Sea cucumber hatchery seed production in Malaysia: From research and development, to pilot-scale production of the sandfish *Holothuria scabra*

Devarajen Vaitilingon[1,2], Stuart Smith[1,2], Guy Watson[1,2], Tim Miller[1], Syed Alattas[3], Keat Ong Hock[2], Jamari Zainoddin[4], Ilias Zaidnuddin[4], Hamzah Azhar[5]*

Abstract

Sea cucumbers in Malaysia have been overexploited for decades, and their stock status in both Peninsular Malaysia and Sabah has dropped to an extreme low, resulting in direct impacts on both coastal communities and marine ecosystems. As a result, the Malaysian government is now looking for alternatives such as fisheries management, conservation and aquaculture to manage the depleting wild stocks. This project is a product of fruitful collaboration between the Malaysian government and an Australian-based private company. Since its inception in July 2014, a hatchery, nursery and broodstock rearing facility has been set up. The first spawning trials were done in February 2015 followed by nine other successful spawning events over a period of nine months. Overall, the spawning success rate was about 60% and the fertilisation rate was 85%. Broodstock are now being conditioned in tanks, with some male and female individuals kept separately. Spawning events produced around 4,300,000 larvae with a survival rate of over 30% during the larval stage. Settlement occurred at day 12 after fertilisation, and about 30,000 juveniles (3–5 mm long) have been produced so far.

Introduction

Sea cucumber (*gamat* in Malaysian) resources in Malaysia have been exploited for decades due to the animal's many health and nutritive benefits. It is traditionally used in local dishes or to derive products of local medicinal value. In addition, dried sea cucumber products are highly sought after by local Chinese communities for culinary purposes, and are also believed to bring good luck, money and fortune, particularly when eaten as part of the famous "treasure pot", during Chinese New Year celebrations. Over the last several decades, there has been an increasing demand from both local and regional markets and this demand has contributed to the dwindling of wild sea cucumber stocks. As a result, the status of sea cucumber stocks in Peninsular Malaysia and mainly in Sabah have dropped to an extreme low, resulting in direct impacts on both coastal communities and marine ecosystems (Choo 2012).

This situation is now worse due to the increased demand for derivative products such as *gamat* water and oil (medicinal values), personal care products (soap, toothpaste, acne cream), and products that were traditionally used in the *kampung* (villages), which are now gaining sales momentum at the commercial level. A survey undertaken in 2012 by a local marketing company revealed at least 13 registered Malaysian companies claiming to be involved in research and development activities to develop derivative products from sea cucumbers, mainly species in the genera *Stichopus* (BioSys Consulting Pty Ltd 2012; Purcell et al. 2014). The Malaysian government is now looking for alternatives such as fisheries management, conservation and aquaculture to manage the depleting wild stocks. This project is a product of fruitful collaboration between the Malaysian Department of Fisheries (DOF), AADCo Projects Malaysia Sdn Bhd[6] (spin-off from research conducted by an Australian-based company, Asia Aquaculture Development Co. Pty Ltd) and a local engineering company (Sesaga Engineering Snd Bhd). The project, led by the spin-off company, aimed at developing and commercialising a sea cucumber aquaculture system for producing premium quality trepang (beche-de-mer). This project is developed under the business incubator programme of the Fisheries Research Institute (FRI), Pulau Sayak, Kedah, Malaysia. Our aim within the next three years is to implement a commercially viable sea cucumber aquaculture facility that will:

- allow a continuous production of good quality trepang;

[1] Asia Aquaculture Development Company Pty Ltd, Suite 4.1/105 Kippax street, Surry Hills, 2010 NSW, Australia
[2] AADCo Projects Malaysia Sdn Bhd, 2980-02 Kompleks Chai Leng, Jalan Baru 13700, Prai, Pulau Pinang, Malaysia
[3] Sesaga Engineering Sdn Bhd, 2980-02 Kompleks Chai Leng, Jalan Baru, 13700, Prai, Pulau Pinang, Malaysia
[4] Fisheries Research Institute Langkawi, Pejabat Perikanan Daerah Langkawi,Bukit Malut, 07000 Langkawi, Kedah, Malaysia
[5] Fisheries Research Institute Pulau Sayak, Kampung Pulau Sayak, 08500 Kota Kuala Muda, Kedah, Malaysia
[6] Sdn Bhd is an abbreviation for sendirian berhad, a Malaysian term meaning "private limited".
* Author for correspondence: dev@aadcoprojects.com

- allow a better quality control on final products;
- achieve higher production rates; and
- supply the increasing worldwide demand with a labeled premium quality product.

