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Growth of the hatchery-produced juveniles of commercial sea cucumber *Holothuria* (Theelothuria) *spinifera* Theel

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Abstract

The present study was conducted to develop the hatchery technology for seed production of the widely exploited commercial sea cucumber *Holothuria spinifera* Theel 1886, to facilitate an effective stock enhancement programme. Broodstock collected by skin divers were used for spawning trials. The embryonic and larval stages were similar to other aspidochirotes. The larval survival rate was 43.5% on day 9, which decreased to 18.3% nearing metamorphosis on day 13, the growth rate was 48 μm day $^{-1}$ and the settlement rate was 3.5%. Algamac® used to induce settlement also served as the food source for the early settled juveniles, followed by *Sargassum* spp. extract ($<$40 μm) for the first month. *Sargassum* spp. powder along with fine sand (1:1) was given to juveniles $>$20 mm. Addition of *Spirulina* spp. along with *Sargassum* spp. powder and fine sand (0.5:1:2) enhanced the growth rate of the juveniles. In the hatchery, the juveniles attained an average size of 1, 30 and 48 on 20, 80 and 120 days respectively.

Keywords: *Holothuria spinifera*, survival rate, Algamac®, *Sargassum* spp., *Spirulina* spp.

Introduction

Sea cucumbers belonging to the families Holothuridae and Stichopodidae form an important part of multispecies fishery, existing for over 1000 years along the Indo-Pacific region, and the processed product is a valuable source of income. Increasing demand and inadequate management of sea cucumber stocks in many countries have resulted in severe overexploitation of commercially important species. Bruckner, Johnson and Field (2003) reported that the holothurians are susceptible to overexploitation because of late maturity, density-dependent reproduction and low rate of recruitment. The release of hatchery-produced juvenile sea cucumbers into the natural habitat is being advocated in many countries as one of the means of rebuilding the wild stock, a process termed as restoration, restocking or reseeding (Battaglene 1999).

The breeding and cultivation of holothurians dates back to 1950, with the first recorded production of juveniles of *Stichopus japonicus* (Imai, Inaba, Sato & Hatanaka 1950). Later, studies on reproduction, early development and metamorphosis of *Cucumaria elongata* (Chia & Buchanan 1969), *C. pseudocurata* (Rutherford 1973), *Aslia lefevrai* (Costelloe 1988), *Psolus chitonoides* and *P. bullatum* (McEuen & Chia 1991) and *C. frondsa* (Hamel & Mercier 1996) were extensively undertaken. Meanwhile in 1985, techniques were developed for producing juveniles of *S. japonicus* in China (Shuxu & Gongchao 1981; Chen 2003) and seed of *Actinopyga echinites* in Taiwan (Chen & Chian 1990).

Following the Chinese method, James, Rajapandian, Baskar and Gopinathan (1988) successfully produced the seed of *Holothuria scabra* for the first time. This technique was later applied for the seed production of sea cucumbers in Australia, Indonesia, Maldives and Solomon Islands (Battaglene 1999). The seeds of *Isostichopus fluscus* and *H. fuscogliva* have been successfully produced in Ecuador and in Kirbati Island (Hamel, Hidalgo & Mercier 2003; Friedman & Tekanene 2005).

In India, the beche de-mer industry mainly depends on two species, *H. scabra* and *H. spinifera* (Chellaram, Samuel & Patterson Edward 2003), and both the species are being processed for export. *Holothuria spinifera*, locally called cheena attai or raja attai, was once rated very high in the market and was in good demand in China. At present, the market value is moderate; the processed ones (60 counts kg^{-1}) fetch US $37.5. Besides Gulf of Mannar and Palk Bay of Indian coast, this species is reported to occur in the Red sea, Persian Gulf, Sri Lanka, North Australia, Philippines and China (James 1995). James and Badrudeen (1997) reported an estimated annual landing of 460 tonnes of fresh *H. spinifera* along the south-east coast of India. Increase in demand and inadequate fishery management has resulted in overexploitation of commercial sea cucumber species in India also; hence, the Ministry of Environment, Government of India, has banned both fishery and export of sea cucumbers since 2001, which has had a severe impact on the livelihood of poor coastal communities subsisting on its fishery and processing. Owing to the role played by *H. spinifera* in the commercial fishery and also due to its indiscriminate exploitation, knowledge of early development, larval and juvenile rearing is essential for the seed production so as to carry out, the captive or wild stock enhancement programme of this species effectively. Hence, an attempt was made to produce juveniles of *H. spinifera* in the laboratory. The present work is the first successful attempt of juvenile production of this species under laboratory conditions.

