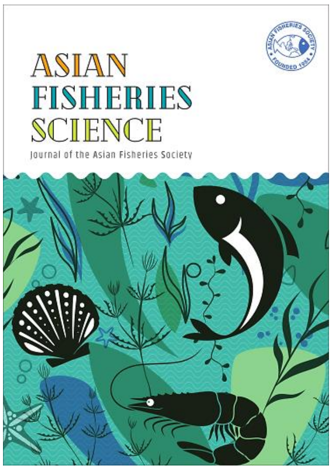


# Evaluation of Hatchery Production from Captive and Wild-caught Sandfish (*Holothuria scabra* Jaeger, 1833) Broodstocks

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## Abstract

The overexploitation of natural populations of sea cucumbers is a threat to the sustainability of sea cucumber aquaculture operations reliant on wild-caught broodstocks. Maintaining wild-caught broodstocks in captivity ensures continuous availability of suitable broodstocks. However, it remains unclear if captive broodstocks are suitable for use in hatchery production. This study evaluates hatchery production of sandfish (*Holothuria scabra* Jaeger, 1833) using captive broodstock, maintained in a pond for 1 year, compared to newly wild-caught broodstock. At the end of the 40-day hatchery cycle, lengths of juvenile sandfish resulting from the wild-caught (3.26 ± 0.13 mm) and captive (3.28 ± 0.16 mm) broodstocks were comparable ($P$ = 0.92). Survival curves of larval and juvenile sandfish differed between the two broodstock sources ($P$ < 0.01), owing to differences in the onset of exponential mortality. However, survival was comparable for juvenile sandfish derived from the wild-caught (1.23 ± 0.27 %) and captive (1.34 ± 0.73 %) broodstocks ($P$ = 0.89) at the end of the hatchery cycle (40-days post-fertilisation). The results of the present study demonstrate that captive sandfish broodstock supported hatchery production comparable to that of wild-caught broodstock.

**Keywords:** sea cucumber, broodstock, aquaculture, Papua New Guinea

## Introduction

Sandfish (*Holothuria scabra* Jaeger, 1833) is a tropical sea cucumber heavily exploited throughout the Indo-Pacific region, and listed as endangered on the International Union for Conservation (IUCN) Red List (Hamel et al. 2001, 2013; Friedman et al. 2011; Purcell et al. 2014). Many sandfish populations have been depleted since the 1990s (Hamel et al. 2001; Hasan 2005; Hair et al. 2016). Despite depleted and declining populations, the high value of sandfish (Purcell et al. 2018) has resulted in continual targeted and opportunistic exploitation of this species across much of its geographical range (Branch et al. 2013; Purcell et al. 2014). Sandfish fisheries are predominantly artisanal (Hasan 2005; Hair et al. 2016; Purcell et al. 2016) and there is widespread interest in restoring production of sandfish, particularly where fisheries can deliver benefits to coastal communities with few alternative income generating opportunities (Bell et al. 2008; Hair et al. 2016; Purcell et al. 2016).

Aquaculture can help restore production of this valuable species in three ways: 1) through sea ranching operations, where cultured juveniles are released to directly supplement fishery catch; 2) through release of cultured juveniles in areas closed to fishing to increase the spawning biomass and improve fishery productivity indirectly; and 3) through farming of sandfish in marine ponds and sea pens until harvest (Battaglene 1999; Agudo 2006; Purcell et al. 2012). A commonality among these approaches is the requirement to source a group of adult, sexually mature individuals (the broodstock) to provide the gametes necessary for successful hatchery production of juvenile sandfish (Battaglene et al. 2002; Agudo 2006; Duy 2010).

