Artificial breeding and larval rearing of three tropical sea cucumber species – *Holothuria scabra*, *Pseudocolochirus violaceus* and *Colochirus quadrangularis* – in Sri Lanka

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Abstract

Overharvesting of sea cucumber species can now be considered a worldwide phenomenon, and artificial breeding and aquaculture practices are possible alternative sources of sea cucumbers for coastal fishing communities. Induced spawning of *Holothuria scabra*, *Pseudocolochirus violaceus* and *Colochirus quadrangularis* was achieved for the first time in Sri Lanka in late 2011. Wild broodstocks of these species were collected and induced through the application of several methods. Thermal stimulation (ambient temperature ± 3–5°C) was found to be the most successful method for spawning initiation of *H. scabra* whilst addition of microalgae was more effective for *P. violaceus*. Spawning of *C. quadrangularis* was initiated through transportation stress. Larval development stages of these three species were very similar, but juvenile growth was much higher in *H. scabra* than in the other two species. *H. scabra* juveniles with an average weight of 11 ± 4 g were transferred to a lagoon pen and they attained an average weight of 207 ± 56 g within a period of four months. The observed average growth rate was 1.7 g day^{-1} and there was 89% survival after four months. Juveniles of *P. violaceus* and *C. quadrangularis*, however, took more than two months to attain an average length of 5–8 mm.

Key words: *Holothuria scabra*, *Pseudocolochirus violaceus*, *Colochirus quadrangularis*, induced spawning, larval rearing.

Introduction

Sea cucumbers (class Holothuroidea) are a group of marine invertebrates that provide an important source of livelihood for many artisanal fishers throughout the world, particularly in developing countries in tropical and subtropical regions (Choo 2008; Friedman et al. 2010). Due to the increasing demand for raw materials, most sea cucumber species, including undersized animals, have been exploited indiscriminately, resulting in overexploitation and depletion of stocks in many parts of the world (Lovatelli and Conand 2004; Conand 2005; Bruckner 2006). To protect their sea cucumber stocks, many nations have implemented various management actions and have developed alternative methods for producing beche-de-mer. Artificial breeding and aquaculture practices have been introduced as an alternative method for producing beche-de-mer to satisfy the increasing market demand, as well as to enhance wild populations through restocking and sea ranching (Ivy and Giraspy 2006; Agudo 2006).

The sea cucumber fishery was introduced to Sri Lanka by the Chinese and for centuries beche-de-mer appears to have been one of the major commodities taken to China (Hornell 1917). As in many other coastal fisheries off Sri Lanka, the sea cucumber fishery is primarily artisanal but provides significant contribution to the livelihoods of coastal fishing communities off the north, east and northwest coasts. There are around 21 commercial sea cucumber species in Sri Lanka and most of these stocks are reported to be at critical level (Dissanayake and Stefansson 2010). Therefore, developing technologies for seed production and sea cucumber culture activities to reduce the fishing pressure on wild populations is timely.

It has been well documented that spawning of tropical and temperate sea cucumbers is usually induced

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through the application of short-term environmental stresses. Temperature change, light intensity, photoperiod, salinity, tidal flux, food availability and change in food type are believed to be the environmental factors involved for controlling gametogenesis and spawning of holothurians (Smiley et al. 1991). Thermal stimulation is the most commonly used, although mature animals will often spawn spontaneously in response to collection and transportation stress (Tanaka 1958; Smiley et al. 1991; Yanagisawa 1998; Morgan 2000; Eeckhaut et al. 2012).

Reliable techniques for induced spawning in holothurians have, however, been developed for relatively few species (Engstrom 1980; James et al. 1994; Hamel and Mercier 1996; Yanagisawa 1998; Morgan 2000; Eeckhaut et al. 2012). The current study attempts to carry out induced spawning in three tropical sea cucumber species: *Holothuria scabra* (sandfish), *Pseudocolochirus violaceus* (sea apple cucumber) and *Colochirus quadrangularis* (thorny sea cucumber).

