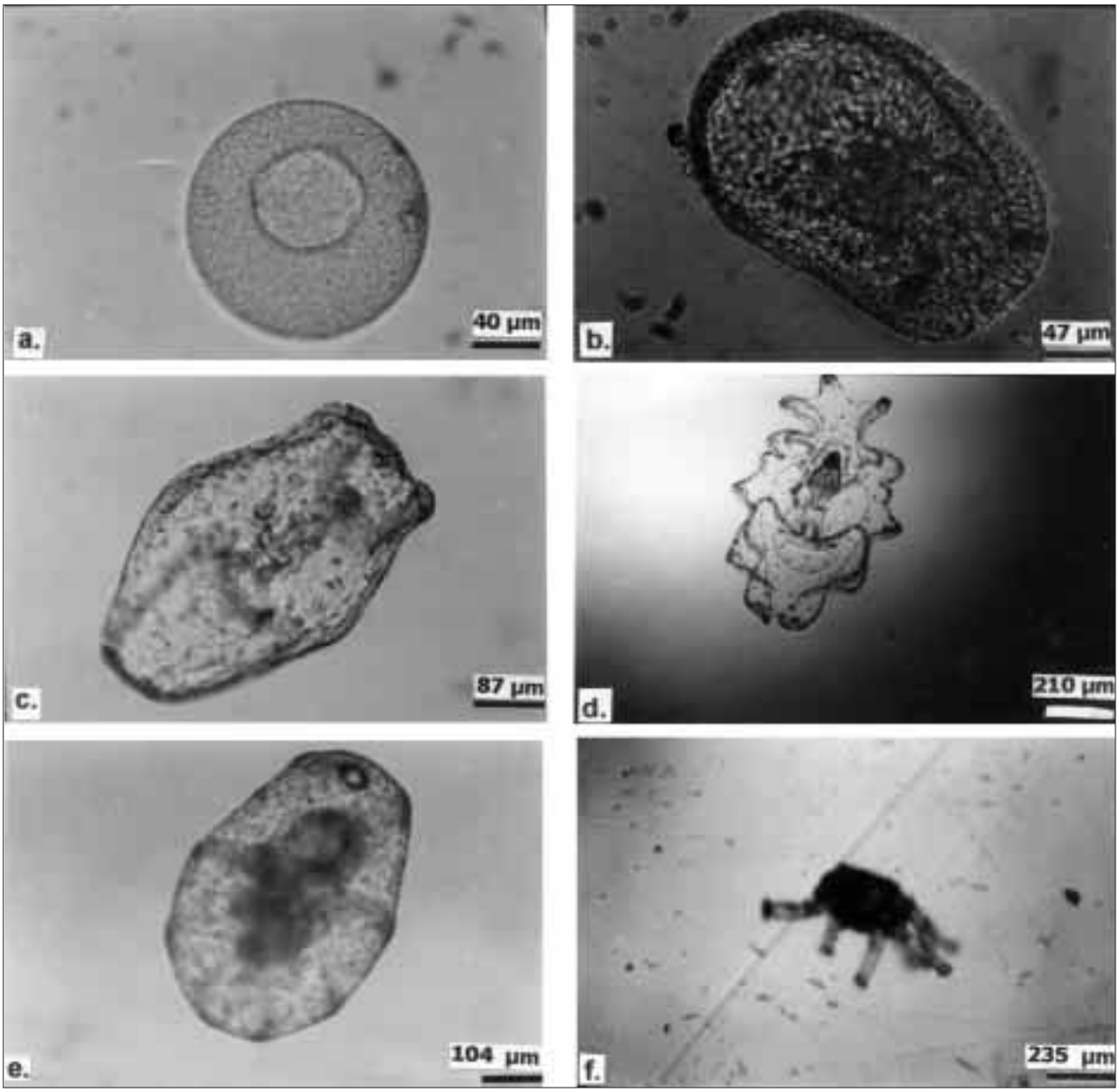

Figure 2. Larval stages of *Holothuria spinifera*:
a. Egg, b. Gastrula, c. Early auricularia, d. Late auricularia, e. Doliolaria, f. Pentactula.

