Advances on spontaneous captive breeding and culture conditions of Caribbean sea cucumber *Stichopus* sp.

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Abstract

The sea cucumber *Stichopus* sp. is a species that inhabits the Colombian Caribbean, and little is known about their reproductive behaviour under controlled laboratory conditions. During 2012 and 2013, from July to October, spontaneous spawning and spermiation events took place in the Aquaculture Laboratory of the Universidad del Magdalena (Santa Marta, Colombia). The mean number of fertilised eggs was 48.4 x 10^6, which developed up to the late auricularia stage. In this study, handling conditions, water quality and limitations associated with reproduction and larval culture are described.

Key words: reproduction, moon phase, ovocyte, *Stichopus*.

Introduction

In Asian countries there is controlled production of sea cucumbers for restocking programmes, conservation strategies and the production of natural health products (Sicuro and Levine 2011). In contrast, in South American countries, studies on the culture of species with commercial importance are emerging (Guzman et al. 2003; Guisado et al. 2012; Rodríguez et al. 2013; Zacarías-Soto et al. 2013). In Colombia, little is known about the sea cucumber species, their biology, taxonomy, population dynamics, fisheries management and culture (Caycedo 1978; Borrero-Pérez et al. 2004; Rodríguez et al. 2013).

It has been found that spawning of sea cucumbers is possible by natural means under controlled conditions. On this subject, several authors report successful results in this process (Sicuro and Levine 2011; Soliman et al. 2013; Zacarías-Soto et al. 2013), while others have obtained eggs by artificial means such as hormones, chemicals, thermal stimulation, *in vitro* fertilisation and photoperiod methods or through controlled food supply (Ong Che and Gomez 1985; Hamel et al. 1993; Conand and Byrne 1993; Morgan 2000; Ramofafia et al. 2000; Fajardo-León et al. 2008; Eeckhaut et al. 2012). That is why the environment and chemicals are considered determining factors for the controlled reproduction of these marine organisms. In addition, the influence of moon phases has been demonstrated by several authors where environmental variables do not produce a response in organisms acclimated in controlled environments (Mercier et al. 2000; Hamel et al. 2001; Battaglene et al. 2002; Asha and Muthiah 2008; Hu et al. 2013).

In spite of the wide existing documentation, information about the reproductive behaviour and spawning methods of Caribbean holothuroids is scarce. This study provides a brief description of the reproduction and larval development of *Stichopus* sp. native of the Colombian Caribbean Sea, with notes on the problems associated through the success of the culture.

Materials and methods

Collection of animals

During their reproductive season (July to October), in 2012 and 2013, two hundred *Stichopus* sp. were purchased from local artisanal fishermen in Rodadero bay in Santa Marta, Colombia (11°13'22,73"N–74°13´32,59"O). The sea cucumbers were rapidly brought to the Aquaculture Laboratory of the Universidad del Magdalena in 20-L plastic tanks filled with seawater. There they were weighed using an analytical scale Ohaus (0.001 g), and their total length was measured with a standard measuring board (in mm). After that, the sea cucumbers were slowly allowed to acclimatise to a 550-L tank filled with ambient seawater (temperature 26°C; pH 7.8) maintained at the Aquaculture Laboratory.

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Broodstock management

After acclimatisation in the laboratory, the animals were randomly separated and put into six circular 550-L plastic tanks at a density of 0.1 ind. L^{-1}, distributed in a recirculation system. The tanks were filled with sterilised seawater, equipped with a biological filter, and aerated by air stones. Laboratory water temperature was maintained at 26.68°C (±0.79 SD). The sea cucumbers were exposed to a 12-h light-dark photoperiod using overhead fluorescent lights. Faeces were siphoned every day and water salinity was adjusted when needed (Fig. 1).

Figure 1. *Stichopus* sp. broodstock.

Eggs and larvae management

The broodstock was constantly monitored to observe reproductive behaviour, such as elevation of the anterior end, prominent gonopore or body wall erection.

