Development of large-scale hatchery production techniques for the commercially important sea cucumber *Holothuria scabra* var. *versicolor* (Conand, 1986) in Queensland, Australia

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Abstract

Overexploitation is an issue affecting sea cucumber fisheries worldwide. Improved management plans for existing sea cucumber populations and/or aquaculture of depleted stocks are considered indispensable to maintaining wild populations and sustainable fisheries. In this context, we evaluated the potential of the commercially important sea cucumber *Holothuria scabra versicolor* (golden sandfish) for mass culture in a hatchery. Adult *H. scabra versicolor* were collected from Hervey Bay (Queensland, Australia) waters by scuba diving and were induced to spawn by increasing the water temperature. More than 46 million eggs were produced from 18 females during 2004 and 2005. Larvae from 9 million eggs were reared to juvenile stage, with more than 300,000 juveniles produced during the 2004 and 2005 hatchery culture period. Juveniles reaching 3 to 5 cm in length three months after settlement are suitable for release in the wild. The present study shows that this species can be hatchery-reared on a large scale to restock depleted populations for sustainable harvesting. Data collected during the two-year trial period indicate that the survival rate increased considerably during the second year, following modifications made to culture techniques. Results obtained are quite promising and considering the market potential, industry value and technical feasibility, this species seems most suitable for stock enhancement.

Introduction

Increasing demand for beche-de-mer along with steady price increases have led to worldwide intensification of sea cucumber harvesting (Conand 2004). The sea cucumber *Holothuria scabra versicolor* (golden sandfish) is one of the most highly sought after species in Asia. *H. scabra* and *H. scabra versicolor* are distributed throughout the tropical Indian and Western Pacific Oceans and their occurrence was noted from Madagascar to the Solomon Islands and New Caledonia (Conand 1998a). Although *H. scabra* and *H. scabra versicolor* are both found over a large geographical range, they often inhabit dissimilar microhabitats, with *H. scabra versicolor* often found in deeper waters than *H. scabra* (Conand 1990). Despite the diverse disparity in its ecological distribution and biological characteristics, *H. scabra versicolor* is considered to be a variety of *H. scabra*, because of a lack of recognizable dissimilarity in spicule structure and internal anatomy (Conand 1998b). However, the speculation that *H. scabra versicolor* is a subspecies or new species (Conand 1990; Massin 1999) has yet to be looked at in more detail. Recent studies on allozyme and 16S mtDNA sequence analyses of *H. scabra* and *H. scabra versicolor* indicates that these two sea cucumbers are distinct but young biological and phylogenetic species (Uthicke et al. 2005).

H. scabra versicolor is a deposit-feeding sea cucumber (Fig. 1) commonly found burrowed in inner rubble reef flats and coastal lagoons, feeding on bottom deposits, rich in nutrients. There are three colour morphs associated with this sea cucumber: black, moderate black spots, and speckled (Conand 1990). Because of the high price and growing demand for sea cucumbers in Asian markets, golden sandfish is fished intensively like other commercial species. High-quality, golden sandfish beche-de-mer fetch more than USD 130 per kg on the export market. Although the processing method for golden sandfish is the same as that for sandfish, the processed product is golden in colour, very different from the grey and wrinkled appearance of sandfish beche-de-mer (Fig. 2).

The worldwide supply of high quality beche-de-mer will not be sufficient to meet the Asian market demand, unless a viable sea cucumber aquaculture develops to partially replace the steady decrease in wild stocks. Aquaculture studies on tropical sea cucumbers have been largely focused on the widespread commercial sea cucumber *H. scabra*. Few studies concern *H. scabra versiclor* (Conand 1990, 1993; Hamel et al. 2001), and little information is available on its biology, in particular, its early life stages. So far, to our knowledge, no attempt has ever been made to captively breed this

