Breeding and larval rearing of the sea cucumber *Holothuria leucospilota* Brandt (*Holothuria vegabunda* Selenka) from the northern Persian Gulf, Iran

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Abstract

Holothuria leucospilota has been induced to spawn by combining two methods: water pressure and thermal stimulation. Larvae were fed using unicellular algae and *Sargassum* extract. Early juveniles were obtained on day 33. Survival rate was 4.2% to the juvenile stage.

Introduction

In a monograph on echinoderms in the "Fauna of Iran" series, Heding (1940) recorded 17 species of holothurians found in the waters around Iran (Table 1). Among commercial species *H. leucospilota* has a low value (Toral-Granda 2006). This species usually spawns over several months (Jayasree and Bhavanabayana 1994; Drumm and Loneragan 2005).

In Iran, holothurians are only co-cultured with shrimps, as Amini Rad (2004) has shown a positive synergism between shrimp and sea cucumbers. There have been no studies to date on the sustainable harvesting and propagating of commercial sea cucumbers in Iran; this work attempts to apply methods used elsewhere in the breeding of sandfish (*H. scabra)* to *H. leucospilota.* In this paper we present the first report of successful *H. leucospilota* larval development in Iran.

Table 1. Seventeen species of holothurians recorded from the Persian Gulf.

	Species	Commercial value
1	*Aphelodactyla irania* Heding	
2	*Colochirus loppenthini* Heding	
3	*Halodeima atra* Jager	Low
4	*H. impatients* Forskal	Low
5	*H. monacaria* Lesson	
6	*H. ocellata* Jager	
7	*H. paradalis* Selenka	Low
8	*H. parva* Lampert	Low
9	*H. spinifera* Theel	
10	*H. leucospilota* Selenka	Low
11	*Protankyra magnihamulae* Heding	
12	*P. pseudo-digitata* Semper	
13	*Stichopus variegatus* Semper	Medium
14	*Stolus sacellus* Selenka	
15	*Thorsonia fusiformis* Heding	
16	*Thyone festina* Koehler and Vaney	
17	*T. dura* Koehler and Vaney	

Material and methods

H. leucospilota for broodstock were collected by snorkeling in depths of 0.5–1.5 m at low tide along the northern Persian Gulf coastal waters of Bandar-e Bostaneh during the summer of 2009. The broodstock was transferred by car to the Persian Gulf Mollusc Research Station at Bandar-e Lengeh, Hormozgan Province, Iran. The work was conducted following the methods outlined in Agudo 2006. Ten sea cucumbers were maintained in a 500-L tank with an 8-cm layer of sand on the floor. The tank was filled with 1-mm filtered and UV-sterilised seawater that was changed daily.

The methods used for inducing spawning in *H. leucospilota* were:

a) Heat shock treatment. Water temperature in the spawning tank was raised by 5°C (by adding warmed seawater) and broodstock held under these conditions for 1 hour.

b) Combination of water pressure and heat shock treatment. Animals were left in the tank at a

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depth of 2 cm for 40 minutes, and then subjected to a powerful jet of seawater for 20 minutes. Subsequently, the water temperature was raised by 5°C for 1 hour. A cover was placed on the broodstock tank and spawning later occurred. After the females spawned, the broodstock tank was left for 1 hour to allow the eggs to be fertilized by sperm from the males.

Spawned eggs were gently siphoned onto an 80-mm sieve into the bucket. The collected eggs were transferred carefully into clean 10-L buckets. To estimate egg density, the water in the buckets was gently stirred to distribute the eggs uniformly. Three 1-mL subsamples were taken from the bucket and eggs were counted under a microscope using a counting cell. Finally, the average density was calculated. The hatching rate was later estimated from the number of early auricularia divided by the number of eggs.