Once the presence of gametes in the culture tanks was observed, these were removed by siphoning and a replacement of 80% of water of the breeding tanks was done. The fertilised eggs were washed with filtered seawater and sterilised, and then selected with a mesh of 60 microns. To estimate the total number of eggs, samples were withdrawn using a 1-mL aliquot. After that, they were incubated in a 50-L aquarium with soft aeration at room temperature (26°C). During the first two days of embryonic development, samples were taken every 30 minutes to monitor the morphological changes by light microscopy observations (Carl Zeiss, Modelo Primo Star), and photographic records were made with a video camera and digital photography (Axiocam ERC 5S).

Feeding practices

Broodstock

Broodstock were fed from the third day of arrival to the laboratory as follows: in 2012, a microalgae powder (5 g per 100 L of powder of *Spirulina* (Artemia-International®) was given; while in 2013, a mixture of marine sediment (previously washed and dried) was used with *Spirulina* powder at a rate of 0.5 g per 100 g of sediment.

Larvae

Two larval feeding protocols were tested. During the 2012 spawning season, powdered algal product, *Spirulina* (Artemia-International®) was used; while in 2013 the larvae were supplied with live algae (*Chlorella* sp.) at a concentration of 5,000 cells mL^{-1}.

Monitoring of the culture conditions

In 2012, water quality parameters were checked daily with a multiparameter Handy Lab: temperature of 25.14°C ±2.02, pH 6.96 ±0.13, salinity 36.4 g L^{-1} ±0.27, and dissolved oxygen 6.40 mg L^{-1} ±0.16. In 2013, water quality parameters were modified in order to ensure better larvae survival: temperature of 28.51°C ±0.17, pH 8.18 ±0.07, salinity 36.96 g L^{-1} ±0.42, and dissolved oxygen 5.56 mg L^{-1} ±0.28. The photoperiod consisted of 12 hours of light and 12 hours of dawn. Aeration was supplied by blowers (Hitachi Iced Serie G).

During incubation and to avoid the appearance of parasites, a daily siphoning of the aquarium was carried out. In addition, 30% of seawater was replaced until the blastula stage was reached and after the start of the auricularia stage, 20% daily seawater exchange was implemented.

Results and discussion

Sea cucumber spawning

From July to October 2012 and from August to October 2013, sixteen spontaneous spawning and sperm releases were presented. Reproductive events occurred within two weeks of the arrival of the sea cucumbers to the laboratory, during the hours of night and dawn. Individuals showed normal behaviour during this time. Sea cucumbers scrolled through the walls of the tanks with a prominent and noticeable genital pore (Fig. 2). Some authors have identified the animal behaviour and the condition of the genital pore as reproductive indicators of species such as *Holothuria scabra*, *Actinopyga mauritiana* and *Stichopus* sp. (Ramofafia et al. 2003; Hu et al. 2010). Since females and males were in the same tank, fertilisation occurred in a free form and subsequent quantification enabled a total average production of 48.4 x 10^6 fertilised oocytes.

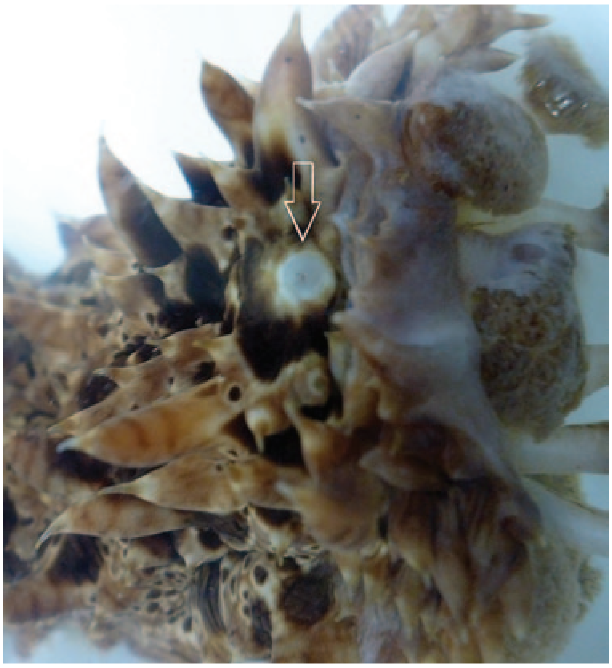

Figure 2. Mature adult with prominent genital pore (arrow).