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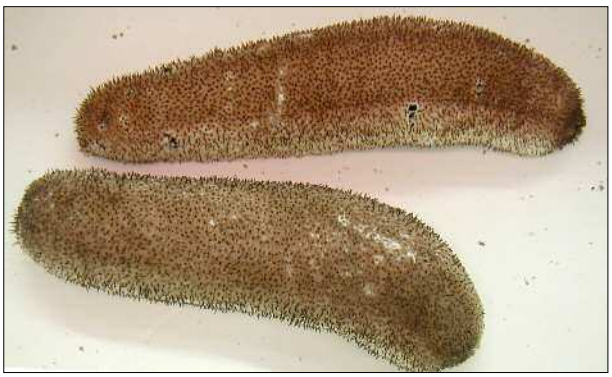

Figure 1. Golden sandfish *Holothuria scabra* var. *versicolor.*

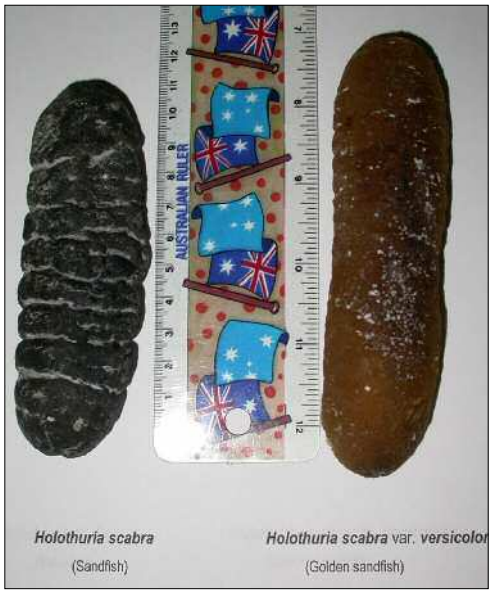

Figure 2. Processed *H. scabra* and *H. scabra versicolor.*

species, other than the preliminary trials carried out at Bluefin Seafoods sea cucumber hatchery, which produced 33,500 juveniles during a 2004 spawning (Giraspy and Ivy 2005).

Bluefin Seafoods Pty. Ltd., Hervey Bay, Queensland has received an innovation grant from the Federal Government of Australia to perfect the hatchery technology for mass production of sea cucumbers for restocking programmes. Under this programme, Bluefin Sea cucumber hatchery has released millions of hatchery produced *H. scabra* juveniles in the designated aquaculture areas of Hervey Bay during the past few years to accelerate the recovery of depleted populations and allow sustainable harvests. With the ongoing research on other species of sea cucumbers, the hatchery is now capable of mass-producing *H. scabra versicolor*. Based on preliminary results obtained during 2004 spawnings, culture techniques have been refined and hatchery technology has been developed for this species. Our results demonstrate that culturing golden sandfish is quite feasible and that it could be used to contribute to the restoration of depleted natural populations and allow, in due course, a sustainable fishery.

Materials and methods

Broodstock collection

Golden sandfish, *H. scabra versicolor,* were collected from Hervey Bay, Queensland, Australia, by divers, between October and December 2004 and during the same months in 2005. They were immediately placed into small 44-L portable bins filled with fresh seawater. A maximum of three animals were placed in each bin equipped with a continuous aeration system. Upon arrival in the port, the broodstock were transported by road to the hatchery facility (Bluefin Seafoods sea cucumber hatchery, Hervey Bay, Queensland). They were then placed in 10,000-L flow-through tanks for a period of nearly 30 minutes before induced spawning was attempted.

Induced spawning

Several spawning trials were carried out using different methods and combination of methods, such as thermal variations, a powerful jet of water on drying animals, addition of Algamac and grinded male gonads, to find out the ideal inducement method for spawning. Thermal stimulation was found to be the most successful and was used thereafter. For each spawning, 10 to 15 animals were gently cleaned and washed to remove sediments and other small organisms attached to their body, and placed in a 1000-L spawning tank. Seawater temperature in the tank was then raised by 3–5° C to induce spawning.

Once the spawning was over the animals were put back in the broodstock holding tanks with flow-through seawater. Enough sand mixed with seagrass powder was added daily to these holding tanks to keep a layer of approximately 1-cm thickness on the bottom.