Access to broodstocks remains a challenge for many aquaculture production sectors, and this is particularly true where wild populations of the target species are overfished (e.g., Chen et al. 2008; Jimmy et al. 2012; Mies et al. 2017). Among developing island nations in the Pacific region, sandfish mariculture relies on wild-caught broodstocks (Battaglene et al. 1999, 2002; Ramofafia et al. 2003a; Hair 2012; Jimmy et al. 2012; Juinio-Meñez et al. 2012; Ceccarelli et al. 2018; Militz et al. 2018). Reliance on wild-caught broodstocks requires technicians to source sufficient numbers of sexually mature, fecund sandfish from wild populations within a narrow time frame before commencing hatchery production (James et al. 1994; Morgan 2000a, c; Hamel et al. 2001; Jimmy et al. 2012). Sourcing the appropriate quantity of quality of sandfish from wild populations can be impeded by many factors, including overfished populations (Jimmy et al. 2012; Purcell et al. 2013), tenure arrangements (Jimmy et al. 2012; Barclay et al. 2016), government policy (Jimmy et al. 2012), transportation logistics (Battaglene et al. 2002; Bowman 2012), and periodicity in gonad maturation (Morgan 2000b; Battaglene et al. 2002; Ramofafia et al. 2003b). The inability to source sufficient numbers of quality sandfish may result in delays to the production cycle (Pitt and Duy 2003) or loss of natural genetic diversity in resulting progeny (Uthicke and Purcell 2004; Purcell et al. 2012; Nowland et al. 2017).

An alternative to reliance on wild-caught broodstocks is for hatcheries to maintain captive broodstocks in tanks or ponds (Pitt and Duy 2003; Agudo 2006; Bowman 2012; Duy 2010, 2012; Kumara and Dissanayake 2017). The utilisation of captive broodstocks offers several production advantages including reduced investment of time and resources to source wild-caught broodstocks, opportunity to select individual sandfish with desirable hereditary qualities, the potential to condition broodstocks for optimal gamete quality, and ensured access to broodstocks (Zamora and Jeffs 2013). Additionally, the use of captive broodstocks eliminates the need for the continued extraction of sandfish and ensures aquaculture is not contributing to the depletion of wild populations (Pakoa et al. 2012).

Despite the many potential benefits of using captive broodstocks, it remains unclear if captive broodstocks can contribute to successful hatchery production of sandfish to the same extent as broodstocks directly sourced from wild populations. Adult sandfish held in captivity for prolonged periods (≥ 1 month) have been reported to lose reproductive condition, and their capacity to function as broodstock was previously found inferior to newly wild-caught sandfish (Morgan 2000a, c). To further evaluate whether captive broodstocks offer potential to replace wild-caught broodstocks, this study undertakes a provisional assessment comparing hatchery production of sandfish resulting from both captive and newly wild-caught broodstocks.

# Materials and Methods

## *Wild-caught broodstock*

Twenty adult sandfish comprising the wild-caught broodstock source for this study were collected by hand from seagrass beds around Kavieng, New Ireland Province, Papua New Guinea (2.6784°S, 150.7980°W). Individuals were weighed immediately after removal from the water, then wrapped in seawater-soaked cloth before being placed into an insulated container. The mean weight (± SE) of the sandfish was 1.2 ± 0.1 kg (range: 0.6 to 2.4 kg). The sandfish were 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 containing 10 cm of beach sand as a bottom substrate. The raceway was provided with unfiltered seawater (salinity: 36 g $L^{-1}$; temperature: 28 °C) from 12:00 to 15:00 daily, and with continuous gentle aeration. The sandfish were held in the raceway for 2 weeks before spawning induction and fed a suspension of powdered *Spirulina* blended with 1 µm-filtered seawater at a ration of 1 g $m^{-2}$ bottom surface area. The sandfish were fed daily in the morning before the start of water exchange.