Larvae were stocked in a 300-L tank filled with 1-mm filtered and UV-sterilised seawater (29°C and 40 ppt salinity) at a density of 0.15 larvae mL^{-1}. Feeding was started on day 3 with *Isochrysis* sp. Subsequently, a mixture of algae (*Chaetoceros muelleri*, *C. calcitrans*, *Tetraselmis* sp.) was fed to a density that was gradually increased from 20,000 to 40,000 cells mL^{-1}. The water in the larval tank was changed, performing a complete water change every second day by siphoning the water through a sieve in a bucket (Fig. 1). Growth and survival data were recorded every second day, when siphoning the larvae onto the sieve finished, by taking three 1-mL subsamples.

Figure 1. Sieving the tank water through a filter.

Larvae were reared to the late auricularia stage using unicellular microalgae. When doliolaria were observed, fibreglass settlement plates coated with *Sargassum* extract were prepared, to be used to induce doliolaria to metamorphose into settled pentactula larvae. *Sargassum* sp. extract was added daily to water in which the plates were immersed (in a separate tank) so that the plates became coated with a fine layer of algal material. These settlement plates were then placed into the larval tank (Fig. 2). After two days, none of the (non-feeding) doliolaria larvae were seen on a sieve during a complete water change. Within a few days, pentactula larvae were formed. At this stage, the larvae were fed daily with *Sargassum* extract.

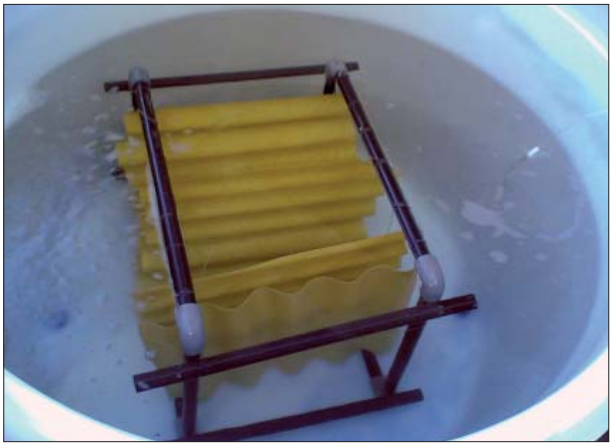

Figure 2. Settlement plates in the larval tank.

Results

Our specimens were only induced to spawn using the combined treatment of water pressure and heat shock treatment. After 1 hour, the males released their sperm; subsequently, 1 female spawned after 10 minutes, releasing about 45,000 eggs. However, 35,000 early auricularia larvae (Fig. 3) were obtained. The hatching rate was 77.7%. The amount of time for larvae to reach the juvenile stage is shown in Table 2.

Table 2. Development of *Holothuria leucospilota* from fertilization to 1mm juveniles.

Stage	Time
Fertilisation	0
Late gastrula	3d
Early auricularia	4–11 d
Mid auricularia	12–14 d
Late auricularia	17–21 d
Doliolaria	22–27 d
Early pentactula	na
Settlement (metamorphosis completed)	na
Juvenile, 1mm (Fig. 4)	33 d

na = data not available

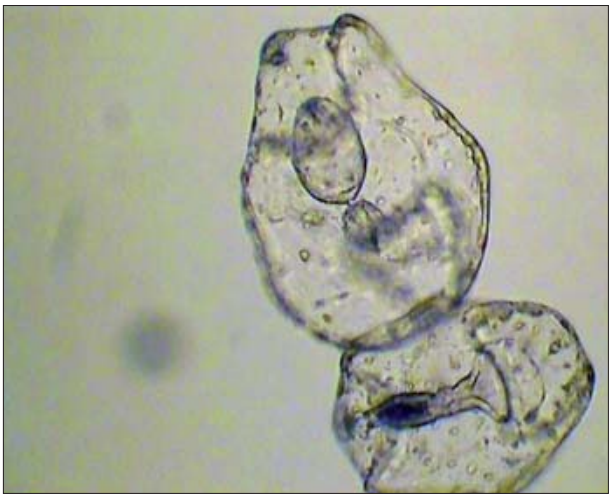

Figure 3. *Holothuria leucospilota* early auricularia larvae.