This study concerns *H. scabra* because it yields one of the largest sea cucumber catches worldwide (Giraspy and Ivy 2005) and it forms an important fishery in Sri Lanka. Further, *H. scabra* appears to have the most potential for aquaculture (James 1996; Battaglene and Bell 1999; Battaglene et al. 1999). The other two species were selected because *P. violaceus* is listed as a protected species under the Fauna and Flora Protection Ordinance in Sri Lanka and there is a possibility of promoting exports of *C. quadrangularis* as a commodity for marine aquarium.

Material and methods

Broodstock collection and induced spawning

Brooders of *H. scabra* and *P. violaceus* were collected from the wild and transported to the Kalpitiya Regional Research Center of the National Aquatic Resources Research and Development Agency (NARA) in late 2011, while *C. quadrangularis* were collected in early May 2012 (Fig. 1). *H. scabra* and *P. violaceus* were conditioned within the hatchery tanks before inducing artificial breeding.

Spawning initiations of these three species were tried using the methods described by Agudo (2006). These methods include thermal stimulation, a powerful jet of water, dry treatment, transportation stress and the addition of increased quantities of microalgae. In thermal stimulation, the water temperature was raised by 3–5°C by adding warm seawater to the spawning tank and maintaining a uniform temperature. Sandfish brooders were introduced into the tank and after around 45–60 minutes the water was replaced with new water at ambient temperature. When the ambient temperature of the seawater was higher than 32°C, a cold shock treatment was given by putting ice bags into the tank to lower the temperature.

When applying a powerful jet of water, the broodstock was kept dry for about 45 minutes and then subjected to a powerful jet of seawater for 15 minutes. Then they were returned to the spawning tank at ambient water temperature. In the dry treatment method, brooders were kept completely dry for about 45–60 minutes and then the tank was refilled with water at ambient temperature. In the last method, *Chaetoceros* spp. (40,000 cells ml^{-1}) was put into the spawning tank until the water became turbid. After one hour, the water was replaced with new water at ambient temperature.

Once the spawning was over, the animals were put back into the broodstock holding tanks. An egg count was made after each successful breeding trial. Fertilisation occurred inside the spawning tanks and the fertilised eggs were collected and introduced into larval rearing tanks. Observations of egg development stages were carried out at different time intervals.

Figure 1. Three sea cucumber species; *Holothuria scabra* (left), *Pseudocolochirus violaceus* (middle) and *Colochirus quadrangularis* (right) used for artificial breeding.

Larval rearing

Feeding of the larvae was started two days after fertilisation. The initial larval stages of the three species were fed with *Chaetoceros* spp. (20,000–40,000 cells ml^{-1}). The latter larval stages of *H. scabra* were fed with grounded shrimp pellets and fine paste of *Sargassum* spp. The larval stages and juveniles of *P. violaceus* and *C. quadrangularis* were fed with *Chaetoceros* spp. (40,000 cells ml^{-1}) continuously. After one month, *H. scabra* juveniles were transferred into an indoor nursery tank and maintained until they reached 1 g in weight (~ 3 cm) and then they were nursed in outdoor fibreglass tanks.

Figure 2. Spawning behaviour of male *H. scabra* (left) and release of sperm (right).

Figure 3. A small bulge formed by female *H. scabra* before releasing eggs (left) and release of eggs (right).

Water exchange and aeration

Water in the larval rearing tanks was changed once a day, in the morning, and dead algae, faeces and dead larvae that had settled in the bottom of the tanks were removed by siphoning. The water of the larval rearing tanks was aerated continuously but gently. The water temperature was maintained in the range of 26–29°C and the dissolved oxygen was above 5.5 mg L^{-1}. The salinity ranged from 33‰ to 37‰ and the pH from 8.0 to 8.3.