An egg count was made after each successful spawning from a 0.5-mL sample of water taken from the spawning tank, using a plankton counting chamber under a stereo-microscope. The egg size measurements were also taken using the microscope with an ocular micrometer. Eggs were

then collected from the spawning tanks using an 80-µm sieve and washed for 10 minutes in 1-µm-filtered UV sterilized seawater to remove excess sperm and dirt.

Larval rearing

The larvae were cultured in 1000-L fiberglass larval rearing tanks with the temperature maintained between 26 and 27° C. During the larval rearing period the salinity ranged between 37.5 and 38 ppt, while pH remained at 8.2. The larval quality (presence of unsatisfactory shape, size and stages of maturity) and the mean larval size were checked regularly by examining samples of 40 larvae under a microscope, using an eye-piece graticule.

Larval diet consisted of *Rhodomonas salina*, *Chaetoceros calcitrans*, *C. mulleri*, *Tetraselmis chui*, *Isochrysis galbana* and *Pavlova lutheri* in different combinations at different stages. The microalgal feed density was gradually increased from 15,000 cells mL^{-1} on day 3 to 35,000 cells mL^{-1} on day 14. The higher microalgal feed density (35,000 cells mL^{-1}) was maintained thereafter until larvae metamorphosed to the doliolaria stage. The larvae were fed two times a day and the food cell density was maintained at appropriate level at all times.

Larvae were collected with sieves every two days and washed for 10 minutes before being transferred to new tanks with filtered and temperature-controlled seawater. On day 17, when they reached the non-feeding doliolaria stage, the larvae were transferred to tanks with different settlement cues such as seagrass extract, seaweed extract, Algamac 2000, Algamac Protein Plus, dead algae, benthic diatoms (*Nitzchia* sp. and *Navicula* sp.) and spirulina, and the flow-through system was maintained. The corrugated settlement plates were covered with settlement cues to facilitate pentacula attachment at metamorphosis. The settled juveniles were initially fed with Algamac 2000, Algamac Protein Plus, seagrass extract, seagrass powder, seaweed extract and seaweed powder. Once they attained an average length of 10-mm, they were fed with fine sand mixed with the above components. The growth rate was monitored for each food type.

Results

The sea cucumbers responded well to thermal inducement and showed pre-spawning behaviours such as twisting and crowding in corners of spawning tanks (Fig. 3). More than 75% of our spawning trials were successful with males, while females shed their eggs in less than 35% of the attempts. Males responded to the heat stress inducement first, raising their anterior end and swaying while releasing sperm. The swaying movement was less vigorous than that of the common sandfish *H. scabra.* After 30 to 90 minutes, females responded by lifting their anterior end and remaining erect for a few minutes before expelling mature oocytes in powerful intermittent jets (Fig. 4). Males stayed upright and continuously spawned for more than an hour in most occasions, while females spent less than 15 minutes in the erect position before releasing their eggs, and went back down afterwards.

The total number of eggs produced from seven females during six spawning events of 2004 was 14.23 million, among which only 3 million were used for larval rearing. During the 2005 spawning season, eleven females produced, in nine successful spawning events, 32.76 million fertilised eggs, among which only 6 million were used for larval rearing trials.

The developmental kinetics of *H. scabra versicolor* larvae at 26–27° C is given in Table 1. The mature

Figure 3. **Spawning behaviour of *H. scabra versicolor.***

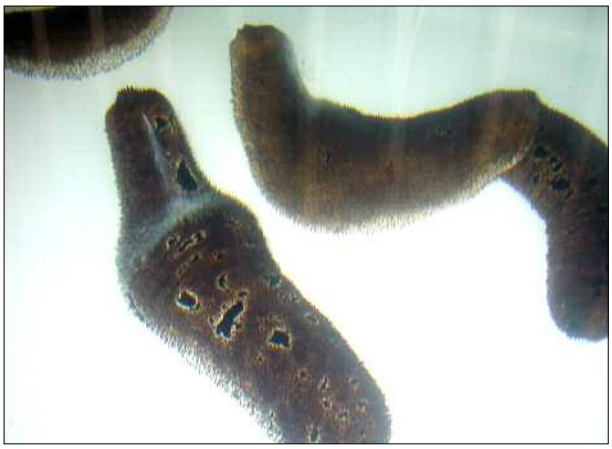

Figure 4 . **Spawning male and female *H. scabra versicolor* in the spawning tank.**

