# Propagation and sea-based growout of sea cucumber stocks in the Northern Territory

**William Bowman**
**Luke Turner**
**Grant Leeworthy**
**Ann Fleming**
**Jens Knauer**
**Michelle Simoes**
**Andrea Birch**
**Mike Gardner**

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**William M. Bowman[1], Luke H. Turner[1], Grant R. Leeworthy[1], Ann Fleming[2], Jens Knauer[2], Michelle Simoes[2], Andrea L. Birch[2], Mike Gardner[3]**

[1] Tasmanian Seafoods P/L PO Box 38795 Winnellie, NT 0820

[2] Darwin Aquaculture Centre PO Box 3000 Darwin, NT 0801

[3] Flinders University School of Biological Sciences GPO Box 2100, Adelaide, SA 5001



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Office Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042
Postal Box 26, Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042
Tollfree 1300 732 213 Phone 08 8201 7650 Facsimile 08 8201 7659
Website www.seafoodcrc.com ABN 51 126 074 048



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**Propagation and sea-based grow-out of sea cucumber stocks in the Northern Territory**

**PRINCIPAL INVESTIGATOR**: William Bowman

**ADDRESS:** Tasmanian Seafoods P/L
PO Box 38795, Winnellie, NT 0820
+61 (4) 00464194
william.bowman@bigpond.com

**PROJECT OBJECTIVES:**

1. Increasing survival of juvenile sandfish production (to average size 25mm) from 5-10% to 30% through manipulative experiments trialling settlement materials, food availability and food type at settlement.

2. Develop a cost-effective system for nursery production of juvenile sandfish measuring growth, survival and labour costs using different nursery systems including raceway systems, open ponds and hapa nets.

3. Develop innovative new methods for releasing and harvesting juvenile sandfish (*H. scabra*) from sea-based growout systems.

4. Demonstrated evidence of the effectiveness of sea ranching through trial releases of juvenile sandfish at Groote Eylandt.

5. Use information gained in larval/nursery production and sea-based production to develop a bio-economic model for sandfish production/ranching.

6. Analyse parental contributions to progeny from commercial scale pooled spawnings of *H. scabra.*

7. Develop breeding protocols that meet the requirements of the genetic management plan.

**OUTCOMES ACHIEVED / ANTICIPATED**

1. Establishment of a pilot hatchery and nursery system to produce up to 300 000 juveniles p.a. for sea ranching.

2. Cooperation with an indigenous corporation on Groote Eylandt leading to a viable ranching system.

3. Planned expansion of ranching activity across at least two more sites in the territory over the next 2-5 years ($120,000p.a.).

**LIST OF OUTPUTS PRODUCED**

1. Site specific Hatchery Manual suitable for large scale production

2. Bio-economic model for sea ranching sandfish

3. Hatchery genetic management plan for NT sea ranching

4. Hatchery capacity and SOPs that enable hatchery runs for ranching to meet the requirements of the genetic management plan

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# 1. Introduction and Background

Sea cucumbers have been fished for the Asian food market world-wide for centuries, with the sandfish (*Holothuria scabra*) being one of the most valuable of these species (van Eys, 1986; Akamine, 2002; Wolkenhauer *et al*, 2010). Increasing demand for dried body wall of sea cucumber, known as bêche-de-mer or 'trepang', has seen depletion of this resource in the traditional fishing grounds close to Asia, and more recently the expansion of the industry to new and distant fishing grounds (Toral-Granda *et al*, 2008).

In the Northern Territory, Australia harvesting of the sea cucumber resource is currently managed by Government through a licencing arrangement that limits effort. It is known as the 'Northern Territory Trepang Fishery'. The Trepang Fishery is likely to be Australia's first export industry, dating back to as early as the 1700s when Macassan fisherman from Celebes (Sulawesi, Indonesia) visited northern Australia to harvest sea cucumber. Government records from 1882 to 1904 reveal that annual catches averaged at least 1000t wet weight of sea cucumber before the catch rate went into a significant decline in response to Government policy limiting resource access to foreign fishers and the impacts of conflicts in Asia (MacKnight, 1976).

Surveys of the sea cucumber resource carried out by the Northern Territory Department of Fisheries (Vail, 1989) led to renewed interest in the fishery, and in 1992 the NT Trepang Fishery was re-established with the NT Fisheries Department adopting a precautionary approach to the management of the fishery, limiting the number of licences, harvest methods, and area of the fishery. The legal jurisdiction of the fishery is from the NT coastline and surrounding islands to 3 nautical miles (nm) seaward of baselines and currently has one operator owning all licences.

Tasmanian Seafoods Pty Ltd (TSF) has been very successful in developing the viability of harvesting, processing, and marketing of sea cucumber products in Australia. Over the last 20 years they have secured a large portion of the Australian wild fishery licenses for this industry, particularly in the Northern Territory and Western Australia where the sea cucumber (*Holothuria scabra*; common name - sandfish) is the dominant species. There are currently around eighteen companies (exporters and contracted harvesters) involved in sea cucumber production in Australia with TSF having the largest interest (between 300 and 400t of product per annum across multiple species). TSF has outlaid significant investment on improving the processing and value adding of the high value species including sandfish. The beach price for sandfish ranges from $3.50-$10.00/kg depending on product form (fresh/ gutted or gutted/ boiled) and logistics of delivery. With a 10:1 processing recovery from gutted/boiled to final dried product, the final processed price can reach $22/kg (beach weight price equivalent). TSF has developed its post-harvest processing systems to a point where they can now achieve a high quality product that brings premium prices on world markets. TSF has more recently, through the Australian Seafood CRC, been investing in innovative ways to increase the overall production capacity of its business.

Releasing sufficient cultured juveniles into the wild to reach the carrying capacity of the habitat is seen as a way to rebuild depleted stocks and potentially to increase harvests beyond historical levels (Munro & Bell, 1997; Battaglene & Bell, 1999; Battaglene, 1999). In commercial hatcheries barriers to the production of tropical sea cucumbers including high mortality rates during larval settlement and the early juvenile stages have hampered large-scale production (Giraspy & Walsalam, 2010).

During the nursery production stage the extreme variability in growth (Gamboa *et al*. 2012; Juinio-Meñez *et al*., 2012; Pitt & Duy, 2004), and uncertainty around feeding requirements (Purcell *et al.* 2012) are also problematic for large scale juvenile production.

Before this project the company had established a pilot scale hatchery and nursery facility for sandfish at the Darwin Aquaculture Centre, successfully producing up to 20,000 juveniles/year. The Australian Seafood CRC project was undertaken to improve hatchery production and significantly scale up to a commercially viable operation, and to assess the feasibility of raising harvest rates through stock enhancement.

## 1.1 Need

Tasmanian Seafoods has identified stock enhancement as a means to improve the viability of sea cucumber harvesting operations in Northern Australia. Successful enhancement of the fishery has the potential increase catches, reduce harvesting time, and improve the operational efficiency and management of the sea cucumber harvesting business in the Northern Territory.

Considerable work has been published on suitable methodologies for the hatchery and nursery production of sandfish (Agudo, 2006). However, much of this work has been developed by hatcheries in the Asia-Pacific region where there is extreme variability in survival and growth. While much research has been published recently on methods for scaling up the production of temperate species of sea cucumber, the development of technology for culturing tropical species has lagged (Purcell *et al.* 2012). Developing improved hatchery and nursery production protocols will compliment a commercial stock enhancement operation through increasing survival rates, increasing production capacity, and ensuring continuity in production year round.

## 1.2 Objectives

1. Increase survival of juvenile sandfish production (to average size 25mm) from 5-10% to 30% through manipulative experiments trialling settlement materials, food availability and food type at settlement.

2. Develop a cost-effective system for nursery production of juvenile sandfish measuring growth, survival and labour costs using different nursery systems including raceway systems, open ponds and hapa nets

3. Develop innovative new methods for releasing and harvesting juvenile sandfish (H. scabra) from sea-based growout systems

4. Demonstrate the effectiveness of sea ranching through trial releases of juvenile sandfish at Groote Eylandt.

5. Use information gained in larval/nursery production and sea-based production to develop a bio-economic model for sandfish production/ranching.

6. Analyse parental contributions to progeny from commercial scale pooled spawnings of *H. scabra.*

7. Develop breeding protocols that meet the requirements of the genetic management plan.

# 2. Methods

## 2.1 Standard Hatchery Production Methods

The following section is a summary of the standard sandfish production methods used. Detailed hatchery protocols can be found in appendix 1. Unless otherwise stated these standard methods were used for trials in this project. The four stages of production and a summary of the research trials undertaken during this project at each are shown in figure 1.

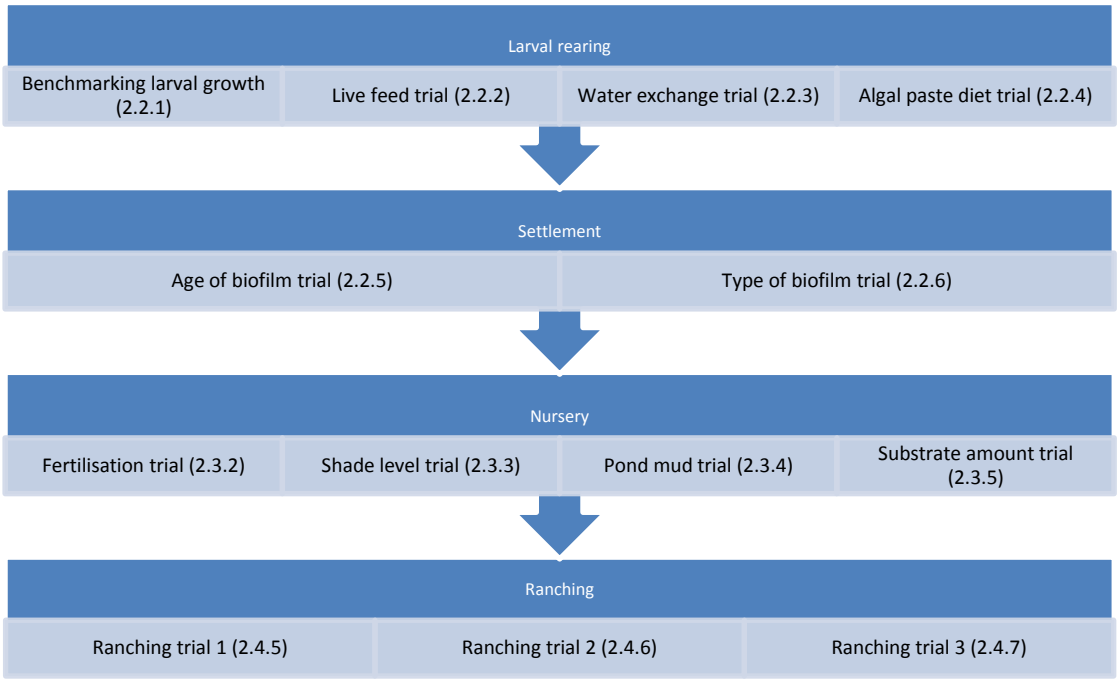



**Figure 1.** Summary of the sequence of experiments undertaken during the project. References to the relevant methods sections are given in parentheses

### *2.1.1 Broodstock Collection*

Broodstock were collected from the intended ranching site to maintain genetic integrity – by hand, using divers. Broostock were maintained in earthen ponds at Darwin, NT for the duration of the project.

### *2.1.2 Spawning*

All larval production took place in the Tasmanian Seafoods hatchery located at the Darwin Aquaculture Centre. Larvae were produced by spawning broodstock using thermal stimulation; methods are outlined in the 'Hatchery Manual' (Appendix 1.). Embryos were collected and held in a 1000L tank of 1 µm, UV-filtered seawater for 48 h prior to stocking.

### 2.1.3 *Larval Rearing*

Larval rearing tanks were supplied with UV sterilised filtered seawater and light aeration throughout larval rearing. The larvae are pelagic for the first 10 to 14 days (auricullariae) (Fig. 2). During the pelagic phase, 30% of the culture water was exchanged daily with larvae fed on the diatom *Chaetoceros muelleri*. The feeding rate started at 10,000 cells mL-1 on day 2 and was increased by 2,500 cells mL-1 each day to a maximum of 30,000 cells mL-1.

After 10-14 days auricullariae reduce their body size and eventually form a barrel-shaped doliolaria (Fig. 2). Once a significant number of doliolariae were observed in the culture tanks, settlement substrates (mussel rope) were added to the larval tanks.

Unless stated otherwise, larvae were measured using a calibrated graticule fitted to a compound microscope. Larvae were placed on a glass slide without a cover slip and measured along the longest axis.

After settlement substrate was added, *C. muelleri* was still added daily at a rate of approximately 30,000 cells mL-1 to account for slow developing larvae. Once larvae transform into the final larval stage, the pentactula (Fig. 2), and all larvae had disappeared from the water column, they were considered settled and tanks were placed on flow-through with no screens at a rate of 200%/day.

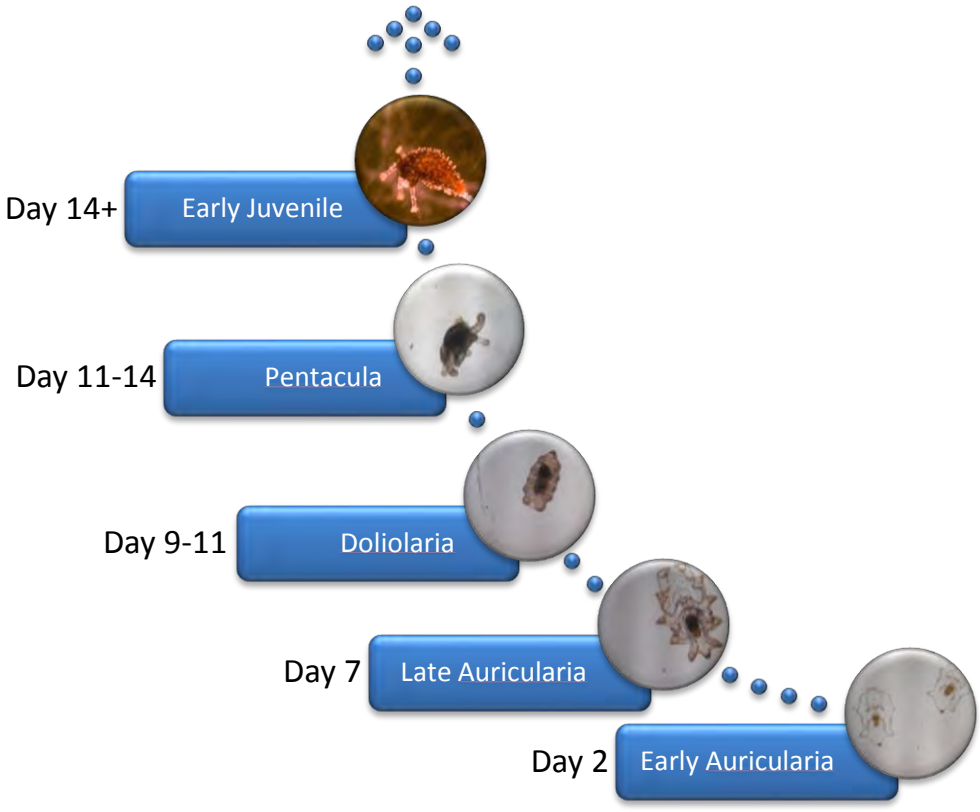



**Figure 2.** Schematic of the larval stages during the hatchery culture of *H scabra.*

Newly settled juveniles (Fig. 2) were supplied with supplementary feed including benthic diatoms (Navicula spp., Nitzschia spp.), Algamac-Protein Plus, and Spirulina.

Once the settled juveniles reached an appropriate size (2-10mm in length) they were harvested from the larval rearing tanks and transferred to Nursery systems. Juveniles were harvested by detachment from tank surfaces and mussel ropes using 1% KCL in seawater solution (Battaglene and Seymour, 1998) and collected on a 250um screen. The collected juveniles were then counted volumetrically by suspending the detached juveniles in 20L container and taking 3 replicate samples of 100ml. The total number of juveniles was estimated by multiplying the average number of juveniles in each replicate sample by 200.

Unless stated otherwise, juvenile sea cucumber were measured using a dissecting microscope fitted with a calibrated graticule from the most anterior part of the body wall to the most posterior part of the body wall.

Juvenile mass was estimated by first blotting dry on filter-paper and then weighing on an analytical balance.

#### 2.1.4 *Nursery*

Juveniles were grown to 1-5g for sea ranching trials in nursery systems consisting of 1000-20000l tanks on flow-through sand filtered seawater under ambient conditions. Juveniles in the nursery systems fed on natural biofilms and were not supplied with additional nutrients unless stated otherwise.

### 2.2 Larval rearing experiment methods

To address objectives 1 and 2 of this project, increasing cost-effective juvenile sandfish production, trials were designed to address some of the constraints on production. Larval rearing experiments were designed to improve feeding regimes, water exchange protocols and improve survival at settlement. Nursery experiments investigated substrate type and fertilisation of substrates on growth and survival in nursery systems.

The following sections explain the rationale behind each trial and detail the methods used where they differ from the standard hatchery methods described above.

#### *2.2.1 Growth, development and size separation of H. scabra larvae.*

Hatchery techniques for sandfish are well established and have been summarised in a number of manuals (James et al. 1994, Agudo 2006, Duy 2010). Generally, fertilised eggs are siphoned from the spawning tank and transferred to larval tanks for hatching. During the entire larval cycle of approximately 12 to 14 days, all larvae are mixed and cultured in one tank irrespective of developmental stage. Once a certain number of doliolariae are observed in a larval tank, settlement substrates are added to facilitate settlement and metamorphosis into juveniles. This means that during the later stages of larval development mid and late auriculariae, early and advanced doliolariae, pentactulae and early juveniles are all mixed in the same tank despite their very different requirements in terms of quantity and quality of food. This ‘one tank fits all’- approach differs completely from larval rearing protocols in other aquaculture industries such as, e.g., South Sea pearl oysters, *Pinctada maxima*.

During a larval run, *P. maxima* larvae are continuously separated according to size and, furthermore, larvae approaching settlement are transferred to separate settlement tanks. This has resulted in significant improvements with regards to efficiencies of larval production (J. Knauer, unpublished data).

To benchmark larval growth during a hatchery run and develop techniques to enable separation or larvae at different stages of development, 2 day old larvae were removed from the hatching tanks by draining. Hatching efficiency was calculated based on the number of remaining larvae on day two compared to the number of initial fertilised eggs. The resulting early auricullariae were transferred to either 1t or 0.45t larval tanks at a density not exceeding 1 larva $mL^{-1}$. Larvae were fed twice daily with bag-cultured *Chaetoceros muelleri* at the standard hatchery feed rate. Following the initial stocking, all larval tanks were drained every second day through different sized mesh screens, separating larvae according to size until day 12, when doliolariae started to develop.

Doliolariae were transferred to settlement tanks that were pre-conditioned by adding a mix of dried *Spirulina* sp. and a plant fertiliser for one week prior to introducing doliolariae. Each settlement tank was provided with four pre-conditioned settlement substrates made from black shade cloth. Once doliolariae disappeared from the water column, all settlement tanks were put on flow-through. Fertilisation using *Spirulina* sp. and a plant fertiliser continued on a daily basis for a further four weeks. On days 42 to 44 all settlement tanks were drained and the number of juveniles counted.

### *2.2.2 Growth and survival of Larval Sandfish fed different Microalgae*

Generally, sea cucumber hatcheries tend to feed larvae diets comprising a number of micro-algal species mixed in different proportions. Depending on the sea cucumber species cultured, the importance of either feeding predominantly *Dunaliella euchlaia* and *Chaetoceros muelleri* (Xilin 2004), or *Rhodomonas sp*. and *Dunaliella sp*. (Mercier et al. 2004) has been emphasised. Similarly, diets comprising a number of micro-algae such as *Chaetoceros spp*., *Isochrysis galbana* (probably *I. aff. galbana* clone T-ISO), *Pavlova salina*, *R. salina*, *Skeletonema spp*. and *Tetraselmis spp*. have been used in varying proportions for culturing larval *H. scabra* (Battaglene et al. 1999; Pitt 2001; Ramofafia et al. 2003; James 2004; Pitt and Duy 2004; Agudo 2006).

Little is known about the relative importance of individual species of micro-algae to larval sea cucumbers. For example, *I. galbana* was found to be the most suitable single-species diet for culturing deep-water redfish, *Actinopyga echinites* (Chen and Chian 1990) and *H. scabra* (James et al. 1994b; James 1999; Morgan 2001). A single-species diet comprising *C. calcitrans* produced the same growth as a mixed, micro-algal reference diet when fed to *S. japonicus* (Ito and Kitamura 1997) and *H. spinifera* (Asha and Muthiah 2006).

Given the contradictory nature of the studies on the nutritional value of micro-algae to sea cucumber larvae it was the aim of this study to assess the effect of *C. muelleri*, *C. calcitrans*, T-ISO, and *P. salina* on growth and survival of *H. scabra* larvae. The flagellate *P. salina*, besides the other three more commonly used species, was chosen since it is easily cultured and known to have a high nutritional value to other

invertebrate larvae such as bivalves and zooplankton (Brown et al. 1997; Knauer and Southgate 1999). Additionally, the effect of a mixed diet comprising *C. muelleri*, T-ISO and *P. salina* on growth and survival of *H. scabra* larvae was evaluated.

Four species of microalgae were used as feed*: Chaetoceros muelleri, Chaetoceros calcitrans, Isochrysis aff. galbana* (T-ISO), *Pavlova salina*, or a ternary microalgal diet (TMD) comprised of 40% *C. muelleri*, 40% T-ISO, and 20% *P. salina.* All microalgal cultures were produced at 24 degrees C under an 18h: 6h light: dark cycle light regime using f/2 medium (Guillard, 1975). Only cultures in the exponential growth phase were used to feed larvae.

Larvae were produced in the Tasmanian Seafoods hatchery and at day 2 were stocked into three replicate 10L aquaria (5000 each) randomly allocated for each diet and an unfed control. All microalgae were fed on an equal dry weight basis and aquaria were lightly aerated with natural photoperiod and water temperature varying from 27.5 to 31.5 °C.

A complete water exchange was carried out every second day and aquaria were washed with 10% sodium hypochlorite solution, thoroughly rinsed with freshwater, then and refilled with 1μm, UV-filtered seawater. To determine growth, length was measured on Days 4, 6, and 8 by randomly selecting 30 larvae, and survival was estimated on Days 4, 8, and 12 by counting the larvae in duplicate 30ml samples taken from each aquaria. Larval biomass was calculated as mmtank$^{-1}$ for each tank on day 8 as a representation of overall productivity using the formula

$$Length\left(Survival \times \frac{5000}{100}\right)$$

The experiment was terminated on Day 12.

### 2.2.3 *Effect of different water exchange protocols on growth and survival of H. scabra larvae.*

The standard protocol for exchanging water in larval tanks in the sandfish industry is a daily, partial exchange of 20 – 30% (Agudo 2006, Duy 2010). In contrast, in the bivalve industry a 100% water exchange is done every two to three days. It was the aim of this study to assess the effect of different water exchange protocols on growth, development and survival of larvae cultured at a low (0.1 larvae mL-1) and high (0.5 larvae mL-1) stocking density.

Nine 100L tanks were set up in a randomised block design in a constant temperature room. Each tank was filled with 1μm-FSW and stocked with either 50,000 (Experiment 1: 0.5 larvae mL-1) or 10,000 day 2 larvae (Experiment 2: 0.1 larvae mL-1). The size (length) at stocking was 431.4±6.8μm and 436.3±8.4μm, respectively (n=30).

In both experiments, the water exchange treatments/protocols evaluated were (i) a daily 30% water exchange, (ii) a batch exchange every second day and (iii) a batch exchange every fourth day with three replicate tanks per treatment. At each water exchange, the water was siphoned from the tank and the larvae were collected on a 90μm mesh and then transferred to a 20L holding bucket. With the exception of the tanks undergoing a daily 30% water exchange, each tank was thoroughly cleaned with freshwater before refilling with 1μm-FSW. The diatom *Chaetoceros muelleri* was

added twice daily to all tanks. The photoperiod throughout the experimental period was 14hL: 10hD.