Since its inception in July 2014, a hatchery, nursery and broodstock rearing facility has been set up. The first spawning trials were conducted in February 2015 and were followed by several other successful spawning events over a period of nine months. An overview of the project's achievements thus far is described in the following sections.

Rearing facilities

Our rearing facility is located within the complex of the Fisheries Research Institute (FRI), Kampung Pulau Sayak, Kedah. It extends over 600 m^2, and half of it is within an existing building while the other half is within an outdoor space. The facility is supplied with filtered seawater via FRI seawater intake and treatment systems, together with other utilities from the main complex. The salinity of the seawater varied between 29 ppt and 32 ppt, depending on the season, with temperatures ranging between 28ºC and 33ºC and pH averaging 8.1.

The building houses a fully biosecure and temperature controlled hatchery, where 85 m^2 is dedicated to larval culture and early juvenile rearing (nursery 1, or N1) and 15 m^2 is for phytoplankton production. The hatchery is equipped with 12 x 1000 L conical bottom tanks used for larval rearing (Fig. 1A), 1 x 4 m^3 (Fig 1B) and 4 x 0.5 m^3 tanks for N1. The room is supplied with 1-μm filtered and ultraviolet (UV) sterilised seawater produced from a filtration bank located outside the room (Fig. 1F) and distributed via an overhead ring. A centralised compressor supplies the hatchery with compressed air. The phytoplankton production room is equipped with a separate recirculating seawater system that continuously supplies filtered (to 1 μm) and UV sterilised seawater for the mass production of *Chaetoceros calcitrans* and *Pavlova* sp. (Fig. 1C). The phytoplankton production capacity is about 300 L of monoculture at any time. The other 200 m^2 is located within a non-biosecure and ambient temperature conditions. The tanks are kept under a roofed but opened space. This space is divided between, nursery 2 (N2, 120 m^2) for juvenile grow-out and broodstock rearing and conditioning (80 m^2). The tanks in N2 (Fig. 1D and E) are supplied with 15-μm sand-filtered seawater while the broodstock tanks are supplied with 30-μm filtered seawater, both running via a flow-through system.

The outdoor space is equipped with 8 x 13 m^3 and 4 x 15 m^3 tanks that are supplied by 30-μm filtered seawater via a flow-through system and compressed air. In the near future, this space will

Figure 1. Sea cucumber rearing facilities. A: Hatchery unit with larval culture tanks; B: Indoor nursery tank with settlement plates; C: Phytoplankton room with *Chaetoceros* sp. cultures; D and E: Outdoor nursery tanks holding juveniles > 10 mm in size; F: Seawater storage and filtration system.

be covered with a greenhouse structure and used to expand the N2 and broodstock conditioning facilities. In addition to the rearing facility is an office for our technical staff, a dry laboratory and storage facilities.

Broodstock management

Sandfish individuals > 25 cm in size (Fig. 2A) were collected on three occasions (October 2014, January and July 2015) along the coast of Pulau Balambangan off Kudat, Sabah, Malaysia. Between 30 and 40 individuals were collected on each occasion, transported by boat to Kudat, and then by road to Kota Kinabalu. They were allowed to settle overnight in tanks continuously supplied with fresh seawater before being packed for airfreight the next day. Eight to ten individuals were placed in a Styrofoam box fitted with two layers of plastic bag and prefilled with a 2–3 cm layer of filtered seawater (Fig. 2B). The bag was then filled with oxygen and sealed. Two ice packs of about 500 g each were placed in each box before being wrapped and prepared for airfreight. Once at Penang airport, the sea cucumbers were transported to our facilities in Kedah and recovered in dedicated broodstock tanks (Fig. 2C and D). The observed survival rates after recovery were 70% after the first shipment in October 2014, and 100% during the last two shipments. Few eviscerated individuals were noted during the first and second shipment, but none on the third shipment.

Broodstock individuals were fed on a regular basis with a mixture of dry algae: *Sargassum* sp., *Gracillaria* sp. and *Ulva* sp. The weight of broodstock individuals was monitored every two weeks and, after each spawning trial, the observed male and female individuals were placed in separate tanks and conditioned to favour gonadal growth and maturation.