Materials and methods

The broodstock of *H. spinifera* collected by skin divers were maintained in 1000 L FRP tanks having 150 mm thickness of fine coral beach sand at the bottom. The water was exchanged daily and sand every fortnight and the brooders were fed with *Sargassum* spp. powder at the rate of 5% of their body weight.

For induced spawning, among the 30 numbers of broodstock, 10 numbers were subjected to thermal stimulation wherein the holothurians were exposed to 3–4 °C above the ambient temperature for an hour. The high temperature was maintained by a heating element controlled by a thermostat. Similarly, inducement by addition of feed constituted by rice bran, Soya powder and *Sargassum* spp. powder (4:1:2) at a rate of 50 g 500 L^{-1} was also attempted to 10 numbers and the remaining 10 numbers were maintained as control.

For holothurian seed production 1 50 000 fertilized eggs, 5700 pentactulae and 2000 juveniles were reared in a lot following the hatchery technique (James *et al.* 1988; Battaglene 1999; Chen 2003). While rearing, periodically representative samples were measured for assessing the growth of larvae and juveniles. The microalgae cultured by the serial dilution technique (Gopinathan 1982) were provided as larval feed as indicated in Asha and Muthiah (2006) and at a concentration specified by Asha (2004).

The doliolaria larvae on 10th day were maintained in a flowthrough system, in which an equal volume of seawater released was let in and were provided with Algamac® (Bio-Marine, Hawthrone, CA, USA) at a rate of 0.5 g 500 L^{-1} for inducing them to settle as pentactulae. The Algamac® was provided for 13 days until the juveniles became visible to the naked eye.

For the newly settled juveniles, *Sargassum* spp. extracts (< 40 μm) were given (10 L 500 L^{-1}) for 4 weeks. When the juveniles attained an average length of 20 mm, a mixture of *Sargassum* spp. powder and fine sand in a proportion of (1:2) was given at 1% of the body weight of juveniles (initially < 80; < 200 μm as the days progressed). From day 30, *Spirulina* spp. (15 mg $juvenile^{-1}$) was provided along with the above feed. Every week, juveniles were taken out using a fine brush and the length and number of live juveniles were noted for assessing the growth and survival rate. Once in a week, the juveniles were transferred to another tank as suggested by Battaglene (1999). The juveniles thus produced from fertilized eggs were reared up to 120 days.

Results and discussion

Spawning and development

The induced spawning on 2 January 2003 occurred in the afternoon, after addition of feed. There was no spawning by thermal stimulation. The female spawned first, followed by three males.

The spawning in *H. spinifera* was induced by addition of feed. Addition of Algamac® at 50 g 500 L^{-1} induced spawning in *H. fuscogilva* (Battaglene, Seymour & Ramofafia 2002). Thermal stress, despite having been effective for *S. japonicus* (Ishida 1979), *H. scabra* (James *et al.* 1988) and *H. scabra* and *A. mauritiana* (Battaglene *et al.* 2002), did not induce *H. spinifera* to spawn. Similarly, Battaglene *et al.* (2002) observed that thermal stress was not effective but addition of dried algae alone yielded induced spawning in *H. fuscogilva*.

In the successful spawning experiment, the females spawned first, followed by the males. Because the released gonadal materials instigate the mass spawning, the blended gonad from mature broodstock may be an effective spawning stimulant (Battaglene 1999).

The spawning behaviour of *H. spinifera* and the occurrence of spawning in the afternoon were similar to the observations on other aspidochirotids (McEuen 1988). The spawning in the late afternoon and evening may be related to a decrease in light intensity as opined by McEuen (1988). Fertilization and early embryonic development and other larval stages of *H. spinifera* are similar to those of other aspidochirote holothurians (Ishida 1979; Maruyama 1980; Chen & Chian 1990).

Larval and juvenile rearing

The chronological developmental stages and the details of the various larval stages up to pentactulae with the time of occurrence and the mean size were as described by Asha and Muthiah (2002).

The doliolaria stage was attained on day 10 in *H. spinifera*, which was similar in *H. scabra* (James *et al.* 1988; Battaglene, Seymour & Ramofafia 1999). It took 15 days at 25–28 °C to reach doliolaria in *A. echinites* (Chen & Chian 1990). During the rearing period of the larvae and juveniles, the seawater temperature ranged from 29 to 31 °C, salinity from 34.8 to 36 ppt, pH from 8.1 to 8.2 and dissolved oxygen from 4.1 to 5.2 mL^{-1}. The attainment of doliolaria on day 10 for *H. spinifera* may be due to the larval rearing at a higher temperature of 29–31 °C. Asha and Muthiah (2005) indicated, a water temperature of 28–32 °C as the optimum for rearing the larvae of *H. spinifera*.