The length of 30 larvae in each tank was measured on day 5 and day 9 in Experiment 1 and day 5 and day 8 in Experiment 2. At the termination of the experiments on day 15 (Experiment 1) and day 12 (Experiment 2), the developmental stage of 100 larvae was assessed and survival estimated. The length data were analysed using ANOVA and survival and developmental stage data were analysed by a Generalised Linear Model with binomial errors. Distribution and variance assumptions were checked with residual analysis, and pairwise comparison of the means for all models was done using Tukey's procedure. Results are expressed as mean ±SE and are considered significant at P≤0.05.

### *2.2.4 Nutritional value of the algal paste Thalassiosira weissflogii as a larval diet for H. scabra*

Due to the costs associated with live algae culture it is commercially desirable to replace live micro-algae with alternative or artificial, off-the-shelf diets. One such potential alternative for *H. scabra* is the diatom *Thalassiosira weissflogii* (TW), which is available as an algal paste (Instant Algae®, Reed Mariculture, Inc, USA). The nutritional profile of TW is very similar to *C. muelleri* and may be an appropriate replacement but, so far, this has only been supported by one anecdotal report (Hair et al. 2011).

This particular algal paste was chosen as its nutritional profile is similar to *C. muelleri*, and it has been widely used in finfish, shellfish and shrimp hatcheries (www.reedmariculture.com). To evaluate the nutritional value of the algal paste as a partial and total replacement of live *C. muelleri*, larvae were either fed live *C. muelleri* (100%C), *C. muelleri* partially replaced with *T. weissflogii* (75%C:25%T, 50%C:50%T and 25%C:75%T) or *T. weissflogii* only (100%T).

Three replicate 100 L tanks were stocked with 41,000 two day old larvae each (0.4 larvae $mL^{-1}$) and fed twice a day. All tanks were filled with 1 µm-and UV-FSW and were randomly allocated to each experimental diet.

On day 10 a sample of the larvae collected and the length of 50 randomly selected larvae from each tank was then measured to calculate growth rate (µm $day^{-1}$). Survival was calculated on day 14 by counting the larvae in duplicate 72 mL samples taken from each tank. On day 16 all larvae from a tank were collected in a 20 L bucket and the total number of larvae and the number of larvae ready for settlement (doliolariae) were counted.

### *2.2.5 Effect of age of biofilm on settlement efficiency of H. scabra*

Natural biofilm has been suggested as a successful settlement inducer for many marine invertebrates (including sea urchins). The effects of natural biofilm have not been extensively researched in regards to *H. scabra*. This trial was designed to investigate the effects of different age biofilm cultures on the survival of *H. scabra* during settlement.

Larvae ready for settlement were separated from the other larvae using a 150µm mesh screen. Larvae were considered competent when at least 50% of the larvae on the mesh were at the doliolaria larval stage. Thirty 5L containers were set up in a randomised block design. Two hundred doliolariae were transferred to a 5L container (0.1 larvae $mL^{-1}$) supplied with 2.5L of UV- and 1µm-FSW on flow-through. The photoperiod was 14hL: 10hD and the light intensity was equivalent across all containers. Each container held a PVC plate (97x37x2mm) conditioned for one of five different periods of time in the same 1000L outdoor tank:

1. unconditioned plates
2. plates conditioned for six days
3. plates conditioned for eight days
4. plates conditioned for 11 days
5. plates conditioned for 18 days

The diatom *C. muelleri* was added (30,000 cells $mL^{-1}$) each morning and the water was turned off for 2h. At the termination of the trial, larvae and juveniles were dislodged from the containers by spraying with a 1% KCl solution. Percentage survival and settlement were evaluated by examination of each individual specimen using a binocular microscope. Larvae were considered to have settled as of the late pentactula stage with two tube feet and no more cilia present (Hamel and Mercier 1996). Settlement efficiency was estimated as percent of larvae that had settled. Additionally, samples of the biofilm from two randomly chosen PVC plates per treatment were collected and stored in 2% formaldehyde. The composition and numerical abundance of micro-algae and cyanobacteria was analysed by Dr Gustaaf Hallegraeff, School of Plant Science, University of Tasmania (this analysis was paid for using a grant allocated to Michelle Simoes by Charles Darwin University). Settlement and survival data were analysed after 6 days by a Generalised Linear Model with binomial errors. Results are expressed as mean ±SE (n=6) and are considered significant at $P \leq 0.05$.

### *2.2.6 Evaluation of different types of biofilm on settlement efficiency*

Metamorphosis and settlement are the two essential components of sea cucumber development and culture (Smiley et al. 1991; Yanagisawa, 1998). Extensive literature provides evidence pertaining to the influence of chemical and physical cues on marine invertebrate larvae to settle and metamorphose on a suitable substratum for juvenile growth and eventual reproduction (Hadfield and Paul, 2001). Larvae will respond to certain settlement cues by attaching to a substrate and metamorphosing into juveniles (Battaglene, 1999). Attachment of the tube feet to a surface is necessary to stimulate metamorphosis (Cameron & Hinegardner, 1974). These settlement cues can include certain bacteria and diatoms (Yanagisawa, 1998). Considerable experimental evidence suggests that chemical cues are very important in substrate selection by larvae (Hadfield and Paul, 2001). In nature, chemical cues may interact with physical or hydrodynamic factors to induce larval settlement (Hadfield and Paul, 2001).

Microbial films have long been recognized as necessary for the settlement of some invertebrate larvae (Hadfield & Paul, 2001; Bourne et al. 2006). In some cases,

specific types of bacteria present in biofilms may be responsible for facilitating settlement and chemicals bound to or released from bacteria may function as settlement inducers (Hadfield & Paul, 2001 Bourne et al. 2006). The bacterial biofilm developing on settlement substrates changes, sometimes dramatically, with age (Bourne et al. 2006). It is therefore possible that substrates conditioned for a different number of days have different effects on settlement efficiencies of larval *H. scabra*. Therefore this trial was developed to investigate the effects of age of biofilm on settlement of *H. scabra* larvae.

Thirty 5L containers were set up in a randomised block design using 1µm filtered seawater (FSW) and were each stocked with two hundred doliolariae. Larvae ready for settlement were separated from the other larvae using a 150µm mesh screen and each container held a PVC plate (97x37x2mm) conditioned with a settlement agent.

The diatom *C. muelleri* was added (30,000 cells $mL^{-1}$) each day and at the termination of the trial, larvae and juveniles were dislodged from the containers by spraying with a 1% KCl solution. Percentage survival and settlement was evaluated after 5 days by examination of each individual specimen using a binocular microscope. Larvae were considered to have settled as of the late pentactula stage with two tube feet and no more ciliae present (Hamel and Mercier 1996). Data were analysed by a Generalised Linear Model with binomial errors. Results are expressed as mean ±SE and are considered significant at $P \leq 0.05$.

*Experiment 1*
The larvae were transferred to the settlement containers on day 16 with 65.0±3.6% at the doliolarial stage. A total of six treatments were assessed (n=5):

1. a control with unconditioned plates
2. plates covered with a natural biofilm
3. plates covered with the diatom *Navicula* cf *jeffreyae*
4. plates painted with re-hydrated *Spirulina*
5. plates covered with an algal paste (*Thalassiosira weissflogii* = TW)
6. plates covered with the green alga *Ulvella* cf *lens*

*Experiment 2*
The settlement containers were stocked with 14 day-old larvae, with 83.0±1.0% being doliolariae. A total of six treatments were assessed (n=5):

1. a control with unconditioned plates
2. plates covered with a natural biofilm
3. plates covered with *N.* cf *jeffreyae* and a natural biofilm
4. plates painted with re-hydrated *Spirulina* and a natural biofilm
5. plates covered with TW and a natural biofilm
6. plates covered with *U.* cf *lens* and a natural biofilm

## 2.3 Nursery Production

### *2.3.2 Effect of different feeds and fertilisers on growth and development of juvenile Holothuria scabra*

In the absence of an established industry standard, a number of readily available feeds and fertilisers were used to culture juvenile *H. scabra* during the early nursery stage

*Juveniles*

Juveniles 40 days old were obtained from a single hatchery tank. They were size graded by washing them through a 2mm and 0.92mm mesh screen and only juveniles collected on the 0.92mm mesh were used for stocking. The size range of the juveniles ranged from 2.0 to 5.0mm in body length. The experimental system comprised 30x45L tanks set up in a constant temperature room, and supplied with two 23cm pieces of mussel rope tied to a ceramic weight. Each tank was filled with 41L of 1µm filtered seawater (FSW) and stocked with 100 randomly selected juveniles. The photoperiod was 14hL: 10hD.

*Feeds*

The feeds evaluated were:

- the algal paste *Thalassiosira weissflogii* (Instant Algae®, Reed Mariculture, USA)
- spray-dried cyanobacteria *Spirulina* (TAAU Australia P/L, Australia)
- pelleted chicken manure (Tropigro P/L, Darwin, Australia)
- plant fertiliser Aquasol (Orica P/L, Australia)
- larval feed Algamac-Protein-Plus (Aquafauna Bio Marine Inc, USA).

There was also an unfertilised treatment as control (n=5). All particulate diets were ground with a pestle and mortar and screened through a 320µm screen. The correct amount of feed was weighed, 50mL of 1µm-FSW added, vigorously shaken on a shaker for 20sec and then added to tank. Just prior to adding the diets water flow to all treatments, including the control, was turned off for 2h.

All tanks were conditioned for one week prior to stocking with the appropriate feed. Feed was supplied once daily at 10% of the initial total wet weight of juveniles throughout the experimental period. A sample of 100 juveniles of the identical size to be stocked was weighed to arrive at an estimate of total wet weight.

*Sediment*

Beach sand was used as sediment for the tanks. Sand was sifted and only particles ranging from 125 - 500μm were kept. The size distribution of the sand was 82% 125 - 300μm and 18% 300 - 500μm. Sand washed with freshwater, dried in a temperature control room, and the total organic load (TOL) measured as described below. A total of 1.13kg of sand was added to each tank, with the sand forming an even layer to a depth of 5mm.

*Total Organic Load*

A sample of sand was scooped from the same area in each tank at the time of stocking and, following that, once a week throughout the experimental period. The scoop was filled completely with sand and, using the same scoop, each sand sample was replaced with cleaned sand. Every week a different area was used for sampling (Fig. 3). The TOL in sand samples was measured as described by Zamora and Jeffs (2011). In order to remove the salt from the sample, each sand sample was washed with 50mL of 0.5M ammonium formate by vigorous mixing on a shaker for 30sec. The samples were then rested for 1h after which the supernatant was discarded. A preliminary trial assessing the suitability of this method was undertaken. The dry weight (DW) was obtained by placing samples in a drying oven for 48h at 60°C. Samples were then weighed and burned in a muffle furnace for 6h at 500°C. TOL was defined as the difference between DW and final weight after combustion.

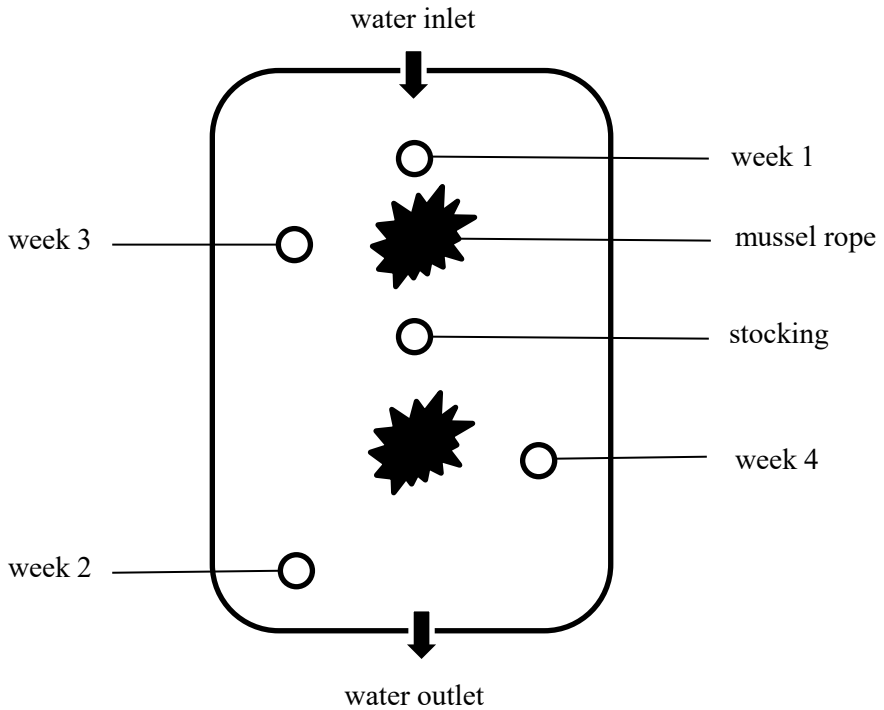



**Figure 3.** Collection points from which a sand sample was taken in each tank every week to assess total organic load.

*Growth and Survival*

At stocking and once a week during the four week trial, the length of 20 juveniles was measured. Ten juveniles were randomly collected from the walls and ten off the sand. At the end of the experiment, 20 juveniles were measured and transferred to an aerated 20L bucket for 24h to purge their gut contents prior to measuring their final wet weight. Wet weight was measured within 1min following removal from seawater. Individuals were dried on a sponge before being weighed (Battaglene *et al.* 1999). Survival was assessed by counting all remaining juveniles in each tank at the termination of the experiment.

*Statistical Analyses*

The data on TOL, the effect of diet on length and wet weight were analysed using ANOVA. Survival data were analysed by a Generalised Linear Model with binomial errors. Distribution and variance assumptions were checked with residual analysis, and pairwise comparison of the means for all models was done using Tukey's procedure. Results are expressed as mean ±SE and are considered significant at P≤0.05.

### 2.3.3 *Effect of shade level on growth and survival of juvenile Holothuria scabra in a commercial scale outdoor system*

Poor survival of newly settled sandfish juveniles was observed in the presence of full sunlight, and high levels of sunlight can also produce undesirable filamentous algal growth in nursery tanks. However, increasing sunlight has also been implicated in increased growth in larger juvenile *H. scabra* (Battaglene *et. al*., 1999). In heavily shaded nursery systems (<7000 lux at midday, Dec 2012) juveniles exhibited acceptable survival, but poor growth. As natural diatom associated biofilms remain the current source of food for these juveniles, a substantial level of sunlight is required, with reduced sunlight likely contributing to poor growth observed in heavily shaded tanks. An optimum level of shading should provide adequate sunlight for diatom associated biofilm growth without impacting on survival or creating unwanted filamentous algal blooms.

Previous nursery system trial methodology showed large variation within treatments, and tank placements indicated that variable sunlight was a major factor in this variability. This trial was designed to assess the effect of different levels of shade on nursery production of *H. scabra.*

Ten raceways were stocked with 1000 juveniles each and shaded. Shadecloth blocking 50% or 90% light was used, with 5 tanks per treatment (Fig. 4). Flow through seawater (3L/min) was filtered to 5µm and monitored daily. Sunlight levels were measured as lux at 8am, 12pm and 4pm each day. Water temperature was recorded daily in all tanks, and logged hourly in one tank per treatment. Juveniles were sampled for weight and length at stocking, weekly during the trial and at termination. Destructive samples to determine dry weights were taken at the beginning and end of the trial. Sampled juveniles were anaesthetized with menthol saturated ethanol (2% working solution) to aid uniformity for length measurements (Yamana and Hamano, 2006). The trial continued for four weeks and was terminated

after the mortality of the majority of any one treatment, leaving too few animals left to enable sampling any further.

**Figure 4.** Experimental shade trial tanks.

### 2.3.4 *Effect of pond mud and enriched pond mud on growth and survival of juvenile sandfish.*

Tasmanian Seafoods (TSF) routinely grow juvenile sandfish in raceway tanks (5.8m L x 1.1m W x 0.4m H) and are fed with the marine sediment that naturally accumulates on the tank floor via the incoming unfiltered sea water. To increase the yield of *H. scabra* juveniles in these tanks, additional feeds have been previously trialled by the DAC to enrich this marine sediment. These trials produced unacceptably high variability in the amount and distribution of sediment between tanks when delivered in this manner. This demonstrated the need to control the amount of the base sediment in future trials.

Sea and pond muds are commonly used in juvenile sea cucumber diets in China (Liu et al. 2009) and India (James 2004) as they are composed of alga, bacteria and other decaying marine plant and animal material that are natural foods of sea cucumbers. Pond mud was selected as the base sediment in this study and was compared with a treatment enriched with 10% inclusion of a formulated diet, and an unfed control.

Thirty 40L tubs were set up in 3 banks with 1000% $day^{-1}$ flow-through exchange of 10µm filtered seawater. Tanks were gently aerated with one small air-stone and shaded with 90% shadecloth. Each treatment group (n=10) was follows: Control (C) - unfed; Pond mud (PM) and Pond mud with 10% formulated diet inclusion (Adam and Amos P/L SA, Australia) (PMA).

Pond mud was sourced from marine aquaculture ponds in Darwin Harbour. It was rinsed with freshwater though a 500µm screen to remove large particles and oven dried for 12 hours at 60°C. Dried mud was ground with mortar and pestle to a particle size of <125µm and stored at 4 degrees C prior to use.

Experimental treatment tanks were conditioned with 3g of each treatment initially, alongside the unfed controls, 3 days prior to stocking juveniles. Feeding was performed again on day 8 across all treatments when juveniles had visibly cleared approximately 50% of the sediment.
Prior to stocking, juvenile sandfish were harvested from the larval rearing tank using 1% KCL in seawater to detach them from substrates. Fifty juveniles (300-500µm) were added to each trial tank. Individual lengths, mean wet weight and mean dry weight were taken from a sample of juveniles in the size group of those that were stocked.
Juveniles (n=20) from each tank were removed weekly by pipette and transferred to petri dish for a maximum of 10 minutes for a photograph under a microscope (Leica M80) for later measurement (Leica Application Suite v.4.2) before being returned to their respective tanks. Individual length (mm) and mean wet and dry weights (g) were taken from all juveniles at the termination of the trial (day 24). Survival was measured by an in-tank count of live juveniles remaining on day 14, and again on day 24. Differences in survival were analysed using Wald's test, mass and length data were analysed using ANOVA.
Temperature in all tanks was measured twice daily (8am and 2pm) to ensure uniformity of flow rate and temperature, and flow rates were adjusted when necessary.

### 2.3.5 Effect of the amount of artificial substrate in *H. scabra* nursery systems on the survival of newly settled juveniles.

Artificial substrate in the water column has been used by TSF and by Purcell and Agudo (2013) to increase surface area for diatom growth and to provide additional shading for juvenile sandfish in nursery systems. TSF has used ropes and pieces of shadecloth attached to ceramic weights for this purpose but its effects on juvenile survival have not been thoroughly tested. Consistently low and variable survival rates of juveniles are a barrier to accurate juvenile production research, and addition of an ideal type and level of substrate has the potential to ameliorate these factors. After several growth trials yielding variability that compromised the statistical significance of findings, it was decided that focus on improving early survival was necessary as a precursor to continuation of more accurate and controlled growth trials. Ropes are successfully used by TSF as larval settlement substrate and so were chosen as a trial nursery substrate at 3 inclusion levels: none, 30cm and 180cm lengths. A lower stocking density (30/tank) than in trial A (50/ tank) was chosen to emulate stocking densities used in TSF commercial raceways, and to potentially avoid density related growth effects indicated in trial A. All tanks were conditioned with dried pond mud as base sediment, found to be beneficial for growth in a previous trial.

Thirty 40L tubs were set up in 3 banks of 10 tubs with 1000% flow through of 10µm filtered seawater. Tanks were gently aerated with a small air-stone and shaded with 90% shadecloth. The control and each treatment group were replicated 10 times each, as follows: Control - no rope (C), 30cm rope (R30) and 180cm rope (R180). Washed ropes were weighed down with ceramic weights to submerge the majority of the ropes in the water column. Dried pond mud (3g), prepared according to the Trial A was added to all tanks after ropes were in place and allowed to settle for one day prior to stocking of juveniles.

Juvenile sandfish were harvested from the larval rearing tank by removing the rope substrates and immersing them in a tub of 1% KCL in seawater, causing the juveniles to disengage with the rope fibres. Juveniles were harvested from the tub,

graded and those that sat between a 500µm and a 900µm screen (individual average was 1.36mm SE=0.08 length, 0.0003g wet weight and 0.00006g dry weight) were used in the trial. Thirty juveniles were assigned to each experimental tank.

On the first day of the trial animals were evenly placed into the tank and allowed to settle on the tank floor and/or ropes. Temperature in all tanks was measured hourly using one temperature logger per bank of 10 tubs throughout the trial. Flow rates were monitored daily and adjusted when necessary to ensure uniformity of flow rate and temperature between tanks. To avoid the impact of sampling stress on juveniles at the lowered stocking rate, juveniles were not removed for measurement during the trial. Instead weekly photos and visual survival counts (at 1, 4 and 7 days post-stocking) were recorded during the trial to track performance. Upon termination of the trial, juveniles in C tanks were harvested by individual removal via pipette. Rope treatment tanks were harvested by gentle removal of ropes and immersion in 1% KCl solution to disengage juveniles. Juveniles found on walls and floor of rope tanks were harvested as per C tanks.

Analysis

The proportion of juvenile *H. scabra* surviving in each treatment was compared with a generalised linear model fit to a binomial distribution with a logit link function.

## 2.4 Sea Ranching

To pursue ranching as an effective management component for the Beche de mer fishery in the Northern Territory, it is essential to gain basic knowledge of the effectiveness of sea cucumber ranching in local waters. Objectives 3 and 4 of this project were developed to gain essential knowledge that can be used to make informed management decisions on sea cucumber ranching in the NT.

To address objectives 3 and 4 of this project the following three trial releases were conducted followed by subsequent surveys. The trials enabled the collection of data on the growth, survival and migration of released cultured juvenile *H. scabra*.

### *2.4.1 Juvenile production, tagging, and transport*

Juvenile sandfish were produced for ranching trials in the Tasmanian Seafoods hatchery located at the Darwin Aquaculture Centre. Broodstock used were sourced from areas nearby the trial site to maintain genetic integrity, using hatchery production methods described by Bowman (2012).

To obtain ranching approvals for juveniles prior to release, whole animal samples were sent to the Berrimah Veterinary Laboratory of Northern Territory Government Department of Resources to screen for disease or poor health following the methods described by Purcell & Eeckhaut (2005). For identification of hatchery released animals all juvenile sandfish were immersed for 12 hours in a fluorochrome Oxytetracycline solution (method outlined in Simoes & Knauer, 2012), to make calcium spicules absorb a fluorescent ‘tag’ and promote juveniles to void their gut contents prior to transportation. Juvenile sandfish were transported to the ranching site in seawater, in plastic lined airline approved foam boxes at densities no greater than 300g per litre.

### 2.4.2 *Release area*

Little Lagoon (Groote Eylandt 13°49'176S 136°49'366E. Fig. 5) is a sheltered lagoon with large seagrass beds and is an area of natural habitat for the sandfish. The lagoon is approximately 20km$^2$ in area with two sites being utilised for trials over the course of the project.