In this study, the new moon seemed to exert a great influence over the sea cucumber reproduction. The greater reproductive peak occurred in September 2012, during the new moon phase (Table 1). In this period, during three consecutive days, natural spawning took placed (Fig. 3). These events recorded the largest number of fertilised oocytes by month (17.1 x 10^6). The reproductive behaviour of this species is similar to that reported for *Isostichopus badionotus*, showing reproductive peaks from July to November, as described by various authors (Guzman et al. 2003; Foglietta et al. 2004; Zacarias-Soto et al. 2013).

Some studies have established that the lunar cycle has a major role in the reproduction of species such as *Stichopus* sp., *H. scabra*, *I. fuscus* and *I. badionotus*, enabling the prediction of reproductive events based on lunar periodicity (Babcock et al. 1992; Mercier et al. 2000). Thus, their influence is possibly related to endogenous rhythms of each species. *Stichopus* sp. spawning occurs between the first and second night after the new moon from May to August, both in captivity and in the wild. It has also been referenced in species such as *A. japonicus*, *H. scabra*, *I. fuscus*, *Polycheira rufescens*, *Pearsonothuria graeffei*, *S. herrmanni* (Kubota and Tomari 1998; Morgan 2000; Hamel et al. 2002, 2003; Mercier et al. 2007; Hu et al. 2013; Soliman et al. 2013), and in the wild, *B. argus*, *Euapta godeffroyi*, *S. chloronotus* and *H. tubulosa* (Babcock et al. 1992; Andrade et al. 2008). Although spawning presented in this study was associated with the moon phase, it is necessary to evaluate a longer period to confirm whether this is a species reproductive pattern, as the moon phases have been associated to the reproduction and may vary among species.

Table 1. Production of fertilised eggs of *Stichopus* sp. under laboratory conditions.

Moon phase	Spawning date	Fertilised eggs x10^6
Third quarter	Jul-10-2012	1.2
New moon	Aug-20-2012	4.0
	Sept-17-2012	12.3
	Sept-18-2012	2.1
	Sept-20-2012	3.3
Menguante	Oct-10-2012	5.3
New moon	Aug-6-2013	2.4
	Aug-7-2013	1.5
	Aug-8-2013	1.7
	Aug-9-2013	1.8
	Sept-5-2013	2.1
	Sept-6-2013	1.8
	Sept-7-2013	3.2
	Oct-5-2013	3.0
	Oct-6-2013	1.2
	Oct-7-2013	1.5

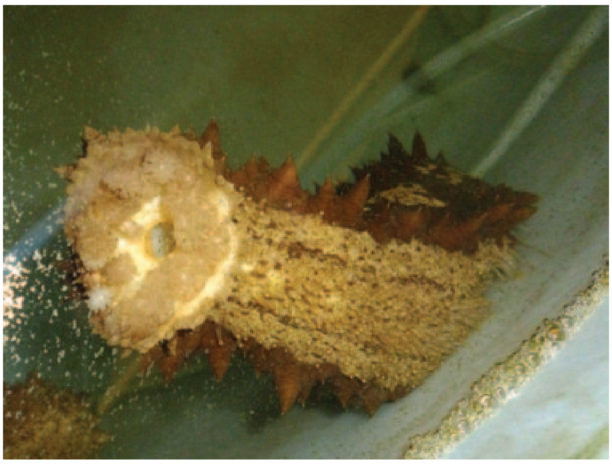

Figure 3. *Stichopus* sp. female spawning.