During the spawning trial, 43.8% of auricularia were hatched out from 1 500 000 fertilized eggs. On day 3, the survival rate was 79.9%, which decreased to 63.9% and 43.5% on days 5 and 9 respectively. On day 11, the survival was 39.6%. Nearing metamorphosis, on day 13, the survival rate was 18.3%.

The larval growth rate was 48 μm day^{-1} and the growth equation indicated a high level of significance ($r = 0.9695$; $P < 0.01$) (Fig. 1).

The metamorphosis and settlement are the critical stages in the development of sea cucumber larvae (Preston 1993). In this study, Algamac® was used as the settlement cue. Battaglene (1999) also observed high settlement using Algamac® in *H. scabra*. In *H. spinifera*, Asha and Muthiah (2002) reported more settlement (40%) of Algamac®-fed larvae, followed by 20% for periphytic diatom-fed larvae. Ito and Kitamura (1997) accelerated the metamorphosis in *S. japonicus* by providing periphytic diatom at > 20 000 cells cm^{-2}. Studies have to be undertaken to evolve suitable cues and their concentration so as to obtain more juveniles.

On day 15, 5700 pentactulae of 0.32 mm length, forming 9.4% of early auricularia, were produced. On day 23, 2000 juveniles of 0.95 mm, forming 3.5% of early auricularia, were reared. On day 31, the juveniles had a mean length of 2.3 mm (Fig. 2a) and the survival rate was 7.5%. On day 48, the length of the juveniles ranged from 6 to 14 mm, with a mean of 10.1 mm (Fig. 2b) and the survival rate was 3.5% (Fig. 3). Adding *Spirulina* spp. along with *Sargassum* spp., a growth rate of 1.5 mm day^{-1} was obtained. On day 85, 56 juveniles produced in the size range of 31–60 mm with a mean of 41 mm (Fig. 2c) were produced. The observed growth rate was 0.64 mm day^{-1} with a 2.8% survival. In the fourth month, the juveniles attained a mean size and weight of 48 mm and 2.84 g respectively (Fig. 2d).

The growth of the juveniles can be described by the following equation:

$$Y = 0.0001x^{2.8513}$$

An $r = 0.993$ indicated a high level ($P < 0.01$) of significance (Fig. 4).

During the juvenile rearing, maximum mortality (92.5%) occurred on day 31 at 2.3 mm length. Battaglene *et al.* (1999) observed that juveniles of < 5 mm holothurians are more susceptible. The juveniles attained an average size of 41 mm (2.3 gm) on day 85, with an average growth rate of 0.64 mm day^{-1}.

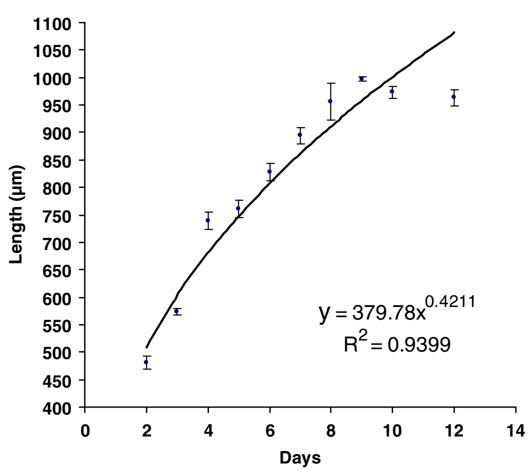

Figure 1 Growth rate of the larvae of *Holothuria spinifera*.

