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Successful large-scale hatchery culture of sandfish (*Holothuria scabra*) using micro-algae concentrates as a larval food source

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ABSTRACT

This paper reports methodology for large-scale hatchery culture of sandfish, *Holothuria scabra,* in the absence of live, cultured micro-algae. We demonstrate how commercially-available micro-algae concentrates can be incorporated into hatchery protocols as the sole larval food source to completely replace live, cultured micro-algae. Micro-algae concentrates supported comparable hatchery production of sandfish to that of live, cultured micro-algae traditionally used in large-scale hatchery culture. The hatchery protocol presented allowed a single technician to achieve production of more than 18,800 juvenile sandfish at 40 days post-fertilisation in a low-resource hatchery in Papua New Guinea. Growth of auricularia larvae fed micro-algae concentrates was represented by the equation *length* (μm) = 307.8 × ln(*day*) + 209.2 (R^2 = 0.93) while survival over the entire 40 day hatchery cycle was described by the equation *survival* = 2 × $day^{-1.06}$ (R^2 = 0.74). These results show that micro-algae concentrates have great potential for simplifying hatchery culture of sea cucumbers by reducing infrastructural and technical resources required for live micro-algae culture. The hatchery methodology described in this study is likely to have applicability to low-resource hatcheries throughout the Indo-Pacific and could support regional expansion of sandfish hatchery production.

1. Introduction

Sandfish, *Holothuria scabra,* is a commercially valuable species of tropical sea cucumber (Purcell, 2014) that is exploited throughout the Indo-Pacific region (Hamel et al., 2001, 2013; Friedman et al., 2011). Although sea cucumber fisheries are predominantly artisanal, exploitation in the Pacific region is considerable and there are at least 300,000 fishers (Purcell et al., 2013, 2016). Declining stocks have prompted management agencies to set moratoria for many fisheries (Purcell et al., 2013; Hair et al., 2016) and has resulted in the listing of sandfish as endangered on the IUCN Red List (Hamel et al., 2013).

There is widespread interest in restoring populations of sandfish, particularly where this can deliver benefits to coastal fishing communities with few other livelihood opportunities (Bell et al., 2008; Hair et al., 2016; Purcell et al., 2016). Aquaculture offers potential to help restore production of this valuable species in three ways: (1) through production and release of cultured juveniles in areas closed to fishing to increase the spawning biomass; (2) through sea ranching operations, where cultured juveniles are released to supplement fishery catch; (3) through farming of cultured juveniles in earthen ponds and sea pens (Battaglene, 1999; Agudo, 2006; Purcell et al.,2012). A commonality among these various approaches is the requirement for successful hatchery production of juvenile sandfish.

Hatchery culture remains a major bottleneck in aquaculture production sectors, and this is particularly true among developing island nations in the Pacific region. Current production methods for sandfish rely on large quantities of live, cultured micro-algae to feed larvae and juveniles up until at least 30 days post-fertilisation (James et al., 1994; Agudo, 2006; Duy, 2010). However, mass culture of adequate volumes of high quality micro-algae is both labour and resource demanding (Coutteau and Sorgeloos, 1992) and has been identified as a hindrance to improved sandfish production (Agudo, 2006; Purcell et al.,2012). Low-resource hatcheries in remote locations often lack the required infrastructure and technical capacity to achieve adequate micro-algae production (Ito, 1999). This issue has prompted research to investigate alternative food sources for larvae, such as phototrophically-grown, highly-concentrated marine micro-algae that are commercially-available as bottled products (Reed and Henry, 2014). These micro-algae concentrate products offer “off-the-shelf” convenience and a nutritionally consistent larval food source (Duy et al., 2016a,b).

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The potential for micro-algae concentrate products to support hatchery culture of sandfish was first realised by Hair et al. (2011). Failing live micro-algae cultures in the midst of hatchery production resulted in an impromptu trial to supplement the limited supply of live micro-algae feed with micro-algae concentrates. This successful hatchery culture of sandfish on a diet composed only partially of live micro-algae prompted further research on the potential of micro-algae concentrate products as a complete replacement for live micro-algae. Duy et al. (2015) showed that sandfish larvae successfully ingest and digest a diversity of micro-algae concentrate products, and that ingestion and digestion of some micro-algae concentrates was comparable to that of live, cultured micro-algae. Successful experimental culture trials with sandfish larvae and early juveniles were subsequently achieved using *Isochrysis* sp., *Pavlova* sp., and *Thalassiosira weissflogii* micro-algae concentrates fed individually and as a ternary diet (Duy et al., 2016a, b, 2017).

While the results of these studies are promising for the development of commercial sandfish hatchery culture without live, cultured micro-algae, an appropriate protocol for large-scale hatchery production of sandfish using micro-algae concentrate products has not yet been reported. Here we present a refined methodology for successful large-scale hatchery culture of sandfish using micro-algae concentrate products.

2. Material and methods

2.1. Broodstock collection and conditioning

Twenty adult sandfish used as broodstock were sourced from the Kavieng lagoonal system (2.6784°S, 150.7980°W) of New Ireland Province in Papua New Guinea. Collection of broodstock involved hand-picking individual sandfish from seagrass beds. Sandfish were immediately weighed when removed from the water and wrapped with cloth soaked in seawater before being placed into an insulated container. The mean weight (± SE) of collected broodstock was 1.2 ± 0.1 kg (range: 0.6–2.4).

