First trials to induce spawning and larviculture of *Holothuria scabra* in Palau

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Introduction

Holothuria scabra, commonly known as sandfish or molech in Palau (Fig. 1), is considered to be one of the most commercially valuable species of sea cucumber that is processed into beche-de-mer (Agudo 2006; Purcell et al. 2017). Since the turn of the century, *H. scabra* has seen a 6- to 12-fold increase in price, with studies indicating that a kilogram of extra-large, premium quality beche-de-mer can fetch up to USD 1800 in Asian markets (Purcell et al. 2018). It is without a doubt that the decline in sea cucumber populations has been driven by their high commercial value. This has led to sea cucumber species such as *H. scabra* being classified as endangered on the International Union for Conservation of Nature (IUCN) red list. A study by Anderson et al. (2011) indicated that although sea cucumbers have been exploited for centuries, only in the last 60 years have the common boom-and-bust patterns appeared, with overexploitation and a lack of fisheries management leading to a decline of 81% of global populations.

The export of *H. scabra* from Palau has been banned since 1994. A study by Pakoa et al. (2009) showed that while sandfish have a limited distribution in Palau due to their particular habitat requirements, aggregations could be found in marine protected areas of Ngatpang State. However, a further study by Pakoa et al. (2014) revealed that *H. scabra* populations in the Palau states of Ngarchelong and Ngatpang had again declined between 2007 and 2012, despite the export ban. This decline was most likely due to uncontrolled and illegal fishing activities. Another study by Kitalong (2008) in the Palauan state of Airai reported a decrease of nearly 80% in *H. scabra* stocks between 1991 and 2008.

The high value of *H. scabra* on the international seafood market, coupled with the relative ease of culturing and the low cost of implementing culture systems, make it an ideal species for aquaculture development (Ferguson et al. 2020). This study focuses on the spawning induction, larval development and nursery establishment of *H. scabra* in order to contribute to the regeneration of declining wild populations and to explore opportunities for commercial aquaculture in Palau.

Methodology

Broodstock collection

Given the local availability of wild *Holothuria scabra* in Palau, 40 individuals were collected from seagrass habitats

Figure 1. *Holothuria scabra* broodstock from Ngarchelong State, Palau. (Image: Lin Tsung Han)

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by local fisherwomen in Ngarchelong State for each spawning induction trial. The broodstock were carefully handled and transported to the hatchery approximately one hour's drive from Koror, in 100-L plastic tanks with just enough seawater to cover the sea cucumbers. An underwater aquaculture sea pen was constructed next to the hatchery facility in a calm sandy area for holding the broodstock. This eliminated the need to maintain long-term broodstock in the hatchery tanks.

Induced spawning

In total, 10 induced spawning trials were conducted between December 2020 and June 2021 (Table 1).

For each spawning trial, 40 *H. scabra* were used, ranging between 157 and 270 g in weight. All of the broodstock were gently rinsed with seawater to remove sediment and other unwanted organisms attached to their body, before being transferred and acclimatised to the spawning tank, two days prior to spawning inductions.

The spawning induction combined three stress treatments (Table 2), as outlined by Agudo (2006). The first treatment used to induce spawning was a dry treatment. This was conducted by draining the spawning tanks and leaving the animals in just one inch of water for 1.5–3.0 hours. The tanks were then filled with water at room temperature before undergoing temperature shock in two steps. First, to induce the cold treatment, ice-filled buckets were added to the spawning tanks to gradually lower the water temperature to 20°C, which was then maintained for three hours.

Boiled seawater was then slowly added to the spawning tanks, increasing the water temperature to 33°C, which was then maintained for two hours. At the same time, spirulina powder at a concentration of 20 g/100 L was added to the tanks. Following the final treatment, water in the tanks was returned to ambient temperature through the removal of hot water and addition of ambient temperature seawater. Timing of the initial spawning behaviour from the male *H. scabra* (Fig. 2) ranged from immediately after the temperature was reduced following the final treatment, to almost one hour after the temperature was returned to ambient, with females spawning, on average, one hour later.

Throughout the treatments, gentle aeration was provided to create some water movement within the tanks, and any waste excreted was removed from the tanks to maintain water quality.

