



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 $length (\mu m) = 307.8 \times \ln(day) + 209.2$ ($R^2 = 0.93$) while survival over the entire 40 day hatchery cycle was described by the equation $survival = 2 \times day^{-1.06}$ ($R^2 = 0.74$). 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

(Battaglione, 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 (\pm 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 μ m 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 (Battaglione 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 μ m 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 μ m 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 μ m) 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 μ m 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.

Day(s)	Product	
	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

administered to larval rearing tanks.

Polycarbonate settlement plates, cut to consistent dimensions (30 cm × 45 cm), were painted with a *Spirulina* paste (20 g powdered *Spirulina* mixed with 70 mL freshwater) and allowed to air dry (Duy, 2010). Twenty settlement plates were added to each larval rearing tank once competent doliolariae accounted for > 25% of the larval population (16 days post-fertilisation).

For the duration of hatchery culture, water temperature was maintained between 26 and 27 °C, salinity between 36 and 37 g L⁻¹, and dissolved oxygen > 4.8 mg L⁻¹. These water quality parameters were assessed twice daily before feeding.

2.4. Data collection and analysis

Embryonic and larval development was monitored daily until first settlement was observed. Samples of developing larvae were taken directly from the water column and concentrated on a 90 µm mesh screen. All samples were fixed using formalin solution in seawater prior to microscopic examination. One hundred embryos/larvae were examined at 40× magnification and classified according to their developmental stage.

Morphometric measurements of embryonic, larval, and juvenile developmental stages were also undertaken on 0, 2, 4, 12, 16 and 40 days post-fertilisation. Unfertilised eggs were collected by precision pipetting of eggs being released from the gonopore of spawning sandfish. Developing larvae were measured opportunistically after assessing development. Measurements of egg diameter and the total larval length of the different larval stages, as per Duy et al. (2016b), were made using digital microscopy measurement software TouView (Version 3.7.2270). Measurements were taken from 50 replicate specimens at a single point in time. Juvenile sandfish were measured at 40 days post-fertilisation *in situ* using vernier callipers.

Survival was monitored temporally in four replicate larval rearing tanks. Survival estimates occurred at the same time as larval measurements by determining larval density in a known volume of water (50 mL) as a proportion of the initial stocking density. At 40 days post-fertilisation the total number of juvenile sandfish was calculated by counting the number of sandfish on half of the settlement plates and half of a tank's surface area. Survival was determined as the number of juvenile sandfish as a proportion of the eggs initially stocked.

Growth rate during early hatchery culture was determined by developing a linear regression model that accounted for the increasing heteroscedasticity in total length as the sandfish larvae grew. This was achieved by employing heteroscedasticity-consistent covariance matrices derived using the *R* function *vcovHC* (package *sandwich*) in general linear hypothesis tests (function *glht*; package *multcomp*). The variance-mean ratio was further constrained by ln_e transformation of the predicting variable (days) prior to fitting the linear regression model.

Survival over the entire hatchery cycle (until 40 days post-fertilisation) was modelled as an exponential decay function (i.e., ln_e transforming both response and predictor variables in the model of survival as a function of time) with a random effect of tank (to account for repeated measures from replicate tanks) using a linear mixed-effects model (*R* function *lme*; package *nlme*).

3. Results

Spermatozoa of sandfish broodstock had a mean (± SE) length of 51.4 ± 0.3 µm and unfertilized eggs had a mean diameter of 164.7 ± 1.0 µm in this study. Fertilisation success of 99.0 ± 0.6% was achieved. Embryos and larvae progressed in development from gastrula, to auricularia larvae, to doliolaria larvae, and then pentactula larvae before final metamorphosis into juvenile sandfish (Fig. 1). The auriculariae were further divided into three stages of development corresponding to the early-, mid-, and late-auriculariae stages

commonly acknowledged (Agudo, 2006; Duy, 2010).

Growth from hatching until 12 days post-fertilisation, when half of the larval population had reached the late-auricularia stage (Fig. 1), was logarithmic (Fig. 2). Growth during this period was adequately modelled using the equation $length = 307.8 \times \ln(day) + 209.2$ which explained 93% of the variance ($R^2 = 0.93$). While growth was initially rapid, it slowed as auriculariae approached metamorphosis. At 13 days post-fertilisation the first doliolaria larvae were observed (2% of population; Fig. 1). Doliolariae measured 16 days post-fertilisation were 34% the size (321.6 ± 4.3 µm) of the late-auriculariae observed four days earlier (936.0 ± 9.5 µm). First settlement occurred at 17 days post-fertilisation, and last settlement occurred at 25 days post-fertilisation. At 40 days post-fertilisation hatching tanks contained entirely early juvenile sandfish, with a mean length of 3.26 ± 0.13 mm.