The early auricularia were developed after 48 hours. They measured on average 498.43 ± 31.53 µm and were slipper shaped, transparent, and pelagic in habit, similar to those of *H. scabra*, except for the posterior loop, which was slightly broader than the anterior one (Fig. 2c). On the ninth day, lateral projections in the auricularia became more prominent, and lipid spheres appeared at the tips of the projections (Fig. 2d), which indicated the larval competency and its readiness to metamorphose in the congenial environmental condition (Battaglene 1999). At this stage it measured a mean size of 809.43 ± 123.29 µm, which is significantly different from the early auricularia ($t = 5.56$, $df = 11$, $P > 0.01$).

On the 10th day, a few auricularia were metamorphosed to the non-feeding, highly motile barrel-shaped doliolaria stage (Fig. 2e). The mean size was 467.57 ± 56.94 µm. A few doliolaria were metamorphosed to the creeping stage, called pentactula, on the 13th day. The composition of the larvae was observed to be auricularia 91%, doliolaria 8% and pentactula 1%. The pentactula were tubular with five tentacles at the anterior end and two podia at the posterior end (Fig. 2f). The colour was greenish brown and size was much smaller than that of *H. scabra*. The mean size at this stage was 330.16 ± 50.11 µm. By the 20th day, tube feet and tentacles became more distinct and spicules could be seen projecting from the skin of three specimens.

Survival to settlement

During the larval cycle, growth rate was progressive during 12 days, at a rate of 49.4 µm/day. The larval survival rate from the 4th to the 6th day remained at 76.9% and then decreased to 34.6% on the 11th day. The maximum mortality was noticed on the 9th day and also during the metamorphosis (Fig. 3). The larval settlement and further growth were affected greatly by the grazing of predators, which could not be controlled properly, forcing the experiment to be stopped.

The trial experiment conducted to test the effectiveness of different settlement cues indicated that the larval settlement can be better induced by Algamac and periphytic diatoms. Forty per cent survival was noticed in the larvae fed with Algamac, and 20% in those fed with periphytic diatoms. There was no settlement among the larvae fed with *Spirulina*, dead algae, or *Sargassum* powder (Fig. 4).

Discussion

The spawning of *H. spinifera* occurring without induction indicated a possible natural spawning period in March. Spawning in captivity has also been observed for *H. atra* (ICLARM Coastal Aquaculture Centre 1993). The sperm released might have induced the females to spawn. Battaglene (1999) has suggested that blended gonad from mature broodstock may be an effective spawning stimulant.

Fertilisation and early embryonic development up to late auricularia were similar to those of other holothuroids (Preston 1993). The time taken to reach the doliolaria stage is the same for *H. spinifera* and *H. scabra* (James et al. 1988) — 10 days. It is less than the 15 days taken by *Actinopyga echinites* (Chen and Chian 1990) and the 20 days taken by *H. atra* (Ramofafia et al. 1995).

Survival to settlement was 5%. Battaglene (1999) observed 1–35% mortality from survival to settlement and high mortality at first feeding and settlement. High mortality of 65.4% was noted on the ninth day, which was mainly due to ciliates.

As the larval and copepod sizes were similar, sieving out copepods was not possible. James et al. (1988) have observed similar hindrance of copepods in the larval rearing of *H. scabra*. Further studies have to be initiated to eradicate copepods in the larval rearing system.

In the settlement cue experiment, better settlement was observed among the larvae fed with Algamac (40%) and periphytic diatoms (20%). Similarly, Battaglene (1999) observed Algamac as a potential settlement cue and food for settled pentactulae of *H. scabra*. In future experiments, the feeding rates of suitable settlement cues will be given importance to enable large-scale seed production for ranching to replenish natural stocks of *H. spinifera*.