## *Captive broodstock*

Twenty sandfish comprising the captive broodstock source for this study were randomly selected from a population of 170 adult sandfish maintained at the NIMRF. Adult sandfish were originally collected from seagrass beds (2.6784°S, 150.7980°W) one year before this study and were maintained in a ca. 400 $m^2$ (22 m × 18 m) marine pond with a water depth of 1.5 m. The pond was constructed using a GSE HPDE pond liner covered with 20 cm of beach sand as a bottom substrate. The pond received no nutrient or feed inputs but did receive a continuous water exchange of 200 to 300 L $min^{-1}$ of unfiltered seawater, sourced from the fringing reef surrounding the research facility. No additional aeration or water agitation was provided to the pond. The mean weight (± SE) of the sandfish selected as the captive broodstock source was 0.6 ± 0.1 kg (range: 0.2 to 1.0 kg). The selected sandfish were transferred to a 2000 L raceway 2 weeks prior to spawning induction. The raceway and sandfish were maintained using the same method described above for the wild-caught broodstock.

## *Spawning induction*

Spawning induction was conducted separately for the wild-caught and captive broodstocks, using the same induction method, a week before the October new moon. This timing corresponds to the seasonal period of enhanced natural spawning reported for sandfish in Melanesia (Ramofafia et al. 2003b). Neither wild-caught nor captive broodstocks were subjected to artificial spawning previously. Spawning induction was initially attempted using thermal stimulation (Battaglene et al. 2002; Agudo 2006; Duy 2010; Militz et al. 2018) in a 200 L spawning tank, where the water temperature was gradually increased, then suddenly reduced over a temperature range of 27 to 34 °C. This procedure was repeated three times between 10:00 and 16:00. Lack of response to thermal stimulation proceeded the next day with spawning induction using food stimulation (Agudo 2006; Militz et al. 2018), where broodstocks were exposed to a high concentration of powdered *Spirulina* (0.1 g $L^{-1}$) in the spawning tank. Spawning of the captive broodstock occurred within 3 h of exposure to *Spirulina*, with four sandfish contributing spermatozoa and two sandfish spawning eggs. Spawning of the wild-caught broodstock occurred 5 h after exposure to *Spirulina*, with six sandfish contributing spermatozoa and two sandfish spawning eggs.

The broodstocks were removed from their respective spawning tanks when spawning activity notably declined. Spawning tanks containing gametes were left undisturbed for a further 2 h but were supplied with gentle aeration during this time. Three replicate aliquots were removed from each spawning tank, and 100 eggs from each were examined to determine fertilisation rate (Militz et al. 2018). Fertilised eggs were then collected in a submerged 90 µm mesh basket through which 1 µm-filtered seawater flowed to remove residual *Spirulina*, before transfer to 20 L containers. The density of harvested eggs was then estimated using three replicate Sedgewick-Rafter counts before they were stocked into 450 L (effective volume 350 L) larval rearing tanks at a density of 0.3 eggs $mL^{-1}$ (Agudo 2006; Militz et al. 2018). Six replicate larval rearing tanks were stocked with cleaned eggs sourced from the captive broodstock and a further six replicate larval rearing tanks were stocked with cleaned eggs sourced from the wild-caught broodstock. All larval rearing tanks were supplied with continuous gentle aeration.

## Larval rearing

Larval rearing methodology followed that of Militz et al. (2018) for hatchery production of sandfish using micro-algae concentrate products, the methodology based on an extension of general protocols for the hatchery culture of sandfish (Battaglene 1999; Agudo 2006; Duy 2010). Two commercially-available micro-algae products (Instant Algae®, Reed Mariculture Inc.) were used in this study; Isochrysis 1800® (mono-culture *Isochrysis* sp.) and Shellfish Diet 1800® (a mixture of 3 % *Chaetoceros calcitrans*, 30 % *Isochrysis* sp., 13 % *Pavlova* sp., 19 % *Tetraselmis* sp., 30 % *Thalassiosira pseudonana*, and 6 % *Thalassiosira weissflogii* on a dry weight basis). Both products were purchased from an Australian distributor and imported to Papua New Guinea. Microalgae concentrates were prepared for use and dispensed to larval culture tanks as described by Militz et al. (2018), and daily micro-algae rations (Table 1) were split between two daily feeds at 09:00 and 16:00. Water temperature, salinity and dissolved oxygen levels in larval culture tanks were maintained at 26-27 °C, 36-37 g $L^{-1}$, and > 4.8 mg $L^{-1}$, respectively, throughout the study.