Egg collection and larviculture

Fertilisation of the eggs was checked one hour after the first female was observed spawning (Fig. 3). Egg collection then took place two hours after fertilisation by removing the aeration stones and syphoning the eggs from the bottom of the tank into a 200-mesh net[2] placed in a bucket. The eggs

Table 1. Dates and corresponding lunar phases of *Holothuria scabra* spawning trials.

Spawning date	Lunar phase
20 December 2020	9 days before full moon
11 January 2021	2 days before new moon
27 January 2021	Full moon
09 February 2021	2 days before new moon
26 February 2021	Full moon
18 March 2021	9 days before full moon
31 March 2021	5 days after full moon
26 April 2021	Full moon
11 May 2021	New moon
11 June 2021	2 days after new moon

Table 2. Timetable of spawning inducing treatment.

Time	Treatment
09:00–11:00	Dry-out treatment
11:30–14:30	Cold treatment
15:00–16:30	Hot treatment with spirulina bath (20 g/100 L seawater)
17:00–17:30	Ambient temperature/sunset
17:30–19:30	Broodstock spawning

[2] https://www.kmizeolite.com/mesh-chart/

Figure 2. Male *Holothuria scabra* spawning. (Image: Tsung Han Lin)

were then rinsed with fresh sea water to remove any dirt or excess sperm, before being placed into concrete rectangular raceways (8.8 m x 1.75 m x 1 m) at a concentration of 0.5 eggs/ml.

Prior to the addition of eggs, the raceways were filled with 1µm-filtered and UV-filtered seawater. Because the raceways are located outside and under cover, no heaters were required to maintain the temperature, which ranged between 28.0 and 29.5°C, with a salinity of 33–35 ppt. This also meant that lighting followed natural day and night cycles and there was no need for artificial lighting. Aeration was distributed evenly throughout the tank to provide gentle water circulation. No water changes or filtration of the tank water were conducted until day 15, when the flow-through system was opened and 1µm-filtered, and UV-filtered seawater was added at a rate of 1 L/min.

On day 2 when the first larvae were observed at the early auricularia stage (Fig. 3), live *Tetraselmis* sp. were used as feed. This was supplemented with 0.5 g of *Spirulina* and *Chlorella* powder daily per raceway, until day 15 when the majority of doliolaria larvae were observed disappearing from the water column, metamorphosing into the benthic pentactula stage (Table 3). At this point, naturally conditioned settlement plates covered with diatoms were deployed into the tanks to provide a greater surface area for settlement.

Table 3. Timetable of the life stages of *Holothuria scabra* from trials conducted, including size ranges.

Stage	Time after fertilisation (days)	Size (µm)
Fertilised eggs	0	177–191
Blastula	1	149–170
Gastrula	2	284–330
Early auricularia	2–3	469–501
Mid auricularia	4–5	708–719
Late auricularia	6–9	718–1131
Doliolaria	10–14	478–632
Pentactula	15–23	362–433
Early juveniles	24	>500