Results

Induced spawning

Thermal stimulation (ambient temperature ± 3–5°C) was found to be the most successful method for spawning initiation of *H. scabra* and three successful breeding trials have been carried out since October 2011. The males spawned before the females and pre-spawning behaviour included rolling and twisting on the substrate. Males raised the anterior end of their bodies perpendicularly prior to spawning and often swayed from side to side, releasing a steady stream of sperm from a single gonopore at the top of the anterior end (Fig. 2). Males remained erect and spawned continuously for several minutes or hours, even when they were disturbed.

Females started spawning about an hour after the first male, by raising their bodies off the substrate, in a similar manner to males. They formed a bulge in the anterior part of their body and released pale yellow eggs in short powerful bursts which lasted for 20–30 seconds (Fig. 3). Immediately after spawning, the females returned to a horizontal position. In *H. scabra*, around 0.7, 0.38 and 1.8 million eggs were produced in the first, second and third breeding trials respectively.

Spawning of *C. quadrangularis* was initiated by transportation stress, while the addition of increased quantities of microalgae (*Chaetoceros* spp.) was more effective for *P. violaceus*.

Two successful breeding trials were carried out for *P. violaceus* in April and May 2012, and one trial for *C. quadrangularis* in May 2012. In both species, males spawned first and females reacted about 40–50 minutes after the first male. Males spawned continuously for around 35 minutes and females for 20–30 seconds. Around 0.52 and 0.09 million eggs were produced by *P. violaceus* in the two breeding trials and around 0.73 million eggs were produced by *C. quadrangularis*. High mortality rates were observed at the end of the larval rearing process and for all these species the survival ranged from 0.7% to 1.5%.

Larval development and larval rearing

After fertilisation, cleavage started and the embryo further developed inside the fertilisation membrane. The cleavage was complete and holoblastic. The blastula stage occurred within an hour after fertilisation and it became a typical gastrula stage after one day. The larval development of *H. scabra* consisted of auricularia larva, which is a feeding stage, and then the non-feeding doliolaria larval stage and finally the pentactula larval stage. Auricularia is a slipper-shaped, transparent larva with ciliated bands around the body. It has a pre-oral loop in the anterior part and an anal loop posterior. Three larval developments stages – early, middle and late auricularia – can be identified. Early auricularia larvae were formed after two days and late auricularia were formed after five or six days and remained for 10–13 days. The digestive tract, comprising foregut, mid gut, hindgut and larval anus, was observed in the late auricularia stage. Auricularia larvae are pelagic in habit and feed on microalgae.

Doliolaria larvae are smaller and more compact than auricularia larvae. They have a barrel-shaped, dark-brown body with five ciliated bands (Fig. 4). In this stage, rapid changes can be observed inside their body and all adult features begin to form. This stage remained for 2–3 days (day 14 to 16 in their life cycle) and plastic sheets (PVC) were transferred to the larval tanks as soon as the first doliolaria larva was observed, as they need favourable substratum to settle on and metamorphose into pentactula larvae.

Pentactula larvae were observed after 16 days. It is a tubular-shaped larva with five tentacles at the anterior end and a single posterior foot. The body colour is dark with a greenish-grey tinge. Tube feet develop all over the body. The pentactula creeps over the sides and bottom of the tank and they actively feed on benthic algae and other detritus. After one month they become typical sea cucumbers with the same body shape as adults, but in the early juvenile stages there are two long tube feet at the posterior end. In this study, they were tightly attached to the settlement substrata but could perform slow activities. The juveniles produced in the indoor hatchery tanks (~ 3 cm length) were nursed in outdoor fibreglass tanks until they reached around 5–10 g (Fig. 4). The nursed juveniles were grown out in a lagoon pen.

The development stages of *P. violaceus* and *C. quadrangularis* are very similar to those of *H. scabra*. In both these species, the non-feeding doliolaria stage appeared around 13–14 days but juvenile growth was very low compared to *H. scabra* juveniles. Juveniles of these species took more than two months to attain an average length of 5–8 mm and *P. violaceus* juveniles attained an average length of 1 cm only after four months (Fig. 5).