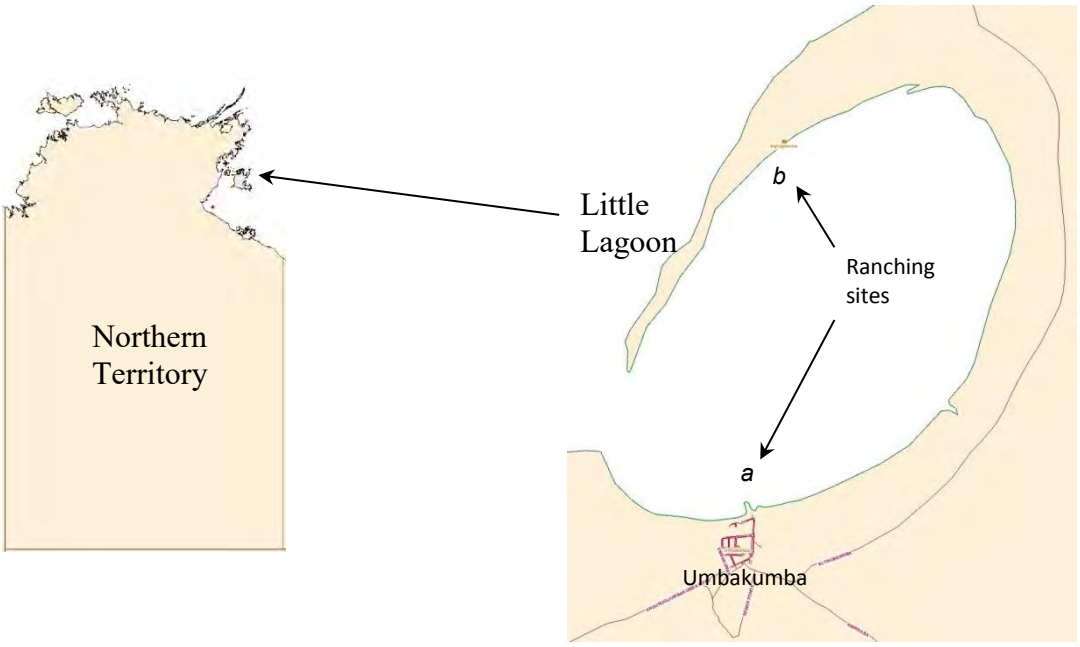



**Figure 5.** Location of sites for sandfish ranching trials in Little Lagoon – Groote Eylandt

### 2.4.3 *Survey methods and identification of released juveniles*

Sandfish abundance and size frequency was determined by using the 'hip-chain' transect method (Leeworthy & Skewes, 2007) where belt transects 1.5m x 50m (75m$^2$) were used to monitor each site during low spring tides. To identify released hatchery produced juvenile sandfish from wild recruits, skin samples were collected using a scalpel and excising a small sample (approx. 5-10mm$^2$, 1mm deep) from the edge of the ventral surface of the body wall. Skin samples were placed in labelled vials, sealed from light, and stored in a freezer for later processing in the laboratory.

In the laboratory spicules were extracted from the skin samples by dissolving the tissue in 12% bleach ($NaHClO_4$) and rinsing in freshwater. The extracted spicule solution was subsequently examined on a slide with a microscope fitted with a fluorescent light to identify individuals with marked calcium spicules (Purcell & Blockmans, 2009).

### 2.4.4 *First release*

The first release was carried out on intertidal seagrass beds adjacent to the community of Umbakumba (trial site '*a*'). Two sites were used separated by a geographical barrier, one as the trial site and one as a control site. 10,000 juvenile sandfish were released onto the trial site in July 2011. Prior to release site surveys were carried out assessing sandfish abundance and generating baseline weight data. Juveniles were spread out across the release site including in four 100m$^2$ release pens that were set up to act as reference points for accurate estimation of hatchery released juvenile growth and survival (Fig. 6.).

**Figure 6.** Seabed pen on the ranching trial site.

Monitoring was carried out in August, October, December 2011, and June 2012 to identify hatchery produced animals and assess growth and survival using the survey methods described.

### *2.4.5 Second release*

The second release site (trial site '*b*') had only a small amount of seagrass, and a greater exposure to wave action than the first site. Initial abundance survey of the second release site showed very low standing stocks (<5 individuals/ha).

5000 juveniles were released into a small area (5m x 5m) on the seagrass in January 2012 with the intention of generating comparative growth data and information on the movement of released hatchery produced sandfish. Due to unfavourable tides and weather we were unable access the site again until June 2012, following that we were able to access the site again to conduct monitoring in August and November 2012. During monitoring events growth data was recorded, and during the August survey locational work was carried out using a GPS to record the location of hatchery produced juveniles in relation to the release point. This generated information on the direction and how far some individuals had moved from the release point after eight months.

### 2.4.6 *Third release*

Research conducted during the third release focussed on assessing the effectiveness of sea ranching by conducting an analysis comparing the performance in terms of growth of released juvenile sandfish and wild recruits.

An analysis of length, weight, and growth was done using data from previous ranching and grow-out work, and fishery catch data, to develop a generic growth model and provide a benchmark for sandfish growth under Northern Territory conditions. Monthly or bi-monthly monitoring of sandfish was conducted between June 2012 and January 2013 using the survey methods described on both a release site and a control site at trial site '*a*', establishing a control site and a trial site that was surveyed before and after hatchery produced juvenile sandfish were released. Modal progression analysis on monitoring data to follow both wild recruits and hatchery released juveniles was carried out to compare growth rates between the two stocks.

To compare to growth between wild recruits and hatchery produced juveniles, the generic growth model was used to assign a relative age to the wild recruits.

## 2.5 Hatchery Genetics

As part of the industries sea ranching development program, Tasmanian Seafoods P/L commissioned a survey of the genetic structure of *H. scabra* across the species Northern Territory range. This work was done by Flinders University supported by the Australian Seafood CRC project 2008-733. The survey identified two genetically distinct populations in the NT (East and West) with little gene flow between the two populations. A second part of this project was to analyse the genetic diversity of the progeny produced by a standard hatchery spawning. This pilot analysis showed that in a pooled spawning 75% of the 40 broodstock contributing to the progeny. However, only five broodstock contributed to the majority (71%) of the progeny. It was noted that the conditions for the spawning for this work were not optimal and therefore may not reflect the true diversity for hatchery diversity. Therefore a second, more comprehensive study was commissioned as part of the current project to collect more data on the broodstock contributions to hatchery spawnings.

*Samples analysed*

The samples of broodstock and progeny from 3 different spawning attempts were genotyped for 19 loci previously developed (Fitch et al 2013). The data consisted of individuals from each of two time periods, one nursery sample (30-43 days old) and another hatchery sample collected at 2 months old. Genotyping of samples from an additional time period (larval sample) was attempted but failed. DNA extraction methods akin to forensic DNA might have resulted in successful genotyping of these larval samples, but these were not attempted due to time and monitory constraints.

These genotype data were cleaned to remove loci that were identified as being problematic when scoring. Additionally, progeny that genotyped at only 6 or less loci were removed. This left 14 loci for determining the contribution of broodstock to the progeny. The number of progeny individuals that were successfully genotyped for 7 or more loci was: 100 nursery and 98 hatchery samples from Batch 04/13 with 6/7 broodstock; 92 nursery and 87 hatchery samples from Batch 02/13 with 8/10 broodstock; 47 nursery and 92 hatchery samples from Batch 03/13 with 3/7 broodstock. Unfortunately, the broodstock genotypes were fairly incomplete, limiting the identification of particular broodstock. However analysis of parental progeny

arrays was able to be performed with and without reference to the incompletely genotyped broodstock.

*Method use to assess parental contributions*

The program COLONY was used to examine the sib groups within the progeny data (Jones and Wang, 2009). This was done in two ways. Firstly the genotypes of the progeny were examined for sib-groups within each of the 3 batches, pooling the different time samples. The resulting best likelihood results, indicating the most likely parental contributions, were then plotted using Pedigree Viewer. Secondly the broodstock were included in the analysis of sib groups and parental contributions of the broodstock to the progeny were identified.

# 3. Results

## 3.1 Hatchery production

### *3.1.1 Growth, development and size separation of H. scabra larvae.*

Survival of larvae up to day 12 was 67% (Fig. 7). The % distribution of larvae on screens (Table 1) and their size (Table 2) show that it was possible to achieve meaningful separation of larvae using mesh screens. Moreover, based on this first set of data it will be possible to reduce the number of mesh screens used during a larval run. Larvae separated on a 100µm mesh on day 2 did not differ in size from larvae collected on an 85µm mesh; similarly, a 320µm mesh did not separate larvae any bigger than larvae caught on a 250µm mesh (Table 2).

On day 12, early doliolariae were first observed swimming in the upper water column of the larval tanks containing larvae collected on a 250µm mesh. Starting on day 13, we collected other larval stages on a 250µm mesh and doliolariae on a 210µm mesh. Unsurprisingly, larvae collected on a 210µm mesh were not all at the same developmental stage and ready for settlement. The decision to transfer larvae to settlement tanks or return them to the larval tanks depended on two factors. First, the proportion of doliolariae present was assessed and secondly, the developmental status of the non-doliolaria stages in the sample. The efficiency of separating doliolariae from other larval stages was 40% on day 13, increased to 73% on day 14, decreased slightly to 67% on day 15 and, finally, decreased further to 46% on day 16 (Fig. 8). If the majority of non-doliolariae were in the late auricularia stage with well-developed hyaline spheres, all the larvae were counted as doliolariae and transferred to a 1t settlement tank. As of day 17, growth and development of the remaining larvae in the larval tanks appeared to be arrested and they were discarded. A total of 14% of the original day 2 larvae were transferred to 1t settlement tanks, each stocked with 50,000 doliolariae.

Settlement efficiencies varied from 8 to 41% with an average of 23% of transferred doliolariae successfully metamorphosing into juveniles.

In summary, the hatchery run showed that size separation of larvae during a larval run as well as transferring doliolariae to separate settlement tanks is achievable. This allows for the collection of accurate data on larval survival, growth and development.

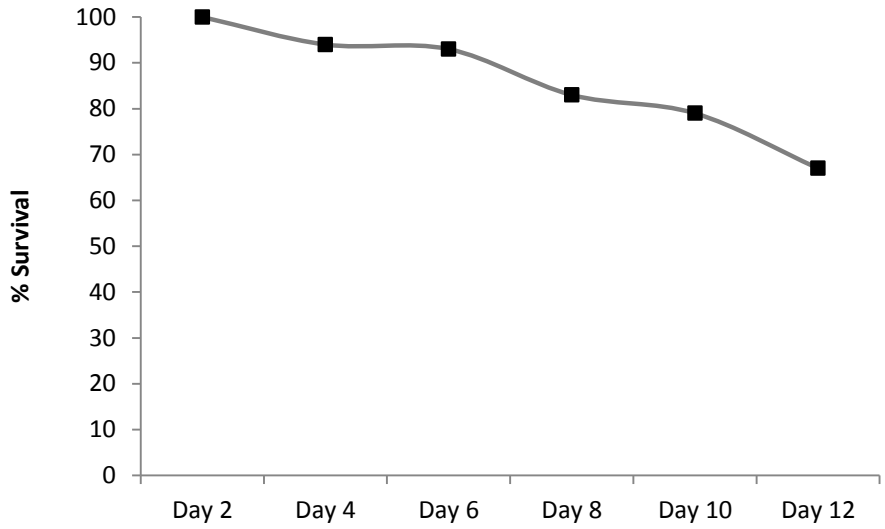



**Figure 7.** Cumulative survival (%) of *Holothuria scabra* larvae (day 2 larvae numbered $2.8x10^6$).

**Table 1.** Percentage distribution of larval *Holothuria scabra* on mesh screens.

| | Mesh screen (µm) | | | | | | |
|---|---|---|---|---|---|---|---|
| Day | 85 | 100 | 120 | 150 | 210 | 250 | 320 |
| 2 | 21.7 | 29.6 | 48.7 | | | | |
| 4 | 3.0 | | | 47.7 | 49.3 | | |
| 6 | | | | 9.5 | 53.0 | 31.6 | 5.9 |
| 8 | | | | | 31.6 | 68.4 | |
| 10 | | | | | 16.6 | 83.4 | |
| 12 | | | | | | 100.0 | |

**Table 2.** Total length (µm) of larval *Holothuria scabra* on mesh screens. Data are the mean± SE (n=50).

| | Mesh screen (µm) | | | | | | |
|---|---|---|---|---|---|---|---|
| Day | 85 | 100 | 120 | 150 | 210 | 250 | 320 |
| 2 | 435.0±13.7 | 429.2±9.9 | 547.5±10.2 | | | | |
| 4 | 512.9±17.3 | | | 588.8±21.0 | 660.8±11.3 | | |
| 6 | | | | 675.4±22.9 | 863.8±15.0 | 947.1±12.4 | 919.6±16.4 |
| 8 | | | | | 912.9±24.2 | 1,029.6±15.7 | |
| 10 | | | | | 922.9±22.9 | 1,225.0±15.7 | |
| 12 | | | | | | 1,183.3±20.4 | |

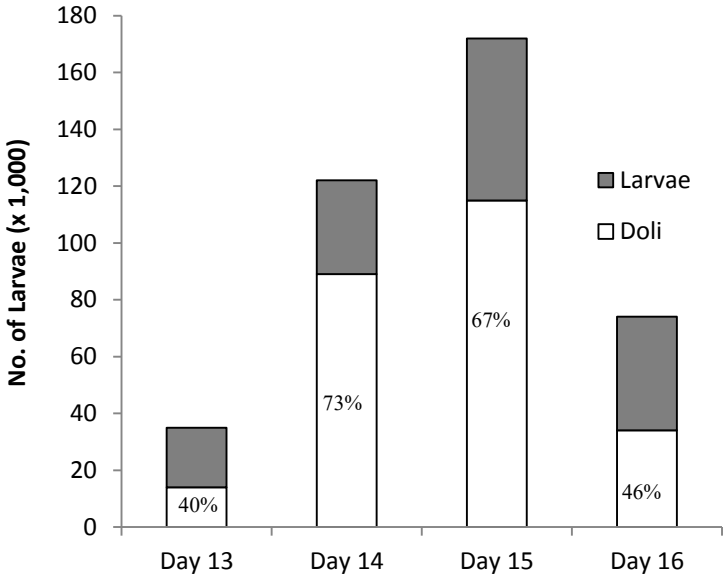



**Figure 8.** Total number of *Holothuria scabra* larvae transferred to settlement tanks (□ = doliolariae, ■ = non-doliolariae). Percentages refer to the proportion of doliolariae in the total sample.

### *3.1.2 Growth and survival of Larval Sandfish fed different Microalgae*

The growth data of *H. scabra* larvae fed different micro-algae are presented in figure 9. On day 8 larvae fed the diatoms *C. muelleri* (883.8±13.2µm) and *C. calcitrans* (847.1±10.5µm) were significantly larger compared to larvae fed the other microalgae species. Larvae fed the TMD (890.8±12.9µm) also had a significantly greater length than larvae fed the other diets with the exception of larvae fed *C. muelleri.* Larvae fed T-ISO (725.4±14.8µm) and *P. salina* (678.8±10.6µm), and all fed larvae were significantly larger than unfed larvae (570.8±9.3µm). Length of day 2 larvae at stocking was 441.7±7.4µm (n=50).

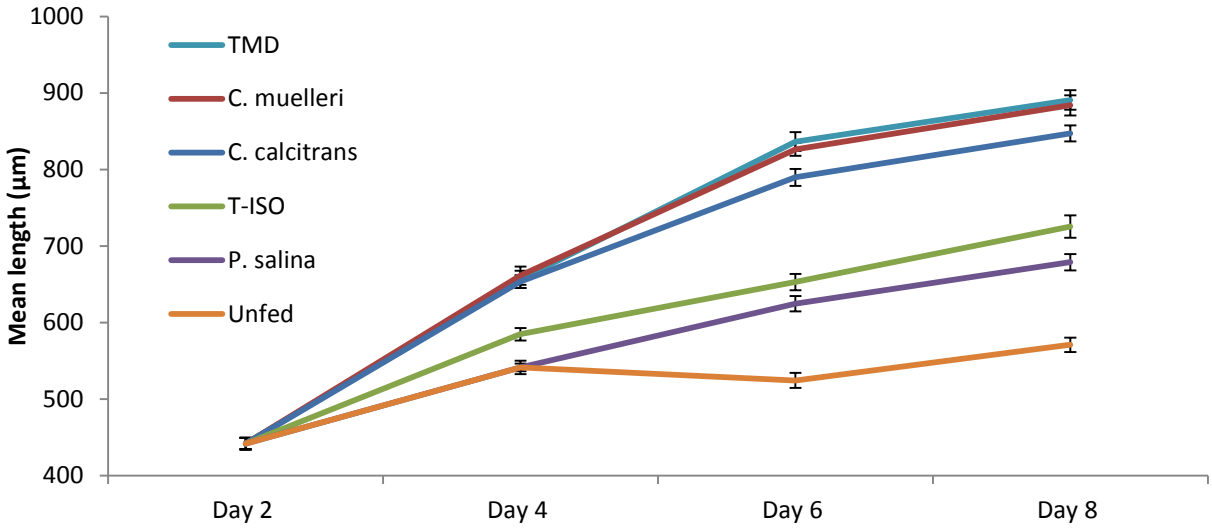



**Figure 9.** Length of *H. scabra* larvae fed either a single micro-alga or a ternary micro-algal diet (TMD) comprised of *Chaetoceros muelleri* (40%), T-ISO (40%) and *Pavlova salina* (20%). Values are the mean±SE (n=3).

Survival of larvae on days 4, 8 and 12 is shown in Figure 10. On day 12, survival of larvae fed; *C. muelleri* (58.2±2.0%), the TMD (55.2±2.6%) or *C. calcitrans* (50.2±2.6%) was significantly higher than that of larvae in all other treatments. However, there was no significant difference in survival between larvae fed either T-ISO (35.1±1.0%) or *P. salina* (33.1±4.6%) and unfed larvae (29.1±1.0%).

Overall larval biomass was highest in tanks with larvae fed *C. muelleri (*2.93 $x10^6$±0.12$x10^6$µm), the TMD (2.683$x10^6$±0.1$x10^6$µm) and *C. calcitrans (*2.592 $x10^6$±0.05$x10^6$µm). Biomass in the tanks supplied with T-ISO (1.527$x10^6$±0.03$x10^6$µm) and *P. salina* (1.217$x10^6$±0.07$x10^6$µm) were lower and biomass in tanks supplied with *P. salina* was not significantly different from tanks with unfed larvae (1.138$x10^6$±0.06$x10^6$µm) (Fig. 11).

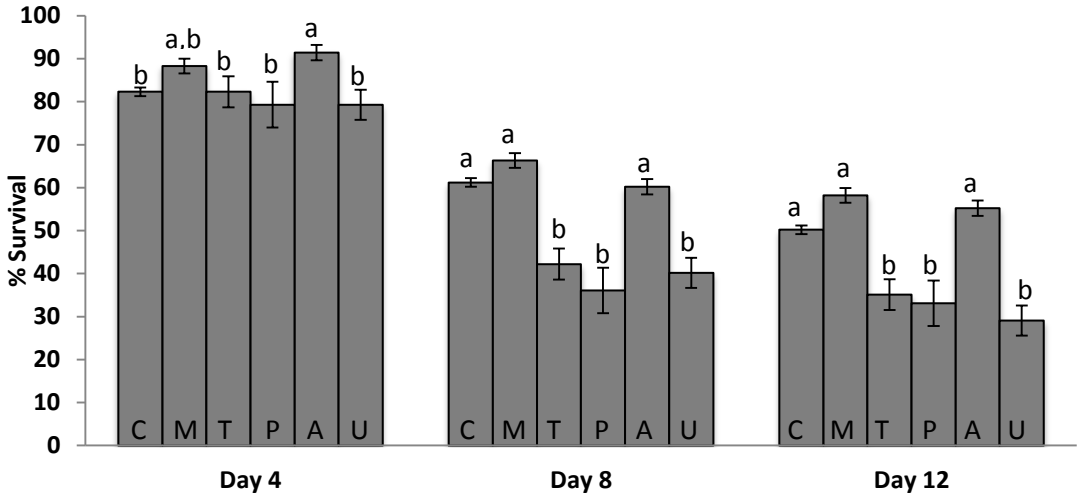



**Figure 10.** Cumulative survival of sandfish, *Holothuria scabra*, larvae fed either a single micro-alga (C = *Chaetoceros calcitrans*, M = *C. muelleri*, T = T-ISO, P = *Pavlova salina*, U = unfed control) or a ternary micro-algal diet (A) comprised of *C. muelleri* (40%), T-ISO (40%) and *P. salina* (20%). Values are the mean±SE (n=3). Significant differences between treatments are indicated by different superscripts ($P \leq 0.05$).

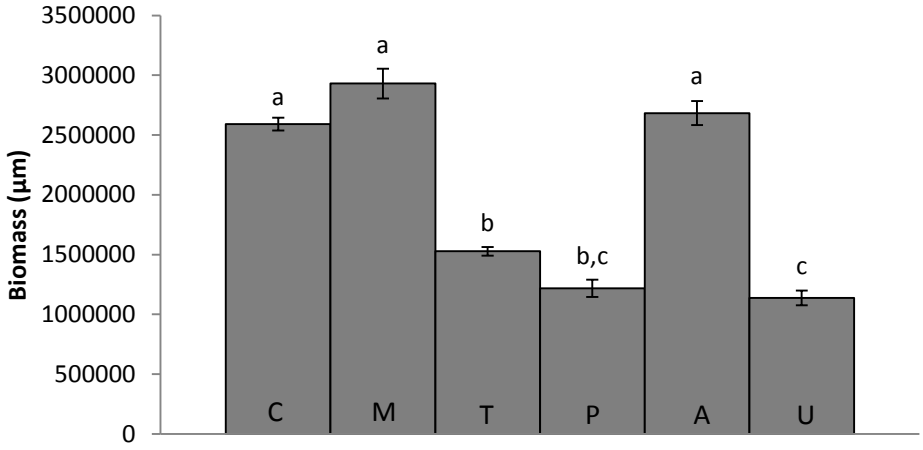



**Figure 11.** Larval biomass (µm) on day 8 for *Holothuria scabra* larvae fed either a single micro-alga (C = *Chaetoceros calcitrans*, M = *C. muelleri*, T = T-ISO, P = *Pavlova salina*, U = unfed control) or a ternary micro-algal diet (A) comprised of *C. muelleri* (40%), T-ISO (40%) and *P. salina* (20%). Values are the mean±SE (n=3). Significant differences between treatments are indicated by different superscripts ($P \leq 0.05$).

The percentage of competent doliolariae on day 12 in larvae fed *C. muelleri* (96.0±2.2%) was not significantly higher than in larvae fed the TMD (90.9±2.8%); however, *C. muelleri*-fed larvae did have a significantly higher percentage competent doliolariae compared to larvae fed *C. calcitrans* (84.7±2.7%) (Table 3). Neither the larvae fed T-ISO or *P. salina* nor the larvae from the unfed control treatment developed into competent doliolariae (Table 3).

**Table 3.** Percentage of competent doliolariae (day 12) of sandfish, *Holothuria scabra*, fed either a single micro-alga or a ternary micro-algal diet (TMD) comprised of *Chaetoceros muelleri* (40%), T-ISO (40%) and *Pavlova salina* (20%). Values are the mean±SE (n=3). Significant differences between treatments are indicated by different superscripts ($P \leq 0.05$).

| Diet | Competent doliolariae (%) |
|---|---|
| *C. calcitrans* | $84.7 \pm 2.7^{b}$ |
| *C. muelleri* | $96.0 \pm 2.2^{a}$ |
| T-ISO | $0.0 \pm 0.0^{c}$ |
| *P. salina* | $0.0 \pm 0.0^{c}$ |
| TMD | $90.9 \pm 2.8^{a, b}$ |
| Unfed | $0.0 \pm 0.0^{c}$ |

### *3.1.3 Effect of water exchange on larval growth and survival*

*Experiment 1*

The length of larvae from all three treatments was not significantly different on day 5. However, after nine days larvae undergoing a 30% daily water exchange were significantly longer than larvae from the other treatments (Table 4).

**Table 4.** Total length (µm) of larval sandfish, *Holothuria scabra*, cultured in tanks with different water exchange protocols at a stocking density of 0.5 larvae $mL^{-1}$. Values are expressed as mean±SE (n=3) and significant differences within columns are indicated by different superscripts ($P \leq 0.05$).

| Treatment | Day 5 | Day 9 |
|---|---|---|
| 30% daily | $668.1 \pm 11.4^{a}$ | $873.1 \pm 16.4^{a}$ |
| Second day | $671.7 \pm 7.8^{a}$ | $827.2 \pm 13.7^{b}$ |
| Fourth day | $664.2 \pm 9.9^{a}$ | $829.7 \pm 13.2^{b}$ |

[1] Size at stocking was 431.4±6.8µm

The proportions of larvae at different developmental stages as measured on day 15 are shown in Figure 11. The percent doliolariae in the 30% daily exchange treatment was significantly higher compared to the two other treatments. Moreover, the percent doliolariae in the fourth day batch exchange treatment was significantly higher than in the second day exchange treatment.