eggs of *H. scabra versicolor* were spherical and visible to the naked eye with a mean size of 205.36 ± 17.54 µm (n = 40). The auricularia larvae constituted the first feeding stage and they began to appear nearly 48 hours after fertilisation. The larvae are transparent and they feed well during their pelagic phase. The newly hatched early auricularia larvae were 409.48 ± 11.5 µm in length. Auricularia larvae developed rapidly, reaching the middle auricularia stage on day 8 and the late auricularia stage around day 14. The middle auricularia measured 954.72 ± 12.23 µm in length.

During this progressive growth the larvae accumulated hyaline spheres in their body. After 13 to 15 days they reached the late auricularia stage with a maximum size of 1.25 to 1.31 mm. The late auricularia is transformed to the non-feeding doliolaria stage on the day 17, with a mean size of 853.82 ± 7.74. The doliolaria metamorphosed to creeping stage pentacula on the day 19, and the pentacula larvae possessed five well-developed primary tentacle and a single ventroposterior podium. The pentacula develop tentacles and tube feet and form the juvenile with more distinct spicules. The survival and development of larvae up to the pentacula stage is shown in Figure 5.

The culture success based on the survival rate of fertilized eggs across the different larval stages increased markedly between 2004 and 2005 (Table 2). The larval development, settlement and juvenile growth were asynchronous, as different stages and sizes of larvae and juveniles could be seen at the same time in a batch. The overall survival at the juvenile stage was 1.12% in 2004 and 4.53% in 2005. The larval settlement experiments showed that the juveniles attached to the settlement plates or to the tank base and fed on the biological film that had developed.

The best larval settlement was obtained with a mixture of *Nitzschia* sp. and *Navicula* sp., followed by the single use *Navicula* sp., *Nitzschia* sp., Algamac 2000 and Algamac Protein Plus (Fig. 6). The first settled juveniles were clearly visible on the settlement substrate after 25 days of culture and measured 1–1.5 mm in length. Wide variation in growth was

Table 1. **The developmental kinetic based on the observations of four spawnings of *H. scabra versicolor* during 2005.**[a]

Time for fertilization	Stage	Remark
0	Fertilized egg	Size: 205.36 ± 17.54 µm
40 min	1st cleavage	2 cells
2 h 10 min	2nd cleavage	4 cells
3 h 40 min	3rd cleavage	8 cells
9 h	Blastula	Rotary motion
2 d	Late gastrula	Gastrula – auricularia transition
3 d 12 h	Early auricularia	Pre and post oral lobes formation
8 d	Mid auricularia	Lateral processes extension
14 d	Late auricularia	Distinct hyaline spheres
17 d	Doliolaria	Five ciliary rings
19 d	Pentacula	Disappearance of ciliary bands and benthic life
22 d	Early juvenile	Feeding benthic diatom and detritus.

a. Larvae were considered to have reached a developmental stage when more than 50% of the larvae had accomplished the specified stage.

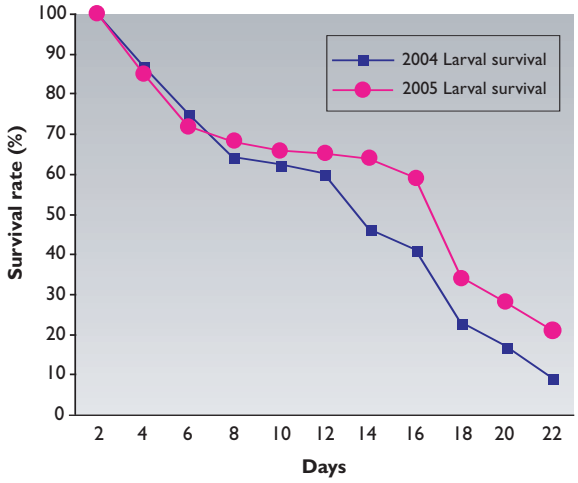

Figure 5. **Survival rate of *H. scabra versicolor* larvae.**

Table 2. **Details of 2004 and 2005 spawning trials and respective larval survival obtained**

