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Spawning, larval development and juvenile growth of the tropical sea cucumber *Holothuria leucospilota*

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ABSTRACT

The tropical sea cucumber *Holothuria leucospilota* (Echinodermata: Holothuroidea) is one of the most widespread sea cucumber species with high economic value. To develop the artificial breeding methods of *H. leucospilota*, we conducted the first detailed study on the processes of spawning, embryonic and larval development, and juvenile growth of the species. The results showed that the suitable breeding season of *H. leucospilota* in the Daya Bay might be from June to October and that at least 30 days were needed for embryos to develop into juveniles at a temperature of 29 – 33 °C, a salinity of 27 – 30‰, and a pH of 7.9 – 8.2. The survival rate of the juveniles were approximately 8.5%, the average growth rate during days 31 – 49 of culture was 0.11 mm per day and increased to 0.72 mm per day during days 73 – 120. The body color of the juveniles was transparent yellow at approximately 55 days of culture, and it began to darken at 79 days later. These results indicated that an artificial culture method of *H. leucospilota* was realized. Our research might contribute a feasible way to the artificial breeding, natural resource restoration, and sustainable use of tropical sea cucumbers.

1. Introduction

Holothuria leucospilota (Echinodermata: Holothuroidea, *H. leucospilota*) is a widespread tropical sea cucumber species that is naturally distributed near boulders, corals, and seaweed clumps in the Indo-Pacific region (Bonham and Held, 1962; Liao, 1997). *H. leucospilota* play a key role in the nearshore marine ecosystem as sediment transporters and seabed scavengers that swallow and pass a significant quantity of sand through their guts (Bonham and Held, 1962; Dzeroski and Drumm, 2003). When feeding on organic carbon-enriched sediments, the digestive tract of *H. leucospilota* may dissolve the contained organic-carbon, promoting the balance of seawater acidity/alkalinity and the maintenance of coral reef ecosystems (Bonham and Held, 1962; Schneider et al., 2011).

H. leucospilota is one of the most important edible and usable economic sea cucumber species. The gonads of *H. leucospilota* are an important traditional food in the Rarotongan (Cook Islands) culture (Drumm and Loneragan, 2005). Regarding commercial applications in medicine, a variety of active substances, such as polysaccharides and saponins, have been identified from the body wall of *H. leucospilota* with biological and pharmacological functions, including antioxidant, antithrombotic and antitumour activities (Bordbar et al., 2011; Soltani et al., 2015; Zhang et al., 2009; Zhong et al., 2015). In addition, sea cucumbers may improve aquaculture environments when co-cultured with other aquatic species by effectively reducing excess organic-nutrients in the water (Zheng et al., 2009; Yu et al., 2012).

Recently, with the continuously increasing market demand for sea cucumber, overfishing has posed a severe risk to wild sea cucumber resources (Conand, 1997). Artificial breeding of sea cucumbers is considered a useful strategy to solve the crisis (Hu et al., 2013). By releasing artificially cultured sea cucumbers into the wild, the natural sea cucumber populations may be restored (Hu et al., 2013). To date, several sea cucumber species have been successfully cultured and propagated (Laxminarayana, 2005; Hu et al., 2013; Qiu et al., 2015). However, artificial breeding and culture methods for *H. leucospilota* were still limited. Researchers in the Persian Gulf have produced juvenile *H. leucospilota* but reports lacked details of embryonic and larval development (Dabbagh et al., 2011). In the present study, for the first

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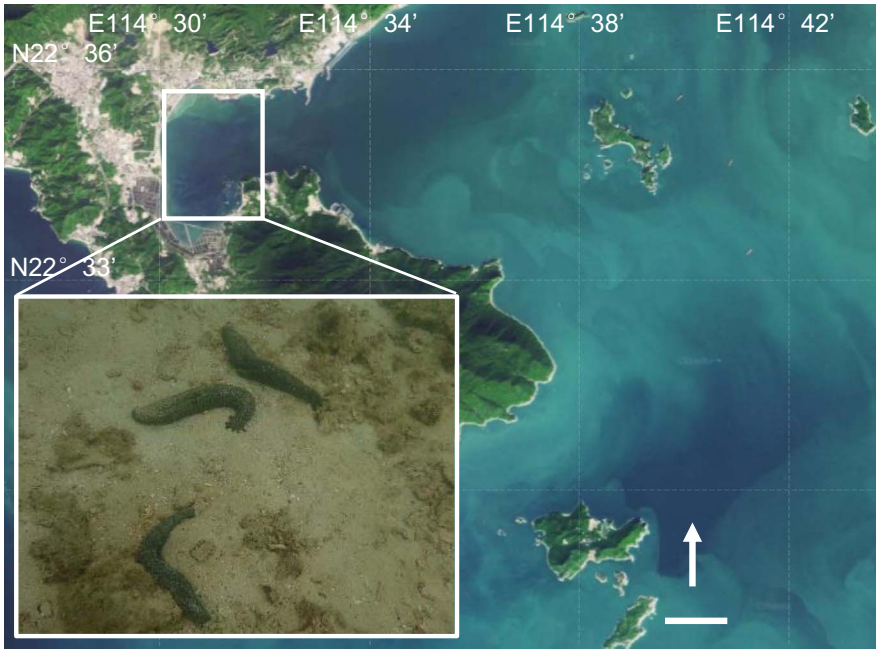