Spawning trials

Thermal stimulation was used to induce spawning in sandfish and a rise in seawater temperature between 3°C and 5°C was observed to be enough to trigger spawning in mature individuals. Spawning was trialled at least twice a month and each time, between 20 and 45 individuals were induced. During each spawning event observed, males always spawned first, triggering females to spawn 30 minutes to 2 hours later (Fig. 3 A and B). Each female spawned, on average, about 1 million eggs. So far, two males and one female from the January 2015 batch have spawned twice over a five-month period.

Spawning success varied over the nine-month trial period (Fig. 4). Out of 14 spawning trials, 9 resulted in successful fertilisation (64%). From February to May 2015, the spawning success rate averaged around 25%, while no females spawned during June, July and August. Spawning in both males and females was observed again as in September. This observation could be due to the seasonality in reproductive cycle of the sandfish individuals.

Larval stage

Upon release of gametes following a spawning event, fertilisation was allowed to occur in the spawning tanks while care was taken to avoid polyspermy. Broodstock individuals were then removed and fertilised eggs were transferred to larval culture tanks at a density of 200–300 eggs per liter (L^{-1}). Larval culture tanks were prefilled with 1-μm filtered seawater the day before in order to allow the water temperature to reach the hatchery room temperature, which was set at 27ºC. Each larval culture tank was supplied with air via a diffuser or airstone.

Figure 2. *Holothuria scabra*. A: Adult sandfish (> 25 cm); B: Packaging and conditioning for airfreight; C: Sandfish individuals recovering in our broodstock tank (inset D).

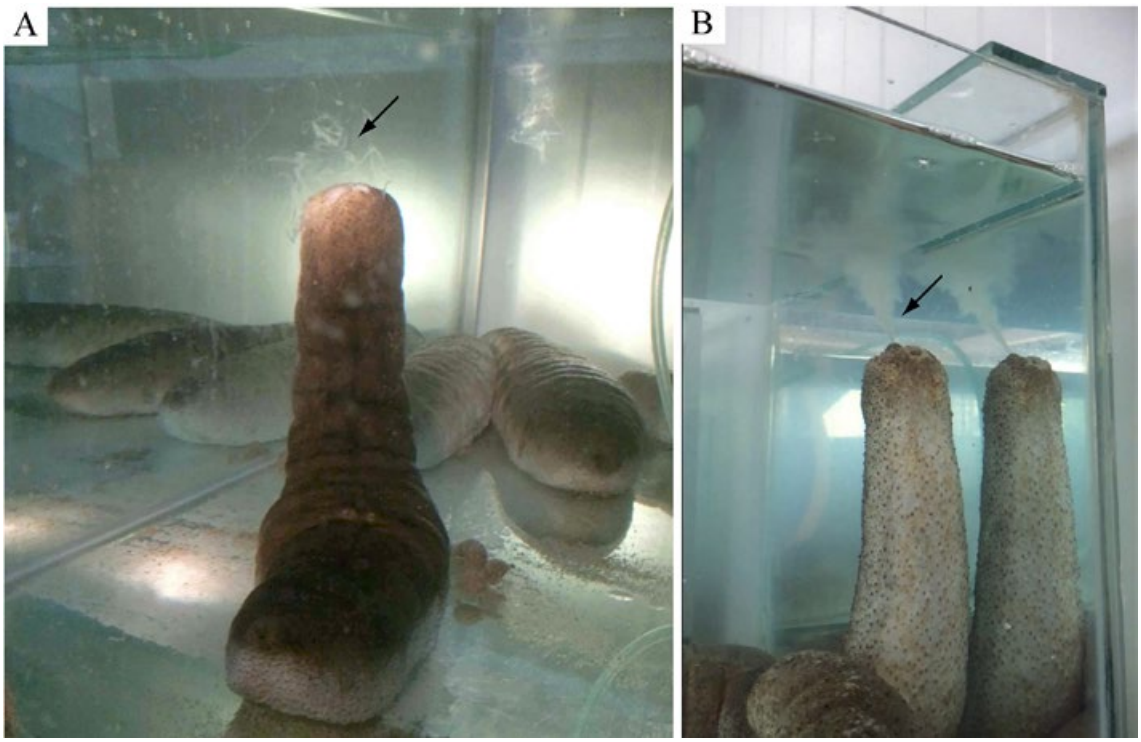

Figure 3. *Holothuria scabra* spawning events. A: Male; B: Female.