Table 1. Feeding regime (cells $mL^{-1}$) for *Holothuria scabra* larvae and juveniles during hatchery production. Daily rations for each Instant Algae® product were divided and fed to larvae twice daily. Adapted from Militz et al. (2018).

| 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 | 5,000 | 20,000 |
| 25-40 | - | 30,000 |

## Data collection and analysis

Larval development was monitored daily until the first settlement was observed. Larvae were collected from culture tanks using a 90 µm mesh screen and fixed using dropwise additions of formalin before microscopic examination. One hundred embryos or larvae were examined at 40× magnification and their development stage classified (Militz et al. 2018). Morphometric measurements of embryonic, larval, and juvenile developmental stages were also undertaken on 0, 2, 4, 12, and 40-days post-fertilisation. Unfertilised eggs were collected on release from the gonopore of spawning sandfish. Developing larvae were measured opportunistically after assessing development. Measurements of egg diameter and total larval length of the different larval stages (Duy et al. 2016) were taken from 50 replicate specimens at each point in time using the digital microscopy measurement software ToupView (Version 3.7.2270). Juvenile sandfish were measured using a vernier calliper at 40-days post-fertilisation.

Survival was monitored temporally in four replicate larval rearing tanks for each of the two treatments. Survival estimates were undertaken on 1, 2, 4, 7, 16, and 40-days post-fertilisation by determining larval density in a known volume of water (50 mL) as a proportion of the initial stocking density (0.3 eggs $mL^{-1}$). At 40-days post-fertilisation, the total number of juvenile sandfish was calculated by counting juveniles present on half of the total surface area available to them within each larval rearing tank. Survival was determined as the number of juvenile sandfish as a proportion of the eggs initially stocked into each tank (105,000 eggs).

Estimates of fertilisation rate were compared between the two treatments with $\chi^2$ analysis. For both treatments, growth rates during hatchery culture were determined by developing a linear regression model that employed heteroscedasticity-consistent covariance matrices derived using the *R* function *vcovHC* (package *sandwich*) in general linear hypothesis tests (function *glht*; package *multcomp*) to account for the increasing heteroscedasticity in total length as the sandfish larvae aged. The variance-mean ratio was further constrained by $\ln_e$ transformation of the predicting variable (days) before fitting the linear regression model.

For both treatments, survival over the hatchery cycle was modelled as an exponential 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 models (*R* function *lme*; package *nlme*).

## Results

The mean (±SE) diameters of eggs spawned by wild-caught (164.7 ± 1.0 µm) and captive (162.9 ± 1.6 µm) broodstocks were similar ($t_{2,98}$ = 0.92, *P* = 0.36) as were the lengths of spermatozoa released from wild-caught (52.2 ± 0.3 µm) and captive (52.1 ± 0.3 µm) broodstocks ($t_{2,98}$ = - 0.05, *P* = 0.96). The resulting fertilisation success of gametes from the wild-caught (99.0 ± 0.6 %) and captive (97.3 ± 0.7 %) broodstocks were also similar ($\chi^2$ = 0.13, *P* = 0.71).

Embryos and larvae progressed in development from gastrula, to auriculariae larvae, to doliolaria larvae, and then pentactula larvae before final metamorphosis into juvenile sandfish. The auriculariae stage was further divided into the commonly acknowledged early-, mid-, and late-auriculariae stages (Agudo 2006; Duy 2010; Militz et al. 2018). Developmental progression occurred more rapidly in larvae resulting from captive broodstock with hyaline spheres, indicative of late-auriculariae, first observed 9-days post-fertilisation, doliolariae were first observed at 12-days post-fertilisation, and the first settlement was observed 16-days post-fertilisation (Fig. 1). In contrast, larvae resulting from wild-caught broodstock achieved these development milestones on 12, 13, and 17-days post-fertilisation, respectively (Fig. 1).