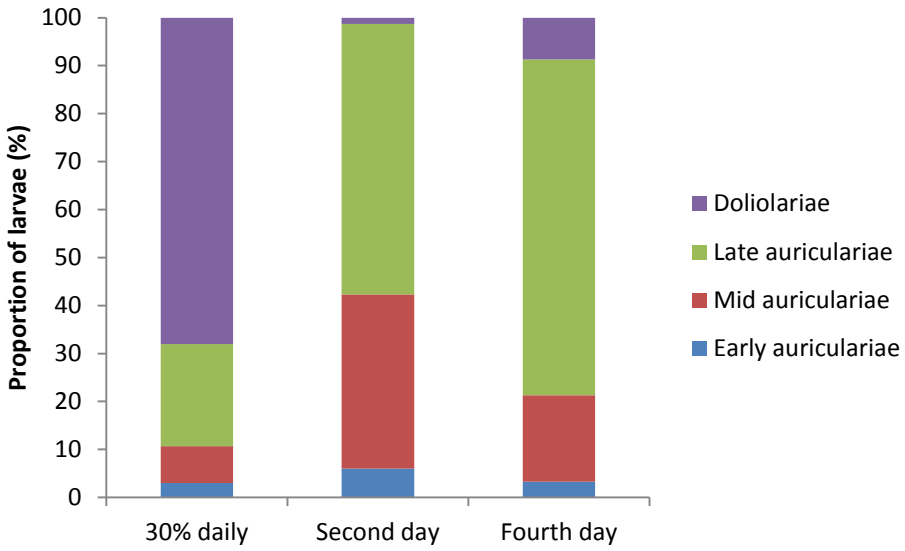



**Figure 12.** Developmental stages (%) of larval *H. scabra* after 15 days culture with different water exchange protocols at a stocking density of 0.5 larvae mL-1. Values are expressed as mean (n=3).

The different water exchange treatments did not have a significant effect on survival, which varied from 71.7±3.3% in the 30% daily exchange treatment to 78.3±4.4% in the fourth day batch exchange treatment (Fig. 12).

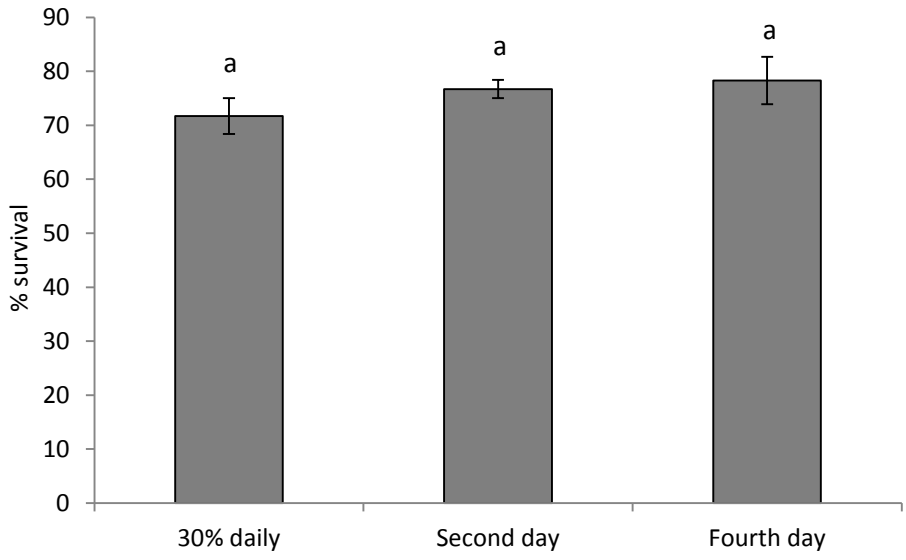



**Figure 13.** Survival (%) of larval sandfish, *Holothuria scabra*, cultured in tanks with different water exchange protocols at a stocking density of 0.5 larvae $mL^{-1}$ for 13 days. Values are expressed as mean ±SE (n=3) and significant differences within columns are indicated by different superscripts (P≤0.05). All tanks were initially stocked with 50,000 larvae.

*Experiment 2*

The length of larvae is listed in Table 5. Water exchange treatment did not have a significant effect on larval length on day 5. On day 8, however, larvae in treatments undergoing either 30% daily water exchange or a fourth day batch exchange were significantly larger than larvae from the second day batch exchange.

**Table 5.** Total length (µm) of larval sandfish, *Holothuria scabra*, cultured in tanks with different water exchange protocols at a stocking density of 0.1 larvae $mL^{-1}$. Values are expressed as mean±SE (n=3) and significant differences within columns are indicated by different superscripts (P≤0.05).

| Treatment | Day 5 | Day 8 |
|---|---|---|
| 30% daily | 862.2 ± 12.4[a] | 1,080.8 ± 10.2[a] |
| Second day | 883.3 ± 8.8[a] | 1,007.2 ± 13.5[b] |
| Fourth day | 864.8 ± 9.6[a] | 1,092.8 ± 12.1[a] |

[1] Size at stocking was 436.3±8.4µm

On day 12 the percent doliolariae in the 30% daily treatment was significantly higher than in the two other treatments. The percent doliolariae in larvae undergoing a batch exchange every fourth day was also significantly higher than in the second day batch exchange treatment (Figure 13).

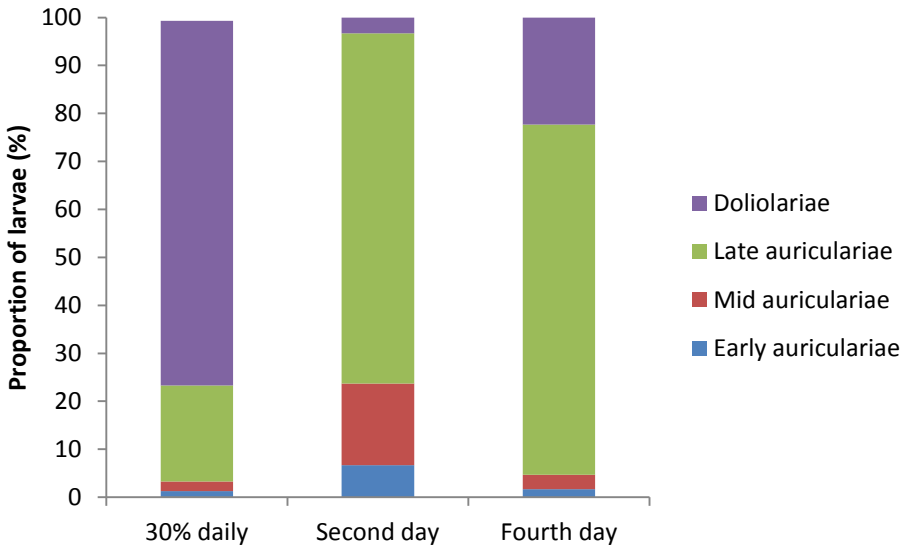



**Figure 14.** Developmental stages (%) of larval *H. scabra* after 12 days culture with different water exchange protocols at a stocking density of 0.1 larvae mL-1. Values are expressed as mean (n=3).

Survival of larvae ranged from 65.0±3.9% in larvae from the fourth day exchange treatment to 75.0±4.4% in the 30% daily exchange treatment. There were no significant differences in survival (Fig. 14).

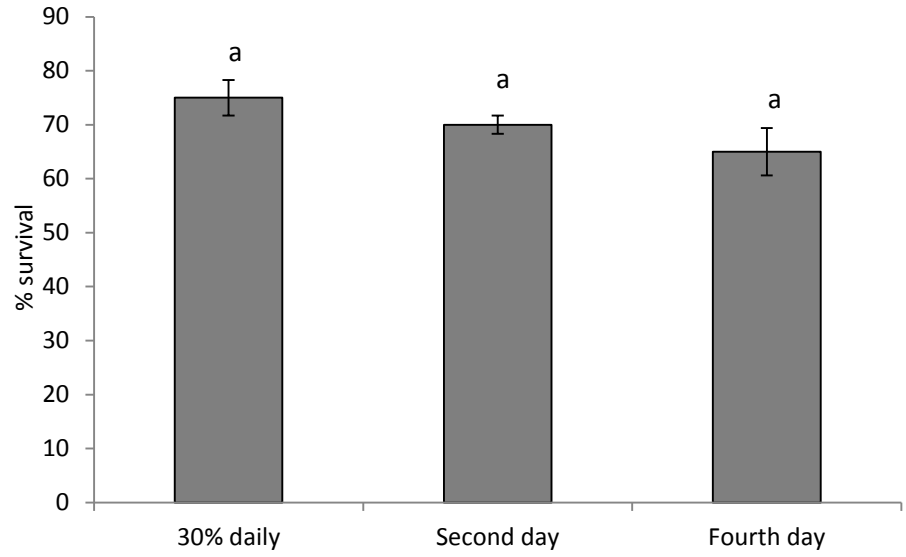



**Figure 15.** Survival (%) of larval sandfish, *Holothuria scabra*, cultured in tanks with different water exchange protocols at a stocking density of 0.1 larvae $mL^{-1}$ for 10 days. Values are expressed as mean ±SE (n=3) and significant differences within columns are indicated by different superscripts (P≤0.05). All tanks were initially stocked with 10,000 larvae.

### *3.1.4 Effect of age of biofilm on settlement efficiency*

The data for survival (%) and settlement (% juveniles) after the 6 day settlement period are shown in figure 15. Survival of larvae in the control treatment was significantly lower compared to larvae from the other treatments, with the exception of larvae settling on plates conditioned for eight days.

The proportion of juveniles on plates conditioned for 11 days was significantly higher than that of any other conditioning period (6 day, 8 day and 18 day). Furthermore, the proportion of juveniles on plates conditioned for either eight or 18 days was significantly higher than on plates conditioned for six days and unconditioned plates.

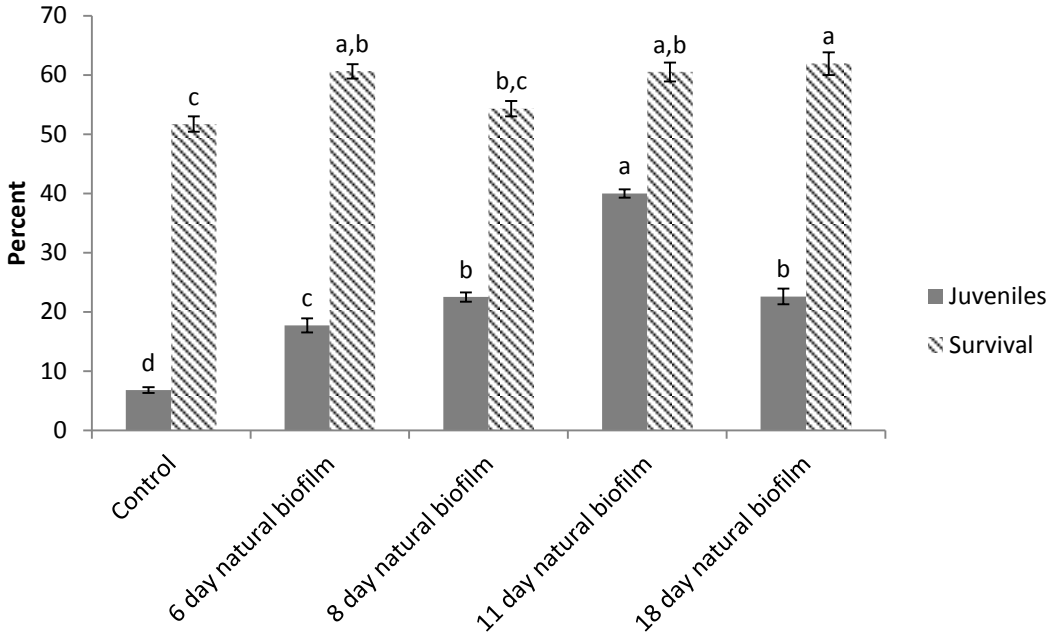



**Figure 16.** Percentage juveniles and survival of sandfish (*Holothuria scabra*) larvae settled on plates containing natural biofilms conditioned for a different number of days. Results are expressed as mean ±SE (n=6). Significant differences between treatments are indicated by different letters above columns (P≤0.05).

A great number of genera of diatoms and cyanobacteria were present on the plates and the distribution of genera was generally similar on plates of different ages. However, the diatom genus *Mastogloia* was only found on both plates with an 11 day-old biofilm.

### *3.1.5 Nutritional value of the algal paste Thalassiosira weissflogii*

The growth rate of larvae fed 100%C (82.0±5.4 µm day$^{-1}$) was significantly higher than that of any other treatment (Table 6). Every 25% drop in the proportion of dietary *C. muelleri* produced a significant decrease ($r^2$ = 0.97983, P≤0.002) in growth rate from 70.3±0.4 µm day$^{-1}$ (75%C:25%T) to 55.4±4.1 µm day$^{-1}$ (50%C:50%T), 31.8±1.2 µm day$^{-1}$ (25%C:75%T) and 9.5±0.4 µm day$^{-1}$ (100%T) (Table 6).

Larvae fed 100%C had a significantly higher percent doliolariae (58.6±1.6%) on day 16 compared to all other treatments. The percent doliolariae in larvae fed 75%C:25%T (42.5±3.7%) was significantly higher than in the 50%C:50%T-fed larvae (3.2±0.5%), and both of these were significantly higher than larvae fed 25%C:75%T (0.0%) and 100%T (0.0%) (Table 6). The relationship between % dietary *C. muelleri* and % doliolariae was significant ($r^2$ = 0.82946, P≤0.05, Fig 16).

**Table 6.** Growth rate (µm day$^{-1}$) and % doliolariae of larval sandfish (*Holothuria scabra*) fed various combinations of live *Chaetoceros muelleri* (C) and an algal paste (*Thalassiosira weissflogii* (T)). Values are the mean ± SE (n=3). Significant differences in a column are indicated by different superscripts (P ≤ 0.05)

| Diet | µm day$^{-1}$ | % doliolariae |
|---|---|---|
| 100%C | 82.0 ± 5.4$^{a}$ | 58.6 ± 1.6$^{a}$ |
| 75%C:25%T | 70.3 ± 0.4$^{b}$ | 42.5 ± 3.7$^{b}$ |
| 50%C:50%T | 55.4 ± 4.1$^{c}$ | 3.2 ± 0.5$^{c}$ |
| 25%C:75%T | 31.8 ± 1.2$^{d}$ | 0.0$^{d}$ |
| 100%T | 9.5 ± 0.4$^{e}$ | 0.0$^{d}$ |

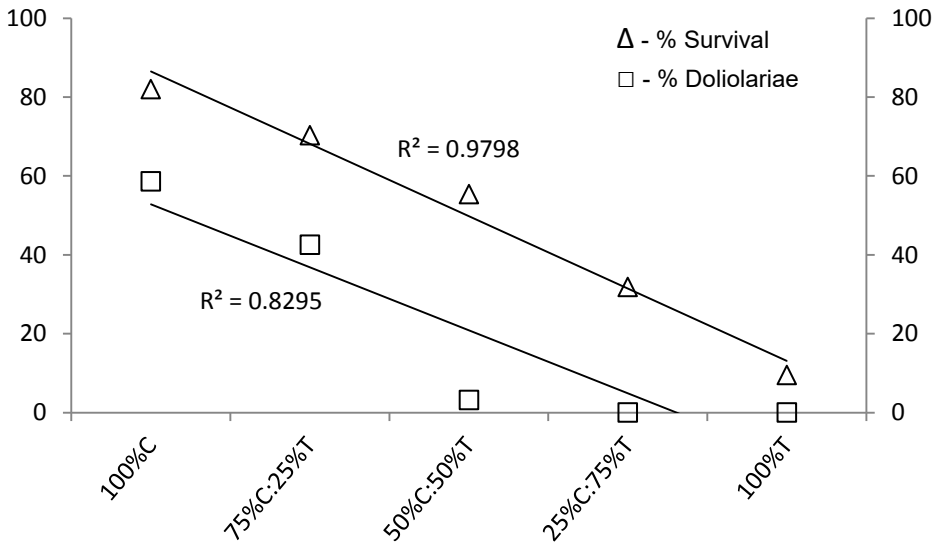



**Figure 17.** Percentage survival of *H. scabra* fed 100%C (65.7±3.7%) and 50%C:50%T (60.5±0.9%) was significantly higher than in 100%T-fed larvae (46.7±1.3%) over a 16-day culture period. The differences in survival of larvae fed 75%C:25%T (53.9±4.0%) and 25%C:75%T (52.9±2.5%) were not significant compared to the other treatments. There was no significant relationship between % dietary *C. muelleri* and survival after 16 days ($r^2$ = 0.70799, P>0.05).

No significant effect of diet treatment on water quality was observed with temperature being 28.4°C, pH 8.1 and DO 6 mg L-1 in all treatments.

### *3.1.6 Evaluation of different types of biofilm on settlement efficiency*

*Experiment 1*

The data for the effects of biofilm type on settlement are shown in figure 17. Survival of larvae ranged from 13.6±1.1% in the *Spirulina* treatment to 18.6±1.4% in the TW treatment, with only these two treatments being significantly different.

The proportion of juveniles varied from 2.7±0.5% in the control treatment to 13.3±1.0% in the TW treatment. All plates conditioned with either a live biofilm, alga

or painted with an alga resulted in a significantly higher proportion of juveniles compared to the unconditioned control. Moreover, the difference in the proportion of juveniles between plates painted with *Spirulina* (8.9±0.4%) and TW was significant.

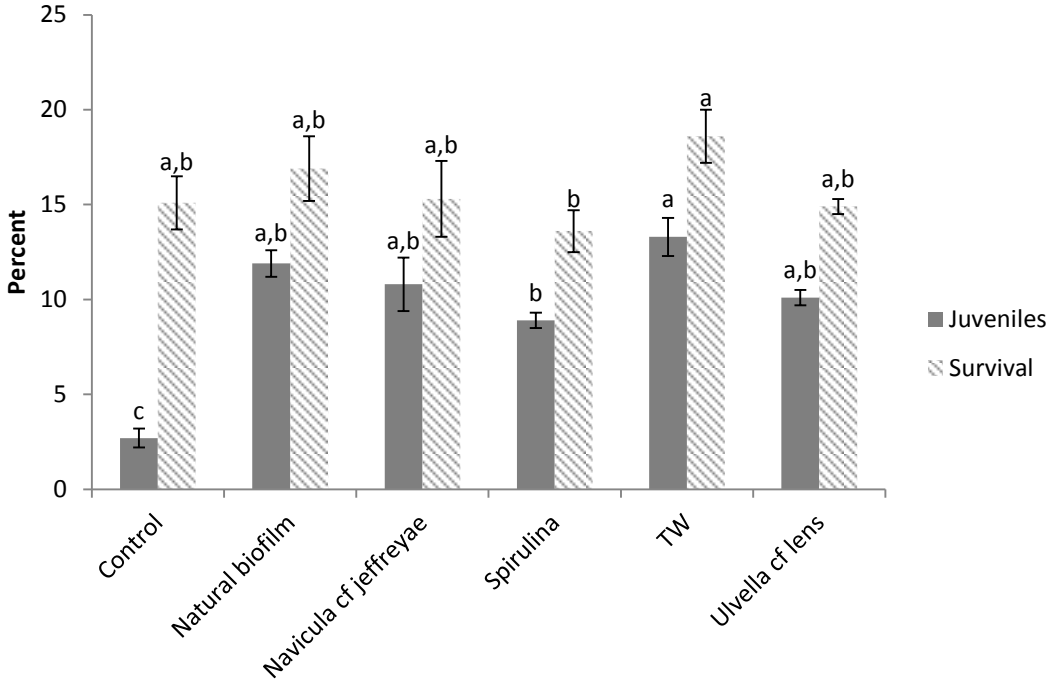



**Figure 18.** Percentage juveniles and percentage survival of sandfish (*Holothuria scabra*) larvae settled on different types of biofilms. Results are expressed as mean ±SE (n=5). Significant differences between treatments are indicated by different letters above columns (P≤0.05).

*Experiment 2*

The data for the second experiment are shown in Figure 18. There were no significant differences in survival of larvae, ranging from 59.8 ± 1.5% in larvae settled on plates conditioned with *Spirulina* and a natural biofilm to 66.2 ± 2.9% in the control larvae.

All conditioned plates resulted in a significantly higher proportion of juveniles compared to the unconditioned control treatment. However, there were no significant differences in percent juveniles among any of the other treatments.

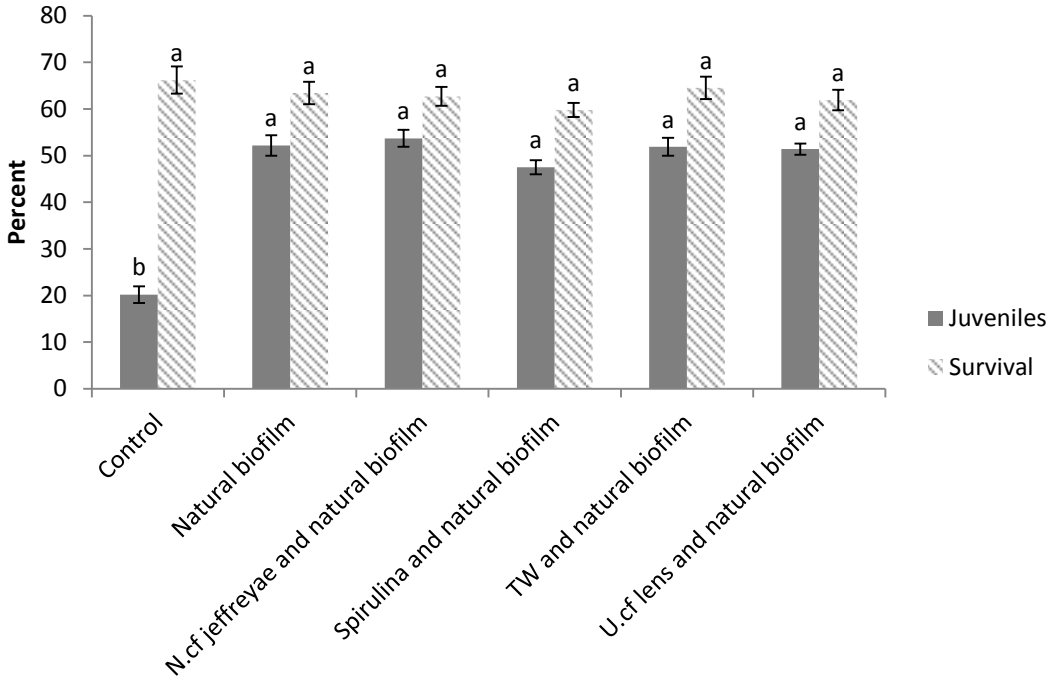



**Figure 19.** Percentage juveniles and percent survival of sandfish (*Holothuria scabra*) larvae settled on different types of biofilms. Results are expressed as mean ±SE (n=5). Significant differences between treatments are indicated by different letters above columns (P≤0.05).

## 3.2 Nursery Production

### *3.2.1 Effect of different feeds and fertilisers on growth and development of juvenile Holothuria scabra*

The length of juveniles in Algamac-fertilised tanks was significantly greater after four weeks compared to all other treatments (Fig. 19). The length of juveniles from tanks fertilised with either manure or Spirulina was also significantly greater than the length of juveniles from either the control or algal paste and Aquasol-fertilised tanks.