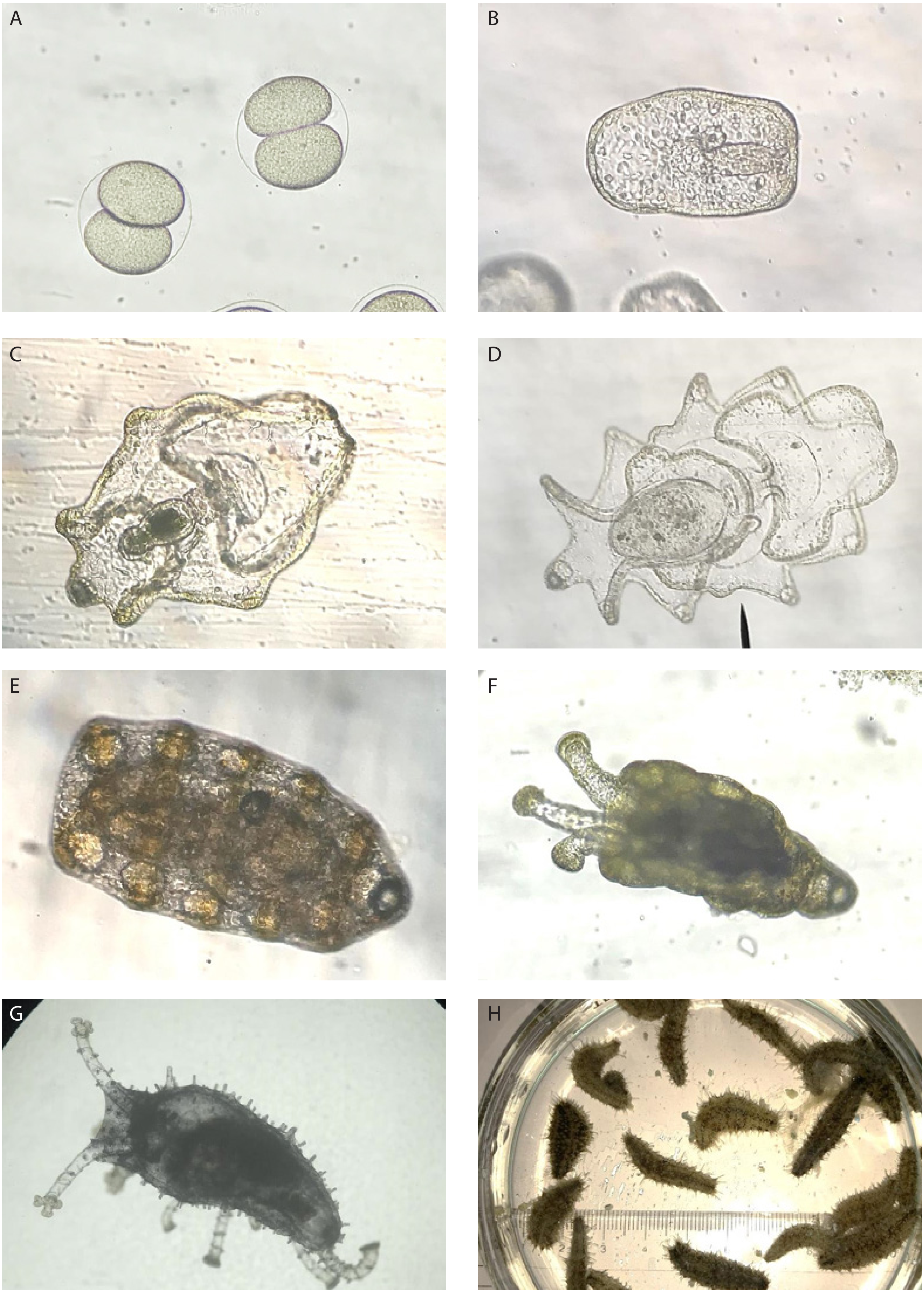

Figure 3. Larval development of *Holothuria scabra*: A) fertilised eggs; B) gastrula; C) early auricularia; D) late auricularia; E) doliolaria; F) pentactula; G) and H) juveniles. (Image: Lin Tsung Han)

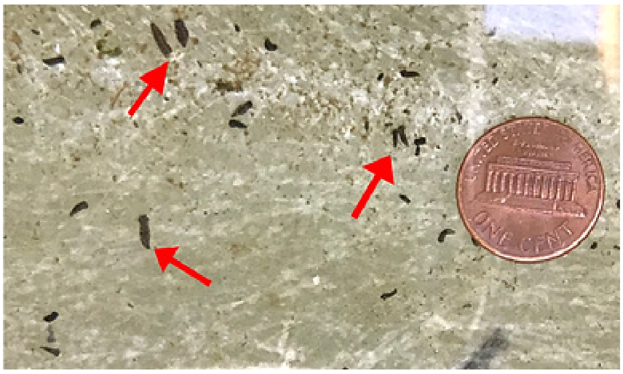

Figure 4. Thirty-day-old *Holothuria scabra* juveniles on settlement plates. (Image: Tsung Han, Lin)

Figure 5. A) Captive-bred *Holothuria scabra* juveniles, 60–90-days old (2–4 g); B) and C) *H. scabra* juveniles being reseeded by community members in seagrass beds of Ngarchelong State, Palau. (Images: Tsung-Han Lin)

Nursery stage

Juveniles of 1-mm length were visible on the settlement plates 24 days after fertilisation (Fig. 4). When they reached 1 mm in size, the juveniles were transferred into new raceways prepared with a thin sandy substrate layer. According to Schagerström (2003), juveniles grow faster on a thin sandy substrate layer than when reared in tanks without sediments. We used a sand layer of approximately 2-mm thickness, and juveniles were stocked at a density of 200–300 individuals/m².