Year	No. of spawning	Eggs (10^6)	Hatch (%)	Middle auricularia (%)	Late auricularia (%)	Doliolaria (%)	Days to settle
2004	6	14.23	87	64	46	32	21
2005	9	32.76	93	68	64	46	19

noticed among the juveniles in all batches. After six weeks, more than 45% of the juveniles reach 15 mm in length. The juveniles reached 20–25 mm within eight weeks. But, after metamorphosis, the juveniles took three months to reach an overall average length of 30 mm under optimum stocking density and good feeding conditions.

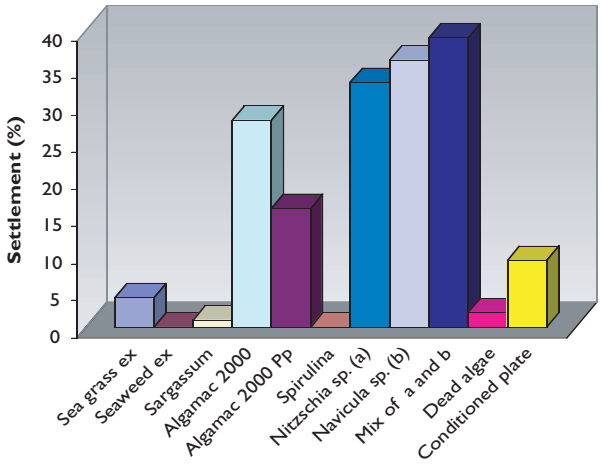

Figure 6. **Settlement of *H. scabra versicolor* larvae in different settlement regimes.**

Discussion

The high demand for beche-de-mer on the Asian market and the systematic overexploitation of wild populations support the call for sea cucumber farming. Aquaculture is a potential alternative source for the market and can also sustain wild harvest fisheries. Over the past decade, efforts to develop hatchery techniques for the culture of commercially important sea cucumber species have increased significantly (James et al. 1994; James 1996a; Ramofafia et al. 1995; Ito 1995; Asha and Muthiah 2002; Lovatelli et al. 2004; Giraspy and Ivy 2005). Several hatchery and grow-out projects have also been undertaken by International agencies with the aim of restocking commercially valuable sea cucumber species.

Among the several commercially important sea cucumbers, spawning and larval rearing in captivity has been successfully achieved only in a few species. In Japan, *Apostichopus japonicus* juvenile production was started nearly 70 years ago (Inaba 1937) and the juveniles were reared in captive conditions (Imai et al. 1950). Later, this species was successfully cultured in China (Shuxu and Gengeheo 1981; Li 1987). Among the tropical holothurians, *H. scabra* is a high-value species and considered as one of the best aquaculture candidates (Battaglene 1999, 2000; Battaglene and Bell 1999). This species has been successfully mass-produced in India (James et al. 1988; James 1996b), Madagascar (Jangoux et al. 2001), Vietnam (Pitt and Duy 2004), and Australia (Giraspy and Ivy 2005). Currently the WorldFish Center project is developing optimal releasing strategies for hatchery-produced *H. scabra* juveniles in New Caledonia (Purcell et al. 2002). But hatchery-raised juveniles of *H. scabra versicolor* have been produced here for the first time and no previous studies exist to compare with.

Thermal stress is a well-known practice used to stimulate spawning in sea cucumbers (James et al 1988; Morgan 2000; Battaglene et al. 1999, 2002; Giraspy and Ivy 2005). The stress on sea cucumbers associated with collection and transportation, powerful water jets on drying individuals (James et al. 1994, 1996) and the addition of dried algae (*Schizochytrium* sp.) (Battaglene et al. 2002) also induced spawning. In all our spawning trials, males spawned first, releasing sperm for more than 30 minutes. Females reacted later with the release of eggs that lasted for less than a minute in most cases. This concurs with other observations showing that, generally, male sea cucumbers spawn first and are easier to induce to spawn (Battaglene et al. 2002), and that females are stimulated by the presence of sperm in the water column (James et al 1994a). A rare observation of *H. scabra versicolor* spawning in the wild was recorded by Desurmont (2005) in New Caledonian waters, three days before full moon and just before high tide.