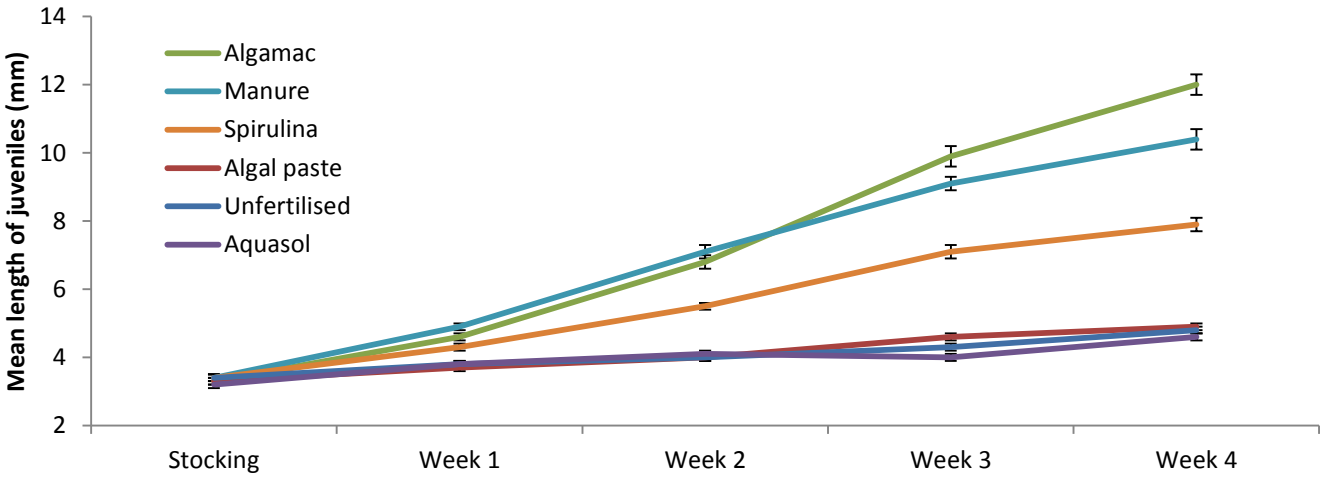



**Figure 20.** Change in body length (mm) of juvenile sandfish, *Holothuria scabra*, reared in tanks fertilised with different feeds. Values are expressed as mean ±SE (n=5)[1] and significant differences within columns are indicated by different superscripts (P≤0.05).
[1] As of week 3 n=4 in Spriulina treatment.

The wet weights of juveniles after the four week feeding trial are shown in figure 20. Algamac-fertilised tanks resulted in juveniles with a significantly greater wet weight than all other treatments. Fertilisation with either manure or Spirulina also resulted in the wet weight of juveniles being significantly higher compared to the control and fertilisation with either algal paste or Aquasol.

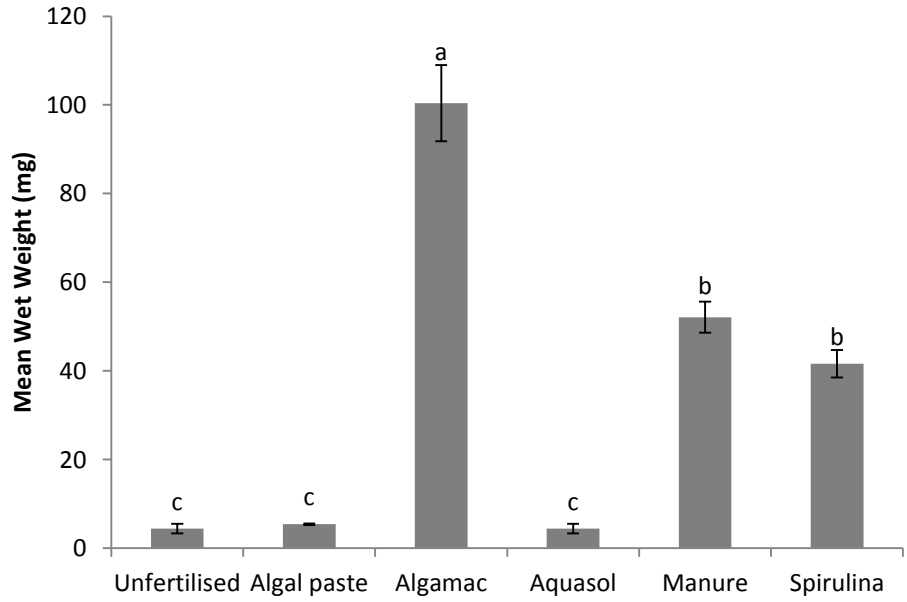



**Figure 21.** Wet weight of juvenile sandfish, *Holothuria scabra*, reared in tanks fertilised with different feeds after four weeks. Values are expressed as mean ±SE (n=5)[1] and significant differences between treatments are indicated by different letters above columns (P≤0.05).
[1] n=4 in Spirulina treatment.

Survival of juveniles after four weeks varied from 58.4±3.6% in unfertilised tanks to 78.4±2.8% in tanks fertilised with Algamac (Fig. 21). Only juveniles in tanks fertilised with Aquasol did not show a significantly higher survival rate compared to the control. The survival rates of juveniles in tanks fertilised with Algamac, manure and Spirulina were significantly higher compared to the other treatments (Fig. 21).

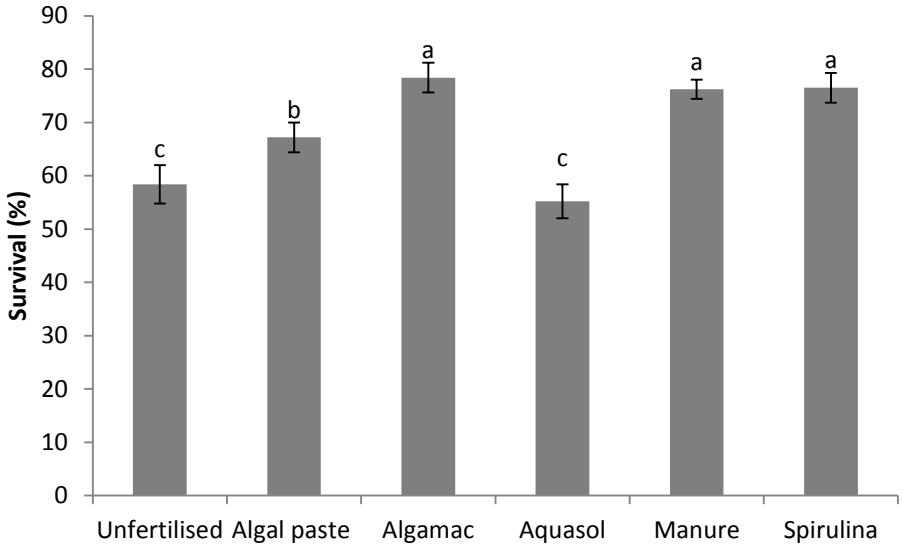
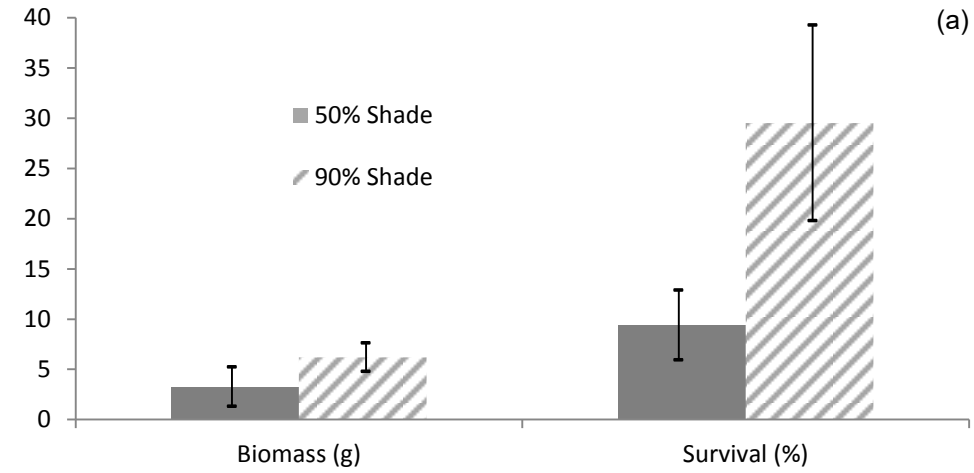
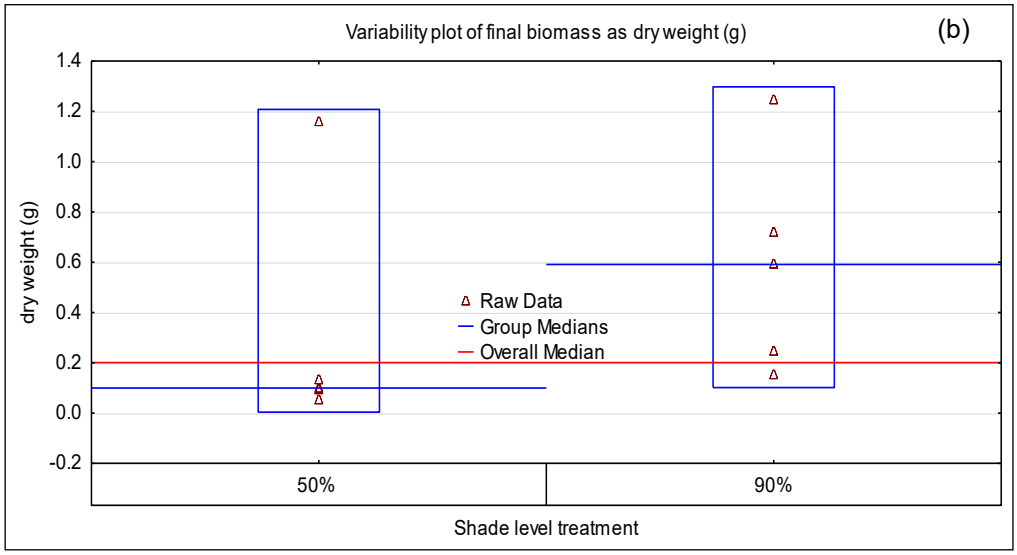



**Figure 22.** Survival of juvenile sandfish, *Holothuria scabra*, reared in tanks fertilised with different feeds for four weeks. Values are expressed as mean ±SE (n=5)[1] and significant differences within columns are indicated by different letters above columns (P≤0.05). [1] n=4 in Spirulina treatment.

### *3.2.2 Effect of shade level on growth and survival of juvenile Holothuria scabra in a commercial scale outdoor system*

Despite mean survival and harvested biomass being lower in the 50% shaded tanks (Fig. 22), statistically there was no significant difference in survival and biomass between treatments (survival t=-1.946, df 8, P >0.19, biomass t=-1.221, df 8, P 0.69). This can be attributed to one extreme result from the 50% shaded treatment which can be seen in the variability plots (Fig. 22).



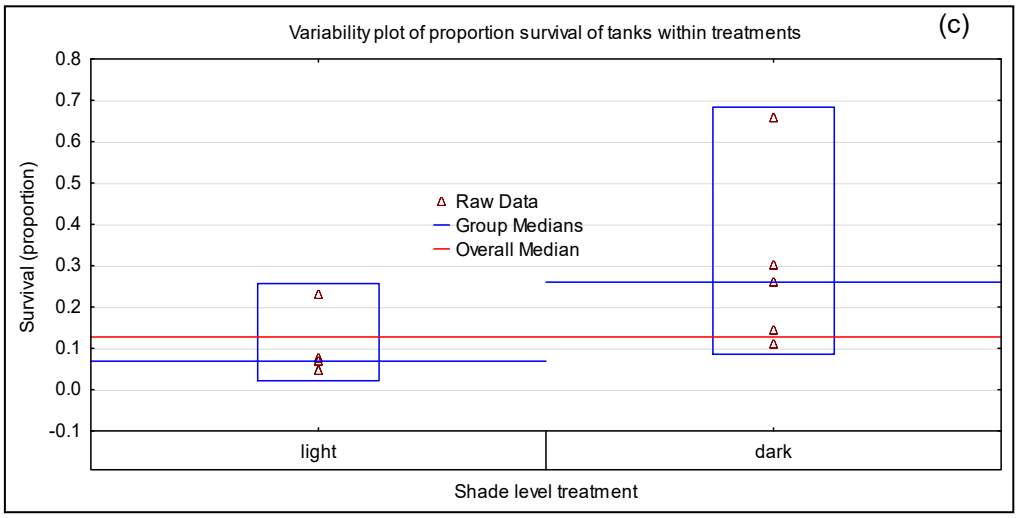




**Figure 23.** (a) Summary of mean biomass and survival ±SE for *H scabra* juveniles reared under 50% and 90% shade. (b) Variability plot of final biomass for each tank categorised into 50% and 90% shade treatments. (c) Variability plot of final survival for each tank categorised into 50% and 90% shade treatments.

### 3.2.3 *Effect of pond mud and enriched pond mud on growth and survival of juvenile sandfish*

The trial ran for 24 days and was terminated when a decrease in survival was apparent across two treatments to prevent further loss of useable data due to variation in stocking density. From day 9 pink bacteria was visible for several days in PMA tanks only. Natural biofilm began to be visible on all tanks after 4 days.

*Survival*

An in-tank survival count at day 14 showed significantly higher mortality ($p<0.0001$) in the PMA treatment than the C and PM tanks (PMA: 41.4%, C: 57.6% and PM: 61.8% survival). There was no significant difference between C and PM survival on day 14. By harvest on day 24, the PM treatment had an increased mortality (38.4% survival) resulting in no significant difference between it and the final PMA treatment (37.6% survival). Both were significantly different ($p<0.0001$) at harvest from the C treatment (51% survival). See figure 23.

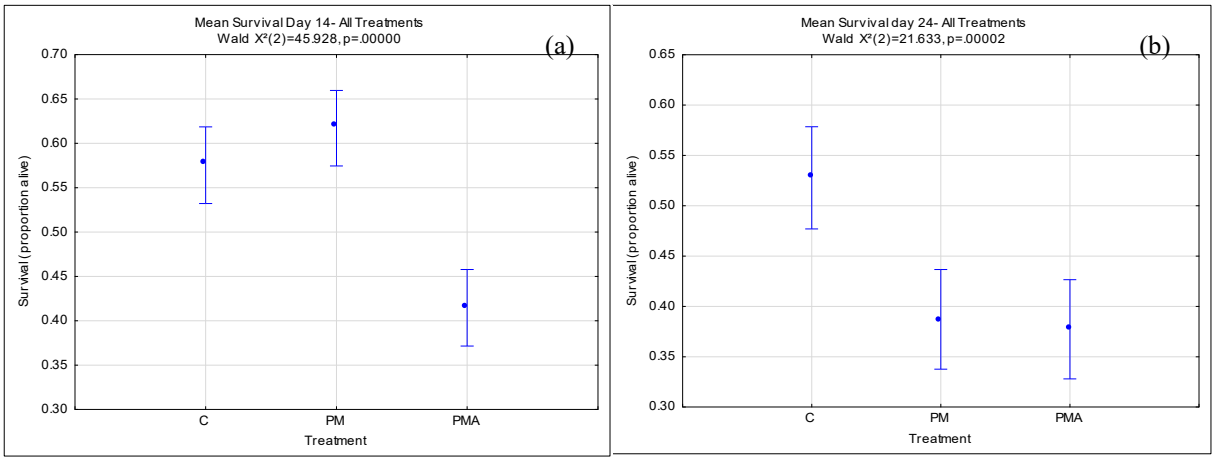



**Figure 24**: Survival of juvenile sandfish cultured with pond mud (PM), enriched pond mud (PMA) or no substrate (C) after 14 days (a) and 24 days (b). Error bars represent 95% confidence interval.

*Mass*

The PMA treatment yielded the highest mean mass at harvest with an average dry weight of 7.16645mg. This was significantly higher ($p<0.0001$) than PM (3.741262mg dry weight) and C (0.0784356mg dry weight) treatments. PM treatment was significantly higher ($p<0.0001$) than the C treatment. The C treatment dry mass at stocking was not significantly different to the C dry mass at harvest.

*Length*

At day 8, *H. scabra* in treatment C were significantly ($p<0.0001$) smaller in length than those in the PM and PMA treatments with a mean individual length of 1.66mm. Mean individual length of PM treatment (2.402mm) and PMA (2.459mm) were not significantly different on day 8. At day 15, *H. scabra* in the PMA treatment had a significantly ($p<0.0001$) larger mean individual length (6.506mm) than those in PM and C treatments. The mean individual length of *H. scabra* in the PM treatment (5.138mm) was also significantly larger ($p<0.0001$) than that of those in the C treatment (2.703mm). At harvest (day 24) *H. scabra* in the PMA treatment again had a significantly ($p<0.0001$) larger mean individual length (11.512 mm) than those in the PM and C treatments. Juveniles in the PM treatment had a mean individual length (8.757mm) that was also still significantly larger ($p<0.0001$) than that of the C treatment (4.640mm). See figure 24.

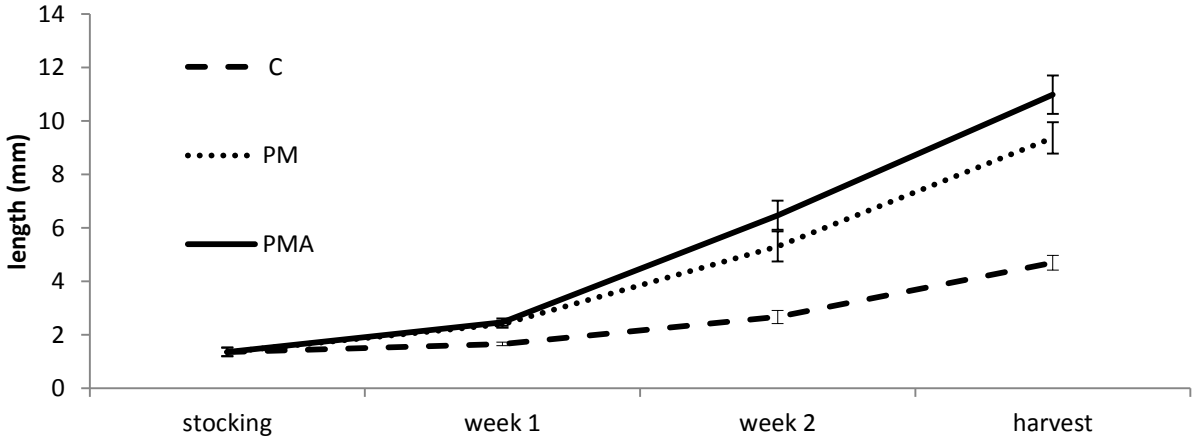



**Figure 25.** Growth of juvenile sandfish cultured with pond mud (PM), enriched pond mud (PMA) or no substrate (C) at stocking and after 7, 14 and 24 days. Error bars represent 95% confidence level.

*Survival vs growth*

Final survival was higher in treatments with lower growth (Figure 25). The PMA treatment experienced an earlier drop in survival than other treatments and higher growth.

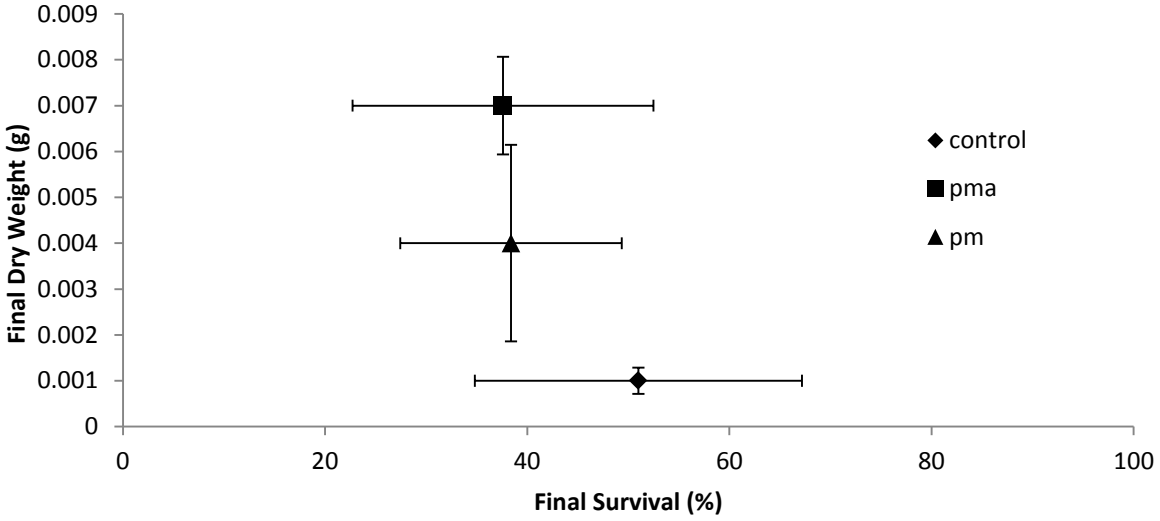



**Figure 26.** The relationship between final survival (%) and final dry weight (g) of juvenile sandfish cultured with pond mud (PM), enriched pond mud (PMA) or no substrate (Control). Error bars represent 95% confidence level.

### *3.2.4 Effect of the amount of artificial substrate in H. scabra nursery systems on the survival of newly settled juveniles*

A natural biofilm developed in all tanks and was readily visible after 4 days. The hourly logged temperature ranged between 23.1°C and 39.0°C with an average of 30.5°C SE=0.045 across all treatments during the trial.

Survival

In-tank survival counts throughout the trial were limited to C tanks only, as juveniles could not be seen on ropes. Survival of juveniles in control tanks at 1, 4 and 7 days post stocking were 93.3% SE=2.48, 83% SE=3.86 and 80.3% SE=4.59 respectively. Upon harvest of rope treatment tanks, the majority of all juveniles recovered were found on the tank walls and floor, and with 44% SE=8.01 (R30) and 33% SE=7.57 (R180) found on ropes. Harvesting from ropes using KCl caused juveniles to 'ball up' for a long period of time, affecting length measurement, and low numbers of animals, many damaged, prevented accurate measurement of weight. Final survival was therefore the only data analysed from harvest. Final survival on day 21 was 30.67% SE=5.21 (C), 27% SE=4.29 (R30) and 31.67% SE=4.01 (R180).There was no significant difference in the proportion surviving from each treatment at harvest (Wald's Chi-square with 2 df =1.42, p=0.492).

3.3 Sea Ranching

*3.3.3 First release*

*Growth*

Juvenile sea cucumbers released in the first release trial grew well with animals reaching a mean wet weight of 327g in 12 months (Fig. 26). The largest animal identified with a positive tag was 508g.

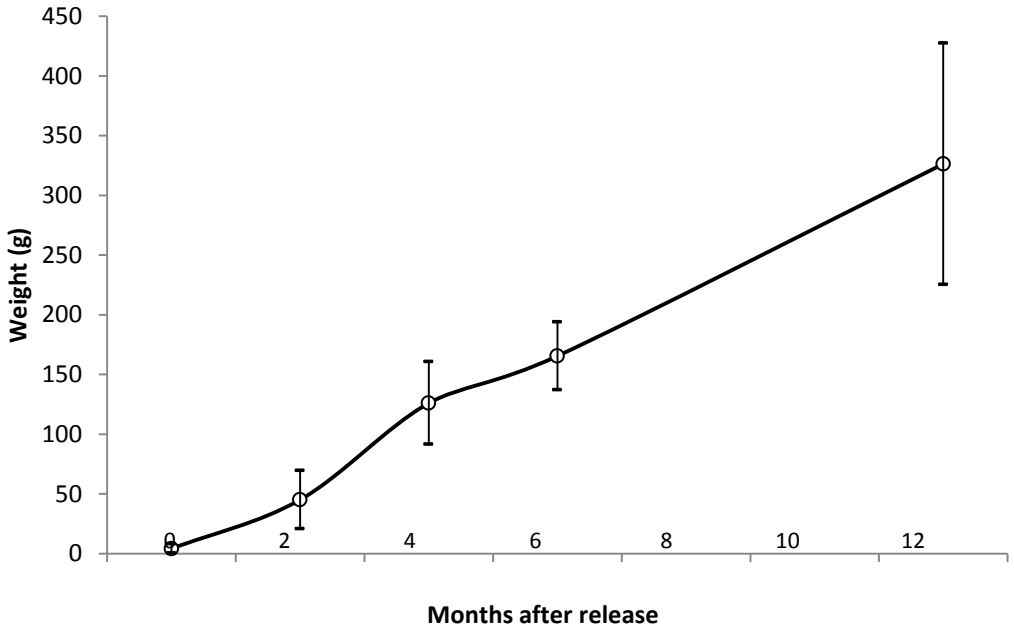



**Figure 27.** Growth of hatchery produced juvenile sandfish released onto the ranching site between July 2011 and June 2012. Bars represent ±SD.