The faster developmental progression was not reflective of faster growing larvae (in terms of total length) resulting from the captive broodstock. The fitted logistic growth models demonstrated statistically similar intercepts (*t* = 0.37, *P* = 0.98) and growth rates (*t* = 1.70, *P* = 0.24) from hatching until 12-days post-fertilisation for the two trials. The equation *length* = 301.0 × ln (*day* +1) + 207.6 adequately modelled growth during this period of hatchery culture for both trials (Fig. 2). This model accounted for 92 % of the variance among total larval lengths of both trials ($R^2$ = 0.92; Fig. 2). At 40-days post-fertilisation, lengths of juvenile sandfish resulting from wild-caught (3.26 ± 0.13 mm) and captive (3.28 ± 0.16 mm) broodstocks were comparable ($t_{2,98}$ = 0.10, *P* = 0.92).

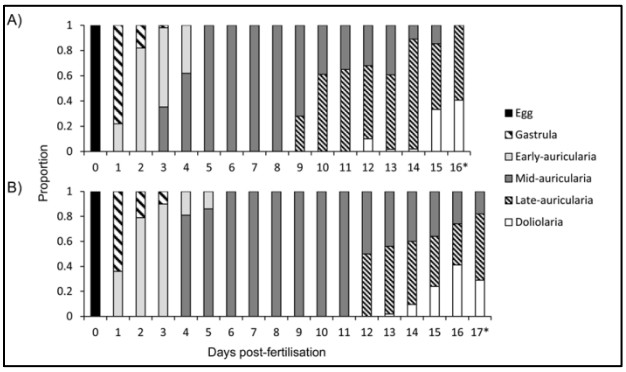


Fig. 1. Development progression of *Holothuria scabra* larvae in hatchery cultures established with gametes from (A) captive and (B) wild-caught broodstocks. Proportions are reported with respect to the given day post-fertilisation. Asterisks (*) indicate the start of settlement.

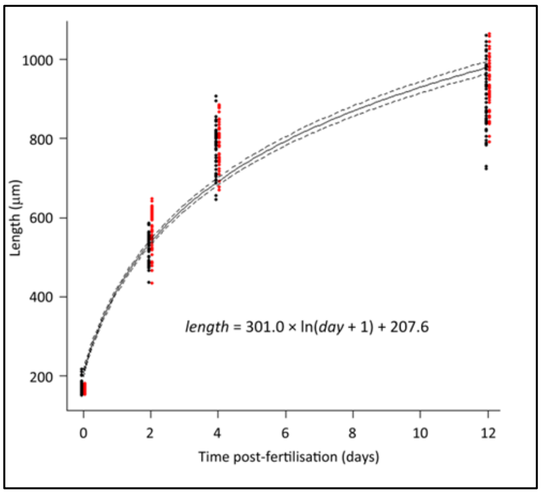


Fig. 2. Temporal change in lengths of *Holothuria scabra* auriculariae cultured from captive (red) and wild-caught (black) broodstocks. The solid line represents the logistic function describing total larval length with an approximate 95 % confidence interval (dashed lines).

Survival over the entire hatchery cycle (i.e., 40-days post-fertilisation) was comparable for larvae from wild-caught (1.23 ± 0.27 %) and captive (1.34 ± 0.73 %) broodstocks ($t_{2,6}$ = 0.15, *P* = 0.89). The fitted survival models demonstrated significantly different intercepts (*F* = 11.37, *P* < 0.01) between the two broodstock sources, but had similar slopes (*F* = 0.76, *P* = 0.39; Fig. 3), and accounted for 57 % of the variance among survival estimates ($R^2$ = 0.57; Fig. 3). The models identified an earlier onset of mass mortality in hatchery cultures established from the captive broodstock, but by 40-days post-fertilisation there was no difference in survival between trials.