The larval cycle of the golden sandfish is similar to most aspidochirote holothurians with early, mid and late auricularia, and subsequent metamorphosis to the non-feeding doliolaria stage before settlement. However, the length of the larval cycle and other larval characteristics differ from other species, even from *H. scabra*. The larvae of *H. scabra versicolor* took 17 days to reach the non-feeding doliolaria stage; *H. scabra* (James et al. 1988), *H. spinifera* (Asha and Muthiah 2002) and *Actinophyga echinites* (Chen and Chian 1990) all take less than 15 days to reach this doliolaria stage, but *H. atra* takes 20 days.

In the present trials, *H. scabra versicolor* larvae were fed with *Rhodomonas salina, Chaetoceros calcitrans, C. mulleri, Tetraselmis chui, Isochrysis galbana* and *Pavlova lutheri* at different proportions at different developmental stages. In a previous investigation Battaglene et al. (1999) fed *H. scabra* larvae with microalgal species such as *Rhodomonas salina, Chaetoceros muelleri, C. calcitrans, P. salinai and Tetraselmis chuii.* While James (2004) used mixed cultures of *Chaetoceros* sp. and *Isochrysis galbana* in his experiments on *H. scabra.*

A stocking level of 0.75 larvae mL^{-1} is found to be suitable for *H. scabra versicolor*. James (1996) suggested stocking levels of 0.5–1 egg mL^{-1} in 800-L tanks with light to moderate aeration. Battaglene and Bell (1999) indicated the suitable larval density as 1 mL^{-1}, which is slightly higher than that used in the present investigation.

In the present study, the larval growth and survival rate was very good at an algal concentration of 4 x 10^4 cells mL^{-1}. The optimal concentration of algae for larval ontogenesis of *S. japonicus*, *H. scabra*, *H. atra* and *H. spinifera* was between 2 and 3 x 10^4 cells mL^{-1} (James et al. 1994; Ramofafia et al. 1995; Asha and Muthiah 2002). Archer (1996) in his experiments on *S. mollis* found that the continued presence of high algal concentrations (above 6 x 10^3 cells mL^{-1}) in the larval culture reduced the ingestion rate of the algae. The optimal growth and good survival rate of *H. scabra versicolor* larvae at comparatively higher algal concentration may be because of their large stomach size when compared with *H. scabra*.

The higher mortality rates were noticed during the larval metamorphosis and settlement stages. Battaglene (1999) in his experiments with *H. scabra* noticed up to 35% mortality from survival to settlement and highest mortality occurred at first feeding and settlement. Settlement cues in sea cucumber culture play two important roles: as a biological signal for the induction of larval metamorphosis, and as a suitable food for settled juveniles. In the present investigation a higher settlement rate was observed in mixed periphytic diatom followed by Algamac Protein Plus.

Algamac is a potential settlement cue and food for settled pentaculae of *H. scabra* (Battaglene 1999). Asha and Muthiah (2002) later observed that Algamac and periphytic diatoms acted as good settlement cues in their experiment with *H. spinifera*. The present results with *H. scabra versicolor* show that the hatchery culture techniques improved significantly over time and also in subsequent batches (1.12% during 2004 and 4.53% during 2005). This improved survival and settlement rate of juveniles during 2005 spawnings were made possible by improved larval rearing protocol, high standards of algal culture, and changes made to post-settlement conditions in the hatchery.

The growth rate of *H. scabra versicolor* juveniles observed in this study is higher than that of *H. scabra*. Battaglene et al. (1999) observed wide variations in growth in their experiments with early-stage juveniles of *H. scabra*. Growth variations among cultured sea cucumbers and also in wild caught juveniles are very common (Ito 1995; James 1996). But there are no previous studies on golden sandfish culture growth to compare with our juvenile growth results. However, consistency in survival and growth rates of juveniles within several batches of cultures indicates that the present growth rate is good for the hatchery production.

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