*Survival*

Prior to releasing juvenile sea cucumber no significant difference between site abundance was observed in May (t=0.708, df 44, P 0.983) however in October after the release, a significant difference in abundance between the ranch site and the control site was observed (t=-2.226, df 34.325, P 0.033) (Fig. 27).

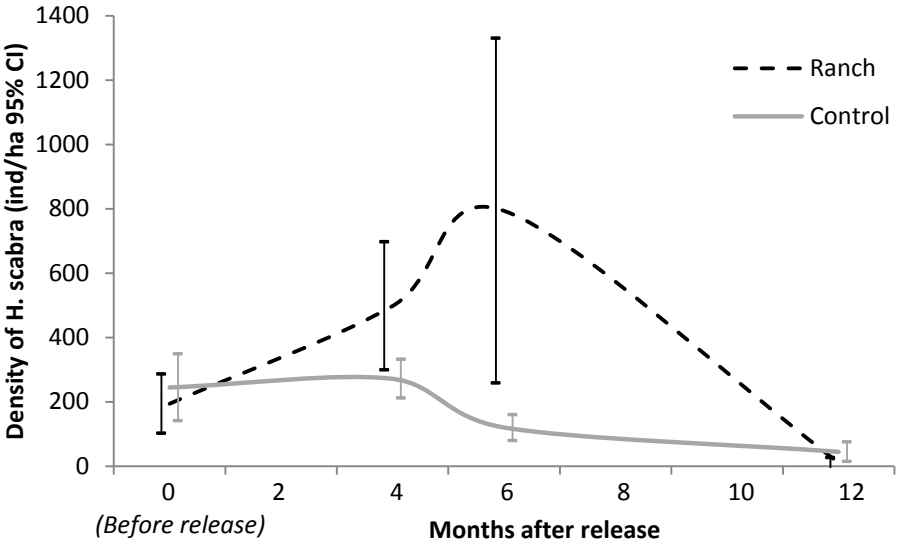



**Figure 28.** Density of sandfish on the release site and the monitoring site June 2011 – June 2012. Bars represent 95% CI.

### *3.3.4 Second release*

The second site, with less seagrass and a greater exposure to wave action, would seem less favourable compared to the first site. However growth rate of tagged animals indicates a comparable growth rate (mean weight 273.4g after 10 months) to what was achieved on the first release site (Fig. 28).

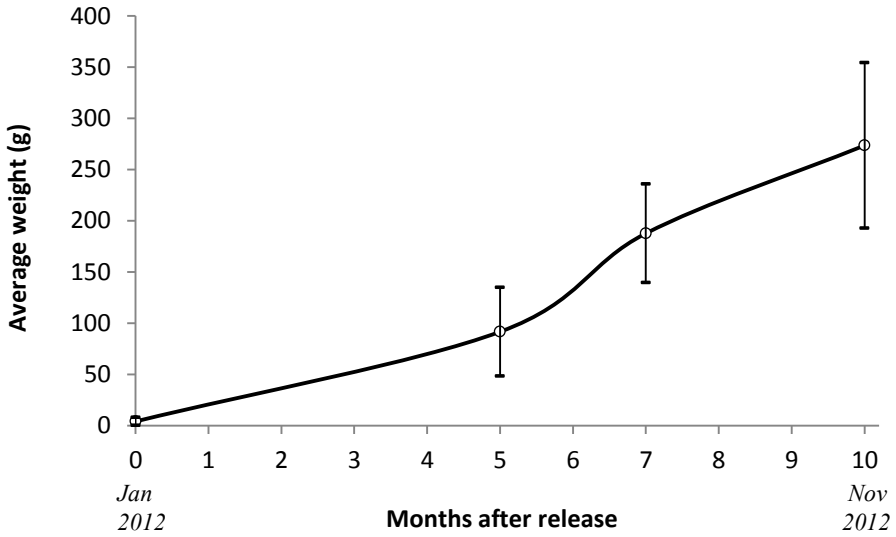



**Figure29.** Growth of hatchery produced sandfish released on the second release site. Bars represent ±SD.

To generate baseline information on dispersal of released juveniles, the second release was conducted in a 5m x 5m area utilising two release cages. The site was chosen for this purpose due to the small area of seagrass for initial protection,

surrounded by a large expanse of sand flat which under suitable conditions, is easy to find and track animals.

After seven months the majority of released animals appeared to remain within 50m from the point of release; however a number had moved up to 190m away (Figure 29). There did not appear to be any specific direction animals were moving from the release point, with some individuals migrating along the gutters adjacent to the beach and some heading straight out to deeper water.

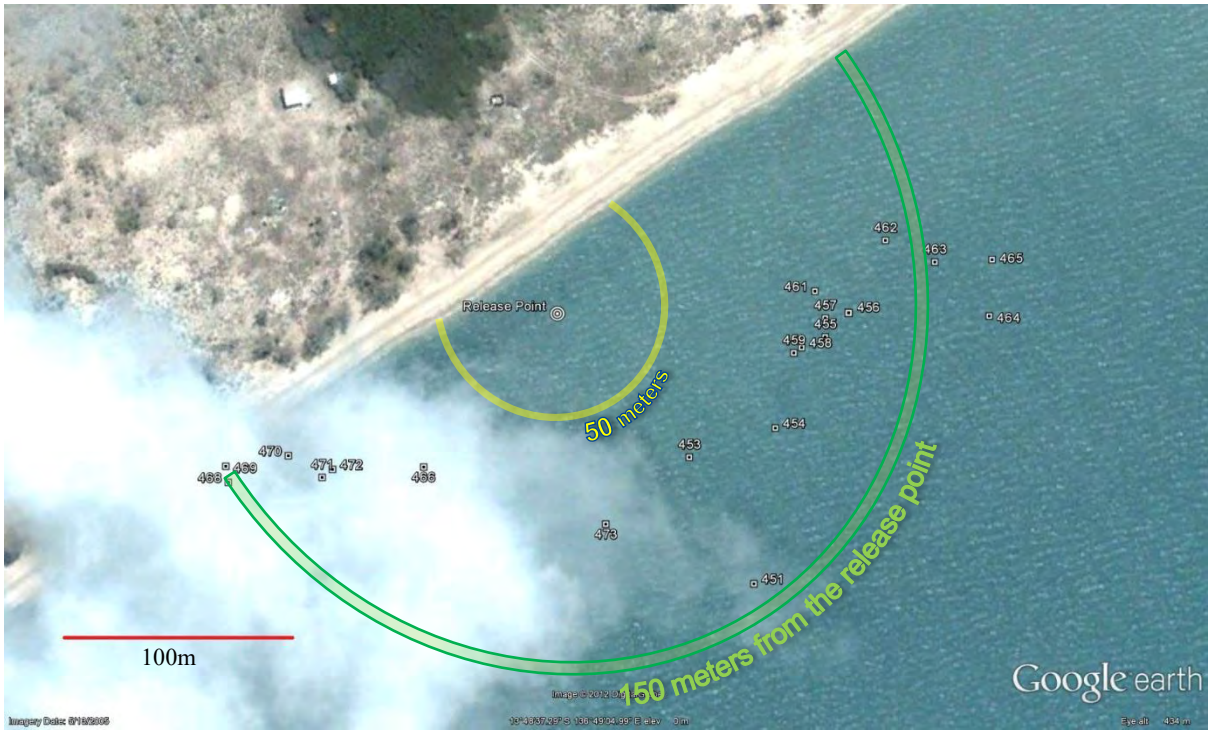



**Figure 30.** Movement of individuals after seven months from point of second release. Each numbered point represents either an individual or a cluster of hatchery released animals.

While this observational work is somewhat subjective, it does provide information on the amount of area required to effectively undertake further movement/migratory studies, and the effect animal movement may have on results of ranching trials.

### *3.3.5 Third release*

*Generic growth model*

Using historical catch data, the $W_\infty$ parameter was estimated to be 1589.9g through a length weight analysis and use of a 'Wetherall plot' (King, 2007). The growth coefficient (*K* parameter) was determined by ordinary least squares regression analysis of mean size at age to be 0.827, indicating sandfish have a relatively fast growth rate, and estimates sandfish will reach full grown size in approximately 20 months (Fig. 30).

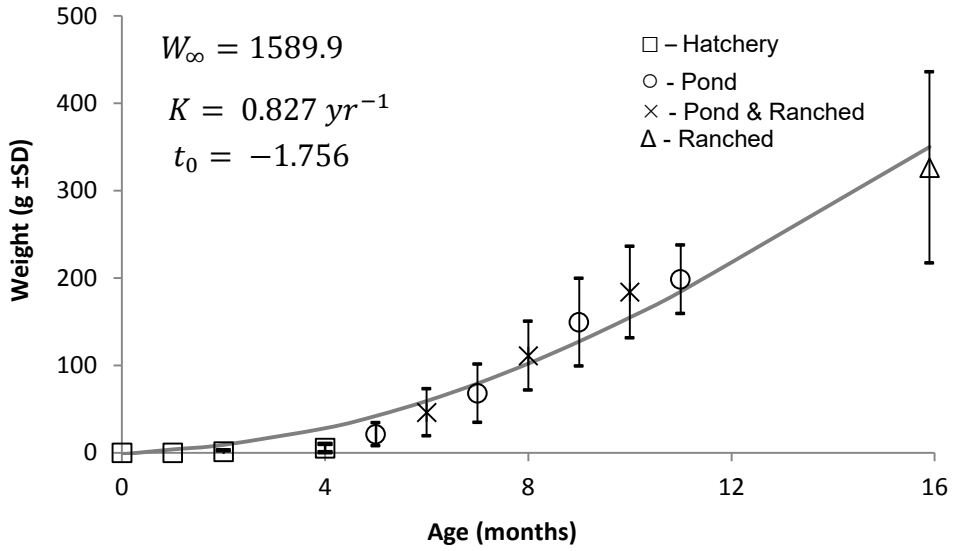



**Figure 31.** von Bertalanffy Plot of known weight at age data for *Holothuria scabra* from the Northern Territory. Bars represent ±SD. Symbols represent data source.

*Modal progression of wild recruits against hatchery produced juveniles*

A total of 346 sandfish were weighed and measured from the monitoring site over six sampling events between June 2012 and January 2013. The weight frequencies over the study period identified a recruitment mode that became detectable in August, and when accounting for known life history durations of larvae and pre-settlement stages, this cohort was likely to be a result of a March / April spawning event, and have been assigned a relative age of 4 months old.

The monthly mean weight of animals sampled on the study site indicated that recruits identified in August were growing with mean weight increasing from 91g, to 176g in November. In the January 2013 survey the mean weight was 257g, however the cohort was not as clearly definable and the distribution became more spread out as individuals in the cohort aged (Fig. 31). An older, small cohort appeared to become identifiable later in the study, and in the January 2013 survey a small number new recruits were observed on the site possibly indicating another light recruitment as a result from a September / October spawning. If this recruitment was derived from the ranched stock spawning would have occurred at approximately 18 months old.

Concurrently monitoring was also being conducted on the adjacent ranching site where 550 sandfish were weighed and measured over five sampling events (June, August, October, November, and January). The pre-release surveys in June and August identified a similar size frequency data set to the cohort detected on the site being monitored for wild recruits.

Released juveniles were spawned in May 2012, making them approximately 2 months younger than the wild recruits, which is reflected in the size distributions. The surveys showed a clear shift in the size frequency distribution with the inclusion of the hatchery produced juveniles onto the ranching site. The November and January survey included sampling for positively tagged animals as shown in Figure 31. By January hatchery released juveniles had reached a mean weight of 186.8g, and mean length of 159.4mm.

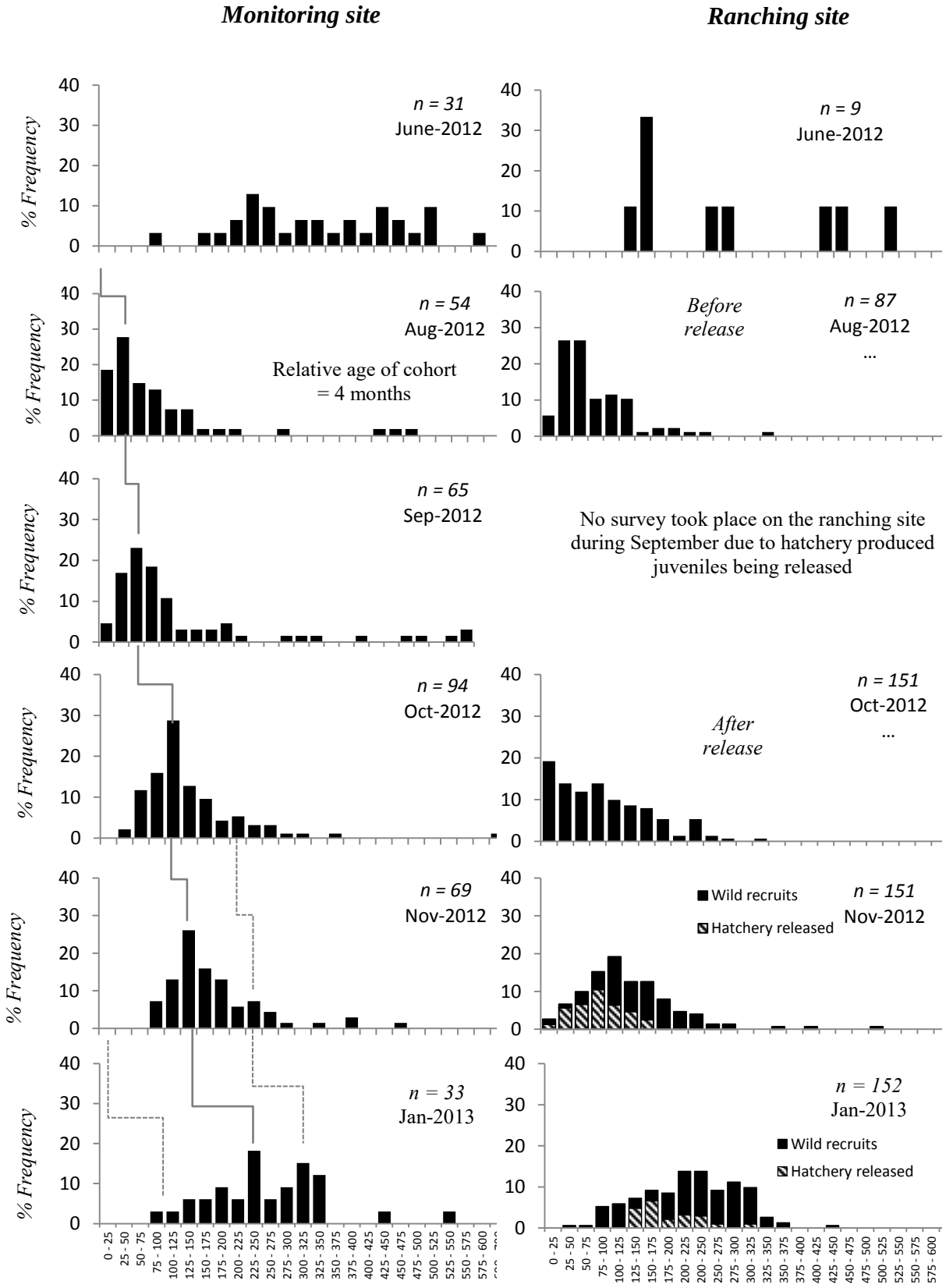



**Figure 32.** Frequency distribution of monthly weight (g) surveys of *Holothuria scabra* from the monitoring site and the ranching site. Surveys conducted between June 2012 and January 2013. Solid line represents the mode of wild recruits; dotted lines represent the modes from possible additional light recruitment events on the monitoring site. Shaded columns represent hatchery produced ranched component of surveyed sandfish on the ranching site.

*Comparison of abundance, biomass and performance*

Density and biomass on both sites was very similar prior to release of hatchery produced juveniles. Mean density of wild sandfish on the monitoring site increased from June onwards as new recruits became detectable, peaking at 1182 /ha ±359 in October before declining to reach 293 /ha ±124 in January. Biomass on the monitoring site also increased from June to plateau between October and November at 165 ±9.99 kg /ha and 166 ±22.95 kg /ha respectively, before declining by January to 81 ±4.59 kg /ha (Fig. 32).

The mean density of sandfish on the ranching site prior to release in August was 853 /ha ±364. In November, after releasing the juveniles the density peaked at 1938 /ha ± 536 before declining to 1076 /ha ±289 in January. The biomass of sandfish on the ranching site also increased rapidly until November, peaking at 257 ±11.77 kg /ha, before plateauing between November and January (Fig. 32).

*Analysis of density and biomass between sites*

A significant difference for both density and biomass was observed between the ranching site and the monitoring site after releasing juveniles in November (density t = 3.714, df 28, P 0.001; biomass t = -4.967, df 218, P <0.001), and January (density t = 5.445, df, 27, P <0.001; biomass t = -11.416, df 183, P <0.001). The ranching site was carrying over twice the number of sandfish per hectare in November and January, and three times the biomass in January compared to the monitoring site (Fig. 32).

*Analysis of density and biomass within sites*

Within both sites there was a significant drop stock density between November and January, (monitoring site t = 5.934, df 28, P <0.001; ranching site t = 2.955, df 27, P 0.006). Between November and January a significant drop in biomass was also detected on the monitoring site (t = 7.246, df 100, P <0.001), however no significant change in biomass was observed on the ranching site over the same period (t = 1.6, df 301, P 0.111).

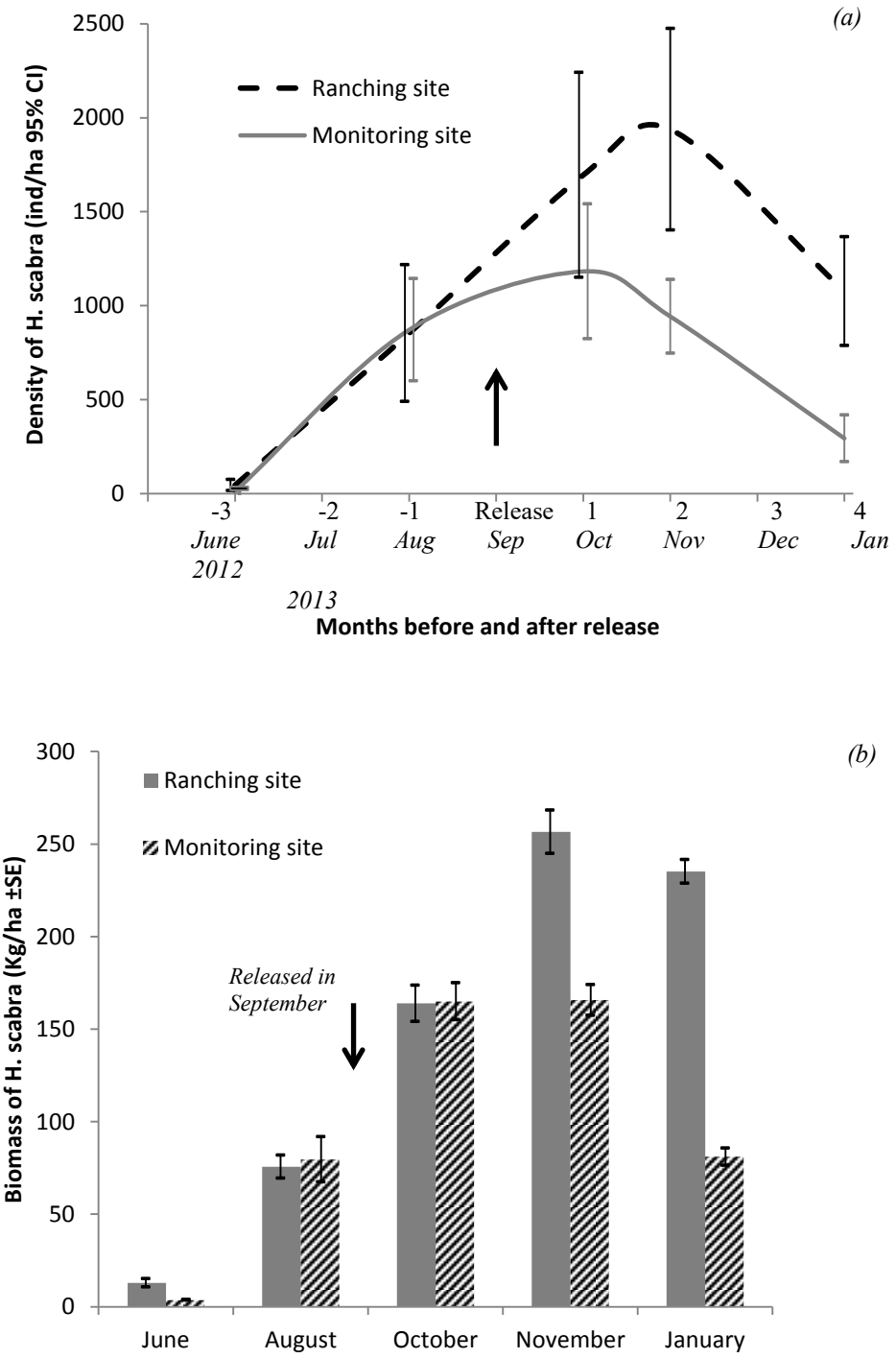



**Figure 33.** (a) Abundances of *H. scabra* on the release site and the monitoring site June 2012 – January 2013. Bars represent 95% CI. (b) Biomass of *H. scabra* on the release site and the monitoring site June 2012 – January 2013. Bars represent ±SE.

*Comparison of size at same age*

Utilising data from the modal progression analysis for wild recruits, and hatchery produced juveniles identified with a positive tag, there was no significant difference in length and weight observed between naturally recruited sandfish and hatchery produced juveniles two months after being released into the wild at 6 months of age (weight $t$ = 1.605, df 76, P = 0.113, length $t$ = -0.993, df 76, P = 0.324). At 8 months of age (four months after being released) a significant difference between the length

and weight of hatchery produced juveniles and wild recruits was observed (weight $t$ = 4.895, df 63, P <0.001, length $t$ = 2.125, df 63, P = 0.038). The mean weight of 8 month old hatchery released juveniles was over 20% greater than the natural recruits at the same age (Fig 33).