Larviculture

Embryonic development of *Stichopus* sp. is shown in Figure 4 and Table 2. In 2012, once the larvae reached the early auricularia stage, they did not continue the metamorphosis and, after a month, 100% mortality was recorded. In addition, during this period, the larvae did not change the stage. In 2013, ten days after fertilisation, the larvae successfully developed until late auricularia (Fig. 4) but an infestation of copepods and protozoa caused 100% mortality. Thus, during larval rearing, mortality, possibly associated with the water quality management, was recorded. The occurrence of protozoan parasites during sea cucumber larviculture is a common problem that has been documented by several authors (Purcell and Eeckhaut 2005; Raison 2008; Hu et al. 2010, 2013). Becker et al. (2009) reported that these microorganisms appear after the hatching period and once the larvae start feeding, they feed on the

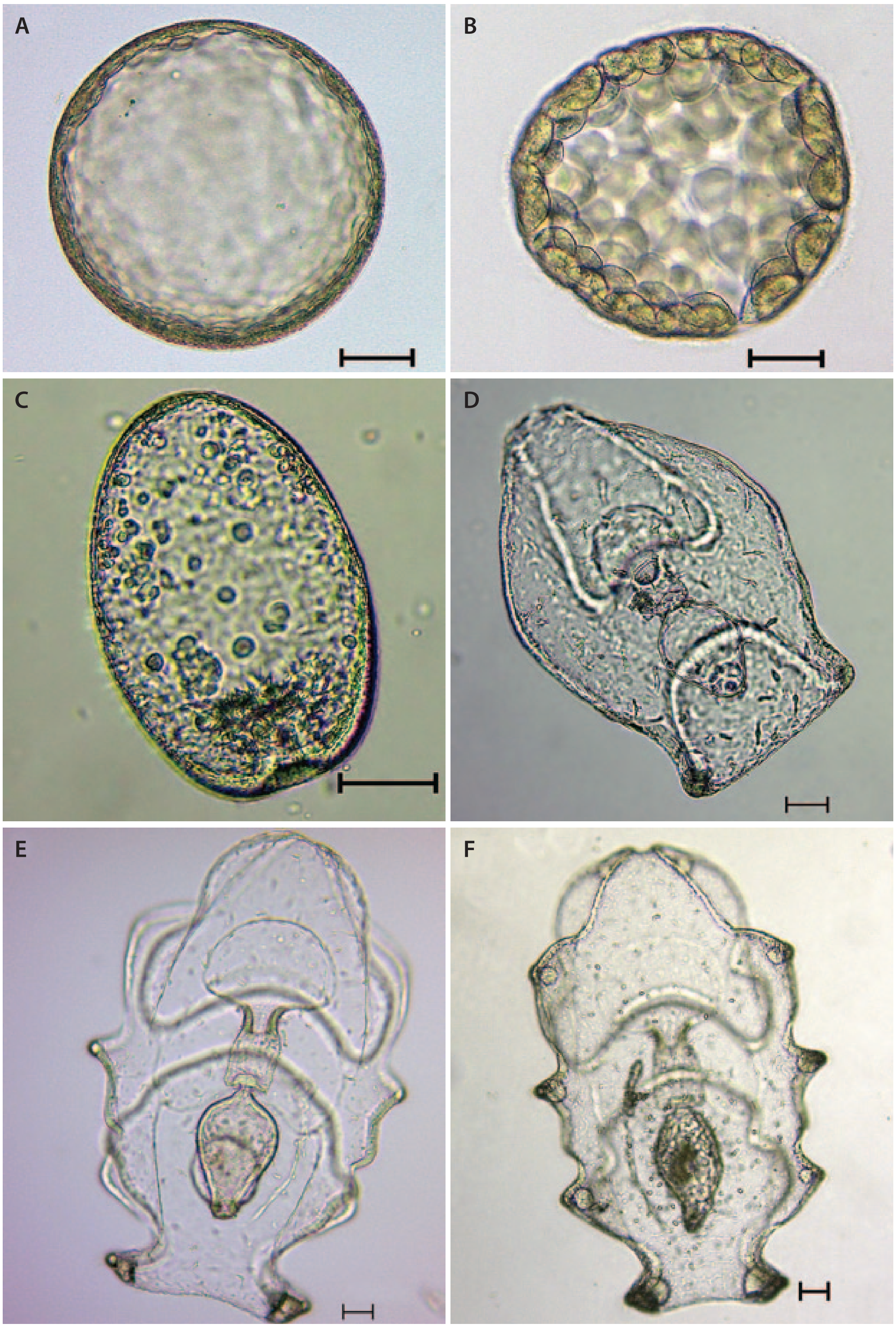

Figure 4. Embryonic and larval development of *Stichopus* sp. Embryonic phases: A) Fertilised egg; B) Blastula; C) Gastrula. Planktonic larval phases: D) Early auricularia; E) Mid auricularia; F) Late auricularia (Scale bar 20 μm).

Table 2. Characterisation of embryonic development of *Stichopus* sp.

Stages	Characteristics	Size (µm)	Time
Fertilised egg	Spherical.	138.02–175.05	0
Blastula	Spherical. It has a cover of cilia that keeps it in constant rotary motion around its axis.	262.72–302.79	4–5 (h)
Gastrula	Invagination of the vegetal pole. Appearance of the rudiments of the digestive tract.	287.40–356.30	8–10 (h)
Early auricularia	Elongation of the larva. Presence of oral cilia, intestine and cloaca.	425.30–476.50	30–40 (h)
Mid auricularia	Larger larvae with larger fold sides and better differentiated and developed digestive tract.	609.28–788.83	50–60 (h)
Late auricularia	Accumulation of hyaline spheres and development and axohidroceloma.	787.59–1,087.66	21–23 (d)