Survival over the entire hatchery culture period was 1.12% which accounted for production of 18,847 juveniles. Our four temporally monitored sample tanks demonstrated comparable survival (1.23 ± 0.27%) to overall hatchery performance. Survival was adequately represented by the equation $survival = 2 \times day^{-1.06}$ which explained 74% of the variance ($R^2 = 0.74$; Fig. 3). This equation identified the major period of mortality during hatchery production to occur in the first 10 days of culture.

4. Discussion

This study emphasises the value of micro-algae concentrate products in facilitating development of aquaculture in remote locations. Our results demonstrate that micro-algae concentrate products were able to support large-scale hatchery production of sandfish in a remote, low-resource hatchery in Papua New Guinea. The methodology reported here allowed a single technician to produce more than 18,800 juvenile sandfish 40 days post-fertilisation in the absence of live, cultured micro-algae.

4.1. Hatchery culture

Our feeding regime corresponded with the developmental progression of the sandfish larvae. Only Isochrysis 1800[®] was fed during the early-auriculariae development stage with co-feeding of Isochrysis 1800[®] and Shellfish Diet 1800[®] occurring during the mid-auriculariae stage through to completion of settlement. Once all larvae had settled, only Shellfish Diet 1800[®] was fed.

This feeding regime avoids offering diatoms and green-flagellates to early-auriculariae, which have a limited capacity to ingest and digest cells of these micro-algae (Duy et al., 2015). However, auriculariae readily ingest and digest diatoms by six days-post fertilisation (Duy et al., 2015), and diets containing *T. weissflogii* or *Chaetoceros* spp. have consistently demonstrated superior hatchery performance compared to single-species diets of golden-brown flagellates (Knauer, 2011; Duy et al., 2016a, b, 2017). It is also broadly acknowledged that diets composed of mixed species of live micro-algae provide a better balance of nutrients for invertebrate larvae than single species diets (Brown et al., 1989; Knauer and Southgate, 1999). It was for these reasons that the multi-species concentrate Shellfish Diet 1800[®] was chosen, offering the capacity to deliver *T. weissflogii* and *C. calcitrans* in combination through use of a single product. This product has not previously been evaluated (Duy et al., 2016a, b, 2017) for its capacity to promote successful hatchery production of sandfish.

Information on the nutritional requirements and preferred diets of post-settlement, early-juvenile sea cucumbers is extremely limited. Recent work by Duy et al. (2017) has shown that continuation of supplemental feeding with micro-algae after settlement achieved improved survival and doubled growth rates of early-juvenile sandfish when compared to unfed juveniles left to feed on accumulated biota in rearing tanks. Benefits of supplemental feeding with micro-algae were found to occur regardless of whether live, cultured micro-algae or

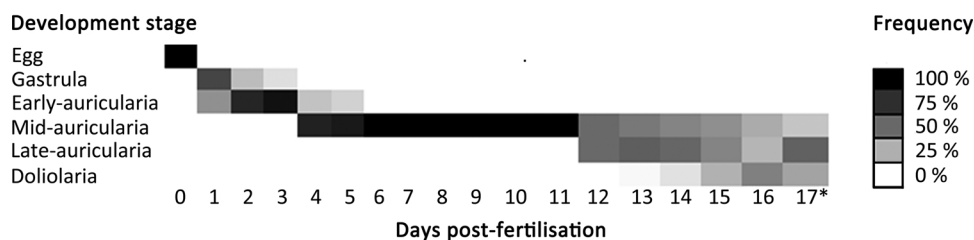


Fig. 1. Frequency chart depicting larval development progression of *Holothuria scabra* fed exclusively with micro-algae concentrates during large-scale hatchery culture. Frequency of occurrence is reported with respect to the given day post-fertilisation. Asterisk (*) indicates the start of settlement.

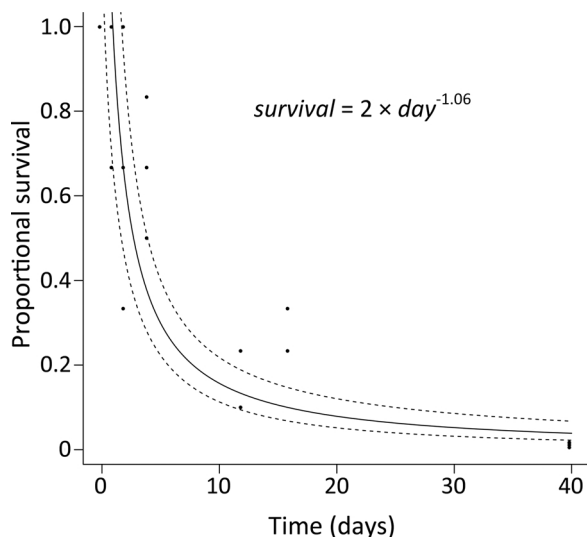


Fig. 2. Proportional survival of *Holothuria scabra* fed exclusively with micro-algae concentrates during large-scale hatchery culture. The solid line represents the exponential function best describing survival with approximate 95% confidence interval (dashed lines).