After 17–21 days of stocking, late auricularia larvae (Fig. 4) were observed. The main features of this stage are that it is transparent, slipper-shaped with ciliated bands, and up to 10 hyaline spheres. Average larval length averaged 1100 mm and sphere diameter 85 mm.

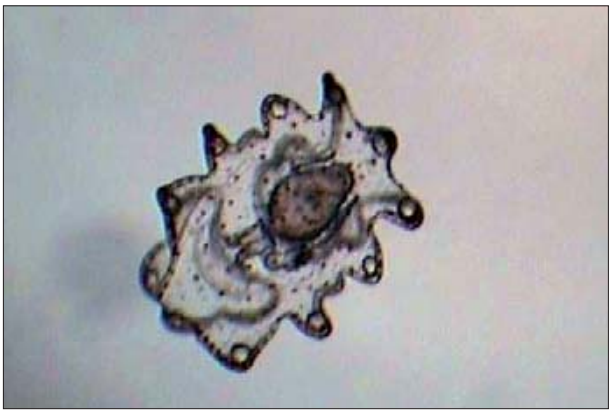

Figure 4. *Holothuria leucospilota* late auricularia larvae.

Auricularia larvae metamorphosed to (non-feeding, pelagic) doliolaria on day 22. Doliolaria larvae were dark-brown, barrel-shaped larvae with five ciliated bands around the body. Length and hyaline sphere diameter averaged 590 mm and 69 mm, respectively.

Discussion

Spawning and larval rearing have only been achieved for some species of sea cucumbers (Laxminarayana 2005). Laxminarayana has also reviewed *Apostichopus japonicus*, for which juveniles were produced for over 60 years ago in Japan and China. Nowadays, the focus of studies has been mainly on the breeding and rearing of commercial sea cucumber species. The development of three commercial sea cucumbers, *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana* was studied by Ramofafia et al. (2003). Hamel et al. (2003) reported larval development of *Isostichopus fuscus*. James (2004) bred *H. scabra* in India. Spawning and larval rearing of *H. atra* were also reported from Mauritius (Laxminarayana 2005). Thermal stimulation methods were used to obtain fertilized eggs in *H. atra* (Laxminarayana 2005) and in *H. scabra* (James 2004; Ivy and Giraspy 2006).

There have been no documented cases so far on the spawning and larval rearing of the sea cucumber *H. leucospilota*. A diet including *Rhodomonas salina*, *Chaetoceros calcitrans*, *C. mulleri*, *Tetraselmis chui*, *Isochrysis galbana and Pavlova lutheri* was used in rearing *H. scabra* in Australia (Ivy and Giraspy 2006). Laxminarayana (2005) fed *H. atra* with unicellular algae (such as *I. galbana* and *C. calcitrans*), algal extract and artificial feeds. Xiyin et al. (2004) used polyethylene film sheets for settling doliolaria larvae of *Apostichopus japonicus*. Rough surface tiles and hard surfaces with available food were used to induce doliolaria larvae to metamorphose into pentactularia (James 2004).

In general, Laxminarayana (2005) reported that *H. atra* larvae that were reared at a salinity of 34–36 ppt reached the pentacula stage by day 20. In the present study, with larvae reared at 40‰, doliolaria larvae were only obtained by day 22. The low growth rate was probably due to high salinity conditions, which prevail in the Persian Gulf. Because no previous rearing work has been carried out on sea cucumbers here, we encountered some problems, such as preparing settlement plates with *Sargassum* extract and determining the density of the *Sargassum* extract. Also in this study, the pentacula larvae were found to be orange. Consequently, it was difficult to distinguish pentacula larvae from juveniles on the plates. In the future, it is hoped to use the experience gained from this study as a stepping stone towards culturing commercial species.

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