Grow-out of juveniles

H. scabra juveniles with an average weight of 11 g ± 4 were transferred into a pen which was constructed in Puttlam lagoon. The size of the pen was

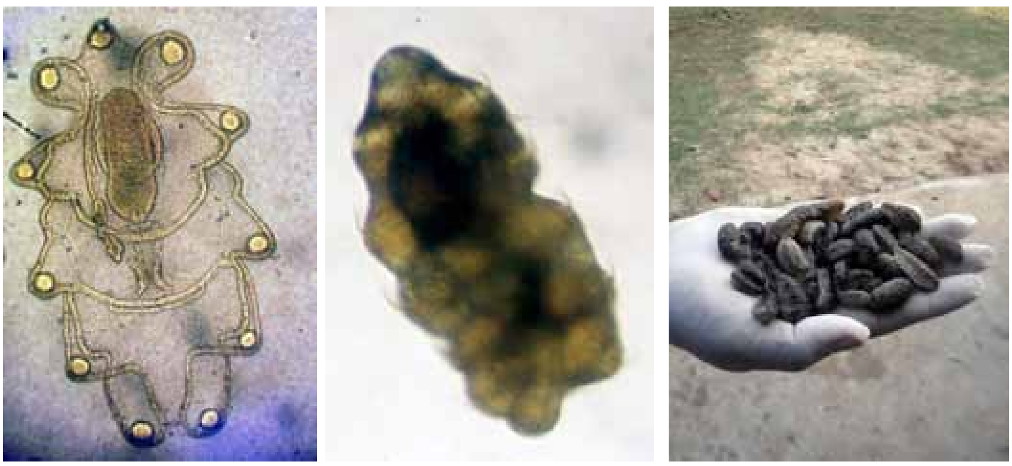

Figure 4.
Different stages of *H. scabra* life cycle: auricularia (left), doliolaria (middle) and juveniles (right).

Figure 5. Larval stages of *P. violaceus* gastrula (left), pentactula (middle) and juveniles (right).

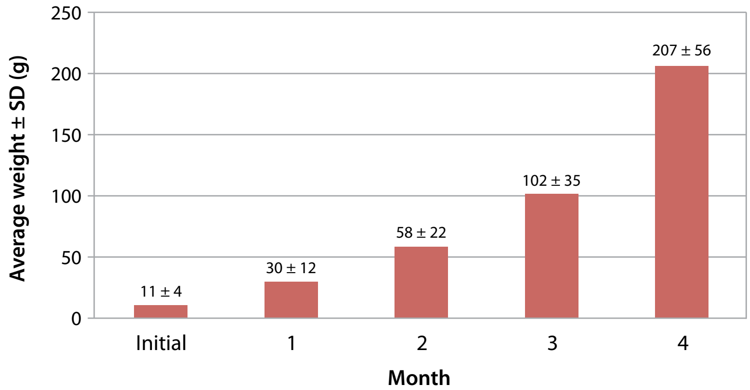

Figure 6. Weight increase of *H. scabra* reared in a lagoon pen.

Figure 7. External appearence of *H. scabra*: initially stocked into pen (left), after one month (middle) and after 3 months (right) of stocking.

56 m^2 and the stocking density was 3 ind. m^{-2}. These juveniles were weighed each month and attained an average weight of 207 g ± 56 within a period of four months (Fig. 6 and 7). There were differences in growth rate with time. In first month the reported growth rate was 0.8 g day^{-1} and this increased gradually, attaining the highest growth rate of 3.6 g day^{-1} in the fourth month. The average growth rate during the four-month period was 1.7 g day^{-1} and the survival rate after four months was 89%.