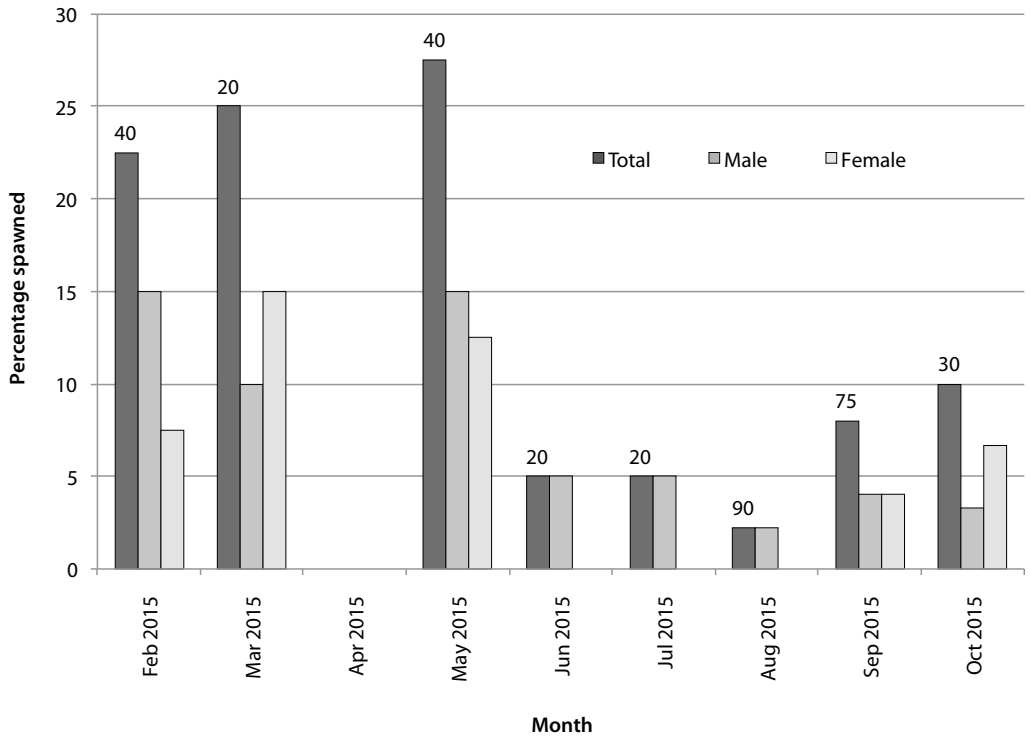

Figure 4. The success rate in spawning *Holothuria scabra* over a nine-month period. Total number of individuals induced to spawn is indicated above bars. Note: No trial was conducted in April.