gut contents and tissues, causing shrinkage and rupture of the intestinal wall, killing the larvae within one to three days. Two preventive actions in *I. fuscus* culture are a decrease in water temperature and increased aeration, but low temperatures and strong agitation generate an adverse effect, which is noticeable in the delay or interruption of larval development (Becker et al. 2009; Mercier et al. 2012). Further to this, in Japan and India, chemical treatments are implemented to remove copepods in hatcheries (James 1994; Ito 1995; Yanagisawa 1998; Ito and Kitamura 1997). As stated by Batagglene and Bell (2004), Trichlorphon or Dipterex are used to control copepod infestation. In this study, sterilisation and filtering of seawater were insufficient to control the problem. Therefore, tests must be performed with different doses of Trichlorfon / Dipterex to find out the optimal concentration required for controlling copepod infestation.

Several authors have noted that larval development is directly related to water quality management as temperature, pH, or salinity. In addition, the quantity and quality of the food supply are key factors in the success of hatchery sea cucumber. As noted by James (1994), Ramofafia et al. (1995), and Battaglene (1999), who studied holothuroids (*H. scabra*, *A. echinites* and *H. atra* respectively), optimal water temperature for tropical sea cucumber larvae is between 27 and 30°C, values which are within the range of the larvae cultured in this study. Authors such as Hamel and Mercier (1996) or Asha and Muthiah (2005) state that a pH of 7.8 to 8.0, is an appropriate value for optimal larval growth in *C. frondosa* and *H. spinifera*; in our study, and in 2012, this factor remained below the optimal range suggested by other authors. In this study, it was not possible to determine whether this factor directly influenced the development and survival of larvae, although in 2013, pH value remained constant (8.18), and culture conditions were improved. Additionally, salinity is also an important factor for larval development of sea cucumbers as the larvae cannot tolerate values below 32 g L^{-1}; such values can cause deformities and high mortality, as has happened in species such as *H. spinifera*, *A. echinites* and *A. japonicus* (Chen and Chian 1990; Asha and Muthiah 2002; Kashenko 2002). In this study, salinity did not have any effect on the larvae; this parameter remained constant and optimal for both hatchery periods (2012 and 2013).