Discussion

Among the tropical sea cucumbers, *H. scabra* is considered one of the best species for aquaculture

(Battaglene 1999; Battaglene and Bell 1999). Artificial breeding and larval rearing of *H. scabra* have been conducted in several countries, including Australia (Bell et al. 2007; Morgan 2001), Fiji (Hair et al. 2011), India (James 2004), Madagascar (Lavitra et al. 2009), New Caledonia (Giraspy and Ivy 2005), Solomon Islands (Battaglene et al. 1999), Vietnam (Pitt and Duy 2004) and Iran (Dabbagh and Sedaghat 2012). Thermal stress is a well-known practice used to stimulate spawning in sea cucumbers (James et al. 1988; Battaglene et al. 1999, 2002; Morgan 2000; Giraspy and Ivy 2005; Eeckhaut et al. 2012) but in the present study thermal stress was successful only for *H. scabra*. The addition of microalgae and transportation stress are two other methods that can be used to induce spawning of sea cucumbers (Dolmatov and Yushin 1993; Reichenbach 1999) and these methods were successfully adopted for *P. violaceus* and *C. quadrangularis* respectively in this study.

Previous studies have shown that spawning of sea cucumbers can be seasonally limited. For an example, in Vietnam, *H. scabra* broodstock could be induced to spawn throughout the year (Pitt and Duy 2004) but in Iran spawning peaks are in early and late summer. It is, however, difficult to conclude reproductive periodicity of *H. scabra*, *P. violaceus* and *C. quadrangularis* in Sri Lanka as we did not make any attempt to induce them throughout the year.

In all our spawning trials, *H. scabra* males spawned first and the females reacted around one hour later. The time between the first spawning males and females among the three species that we studied was usually less than one hour. This is in accordance with previous observations made on several sea cucumber species. According to James et al. (1994), females are stimulated by the presence of sperm in the water column. As observed in our experiments, most aspidochirotids lift the anterior end of the body from the substratum during spawning to facilitate dispersal of gametes and fertilisation (McEuen 1988). Releasing of the eggs in short powerful bursts is typical of many holothurians; it aids the release of eggs into the water column, their dispersion and fertilisation (Battaglene et al. 2002).

The larval development stages of these three species seem to be very similar. However, according to Agudo (2006), there may be differences in the length of the larval cycle from species to species and even within the same species in relation to geographical location. In tropical waters, *H. scabra* (James et al. 1988), *H. spinifera* (Asha and Muthiah 2002) and *Actinopyga echinites* (Chen and Chian 1990) have taken less than 15 days to reach the non-feeding doliolaria stage and, in this study, the doliolaria of these three species also appeared in less than 15 days. As this is the first report of induced spawning and larval rearing of *P. violaceus* and *C. quadrangularis*, there are no published works to make any comparison of larval development stages, larval rearing, growth and survival of these species.

Previous studies have reported that settlement of sea cucumber larvae can be induced by adding suitable food items. Algamac has been identified as a potential settlement cue and food for settled pentaculae of *H. scabra* (Battaglene 1999). Furthermore, Asha and Muthiah (2002) have observed that Algamac and periphytic diatoms acted as good settlement cues for *H. spinifera*. However, in our experiments we did not use this kind of settlement cue and this may be a reason for the observed high larval mortality rates.

In Vietnam, *H. scabra* juveniles with an average weight of 84 g stocked at 0.73 juveniles m^{-2} grew at a rate of 1.05 g day^{-1} over five months (Agudo 2006). This study revealed a much higher growth rate (1.7 g day^{-1}) for this species with stocking density of three juveniles m^{-2}. However, in Iran, *H. scabra* juveniles reared in a sea pen attained up to 22 g after one year (Dabbagh and Sedaghat 2012).

According to preliminary observations, there is a high potential for commercial sandfish culture in Sri Lanka. However, as this is the first attempt to breeding and rear the larvae of any sea cucumber species in Sri Lanka, there needs to be more research to improve the larval rearing and grow-out facilities before starting any commercial activity.

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