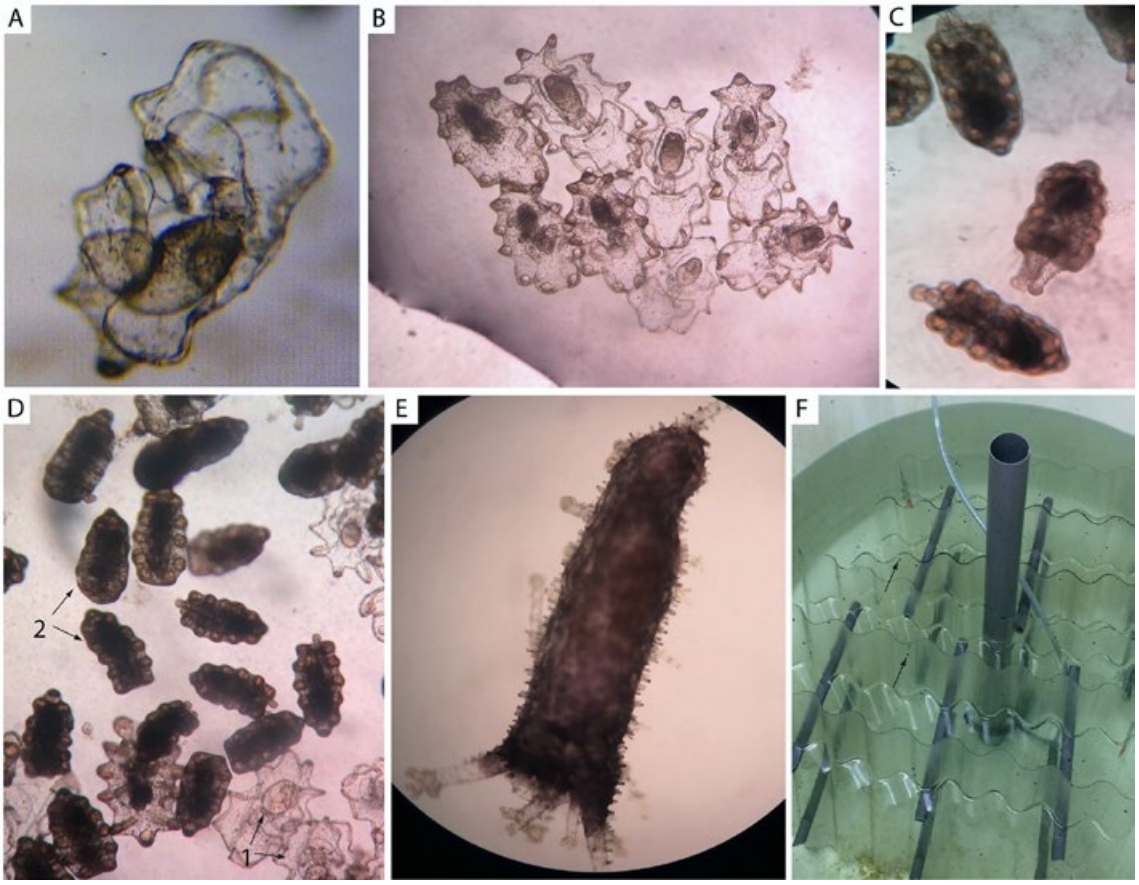

Figure 5. Larval developmental stages of *Holothuria scabra*.
A: Early auricularia; B: Late auricularia; C: Doliolaria;
D: Mixed doliolaria (2), and later auricularia (1);
E: Pentactula; F: Early juveniles (arrow) on settlement plates.

Depending on the number of females that spawned and the number of fertilised eggs collected, three to six larval culture tanks were filled with larvae at each trial.

The onset of feeding occurred two days after fertilisation and the resulting larvae were fed on a daily basis with 1 L of diatom *Chaetoceros calcitrans* per tank, cultivated at a density ranging between 4 x 10^6 and 6 x 10^6 cells per milliliter (ml^{-1}). The different larval stages are shown in Figure 5 (A–E) and larval development was completed within 12–15 days after fertilisation. Doliolaria larvae were allowed to settle on preconditioned settlement plates (Fig. 5F), and were introduced into the larval culture tanks eight days after fertilisation. The resulting pentactula postlarvae were reared in the same tank for another 20–30 days, and early juveniles were then transferred to the N1 tanks. About 50% of the water in the larval culture tanks was exchanged once a week, and after all larvae had settled the feeding ration was reduced to 500 ml, only until all early juveniles were transferred to nursery tanks. Throughout all the larval, postlarval and early juvenile rearing, water parameters such as salinity, temperature, pH and dissolved oxygen were monitored on a daily basis.

Juvenile stage

Figure 6 shows the early juvenile stages from N1 and N2 tanks. About 35–45 days after fertilisation, the resulting juveniles were transferred to the N1 tanks (Fig. 6A and B). The rearing density was about 500 individuals per m^2 (ind. m^{-2}). Their average size ranged between 1 mm and 5 mm, although some fast-growing individuals were observed to be around 10 mm long. Settlement plates that have been preconditioned to favour biofilm growth were added in N1 tanks to increase the surface area. The juveniles were fed every two days with both phytoplankton cultures (2 L for the 4-m^3 tank

and 0.5 L for the 0.5-m^3 tanks) and a supplement of dry algal powder (mixture of *Sargassum* sp., *Ulva* sp. and *Gracillaria* sp.). About 50% of the water in N1 tanks was exchanged every two days before feeding. Once individuals reached 10–15 mm in length, they were transferred to N2 tanks. Some of the fast-growing juveniles from the larval culture tanks were transferred directly to N2 tanks.

N2 tanks had a 3–5 mm layer of sand at the bottom and water was supplied continuously to the tanks (Fig. 6C to F). The rearing density in those tanks varied from 450 ind. m^{-2} for 15 mm-long individuals to 200 ind. m^{-2} for 30 mm-long individuals, and 75 ind. m^{-2} for 60 mm-long individuals. The juveniles were fed every two to three days using the same mixture of dry algal powder as in N1. After nine months of the pilot production trial, our yield was about 30,000 juveniles of 3–5 mm in length, resulting in about 6,000 juveniles of 15–20 cm in length.