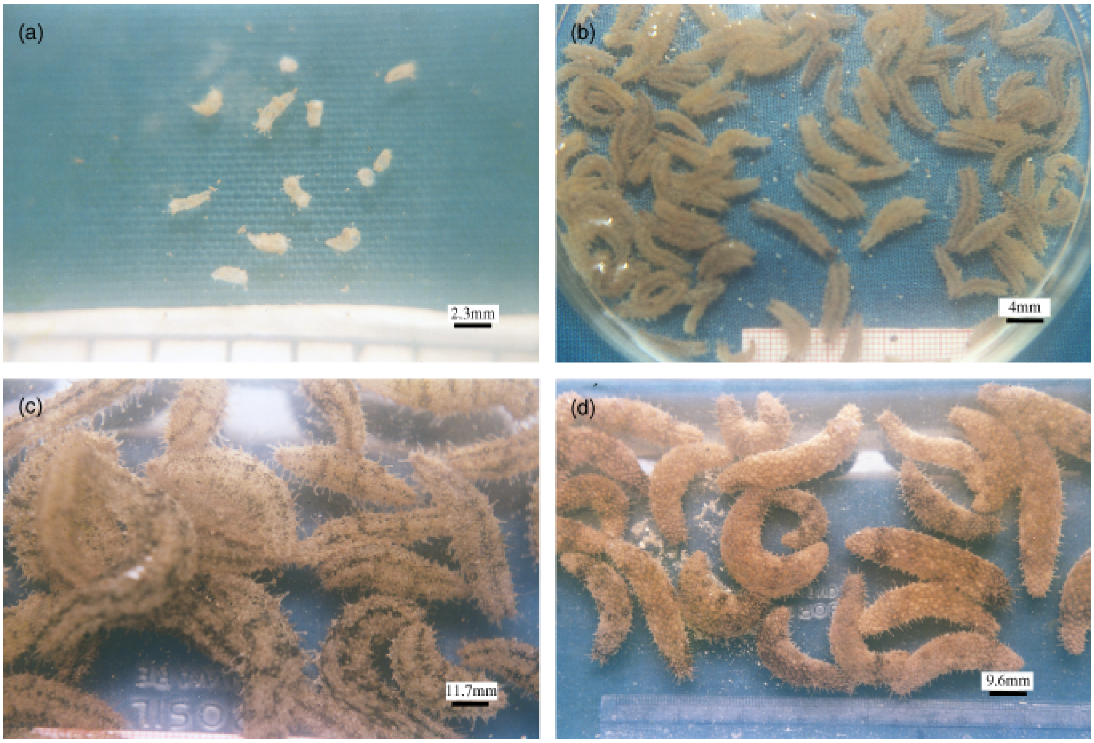

Figure 2 Juveniles of *Holothuria spinifera* (a) 31 days old, (b) 48 days old, (c) 85 days old, (d) 120 days old.

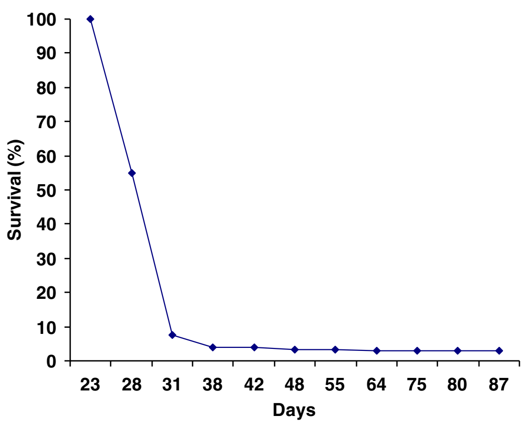

Figure 3 Survival rate of the juveniles of *Holothuria spinifera.*

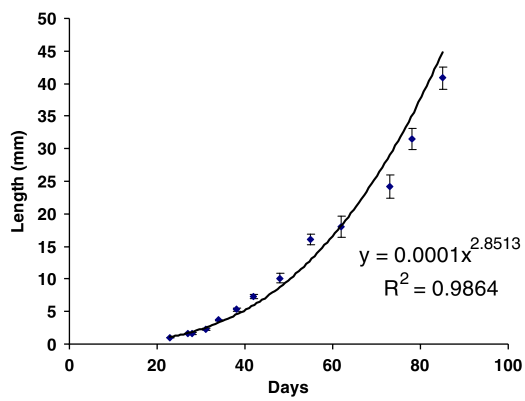

Figure 4 Growth rate of the juveniles of *Holothuria spinifera.*

Battaglene *et al.* (1999) recorded a 0.5 mm growth rate for *H. scabra*. The growth rate of *H. spinifera* was 10–20 mm greater in 3 months and was similar to *I. japonicus* (Ito & Kitamura 1998). The average size of the juveniles sea ranched reported was 20–30 mm by James, Gandhi, Palaniswamy and Rodrigo (1994); < 60 mm and 20 g by Battaglene (1999); and 35 mm by Hamel *et al.* (2003). The juveniles of *H. spinifera* (mean length and weight 48 mm and 2.84 g) produced in this study, after 120 days of growth in the hatchery, were sea ranched into the sea grass beds of Van Island area in Gulf of Mannar at 10 m depth for the first time. In this study, addition of *Spirulina* spp. increased the growth rate to 1.4 mm day^{-1} and it indicated that the probable role of additional protein source in the growth of juveniles and powdered algae is not a major source of food for juveniles (Battaglene *et al.* 1999). The feeding habit of the juvenile sea cucumber in the wild is unknown (Battaglene *et al.* 1999). Moreover, Yingst (1978) opined that many deposit-feeding holothurians do not appear to assimilate algae and have little cellulose activity in their gut. Hence, formulation of a suitable balanced diet for juveniles of sea cucumbers has to be given more thrust in future studies.

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