Broodstock were then transported (< 10 km) by boat to the National Fisheries Authority (NFA) Nago Island Mariculture and Research Facility (NIMRF) where they were held in a 2000 L raceway. The raceway contained 10 cm of beach sand as a bottom substrate and was provided with a continual flow of unfiltered seawater, sourced from the fringing reef surrounding the research facility, from 12:00 to 15:00 daily. The raceway was supplied with continuous gentle aeration. Broodstock were held in the raceway for a period of two weeks prior to spawning. They were fed a daily diet of powdered *Spirulina* (1 g m^{-2} surface area) that was blended with 1 µm filtered seawater (FSW) prior to feeding. Broodstock were fed in the morning before 12:00.

2.2. Spawning

Spawning induction was initially attempted using thermal shock (Battaglene et al., 2002; Agudo, 2006; Duy, 2010) in a 200 L spawning tank. Broodstock were exposed to a gradual increase in water temperature followed by a sudden reduction in temperature, over a range of 27–34 °C, three times between 10:00 and 16:00. Lack of response to thermal shock was the impetus to attempt spawning induction using feed stimulation (Agudo, 2006) the following day and spawning occurred within 3 h of broodstock exposure to a high concentration of powdered *Spirulina* (0.1 g L^{-1}) in the spawning tank. A total of four sandfish contributed spermatozoa and two spawned eggs.

When spawning activity notably declined, sandfish were removed from the spawning tank. Gametes within the spawning tank were left undisturbed for a period of 2 h following broodstock removal to allow fertilisation to occur. The spawning tank was supplied with gentle aeration during this time, but without water exchange. Three replicate samples of 100 eggs were then examined to assess fertilisation success. All eggs beyond first cleavage were considered successfully fertilised. Eggs were then harvested and washed to remove *Spirulina*. Washing involved transferring eggs with a 1 L beaker into a submerged 90 µm mesh basket through which a gentle flow of FSW was passed to remove the *Spirulina*. Cleaned eggs were then transferred to a container of known volume and the quantity of harvested eggs was estimated using replicate Sedgewick-rafter counts before stocking sixteen, 450 L (effective volume 350 L) larval rearing tanks at a density of 0.3 eggs mL^{-1} as per Agudo (2006). All larval rearing tanks were supplied with continuous gentle aeration.

2.3. Larval rearing

Larval rearing tanks were cleaned every second day prior to water exchange, starting 2 days post-fertilisation. Cleaning involved siphoning bacterial films, moribund and dead larvae, and uneaten food from the bottom of the tanks. Siphon effluent was collected in a submerged 90 µm mesh to ensure retention of any live larvae accidentally removed. Mesh contents were returned to the larvae rearing tank after siphoning.

Water exchange proceeded cleaning and occurred every second day from 2 days post-fertilisation. Water was removed from larval rearing tanks by siphon, where a large surface area screen (90 µm) was attached to the siphon head to more evenly distribute suction force. This was necessary to prevent larvae from being drawn into contact with the screen during water exchange. New FSW was added to larval rearing tanks simultaneously to prevent a reduction in tank water volume. Water exchange ceased when the total volume of water exchanged equated to half the tank volume (~175 L). This method of water exchange continued until completion of larvae settlement (25 days post-fertilisation) when the same regime continued without screening.

Feeding of larvae began 2 days post-fertilisation. Over the 40 day period of hatchery culture, two commercially-available micro-algae concentrate products (Instant Algae®, Reed Mariculture Inc.), purchased from an Australian distributor, were used. These were Isochrysis 1800® (mono-culture *Isochrysis* sp.) and Shellfish Diet 1800® (a mix of 30% *Isochrysis* sp., 30% *Thalassiosira pseudonana*, 19% *Tetraselmis* sp., 13% *Pavlova* sp., 6% *T. weissflogii*, and 3% *Chaetoceros calcitrans* on a dry weight basis). The concentrate products were composed of non-viable, but intact, micro-algal cells. The Shellfish Diet 1800® used represents the most recently available formula, a prior formulation of the product did not include *T. weissflogii* or *C. calcitrans* (www.reedmariculture.com). The daily rations of the two micro-algae concentrates fed to sandfish larvae and juveniles during hatchery culture are shown in Table 1. Daily rations for each product were divided and fed to larvae twice daily, at 09:00 and 16:00.

Prior to use, micro-algae concentrates were gently hand-shaken and the required amount of each concentrate was added to a volumetric beaker filled with FSW. The beaker containing the micro-algae concentrates and FSW was stirred to evenly distribute the micro-algae cells and the resulting suspension was passed through a 35 µm screen when

Table 1
Daily rations (cells mL^{-1}) of the two Instant Algae® products fed to *Holothuria scabra* larvae and juveniles during hatchery production. Daily rations for each product were divided and fed to larvae twice daily.

	Product	
Day(s)	Isochrysis 1800®	Shellfish Diet 1800®
2	10,000	–
3	20,000	–
4	20,000	–
5	25,000	–
6–9	12,500	12,500
10–25	5000	20,000
25–40	–	30,000