The growth rates of juveniles (n = 60) in both N1 and N2 are shown in Figure 7. Growth rates recorded among juveniles in N1 tanks showed an average increase in length of 8 mm $month^{-1}$ (or 0.3 mm day^{-1}), which is equivalent to 0.01 g day^{-1}. The arrow in Figure 7 indicates the time when the entire batch of juveniles from N1 was transferred to N2. Juveniles in N2 tanks grew, on average, 5 mm $month^{-1}$ (0.2 mm day^{-1}, 0.02 g day^{-1}).

The allometric relationship between wet weight (g) and length (mm) was investigated using a random sample of 220 juveniles from N1 and N2 tanks. The length of the juveniles was measured to the nearest 0.1 mm and weighed to the nearest 0.01 g. Juveniles were blotted with tissue paper before weight data were taken. Figure 8 shows the relationship between weight and length and the power curve fitted to the data. The relationship observed was highly correlated ($r^2 = 0.9$, $p < 0.01$) and the allometric coefficient obtained was 2.69, which was consistent with previous studies (Agudo 2012; Purcell and Agudo 2013).

Grow-out trial

A small trial for grow-out in sea pens has recently been set up off Pangkor Island, Perak (Fig. 9). The main sea pen is 100 m^2 and constructed with 1-m high high-density polyethylene mesh (6 mm mesh)

Figure 6. Juvenile stage of *Holothuria scabra*.
A: Early juvenile (arrow) in nursery tank; B: Juvenile from one batch showing differences in size;
C and D: 1.5–2.0 cm-long juveniles in outdoor nursery tanks;
E and F: 3–6 cm-long juveniles prior to transplant in sea pens for grow-out.

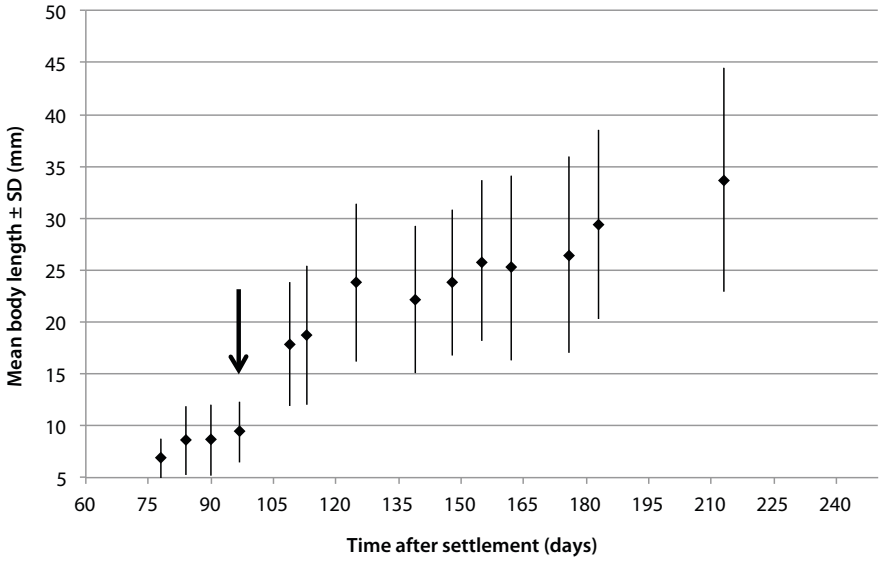

Figure 7. Growth data of early juveniles of *Holothuria scabra* from nursery tanks (n=60). Arrow shows time when juveniles were transferred from indoor N1 to outdoor N2 tanks.