Fig. 1. Position and habitat of *Holothuria leucospilota* in the Daya Bay, Shenzhen, China. The small white box indicated the habitat of *H. leucospilota*, the enlarged white box below showed the status of the sea cucumbers in the situ seabed. The white arrow indicated the North direction. The white bar represented 2 km. The latitude and longitude were signed beside the white broken lines.

time, we obtained and described the embryonic, larval, and juvenile stages of *H. leucospilota*. Our studies have provided the basic artificial technologies for breeding this sea cucumber species and that can be applied and help to enhance the tropical sea cucumber resources in the wild.

2. Materials and methods

2.1. Collection and maintenance of animals

Adult sea cucumbers (*H. leucospilota*) with a total weight of > 400 g and a total length of > 20 cm each (measured in a calm state) were collected from the Daya Bay (N22°35′, E114°31′) in Shenzhen, Guangdong, China (Fig. 1, the geographic map was obtained from online application program http://map.baidu.com/). Gonad maturity of adult sea cucumbers was evaluated midmonth in March, June, August, October, and December. Dissections were performed to calculate gonad maturity. The gonad index (*GI*) was obtained by the formula: *GI* (%) = Gonad weight (*GW*)/Entrails weight (*EW*) × 100 (Gaudron et al., 2008). The gonads were removed from the mature sea cucumbers, fixed in paraformaldehyde solution, embedded in paraffin wax, and sectioned and stained with hematoxylin/eosin.

The candidate spawning sea cucumbers were transported to the aquaculture centre in Maoming (N21°27′, E111°02′), China and raised in 30 m^3 indoor ponds. The sea cucumbers were fed with live microalgae *Amphora* sp. and *Chaetoceros muelleri*. 50% of the bottom seawater was changed for one time a day, all of the seawater used for spawning and larval culture was filtered and disinfected overnight using 20 ppm of trichloroisocyanuric acid with sufficient aeration and neutralized afterwards using 10 ppm of sodium thiosulfate with sufficient aeration until use.

2.2. Spawning and fertilization

Cold, dry and microalgae stimulations were used to induce spawning in *H. leucospilota* as described by Hu et al. (2010, 2013). Specifically, the dry stimulation were conducted as the following steps: candidate spawning sea cucumbers were gently and rapidly caught to the dry tanks, and exposed in the open-air for 2 h in room temperature, then sufficient new seawater were added to the tanks. After spawning and fertilization, eggs were counted and transferred to black hatch tanks (round, ≤1000 l) with the density ranged from 200 to 500 per liter (l) seawater.

2.3. Embryonic and larval cultures

Air stones were placed on the bottom centre of the round hatch tanks to provide sufficient aeration and ensure that the larvae were distributed evenly in the water. The basic environmental data of seawater temperature, pH, and salinity were measured and recorded daily.

After fertilization, embryonic and larval development were observed and imaged with an optical microscope (Eclipse E200, Nikon, Japan). When the functional gut appeared (approximately 36 h after fertilization), the larvae were fed twice a day with a mixture of *C. muelleri* live microalgae and *Saccharomyces cerevisiae* yeast powder. The final concentration of *C. muelleri* ranged from 10,000 to 30,000 cells per milliliter (ml) of seawater; and the quantity of yeast powder ranged from 0.5 to 1.0 g per tonne of seawater. 50% of the bottom seawater was changed with filter (with diameter of 75 μm) for one time, twice a day.

Microalgae of *Amphora* sp. were cultured on wavy polycarbonate plates in 1000 l outdoor tanks using f/2-β growth medium (Guillard and Ryther, 1962). When 50% of the sea cucumber larvae were developed to the doliolaria stage, the live microalgae of *Amphora* sp. were collected from the wavy polycarbonate plates and fed to the sea cucumber larvae.

2.4. Juvenile culture

After attachment, the juvenile sea cucumbers were fed three times a day: in the morning, 1 − 5 l of live microalgae (with a density of 50,000–100,000 cells/ml) were added into a tonne of cultured seawater; in the afternoon, 1 − 3 g of *Spirulina s*p. algae powder were added into a tonne of cultured seawater; at dusk, 1 − 3 g of *Chlorella* sp. algae powder were added into a tonne of cultured seawater. Additionally, the final density of *C. muelleri* was kept in the range of 5000 − 10,000 cells/ml. 25% of the bottom seawater was changed with filter (with diameter of 150 μm) for one time, 3 times a day. Aeration was provided continuously, and the basic environmental data of seawater temperature, pH, and salinity were measured and recorded daily. The body length