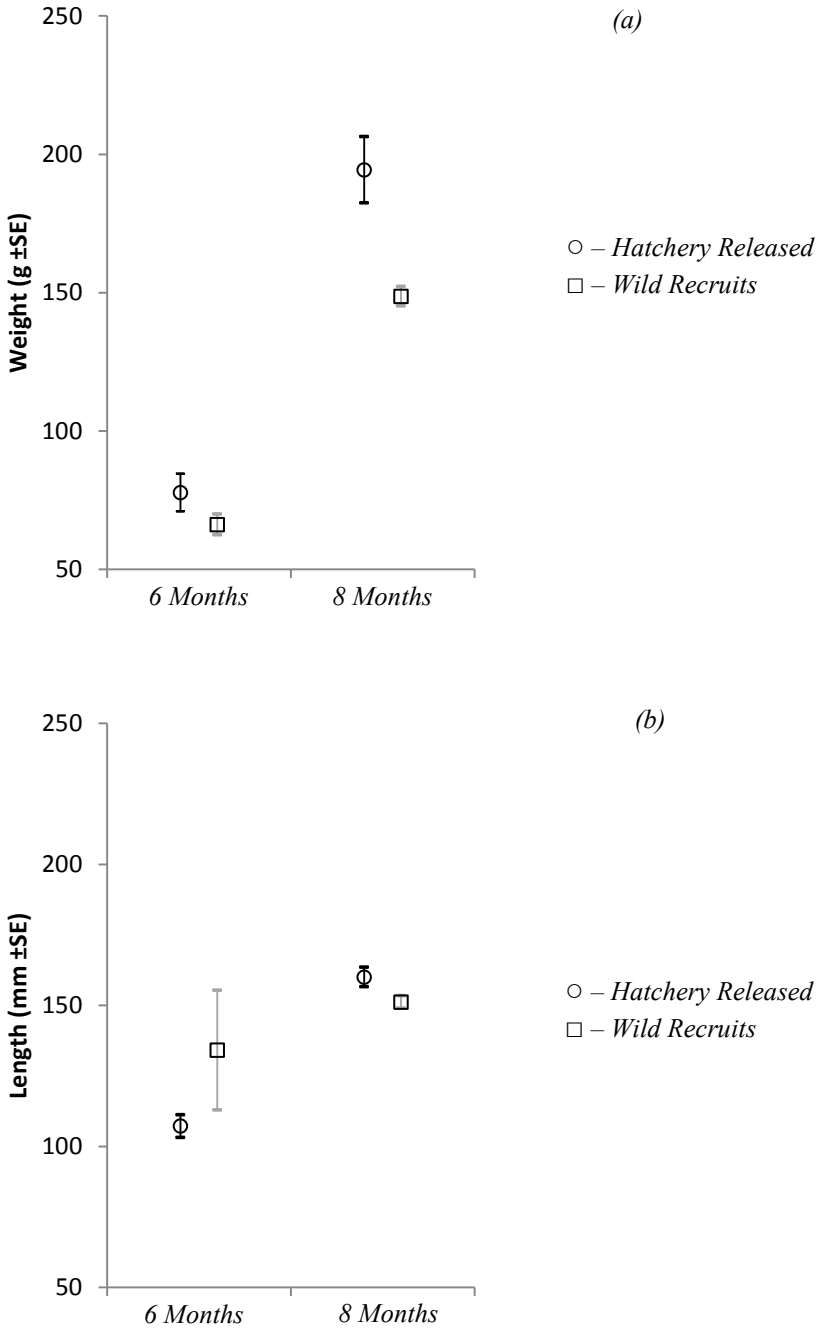


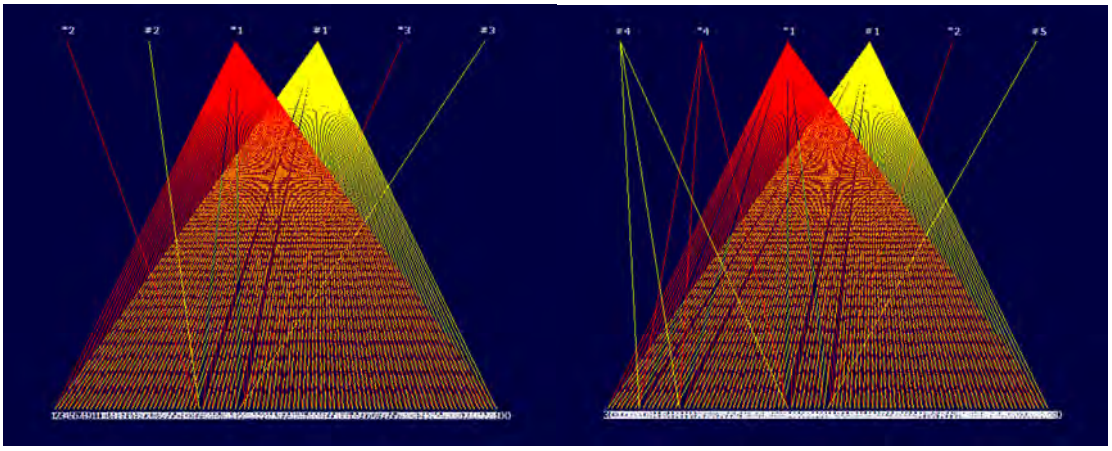

**Figure 34.** Size at age comparison of means between wild recruits and ranched juvenile sandfish. (a) - Weight, (b) – Length. Bars represent ±SE.

*3.4* Hatchery Genetics

Results of the parental contributions are reported below for the three hatchery runs; Batch 04/13, Batch 02/13(2) and Batch 03/13.

*Batch 04/13*

Batch 04/13 consisted of a small spawning from a pool of seven broodstock (SG1-1 to SG1-7). A total of $4.2x10^5$ fertilised eggs were produced.



(a) Nursery (b) Hatchery

**Figure 35.** Analysis of parental contributions of progeny for Batch 04/13. Broodstock were not included in the sib group analysis. Potential parents (only those that have been identified as parents) are represented on the top row and progeny sampled from the nursery (a) and hatchery (b) on the lower row. Lines are drawn from parents to offspring if they have been identified as being a parent/offspring dyad.

When examining the sibling groups of the progeny at different time points i.e. hatchery (31 days) and nursery (60 days) there is no difference between the two collections in terms of contribution of broodstock (Fig. 34).

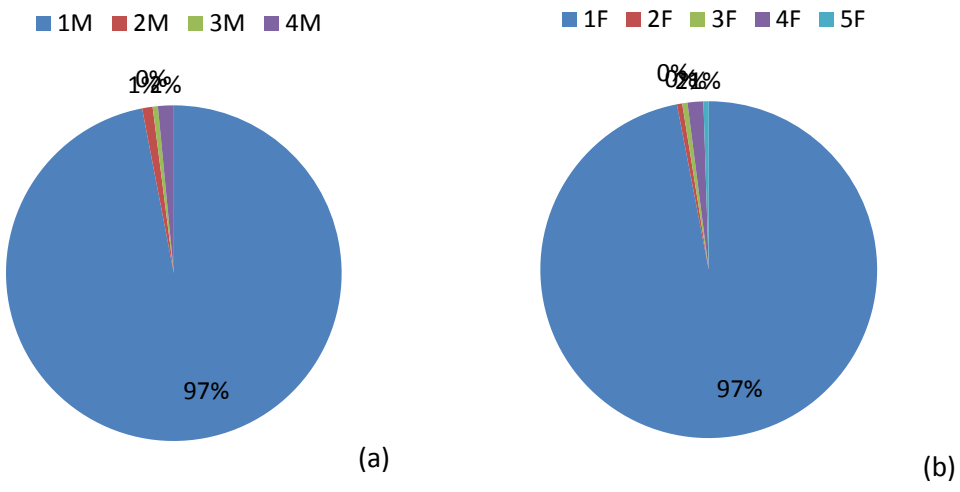


**Figure 36.** Percentage of parental contributions to the progeny for batch 04/13. Both hatchery and nursery progeny have been pooled. The results separated into parent 1 (a) and parent 2 (b). Note that the sex of these parent individuals is unknown.

In batch 04/13 only a single male and a single female have contributed to the vast majority (97/100) of the progeny (Fig. 35). The estimates of effective population size indicate that 2 individuals are involved in contributions to the progeny in batch 04/13 (Table 7).

**Table 7.** Estimates of effective population size from progeny only analysis for batch 04/13

| Estimates by COLONY full likelihood method: Assuming random mating | |
|---|---|
| Alpha | 0.00 |
| Ne | 2 |
| CI95(L) | 2 |
| CI95(U) | 2147483647 |

Analysis of the parental contributions with the parental genotypes included showed no difference to the progeny only analysis with an Ne estimate of 2. There was single paternity and maternity for the offspring fertilized by the broodstock in this batch with broodstock SG1_7 and SG1_4 combining with each other's eggs/sperm as expected.

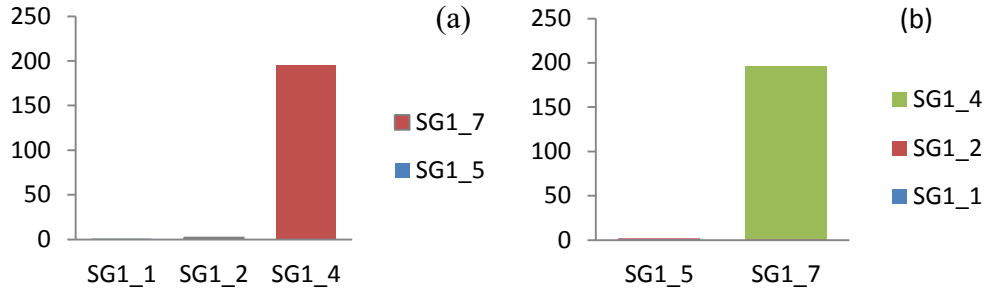
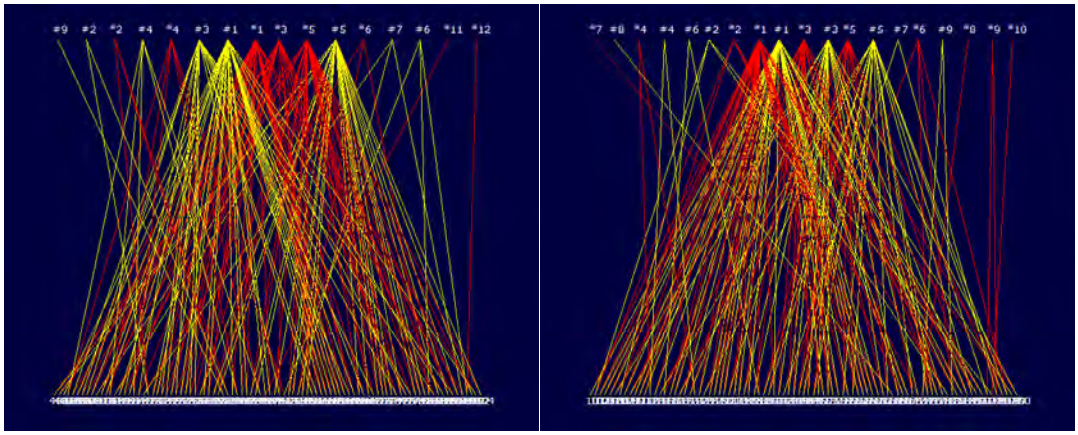



**Figure 37.** Contribution of different broodstock to the progeny from parent 1 (a) and parent 2 (b). Raw numbers are presented.

*Batch 02/13(2)*

Batch 02/13(2) consisted of a larger spawning than batch 04/13 with the production of $3.8 \times 10^6$ fertilised eggs from a pooled spawning of ten broodstock.



Nursery (a) Hatchery (b)

**Figure 38.** Parental contributions of progeny for Batch 02/13(2). Broodstock were not included in the sib group analysis. Potential parents (only those that have been identified as parents) are represented on the top row and progeny sampled from the nursery (a) and hatchery (b) on the lower row. Lines are drawn from parents to offspring if they have been identified as being a parent/offspring dyad.

When examining the hatchery (day 43) and nursery (60 day) progeny separately (Fig. 37) there is no difference between the two collections in terms of contribution of broodstock as was the case for batch 04/13.
There was a greater diversity in parental contribution of sib groups with six parents contributing to the majority of offspring.

In batch 02/13(2) three males and three females contributed significantly to the progeny (Fig. 38).

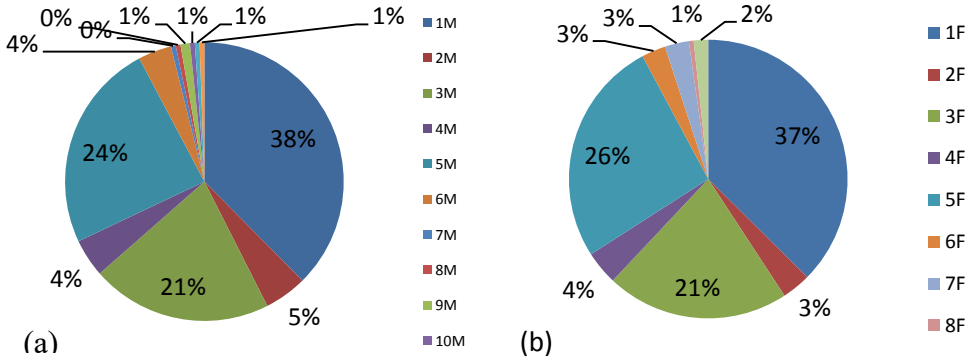



**Figure 39.** Percentage of parental contributions to the progeny for batch 02/13(2). Both hatchery and nursery progeny have been pooled. The results separated into parent 1 (a) and parent 2 (b). Note that the sex of these parent individuals is unknown.

The estimate of effective population size for this batch using only the progeny genotypes indicates that 8 individuals were involved in contributions to the progeny in batch 02/13(2) (Table 8).

**Table 8.** Estimates of effective population size from progeny only analysis for batch 02/13(2)

| Estimates by COLONY full likelihood method: Assuming random mating | |
|---|---|
| Alpha | 0.00 |
| Ne | 8 |

| | |
|---|---|
| CI95(L) | 4 |
| CI95(U) | 23 |

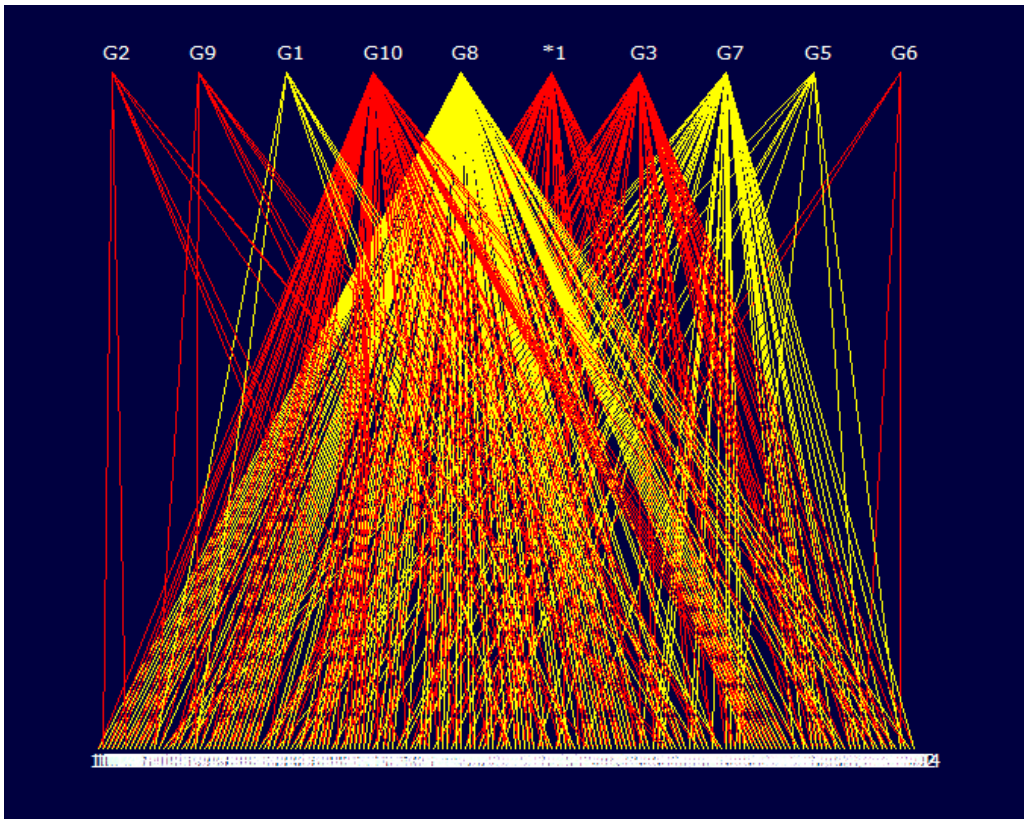



**Figure 40.** Analysis of parental contributions of progeny for Batch 02/13 when broodstock were included in the sib group analysis. Both hatchery and nursery samples have been pooled in this analysis. Broodstock (only those that have been identified as parents) are represented on the top row and progeny on the lower row. Lines are drawn from broodstock to offspring if they have been identified as being a parent/offspring dyad.

Including the broodstock genotypes in the analysis we can see that five individuals contribute the majority of genetic material to the progeny (Fig. 39). This is slightly different to the results when not using the broodstock genotypes but only by a single parent.

In this batch 50% of the broodstock significantly (≥10%) contributed to the progeny; approximately double that observed for batch 04/13. An estimate of the effective population size with the broodstock genotypes included indicates that 5 individuals are involved in contributions to the progeny in batch 02/13(2) (Table 9).

**Table 9**. Estimates of effective population size for batch 02/13 when using broodstock in the analysis.

| Estimates by COLONY full likelihood method: Assuming random mating | |
|---|---|
| Alpha | 0.00 |

| | |
|---|---|
| Ne | 5 |
| CI95(L) | 2 |
| CI95(U) | 20 |

Limited multiple paternity and maternity of released eggs and sperm in batch 02/13(2) was observed. The only broodstock who had a large number of their gametes combine successfully with multiple opposite sex parents was G8. All other progeny were the result of single paternity/maternity batches (Fig. 40).

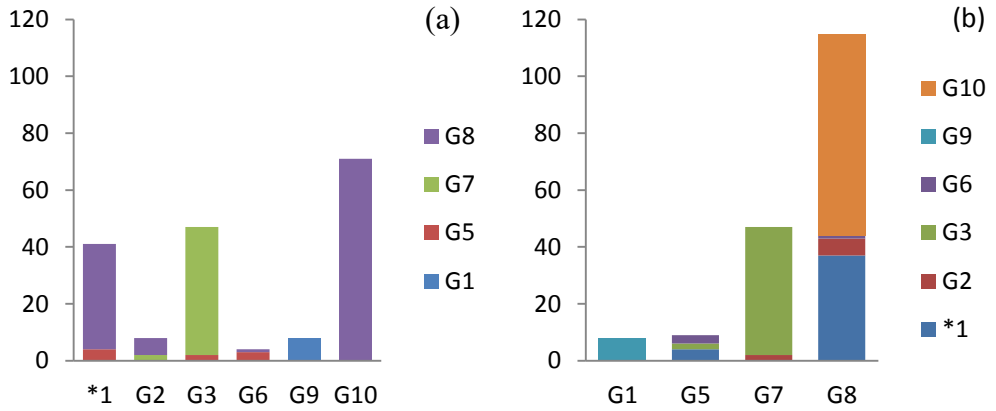
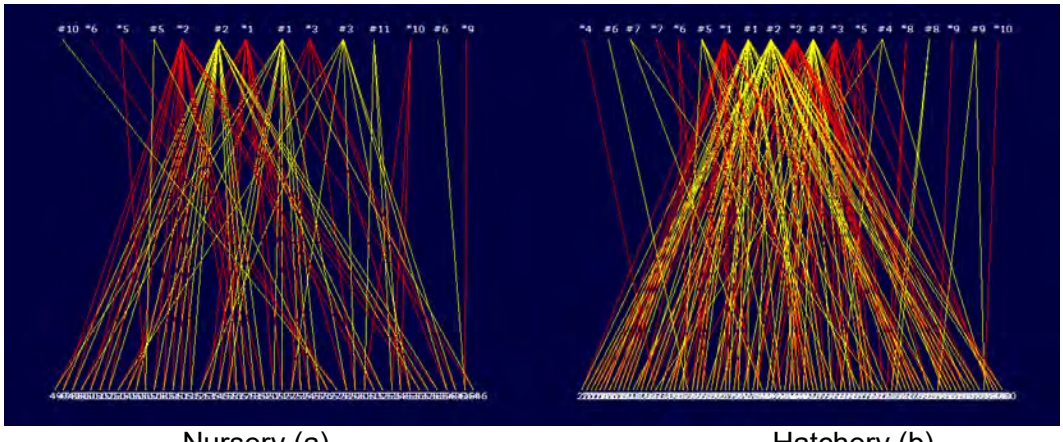



**Figure 41.** Contribution of different broodstock from batch 02/13(2) to the progeny from parent 1 (a) and parent 2 (b). Raw numbers are presented.

*Batch 03/13(2)*

Batch 03/13(2) consisted of a larger spawning than batch 04/13 but a smaller spawning than batch 02/13(2) with the production of $1.08x10^6$ fertilised eggs from a pooled spawning of seven broodstock (SG3-1 to SG3-7).



Nursery (a) Hatchery (b)

**Figure 42.** Analysis of parental contributions of progeny for Batch 03/13. Broodstock were not included in the sib group analysis. Potential parents (only those that have been identified as parents) are represented on the top row and progeny sampled from the nursery (a) and hatchery (b) on the lower row. Lines are drawn from parents to offspring if they have been identified as being a parent/offspring dyad.

When examining the hatchery (day 30) and nursery (day 60) progeny separately there is no difference between the two collections in terms of contribution of broodstock for batch 03/13(2) (Fig. 41).

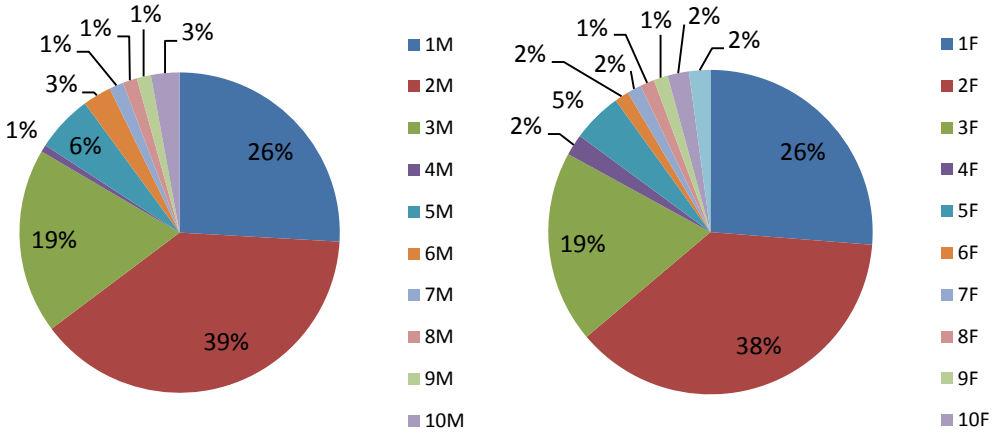



**Figure 43.** Percentage of parental contributions to the progeny for batch 03/13(2). Both hatchery and nursery progeny have been pooled. The results separated into parent 1 (a) and parent 2 (b). Note that the sexes of these parent individuals is unknown.

The progeny-only result for batch 03/13(2) indicate that three males and three females have contributed significantly to the progeny of batch 03/13. The estimated effective population size based on this analysis is eight individuals (Table 10).

**Table 10.** Estimates of effective population size from progeny only analysis for batch 03/13

| Estimates by COLONY full likelihood method: Assuming random mating | |
|---|---|
| Alpha | 0.00 |
| Ne | 8 |
| CI95(L) | 4 |
| CI95(U) | 23 |

With the broodstock included in the analysis, five individuals contribute the majority of germ cells to the progeny in batch 03/13 (2) (Fig. 43). This is slightly different to the results when not using the broodstock genotypes but only by a single parent. The individuals *1 and #1 were identified as parents possibly due to poor genotyping of the actual broodstock who these individuals represent.

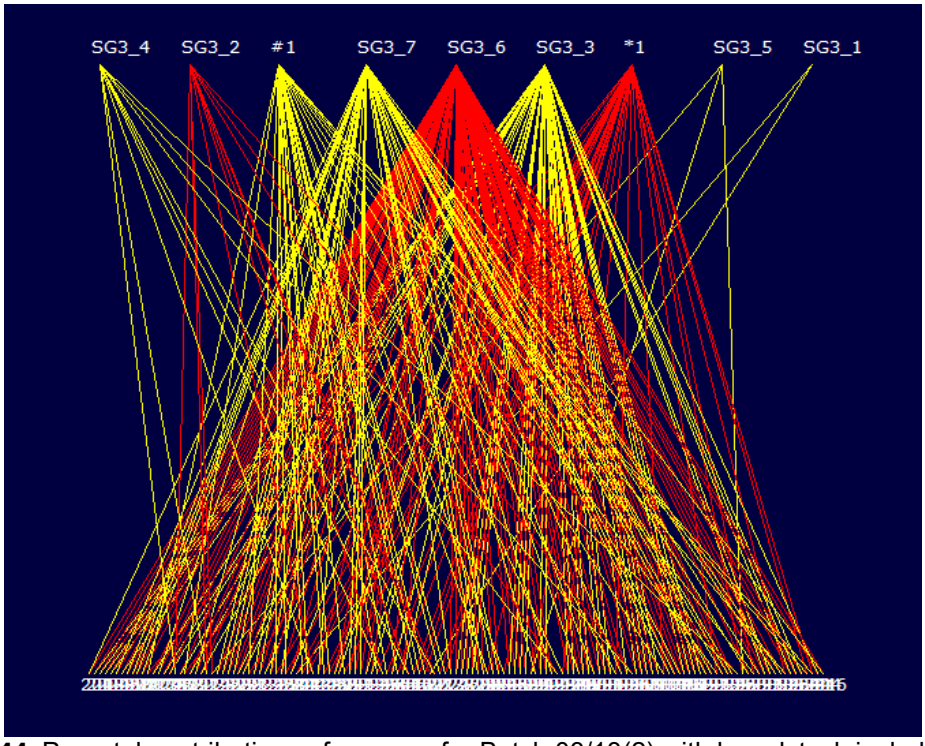



**Figure 44.** Parental contributions of progeny for Batch 03/13(2) with broodstock included in the sib group analysis. Both hatchery and nursery samples have been pooled in this analysis. Broodstock (only those that have been identified as parents) are represented on the top row and progeny on the lower row. Lines are drawn from broodstock to offspring if they have been identified as being a parent/offspring dyad.