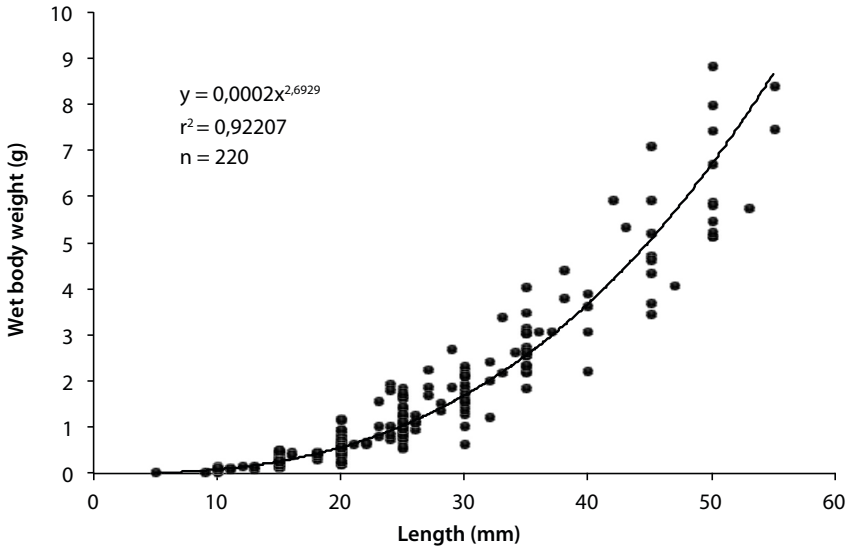

Figure 8. Allometric relationship between length and wet weight from 220 early juveniles of *Holothuria scabra*.

supported by wooden spikes. Within that surface area, sections were partitioned into smaller pens of about 3 m^2 with iron structures. These were for juvenile grow-out.

In October 2015, a small batch of 100 juveniles (length: 38 ± 10 mm; weight = 3.50 ± 2.19 g) was released in one of the small pens. After a month trial, the survival rate was 77%. Growth was very promising, with an average increase in length of 18% and a 48% gain in wet weight within one month of trial at sea (0.4 mm day^{-1}, 0.1 g day^{-1}).

Discussion

The results obtained after nine months of operation are very promising, but have also highlighted some bottlenecks in production. These are: 1) apparent seasonality in spawning success; 2) low fecundity of females; and 3) low survival rate of juveniles from 0.3 mm to 1.5 cm long as shown in Table 1 below.

The reproductive cycle of *Holothuria scabra* in the Sabah region is unknown, although it is generally accepted that spawning patterns in sandfish

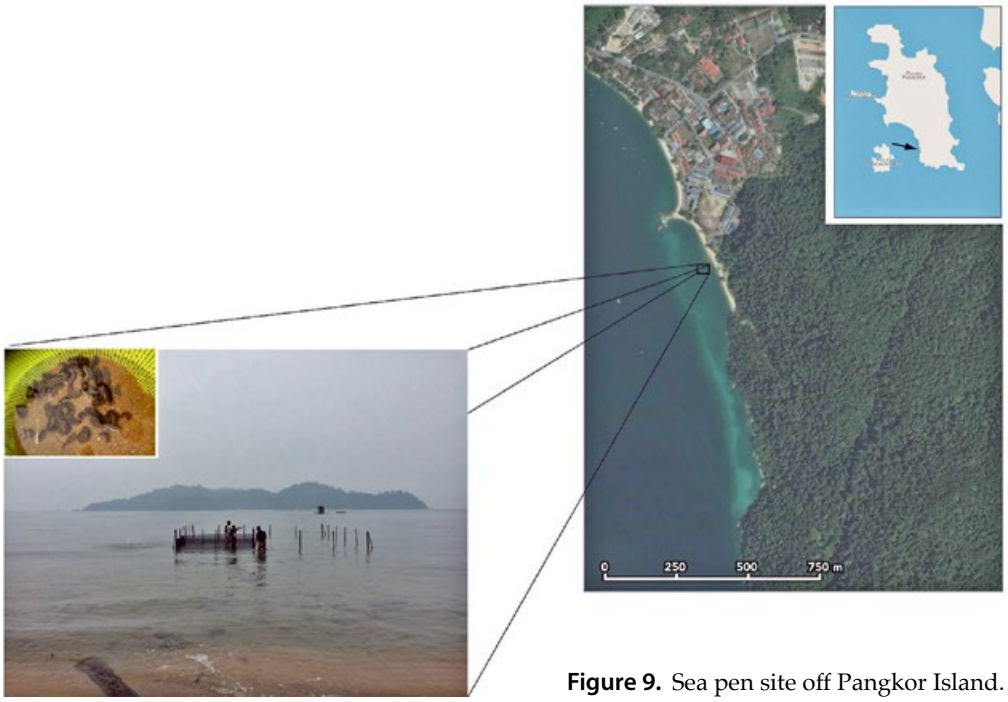

Figure 9. Sea pen site off Pangkor Island.

occurring at low latitudes are not seasonal but instead show continuous spawning throughout the year (Ramofiafia et al. 2003; Tuwo 1999). Although no analysis of the reproductive organs has been performed on our broodstock, indirect observations on spawning rates from the three samples obtained from Sabah (October 2014, January 2015 and July 2015) showed an apparent reproductive cycle, with low spawning rates among males and no spawning at all among females between June and August 2015. A proper study using histological analysis of the gonads will be required to confirm those observations. From our conditioned stock, only two males and one female spawned on two occasions within a five-month period. This indicates that our conditioning system will require further optimisation, in terms of water quality, stock density and feed types.