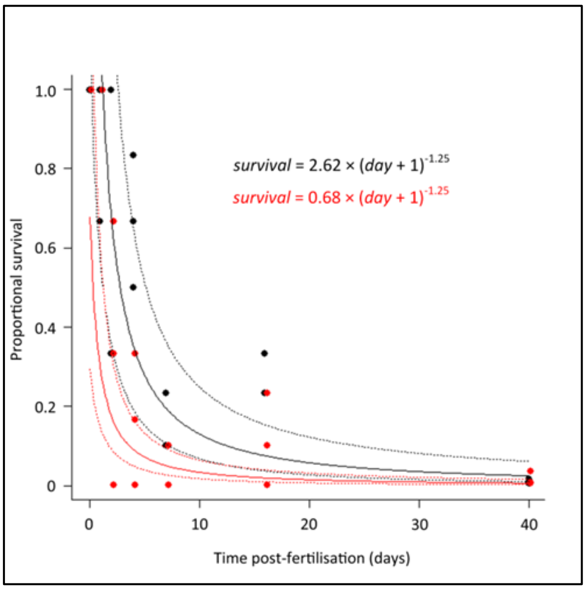


Fig. 3. Proportional survival of *Holothuria scabra* cultured from captive (red) and wild-caught (black) broodstocks to 40-days post-fertilisation. Solid lines represent the respective exponential function describing survival with approximate 95 % confidence intervals (dashed lines).

# Discussion

This study demonstrated the potential in using captive broodstocks for hatchery production of sandfish. The results show that broodstock sourced from a marine pond after a year in captivity were able to support comparable sandfish hatchery production to that achieved with broodstock collected directly from the wild. The results reported here indicate that technical difficulties associated with sourcing wild sandfish as broodstocks (Jimmy et al. 2012) could be eliminated or reduced, through the use of broodstocks maintained in captivity, without compromising hatchery production.

## *Culture performance*

Hatchery culture progression of larval and juvenile sandfish resulting from the gametes of captive and wild-caught broodstocks differed, and survival of embryos and auriculariae took different trajectories. Larvae resulting from the captive broodstock experienced a more rapid onset of mortality, with substantial mortality occurring during embryo and early-auriculariae development. The rapid onset of mortality occurred despite comparable egg size and fertilisation success of gametes from either broodstock source. This disparate early survival may, therefore, reflect differences in the quality, rather than quantity, of maternal reserve partitioning in the eggs (Peters-Didier and Sewell 2017), and identifies assessment of egg quality among broodstock sources and its relationship to hatchery production as an avenue for further research.

Despite differences in culture progression and survival trajectories, production metrics of juvenile sandfish resulting from both broodstock sources were similar. Early survival of sandfish larvae was not a determinant of hatchery performance post-settlement, as reported elsewhere (Ramofafia et al. 2003a). Given that environmental conditions in which sea cucumber broodstocks are maintained has a direct bearing on the environmental tolerance of progeny, superior hatchery culture performance of juveniles resulting from the captive broodstock was anticipated. However, a number of abiotic and biotic environmental factors influence sandfish survival during hatchery culture (Ramofafia et al. 2003a; Knauer 2011; Duy et al. 2016), and it is possible that established hatchery culture methods are not yet sufficiently optimised (Purcell et al. 2012; Militz et al. 2018) for the source of broodstocks to have a measurable effect on hatchery production.

When considering overall hatchery performance, our study further validates the effectiveness of using micro-algae concentrate products for complete replacement of live, cultured micro-algae for hatchery culture of sandfish (Duy et al. 2015, 2016, Militz et al. 2018). The hatchery culture method employed in the present study achieved comparable production of juvenile sandfish, 40-days post-fertilisation, to past studies employing micro-algae concentrate products (Militz et al. 2018) and live, cultured micro-algae (Ramofafia et al. 2003a; Agudo 2006; Gamboa et al. 2012; Juinio-Meñez et al. 2012; Purcell et al. 2012).