In this study, larval development was influenced by temperature and food supply: during the first spawning, obtained between July and September 2012, larval development stopped at early auricularia, and larval sizes were inferior to all of those achieved in 2013 (August–October). That year, live microalgae (*Chlorella* sp.) supply and higher temperature (28°C) resulted in improved conditions for larval culture and advanced the development of the larvae until late auricularia stage. The sizes of the larvae are shown in Figure 5.

Becker et al. (2009), state that growing larvae need to be fed with abundant and high quality live microalgae, especially during the auricularia stage. In case of failure in this requirement, larvae growth and metamorphosis are significantly delayed for long periods, as happened in the first year of our study. Some authors have suggested the use of some algae of the genus *Spirulina* for food in the early stages (Agudo 2006; Zacarías-Soto et al. 2013) but this is only useful during pentactula or juvenile stages because they are benthic (the added *Spirulina* falls to the bottom of the tank). Therefore, in 2013, *Chlorella* sp. was cultured and added as feed during the auricularia stage. As reported by Xilin (1986), Asha (2004) or Asha and Muthiah (2006), its use mixed with other algae (such as *Dunaliella euchlaia*, *Chaetoceros gracilis*, *C. muelleri*, *Isochrysis galbana*, *Nanochloropsis salina*, *Dicrateria zhanjiangensis*, *Pavlova lutheri* and *Tetraselmis chuii*) is a key factor during this stage of culture development.

In 2013, we established that food was ingested by the larvae due to the green gut colouration (Fig. 6). At day ten of the culture, these larvae reached a