Water was changed daily using a water flow through the raceway at a rate of 1 L every 20 seconds, thereby reducing unwanted algal growth and copepod outbreaks that can severely affect survival rates. No supplemental food was added to the tanks during this period because natural sunlight and water conditions provided an environment for sufficient diatom and algal growth, which the young *H. scabra* can consume. Under this regimen, juveniles reached a weight of 3 g (approx. 4–5 mm in length) between 60 and 90 days after fertilisation, at which stage they were of sufficient size to distribute to local coastal communities, with the aim of restocking wild populations previously depleted from overfishing (Fig. 5) (Purcell and Simutoga 2008).

Results and discussion

After this first successful attempt at inducing spawning and rearing of *Holothuria scabra* in Palau, it is clear that there is potential for the development of sea cucumber rearing and restocking of depleted wild populations. However, it is also apparent that our current methods need refining in order to optimise larval culture and juvenile rearing in Palau.

The combination of the three treatments to induce spawning was effective, with a 100% success rate, indicating that protocols developed with *H. scabra* populations in other countries work in Palau as well. Comparing growth rates of the larval stages experienced in these trials appeared to be on par with existing literature (Agudo 2006; Dabbagh and Sedaghat 2012) with pentactula larvae appearing, on average, between 15 and 23 days after fertilisation (Table 3).

However, differing growth rates were observed with some batches during these trials, with late auricularia larvae taking up to seven weeks to progress to the doliolaria stage. Our overall survival rate from fertilisation to early juvenile stage was lower (<1 %) than the 1–2% survival rate reported by Agudo (2006) and Militz et al. (2018). There may be several reasons for these results, such as our limited sources of live algal cultures for feeding, while most publications recommend the use of varying concentrations of live and dry feed (James 2004; Agudo 2006). Furthermore, the concentration of live algae feeds was not measured, which may have resulted in over or underfeeding. High algal concentrations (>40,000 cells/ml) are known to inhibit larval growth during the auricularia stage (Agudo 2006). It must also be noted that we did not conduct any water changes during the first 15 days of larval rearing, which may have resulted in poor water quality, resulting in suboptimal conditions for larval development (Agudo 2006; Ivy and Giraspy 2006; Ito 2015). Despite these setbacks, our trials did reveal that whilst many *H. scabra* populations have spawning peaks in specific seasons, which also follow lunar cycles (James 2004; Dabbagh and Sedaghat 2012; Penina 2017), it was possible to induce spawning irrespective of the lunar cycle or season in Palau (Table 1).

Following the successful spawning and rearing effort conducted at the hatchery of the Bureau of Fisheries, juvenile *H. scabra* were released into a few selected areas in collaboration with the Ebiil Society. The sites chosen were located inside marine protected areas in order to minimise the possible impact of fishing activities on the survival of the released juvenile *H. scabra*. The releasing methodology and timings were based upon those outlined in existing literature. Purcell and Simutoga (2008) indicate that *H. scabra* juveniles between 3 and 10 g are of sufficient size to be released with a 7–20% survival rate to market size. Purcell (2004) also indicates that releasing juvenile *H. scabra* at this size is the most cost effective.

In total 11,000 juveniles were released between February 2021 and July 2021, with these events also being used as educational opportunities for the local community and school children to further the understanding of sea cucumbers within the marine environment.

In the future, our goal is to refine our techniques, including quantifying survival rates, monitoring water quality, and expanding and diversifying our live feed production, to achieve higher yields. It would allow to accelerate the restocking of wild populations, and a possible distribution of juveniles to local aquaculture farms for commercial rearing. It is also hoped this will provide the basis for larviculture of other domestic sea cucumber species, such as *Actinopyga miliaris* and *Actinopyga* sp., which also play important roles in Palau's sea cucumber fisheries.

Acknowledgements

I would like to thank the Bureau of Fisheries (BOF) Director Ms Adelle Lukes Isechal, Aquaculture Division Chief Mr Percy Rechelluul, and all BOF colleagues, in addition to Executive Director of Ebiil Society Inc. Mrs Ann Singeo for their support during the project and providing materials to carry out this experiment. Thanks also to Mr Alex Ferrier-Loh for giving instructions on the sandfish spawning technique and article editions.

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