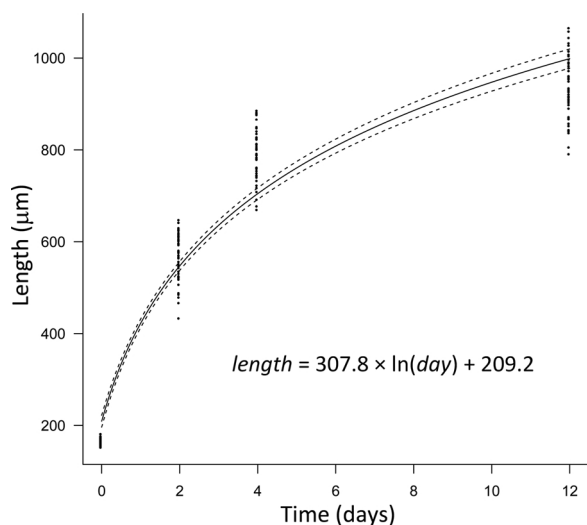


Fig. 3. Temporal change in length for *Holothuria scabra* auriculariae fed exclusively with micro-algae concentrates during large-scale hatchery culture. The solid line represents the logistic equation best describing total larval length with approximate 95% confidence interval (dashed lines).

micro-algae concentrates were offered to juveniles (Duy et al., 2017). For this reason, our protocol continued provision of Shellfish Diet 1800° until 40 days post-fertilisation when juvenile sandfish are of appropriate size (≥ 3 mm) for transfer to early nursery culture systems (Agudo, 2006; Duy, 2010; Purcell et al., 2012).

Use of live, cultured micro-algae for hatchery culture of sandfish rarely achieves survival higher than 1% up to the early-juvenile stage (Agudo, 2006; Purcell et al., 2012). Our study shows that comparable

survival (1.12%) of juvenile sandfish can be achieved 40 days post-fertilisation with use of Isochrysis 1800° and Shellfish Diet 1800° as a complete replacement for live, cultured micro-algae. Our modelled survival curve also indicates that the multi-species micro-algae concentrate diet achieved comparable survival (6.6%) at 25 days post-fertilisation to experimental sandfish feeding trials with Isochrysis 1800° ($6.7 \pm 0.3\%$) and Pavlova 1800° ($6.7 \pm 0.6\%$), fed as single-species diets (Duy et al., 2016a). However, survival of larvae in our study was slightly lower than that reported by Duy et al. (2016a) when feeding a ternary diet of Isochrysis 1800°, Pavlova 1800°, and TW 1200° ($8.5 \pm 1.3\%$) or where TW 1200° was fed as a single-species diet ($13.7 \pm 0.7\%$).

Growth comparisons at days 3, 5, 7, 9, 11, and 13 post-fertilisation show that the larvae in our study were consistently smaller by 50–100 µm than the experimentally-reared larvae of Duy et al. (2016a). However, despite this difference in growth, developmental progression was more rapid in our study, with doliolariae being first observed 13 days post-fertilisation. When compared to the best performing live, cultured micro-algae diets trialled by Knauer (2011) with sandfish larvae, our larvae were also slightly smaller (ca. 20–40 µm) on 4, 6, and 8 days post-fertilisation and demonstrated slower development progression. To some extent, the discrepancy in performance may be attributed to the different scales of production (i.e., experimental versus large-scale culture) and differences in water temperatures between studies. Our hatchery production occurred at slightly lower temperatures (26.0–27.0 °C) compared to the experimental studies of Duy et al. (2016a, b) (26.5–27.5 °C) and Knauer (2011) (27.5–31.5 °C). Activity level, food intake, and growth of sandfish have all been positively correlated with increasing temperature up to 31 °C (Mercier et al., 1999; Wolkenhauer, 2008; Lavitra et al., 2010).