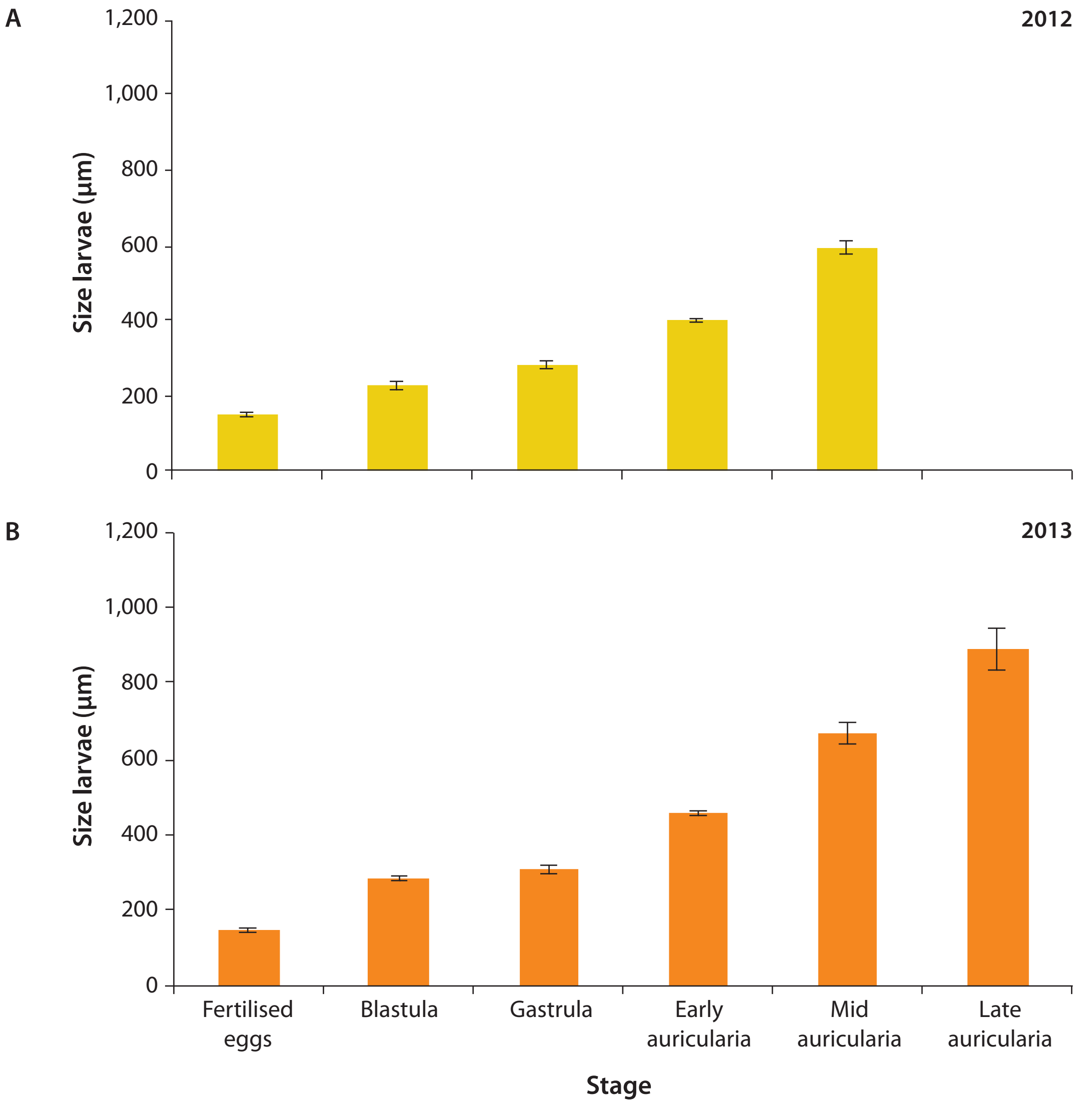

Figure 5. Larvae sizes in each of the reproductive periods under culture conditions:
A) 2012: Temperature 26°C and feeding with *Spirulina* powder;
B) 2013: Temperature 28°C and feeding with *Chlorella* sp. at a density of 5,000 cells mL^{-1}.

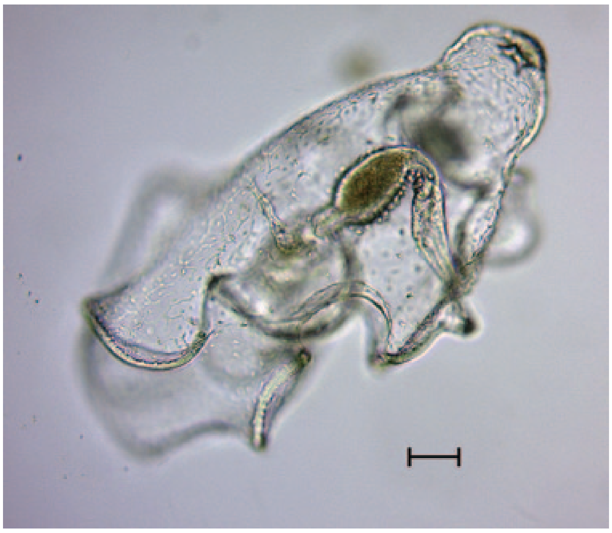

Figure 6. Late auricularia feeding microalgae (*Chlorella* sp.). Note green gut. Scale bar 20 μm.

maximum size of 888.71 μm ±106.71, when development stopped due to new copepod infestation that stopped the larval cycle and as well larvae metamorphosis.

Conclusion

Stichopus sp. reproduction is feasible under laboratory conditions since the species is easy handling and adapts to captivity. In addition, their spawning is continuous from July to November. Our findings will provide the basis for their reproduction under laboratory conditions and in turn promote the development of other related larval feeding studies to optimise sea cucumber production and survival and guarantee larvae requirements for growth and metamorphosis.

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