## *Application*

More than fifteen hatcheries actively produced sandfish over the last decade (Purcell et al. 2012), of which the majority were located in regions of overexploited or depleted sea cucumber stocks (Purcell et al. 2013). In the Pacific, most of these sandfish production centres rely on wild-caught broodstocks (Battaglene et al. 1999, 2002; Ramofafia et al. 2003a; Hair 2012; Jimmy et al. 2012; Juinio-Meñez et al. 2012; Ceccarelli et al. 2018; Militz et al. 2018), despite this approach to broodstock management being inherently limited in supporting sea cucumber aquaculture expansion (Zamora and Jeffs 2013). Reliance on wild-caught broodstocks prevents the introduction of selective breeding through careful selection of individuals from superior-performing aquaculture stock. For locations with seasonal spawning periodicity, reliance on wild-caught broodstocks also limits hatchery activities to natural breeding seasons (Morgan 2000b; Battaglene et al. 2002; Ramofafia et al. 2003b), resulting in inefficient use of hatchery infrastructure. Maintenance of captive broodstocks provides an opportunity to address these limitations in sandfish aquaculture, and may have further advantages where reliable access to wild-caught broodstocks is restrained by overfished populations (Jimmy et al.

2012; Purcell et al. 2013), tenure arrangements (Jimmy et al. 2012; Barclay et al. 2016), government policy (Jimmy et al. 2012), or transportation logistics (Battaglene et al. 2002; Bowman 2012).

Hesitation from hatchery facilities to allocate infrastructure and resources to maintain captive broodstocks is understandable given the lack of demonstrable hatchery performance. For this reason, successful hatchery production of sandfish from a captive broodstock is a compelling result and may provide an incentive for sandfish production centres to establish captive broodstocks instead of relying solely on wild-caught broodstocks (e.g., Bowman 2012; Duy 2012). The comparable hatchery performance achieved using captive and wild-caught broodstocks reported in this study shows that use of a captive broodstock will not necessarily impair sea cucumber hatchery production. This assertion applies to the protocol of this study, were adult sandfish were maintained at a low-latitude location in a small marine pond without environmental manipulation or supplementary food inputs. Ponds appear ideal for maintaining appropriately-sized sandfish broodstocks because access to the broodstocks, security, and control of environmental conditions are superior to ocean-based holding enclosures (Duy 2010; Kumara and Dissanayake 2017). Tank-based habitat simulators (e.g., SPC 2014) are an alternative means of maintaining broodstocks in captivity, though varying degrees of success have been reported for the long-term maintenance of broodstocks in tank-based systems (Morgan 2000a, c; SPC 2014).

The results of the present study encourage examination of possibilities to maximise the capacity in which captive broodstocks can contribute to the developing sandfish mariculture sector. For example, manipulating the environmental conditions under which a captive broodstock is held may lead to improvements in gamete quantity and quality, and provide control over spawning periodicity (Morgan 2000a, c; Zamora and Jeffs 2013; SPC 2014; Peters-Didier et al. 2017), leading to improved hatchery production. However, such benefits will only be possible with improved knowledge of the conditions required for conditioning captive broodstocks (Morgan 2000a, c).

## Conclusion

As wild stocks of sea cucumbers become increasingly exploited (Purcell et al. 2016), reduced reliance on wild-caught broodstocks will be required to support sustainability in this developing aquaculture sector. The provisional assessment undertaken in this study has identified value in thoroughly evaluating the capacity for captive broodstocks to replace wild-caught broodstocks in the production of sandfish. Future research avenues include determination of optimal conditions for sandfish broodstocks in captivity, temporally replicating performance studies comparing sources of broodstocks, establishing the extent to which egg quality influences hatchery production, and cost-benefit analyses on maintaining broodstocks in captivity. As these areas of research are addressed, hatchery managers will be better able to assess the advantages and disadvantages of maintaining captive broodstocks for sandfish aquaculture.

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