Despite supporting successful juvenile sandfish production, it is plausible that the combination of Isochrysis 1800° and Shellfish Diet 1800° is not an optimal diet for sandfish larvae. The nutritional value of Shellfish Diet 1800°, for example, could be reduced by the presence of *Tetraselmis* sp. cells. While ingestion of *Tetraselmis* sp. concentrate (Tetraselmis 3600°) by auriculariae readily occurs, this product is poorly digested (Duy et al., 2015). Ingested *Tetraselmis* sp. cells accumulate in the stomach (Duy et al., 2015) and may have impeded further ingestion and digestion of the other dietary components. We also suspect that the *T. pseudonana* component of Shellfish Diet 1800° may not have contributed to the nutritional value of this product. When *T. pseudonana* concentrate (3H 1800°) was fed in isolation to auriculariae larvae, no ingestion was observed (Duy et al., 2015). These potential problems with *Tetraselmis* sp. and *T. pseudonana* indicate that a portion of the cells provided by Shellfish Diet 1800° (49% dry weight) may not have been utilised by auriculariae, and probably accumulated as waste in the culture environment. On this basis, there likely remains scope for further optimisation of micro-algae concentrates as larval sandfish diets.

4.2. Application

Successful, large-scale hatchery production of sandfish in the absence of live, cultured micro-algae is a compelling result, and adds to a growing body of evidence supporting broader application of such products. Complete replacement of live, cultured micro-algae by micro-

algae concentrate products for hatchery culture of sea cucumbers has now been reported in a number of studies with sandfish (this study; Duy et al., 2016a, b, 2017) and with the four-sided sea cucumber, *Iso-stichopus badionotus* (Zacarias-Soto et al., 2013). These results encourage further examination of the possibilities of incorporating micro-algae concentrates into the feeding protocols of other commercially produced holothurians. Routine hatchery production of other invertebrate species, such as pearl oysters (Southgate et al., 2016a) and giant clams (Southgate et al., 2016b, 2017), using micro-algae concentrates instead of live, cultured micro-algae, is already well established.

To put the potential application of our results into perspective, eighteen hatcheries were known to produce sandfish in 2012 (Purcell et al., 2012). However, production among the developing island nations of the Pacific is underwhelming, with hatcheries only known to operate in Fiji, Federated States of Micronesia, and Solomon Islands. Among these active hatcheries, annual production of $\leq 10,000$ 1 g juveniles were reported (Purcell et al., 2012). This limited production continues despite the socio-economic importance of sea cucumber fisheries (Hair et al., 2016; Purcell et al., 2016) which are depleted (Purcell et al., 2013). For many Pacific island nations, moratoria have been set for sea cucumber fisheries (Purcell et al., 2013; Hair et al., 2016) and there is great interest in restocking depleted populations through aquaculture production (Battaglione, 1999; Agudo, 2006; Purcell et al., 2012).

Sea cucumber aquaculture in the Pacific has been constrained by unreliable and generally low survival rates in the hatchery and high operational costs (Purcell et al., 2012). The ongoing cost of live micro-algae culture is a major limitation for low-resource hatcheries that typify the scale of investment achievable for developing island nations of the Pacific region (Agudo, 2006; Purcell et al., 2012). Low-resource hatcheries in remote locations also typically lack the technical capacity to achieve the level of live micro-algae production required to support hatchery culture (Coutteau and Sorgeloos, 1992; Purcell et al., 2012). Furthermore, costs associated with the necessary infrastructure for establishing micro-algae culture in regional hatcheries create an economic barrier to start-up (Ito, 1999). Minimising or eliminating the requirement for live micro-algae culture would reduce the scale of investment and recurring costs of production to some degree and facilitate expansion of hatchery production of sandfish.

4.3. Conclusions

A number of micro-algae concentrate products have been shown to support successful sandfish hatchery culture under experimental conditions (Duy et al., 2016a, b, 2017). Our study confirms similar performance when these products are used for larger-scale hatchery production. The methodology presented here is likely to be applicable to low-resource hatcheries throughout the Pacific aiming to culture sandfish and other sea cucumber species. Further improvements to hatchery production can be expected with refinement of the methodology presented here. For example, Shellfish Diet 1800® may not be an optimal food choice due to the *Tetraselmis* sp. and *T. pseudonana* dietary components. Examining ontogenetic changes in dietary requirements of sandfish larvae has received little research attention, but will help identify which micro-algae concentrate products, or combination of products, best matches larval dietary requirements. Identification of an optimal micro-algae concentrate-based diet will reveal the full extent to which these products can support sandfish production, and future cost-benefit analyses of sandfish hatchery production using micro-algae concentrates will help to quantify the economic benefit presumed to be associated with elimination of live micro-algae culture from sandfish hatcheries (Purcell et al., 2012; Duy et al., 2015, 2016a,b).

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