Although low production of eggs by females that have been artificially induced to spawn has been reported in other studies (Agudo 2006), however, sandfish from Madagascar that have been thermally induced to spawn did yield up to 4 million eggs (Devarajen Vaitilingon, pers. obs.). The fact that females are producing only about 1 million eggs per spawn is a limitation to our production. In order to operate the hatchery at full capacity (with a density of 200 eggs L^{-1}), we will need eggs from at least six females to be fertilised and this can be an issue if space for broodstock conditioning is limited. Spawning trials with other sandfish populations (namely from Thailand and Indonesia) are now underway to see if the low fecundity is inherent from the Sabah population.

Table 1 shows our output data from a typical production when the hatchery is running at full capacity. Survival rates of juveniles obtained during transition from larval culture tanks to N1 tanks are low and unpredictable (as seen by the high variability around the mean) and this is a major bottleneck in our production. In addition, the juveniles at this stage also show high variability in sizes (Fig. 6B), with larger individuals showing better survival rates than smaller ones. Future works will be focused on addressing this issue by putting more emphasis on transfer methods but also on broodstock conditioning in our rearing facilities and testing different feed types on broodstock individuals. Echinoderms are known to use egg triglyceride as a major energy lipid to fuel larval development and even early juvenile developmental stages (Byrne et al. 2008). The effect of such maternal provisioning on juvenile sandfish is unknown and will be investigated in the coming months. We hypothesised that by manipulating broodstock feed types and increasing triglyceride lipid in eggs, this might increase the performance of juvenile at this critical stage.

The growth rates obtained from juveniles in our nursery tanks are lower than those reported in pond-based or sea pen trials (Purcell and Agudo 2013). This is most probably due to the difference in water quality and rearing density between a tank-based vs a pond-based system. Our first trial at sea with 3.5 g (38 mm) individuals shows better growth rates of 0.1 g day^{-1}.

The next stage of the project will focus on the optimisation of production by eliminating these bottlenecks

Table 1. Summary of output data on *Holothuria scabra*, based on performances at every developmental stage from a representative production done when the hatchery was running at full capacity.

Rearing unit	Stage	Age (days)	Time (days)	Mean size (length)	Weight (g)	Rearing		Survival from previous stage (mean ± SD)	n	Mean global survival rate (%)	Output data (number of individuals)
						Density	System				
Hatchery	Embryo	2	2	100 µm	-	200 L^{-1}	LC - 12 x 1000-L tank	-	6	100.00	2,400,000
	Auricularia	8	10	800 µm	-	110 L^{-1}	LC- 12 x 1000-L tank	54.8 ± 5.6	6	54.80	1,315,200
	Doliolaria	2	12	450 µm	-	70 L^{-1}	LC- 12 x 1000-L tank	34.4 ± 11.1	6	34.40	825,600
	Pentactula	3	15	0.3 mm	-	-	LC- Settlement plates	68.8 ± 15.2	6	23.70	568,013
	Juveniles	45	60	5 mm	0.01	500 ind. m^{-2}	Nursery 1- Settlement plates	2.0 ± 1.5	22	0.47	11,360
Nursery	Juveniles	45	105	1.5 cm	0.3	450 ind. m^{-2}	Nursery 2 tanks	19.8 ± 6.3	3	0.09	2,249
	Juveniles	75	180	3 cm	2	200 ind. m^{-2}	Nursery 2 tanks	55*	2	0.05	1,237
	Subadults	70	250	6 cm	10	75 ind. m^{-2}	Nursery 2 tanks	80*	2	0.04	990

* Only average value was indicated when n < 2.

and expanding our facilities to the available outdoor area. This will increase space for broodstock rearing and conditioning. The access to the lagoon off Pangkor Island will also bring more opportunities for trialling grow-out at sea as opposed to tank systems.

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