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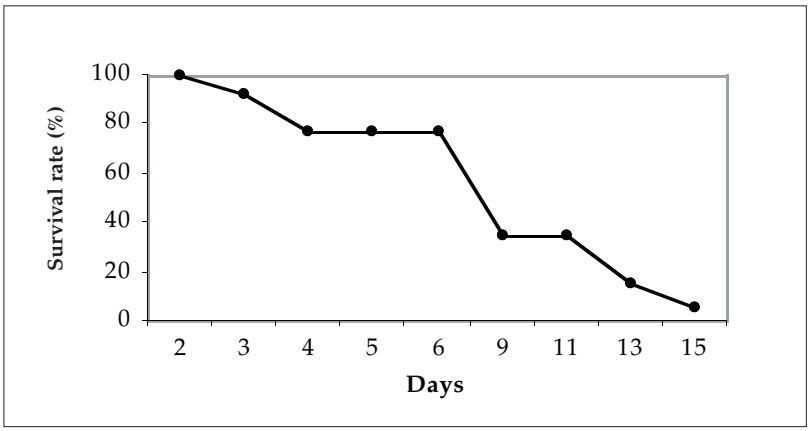

Figure 3. Survival of *H. spinifera* larvae.

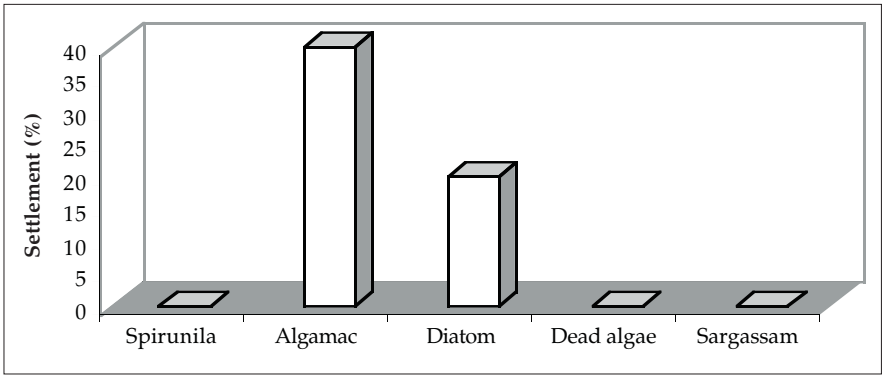

Figure 4. Settlement of *H. spinifera* larvae in different cues.

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Reactions of the larvae of the sea cucumber *Apostichopus japonicus* to sharp desalination of surface water: a laboratory study

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Introduction

Larval development of the sea cucumber *Apostichopus (=Stichopus) japonicus* Selenka to settlement occurs in the water column and lasts for 13–20 days depending on seawater temperature and salinity. Early larvae — blastula and gastrula — occur at the surface water, while later stages — dipleurula, auricularia and doliolaria — move into deeper water. Summer monsoon rains and floods of rivers flowing into Vostok Bay (Peter the Great Bay, the Sea of Japan) considerably reduce the salinity of the surface seawater, thereby affecting larval survival of *A. japonicus* (Kashenko 1992, 1997, 1998) and other invertebrates. Distribution, vertical migrations of marine bottom invertebrate larvae, and their behavioural responses to changing salinity in a stratified water column have been extensively studied. However, the conclusions made by the investigators are not unambiguous (Harder 1968; Mileikovsky 1974, 1981; Seliger et al. 1982; Mann 1986, 1988; Scheltema 1986; Stancyk and Feller 1986; Sulkin and Van Heukelem 1986; Tremblay and Sinclair 1990; Jonsson et al. 1991; Pedrotti and Fenaux 1992; Young 1995; Vazquez and Young 1996; Metaxas and Young 1998; Garrison 1999; Welch et al. 1999).

Reactions of the larvae of the sea cucumber *A. japonicus*, their behaviour, and vertical distribution caused by reduced salinity of surface seawater have not been studied. The aim of this research is a study of this problem under laboratory conditions.

Materials and methods

Experiments were carried out at the Vostok Marine Biological Station of the Institute of Marine Biology FEB RAS (Vostok Bay, the Sea of Japan) during July and August 1992.

Sea cucumbers in the pre-spawning state were collected on 15 July at 6 m of depth, at a temperature of 19.6°C and salinity of 32.7‰. Spawning proceeded on the same day, in separate vessels for females and males. Fertilisation, maintenance to settlement, and all experiments were carried out at a temperature of 22–23°C and salinity of 32‰ (Kashenko 1992). Larvae were reared in three larval cultures. Filtered and UV-sterilised seawater in aquaria was changed every 1–2 days. It was saturated with oxygen and stirred using a microcompressor that supplied air via glass capillaries to the water surface, inflicting no injury to the larvae.

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