The proportion of broodstock contributing significantly (≥10%) to the progeny of this batch was 0.714 the highest observed in the three batches analysed. The estimate of the effective population size with broodstock included is five for batch 03/13(2) (Table 11).

**Table 11**. Estimates of effective population size for batch 03/13(2) when using broodstock in the analysis.

| Estimates by COLONY full likelihood method: Assuming random mating | |
|---|---|
| Alpha | 0.00 |
| Ne | 5 |
| CI95(L) | 2 |
| CI95(U) | 20 |

Once again only limited multiple paternity and maternity of released eggs and sperm was observed. In batch 03/13(2), the only broodstock who had a large number of their progeny from multiple opposite sex parents was broodstock SG3-6. All other major groups were the result of single paternity/maternity fertilisation (Fig. 44).

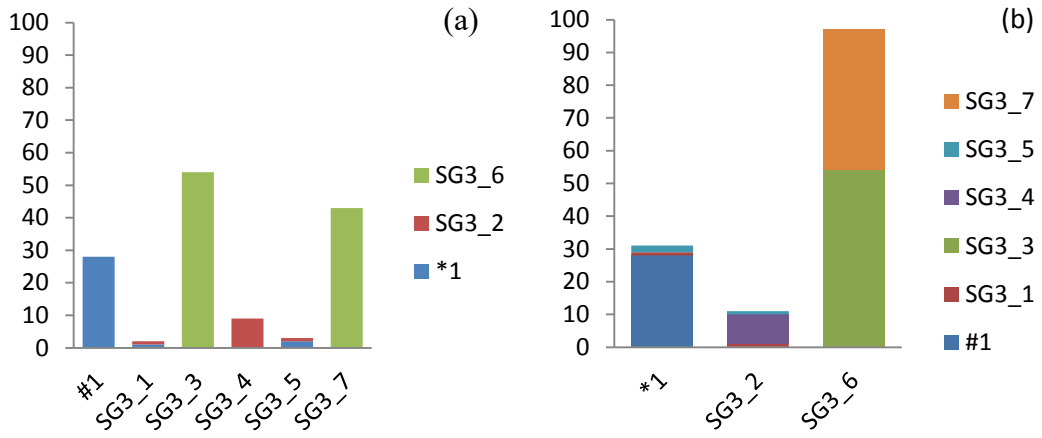



**Figure 45.** Contribution of different broodstock from batch 03/13(2) to the progeny from parent 1 (a) and parent 2 (b). Raw numbers are presented.

# 4. Discussion

## 4.1 Hatchery

*Nutrition*

The sandfish is the most commonly cultured tropical species of sea cucumber; however, research on larval diet, nutrition and feeding has been critically lacking (Purcell *et al.* 2012). Published manuals (Agudo, 2006; Duy, 2010) recommend a mixture of up to four species of microalgae including *Chaetoceros muelleri, Rhodomonas salina* and *Isochrysis galbana* can be used for rearing larval sandfish. Results from research carried out in this project emphasise the efficacy of diatoms (*Chaetoceros sp.*) over the flagellates as the preferred dietary microalgae for culturing sandfish larvae (Knauer, 2011). Of the two diatoms tested in the study, the growth of larvae fed *C. muelleri* did not significantly differ from those fed *C. calcitrans.* However, the percentage of competent larvae reaching the doliolariae stage was significantly greater from larvae fed *C. muelleri* than those fed any other microalgae in the study, including *C. calcitrans*.

More recent assessment of dietary requirements for larval sandfish has focussed on the use of algal concentrates (Hair *et al.* 2011). Algal pastes, comprising refrigerated slurries of one or more micro-algal species, have shown particular promise in aquaculture (Robert and Trintignac 1997), and commercially available algal pastes have been widely used to rear bivalve larvae and spat (Krantz *et al*. 1983; O'Connor and Nell 1992; Troost *et al*. 2009).

In contrast to bivalve production, aquaculture of echinoderms such as sea urchins and sea cucumbers has only recently become commercially significant. The world aquaculture production of 'sea urchins and other echinoderms' increased from 0 t in 2002 to 96,000 t in 2008 (Anonymous 2010), therefore it is not surprising there is little information on the nutritional value of algal pastes for larval echinoderms. Liu *et al*. (2007a, b) found that the larvae of two sea urchin species did not grow and develop normally when fed an algal paste. In contrast, Hair *et al.* (2011) fed an algal paste to sea cucumber larvae and obtained normal growth and development. The primary motive for the Hair (2012) study appeared due to the expense, and the lack of appropriate resources and technical skills for the maintenance of micro algae culture. Under experimental conditions during this project, it was found the use of *Thalassiosira weissflogii* algal paste was not a suitable substitute for live algae. Follow-up pilot studies assessing other algal paste product such as 'Shellfish Diet 1800' (Instant Algae®, Reed Mariculture, USA), which is a mix of *Isochrysis* sp. (30%), *Tetraselmis* sp. (20%), *Pavlova* sp. (20%) and *T. weissflogii* (30%) (www.reedmariculture.com) also did not support the larval development of sandfish.

Given these results establishing the high nutritional value of *C. muelleri* as a microalgal diet for larval sandfish, and the ease of culturing in large volumes such as 1000Lt tanks the use of *C. muelleri* as the preferred diet for larval production is recommended.

*Water exchange protocols*

In order to further standardise hatchery protocols, an assessment of hatchery water exchange protocols was carried out. There are currently two industry standards that

can be used for water exchange in hatchery tanks; a partial (30%) exchange per day (Duy 2010) or a complete exchange (100%) every second day Agudo (2006). Although there were no differences in survival, at both a low and high density larvae cultured using a 30% daily water exchange developed much more rapidly than larvae from the other two treatments.

*Settlement*

It has been established that the number of bacteria in biofilms in larval rearing tanks steadily increase over at least a 13-day period (Bourne *et al.* 2006). It is possible that the uninterrupted development of a stable biofilm has a beneficial effect on larvae, as opposed to the new establishment of a biofilm every two or four days. Moreover, since the larvae in tanks drained every fourth day developed significantly faster compared to larvae from tanks drained every second day this may indicate that, additionally, the physical stress of draining negatively affected the larvae.

Despite the rapid expansion of sea cucumber hatchery production over the last two decades, the process of larval settlement also remains poorly understood. The high mortality rates and large variability in settlement success typically observed in sea cucumber hatcheries is considered a commercial bottleneck and risk to continuity of production. During this project, of the three experiments that were carried out assessing the effect of different settlement substrates on settlement and survival of larval sandfish (Simoes, 2012), only one showed any significant difference. Mature biofilms of 11 days of age had the most beneficial effect on settlement efficiencies. This effect was diminished in younger and older biofilms. Since the diatom genus *Mastoglia* was only present in 11 day old biofilm, it may have contributed to the high settlement efficiency of *H. scabra*. However, it is very likely that the bacterial flora played a significant role in attracting larval *H. scabra*. Importantly, the composition and number of bacteria in biofilms are known to often dramatically change with age in tropical larval tanks (Bourne *et al.* 2006). The potential commercial importance of this finding still has to be evaluated.

Pre conditioning of settlement substrates with natural biofilms appeared to improve settlement and metamorphosis of larval *H. scabra.* Moreover the age of the biofilm could influence the level of settlement. These results suggest that larval rearing methods at settlement should involve some degree of pre-conditioning of settlement substrate and that fine tuning the age of the biofilm on the substrate has potential to improve hatchery production.

## 4.2 Nursery

*Feed, tank fertilisation and substrate*

Little is known about what dietary components are assimilated by newly settled and juvenile sandfish (Purcell *et al* 2012) with current methods generally adopted from those developed for herbivorous gastropods like abalone (Battaglene, 1999). Juvenile sandfish ($\sim$2g) have been shown to grow better on shrimp tank detritus or shrimp faeces than benthic diatoms (Watanabe *et al.* 2012), however using shrimp detritus as a diet becomes impractical when scaling production up for a commercial

operation. The use of 'off the shelf' products to feed sandfish during the nursery stage has obvious advantages for the planning, operation, and management of expenditure in commercial hatcheries. The experiments carried out during this project identified Algamac-fertilised nursery tanks to have a significantly greater length and wet weight in juvenile sandfish compared to all other treatments. Similarly, the survival rate of 78% was the highest obtained in the experiment, although not significantly so, compared to juveniles from tanks fertilised with either manure or Spirulina. Therefore, based on the growth data Algamac is the most suitable diet to use during the early nursery stage of juvenile *H. scabra*. Using this diet on a commercial scale, however, has to take the high cost of the diet ($112.0 $kg^{-1}$) into account. In contrast, the manure used in the trial was two orders of magnitude ($1.3 $kg^{-1}$) cheaper than Algamac. Compared to juveniles in Algamac-fertilised tanks, juveniles in manure-fertilised tanks achieved 87% of the length and 52% of the wet weight with an almost identical survival rate. It may be possible to achieve similar results to fertilisation with Algamac by increasing the amount of manure applied. Given the low cost of the manure used, this is likely to have important commercial implications.

It has been established that stocking density and substrate can affect nursery production (Battaglene, 1999). Battaglene (1999) recommended delaying the transfer of juvenile *H. scabra* onto sand substrate until they reached a length of ≈20mm to maximise survival and growth. He also demonstrated that juveniles transferred to sand grew better than those on hard substrates (fibreglass plates). However, survival was better on hard substrates. In a commercial setting earlier transfer from the hatchery to nursery would greatly benefit productivity. Transfer from the hatchery at 20mm is unrealistic due to density effects in the hatchery post settlement.

The results from this project supported the work of Battaglene (1999) where there was a relationship between survival and growth. Whereby juveniles reared on hard substrates survived better but grew less than those on pond mud substrate. The larger size of juveniles reared on enriched pond mud substrate may suggest some benefit to the enrichment of substrates but over-enrichment may cause high mortality. Further trials which can decouple the effects of density and survival on growth will help to enable better informed decisions on when to transfer juveniles to nursery systems, stocking density and when to provide substrate. As nursery production is the biggest limitation to the number of *H. scabra* produced by a hatchery this area of research has the most potential for increasing the profitability of *H. scabra* aquaculture. It is clear that some level of nutrient is required for optimal nursery production; however, the type of nutrient added to the substrate is important. Algamac and pelletised manure are the most promising additives tested in this trial and therefore are recommended for use as nutrients in *H. scabra* nursery systems.

*Influence of light*

In a study assessing the factors influencing survival and growth of cultured juvenile sandfish, Battaglene *et al.* (1999) found light to have a clear influence on juvenile behaviour and on growth of algae in the nursery tanks. Under small scale experimental conditions, Battaglene *et al.* (1999) found growth was significantly better in tanks that did not have shade; however there appeared to be little difference in survival.

When moving nursery production to large outdoor raceway tanks (5.8m x 1.1m) under direct sunlight at the Tasmanian Seafoods hatchery (Darwin Aquaculture

Centre), high mortality rates were observed in newly stocked juveniles. To address this shade covers were utilised, which did help improve the mortality rate of newly stocked juveniles. To optimise the outdoor nursery system, two readily available shade covers were tested (50% shade, 90% shade) to identify the optimal growing conditions at the hatchery. Results were highly variable across both treatments; however survival and tank biomass did appear better under the 90% shade cover. The results were heavily influenced by one replicate in the 50% shade treatment performing very well in terms of tank biomass, but not survival. It is likely that heavy shade is only required in the very early stages of nursery production, as sandfish out of the TSF hatchery are stocked out from 500μm, and once established they can be exposed to greater levels of light to enhance growth. There are, however, several other factors to consider for newly stocked juveniles such as tank substrate, stocking size and stocking density. Further research is required to develop efficient commercial scale raceway systems however a level of shading between 50% and 90% is recommended for tank based nursery systems.

*Effect of pond mud to nursery tanks*

Juvenile sandfish supplied with pond mud substrate reached a significantly higher average weight and length than juveniles within tanks with only natural biofilm. However, survival could also explain the differences as survival was significantly lower in the pond mud treatments. Density dependant growth may explain the differences in this trial. Stocking density and supply rates of pond mud are important for productivity of a tank based nursery system. Measured visibly, food supply was unlikely to be a limiting factor in any tank, as all tanks had large areas left ungrazed at all times during the trial. Therefore if density had an effect on growth, it is unlikely that competition for food was the primary reason, and rather an alternative social or behavioural factor.

It is apparent that some amount of substrate is required in early nursery rearing of settled juveniles. However, nutrient rich feed additions like the treatment may cause mortality at this early stage due to either virulent bacterial blooms or an over enriched diet. Dried pond mud used in small quantities shows suitability as a controllable, inexpensive addition for newly settled juveniles and has been adopted by TSF for this function. The high growth seen with enriched pond mud, after the early mortality, indicated that its use could have potential for larger and more robust juveniles to boost growth. An analysis of the effects of density and substrate on growth and survival would elucidate the relative importance of each factor in the productivity of the tank based nursery system.

*Effect of substrate on nursery survival*

The poor survival of juveniles reared with mussel rope substrate does not reflect recent observations of artificial substrate used in nursery grow out systems. Purcell and Agudo (2013) found that mesh substrate used in hapas improved growth of juveniles by 15%, though these were stocked larger (average 6mm initial length) than those used in this study (average 1.4mm initial length). It is possible that these larger juveniles were past a hypothetical critical stage for survival that affects the smaller juveniles used in this study. Ropes are successfully used to induce settlement of larvae in TSF larval rearing systems, and so it would be thought possible that ropes would be an appropriate media to enhance survival and growth in newly settled juveniles through to the sizes used by Purcell and Agudo (2013). These examples

show the benefit of substrate in the water for rearing juvenile sandfish, however it wasn't so in this case.

In-tank survival counts during the trial showed high initial survival in the control treatment, suggesting that sub-optimal health condition of juveniles upon transfer to nursery systems was not likely to be a confounding factor on the trial result.

Given the limitations of this experiment no robust conclusions can be made. However given the evidence suggesting a positive effect of substrate in nursery systems further research is recommended; involving higher stocking densities and different types of substrate, perhaps shadecloth or ropes light in colour. It may also be a beneficial commercial option to transfer ropes with newly settled juveniles from larval rearing directly into nursery tanks to minimise stress from harvest.

### 4.3 Sea ranching

*Growth of released juveniles*

Growth of hatchery produced juvenile sandfish released into the wild during this project was variable, however typically faster than other rates of growth reported for the species throughout its range. In release trials conducted in New Caledonia (Purcell & Simutoga 2008), sandfish reached around 150g on average after 12 months however sizes were quite varied. In Fiji, Hair (2012) reported that sandfish reached an average weight of 167g at 8 months before animals were compromised due to a severe weather event. Robinson & Pascal (2012) also observed highly varied growth utilising seabed pens in Madagascar, where in one example sandfish reached an average of 362g in around four months, however other trials achieved 186g in 11 months.

Hatchery produced sandfish released for sea ranching during this project reached 326.3g ±41.3 after 12 months in the first release, 273.4g ±10.4 after 10 months in the second release, and 186g ±11.99SE after four months in the third release. These results clearly indicate that favourable conditions exist in the Northern Territory for juvenile sandfish growth.

*Density and biomass*

Reported densities for populations of sandfish have been highly variable with most studies focussing on adults (Al-Rashdi *et al.* 2007). There have been few studies following the density of juvenile sandfish on seagrass beds, and it is widely acknowledged that sandfish migrate from seagrass beds to deeper, more open areas (Hamel *et al.* 2001).

The fine scale data collected in the present study, relative to previous assessments of juvenile sandfish density on seagrass beds, emphasises the potential effect of tidal movement on juvenile sandfish burying behaviour. In a long term monitoring study in Australia's Torres Strait, Skewes *et al.* (2006) reported juvenile sandfish as being <140mm and occurring in densities from 325.89 /ha, to 7.6 /ha. In the Solomon Islands, Mercier *et al.* (2000) recorded varying densities of juvenile sandfish <100mm depending on the site and substrate, 3475 ±2903 /ha on muddy sand, 2687 ±1856 /ha on mud, and for abundance of all sizes of sandfish on seagrass beds was found to be <20 /ha. In the Vail (1989) survey in the Northern Territory, a beam trawl was used to assess adult stocks. However, one shot was conducted across a seagrass

bed in Gove Harbour. The shot produced only juvenile sandfish (mean weight <20g, mean length 42mm) generating an estimated density of >100 /ha, however the author acknowledged that it was very probable a large number of small individuals went through the net due to the high numbers found stuck in the mesh.

In the present study, densities on the naturally recruiting sites (controls) in the first release and third release peaked at 271 ±61/ha and 1182 ±359 /ha respectively. Interestingly these densities both occurred in the month of October (2011 and 2012), before a significant decline was observed both times leading to a very low standing stock by June 2012 (44 ±30/ha) for the first release, and January (293 ±125/ha) for the third release. Sandfish densities during the release trials reached 793 ±535/ha (December 2011), and 1938 ±536/ha (November 2012) respectively before a significant decline. Similar declines in density observed on the control sites coincided with these declines on the ranching sites.

Density-dependent factors have been shown to affect growth, but not the survival of sandfish (Purcell & Simutoga, 2008; Robinson & Pascal, 2012). Battaglene *et al.* (1999) identified the effects of density dependence for sandfish in land-based systems occurring at 225g /$m^2$, Purcell & Simutoga (2008) found a similar result in New Caledonia during sea ranching trials where growth appeared compromised in sea pens as biomass reached 200-250g /$m^2$. The degree to which local exogenous factors can impact density dependant growth is unclear.

Robinson & Pascal (2012) identified significant variation between carrying capacity for sandfish between sites during sea pen trials in Madagascar. The reported 220g /$m^2$ was achieved at one site, however an alternate site in the trial reached a biomass of 100g /$m^2$ before growth was affected, preventing the sandfish from reaching a harvestable size. Robinson & Pascal (2012) also noted the obvious effect of overcrowding as sandfish stocked in a sea pen at 360g $m^2$ were observed squeezing through the mesh in an effort to disperse. The significant drop in stock abundance on the release sites seems unlikely to be in response to density effects, as observed biomass' in the third release only peaked at 257 kg /ha (25.7g $m^2$).

The similar pattern of a drop in density observed on the release site and the control site in both release trials indicate seagrass beds are only a temporary habitat for the majority of sandfish, providing substrate for larval settlement and juvenile growth before migrating out to sand and mud flats. It is likely the recruitment/migration process created the fluctuating densities and biomass of sandfish observed, as the pulse of settling larvae were recruited to the seagrass bed before migrating out as they approach maturity.

*Comparison of growth between hatchery-produced juveniles and wild recruits*

This assessment was carried out during the third release. While it is reasonable to assume degrees of natural mortality occurred on both sites, this however did not appear to be specifically targeted at hatchery produced juveniles. Released juvenile sandfish dominated their respective mode sizes in both the November and January. The highest mode weight of released juveniles went from 87.5g in November to 167.5g in January, with hatchery produced juvenile sandfish in these modes representing 86% and 71% respectively. When pooling the data across the highest four modes for each sampling month, in November 64% of sandfish between 25g-125g on the ranching site were hatchery produced, and in January 47% of sandfish

125g-225g were hatchery produced. This indicates hatchery produced sandfish were successfully integrating into the existing wild population.

When using the growth model (Fig. 30) to assign a relative age to the wild recruits, the third release identified hatchery produced juveniles being 20% larger than their wild recruit counterparts at eight months of age. It should be noted that direct comparisons are likely to be confounded as data recorded for size at age to compare the growth performance of hatchery released juveniles against the wild recruits were taken at different times. Reported growth of sandfish is fastest during the months of warmer seawater temperatures (Purcell & Simutoga, 2008; Robinson & Pascal, 2012). The July/August months are considered among the coolest period in the Northern Territory, and the period wild recruits were first detected. Releasing hatchery produced juveniles in September 2012 is likely to account for the increase in growth rates as seawater temperatures are generally beginning to increase at that time.

### 4.4 Hatchery Genetics

Genotyping was successful for both nursery and hatchery progeny but less so for broodstock. Given the low incidence of multiple paternities observed in all batches, the number of eggs produced in a pooled spawning is likely an important factor in the number of contributing parents. In addition in a mass spawning environment the low incidence of multiple paternity and maternity suggests that timing or proximity of spawning are important factors for fertilisation.

The analysis of temporal samples provided no evidence for any change in the frequencies of families between the hatchery and nursery environments post settlement. Therefore family groups from the hatchery can be pooled for nursery and grow out without affecting the genetic diversity of the population through dominance of particular families.

The results of this study and the results of the survey of the sandfish population genetic structure (Seafood CRC project 2008-744) were used to develop a hatchery genetic management plan (GMP) for Sandfish ranching in the Northern Territory (Appendix. 3). Estimates of effective population size for contributing broodstock to a standard hatchery run ranged from 2 to 5-8 individuals. The GMP recommended that either, multiple pooled spawning or single pair matings be used to optimise diversity from the hatchery. For a detailed discussion on the implication of hatchery breeding practices on sea ranching see the Hatchery Genetic Management Plan (Appendix. 3).

To meet the requirements set out in the GMP for ranching in the NT some modification to the TSF production systems were necessary. The requirement to maintain at least 5 families in the larval rearing system meant that additional tanks were needed. This was in order to keep the different families separated until they had past the peak mortality bottleneck of settlement to avoid undocumented family drop out. After settlement the families could be pooled with the assumption of no further family related mortality. Based on the data from the hatchery genetics study the family proportions did not change from post settlement to release size. The Hatchery expansion increased the capacity of the hatchery from 23 000l to 36 000l with the capacity to keep at least 7 families in large enough numbers to be able to combine in equal proportions post settlement (Fig 46 (a)). This gives some buffer for the drop out

of families during larval rearing. In the event of family dropout up to two families could crash without effecting the number of juveniles that could be released from a particular hatchery run.

The hatchery genetics study revealed the lower than expected familial contributions of broodstock in pooled breeding events. The target effective population size of 10 could be met by ensuring at least three pooled spawning groups of at least ten individuals are used in each production run. Additionally, TSF have developed a spawning system to allow highly controlled paired mating or individual spawning from which gametes can be combined as required (Fig 46 (d & e)). Using this system multiple paired mating can be used in each run with hatchery capacity to rear 7 separate families, as stated above. A requirement of this breeding system is the supply of mature broodstock in spawning condition. In order to achieve this some degree of conditioning is required. TSF condition broodstock in tanks for short periods prior to spawning. Broodstock must be cultured at low density and synchronicity can be low at times with as little as 10% of the population in spawning condition. This means that a large amount of culture space is required to successfully condition broodstock for spawning. As a guide a minimum of 1 meter squared is required per animal. TSF has conditioning culture systems of a total of 108$m^2$ (Fig 46 (b & c). To address the lack of reproductive synchronicity observed in broodstock outside of the peak spawning times, gonad biopsies can be utilised to select for mature individuals. Maturity is assessed based on oocyte size and sperm activity in samples taken from broodstock.
To increase the success rate of spawning pre-spawning conditioning and gonad biopsy methods are combined with the new spawning technology so that only mature animals of each sex are used for spawning attempts and effective population size targets can be met. Selecting mature individuals greatly reduces the amount of tanks space required to house the broodstock for spawning.
Initial trials using these methods have been promising with one in season spawning producing 160million fertilised ova from 15 broodstock (8 females). In another attempt outside natural spawning period four single pair matings were produced resulting in 2.2million fertilised ova. This was slightly under the required number of ova and the required effective population size. The low number of eggs is most likely a reflection of the spawning condition of the animals used in this trial. On this occasion a total of 30 animals were used in the spawning inductions. Larger numbers of broodstock will be required for effective population size targets to be met outside the natural spawning periods of *H. scabra.*

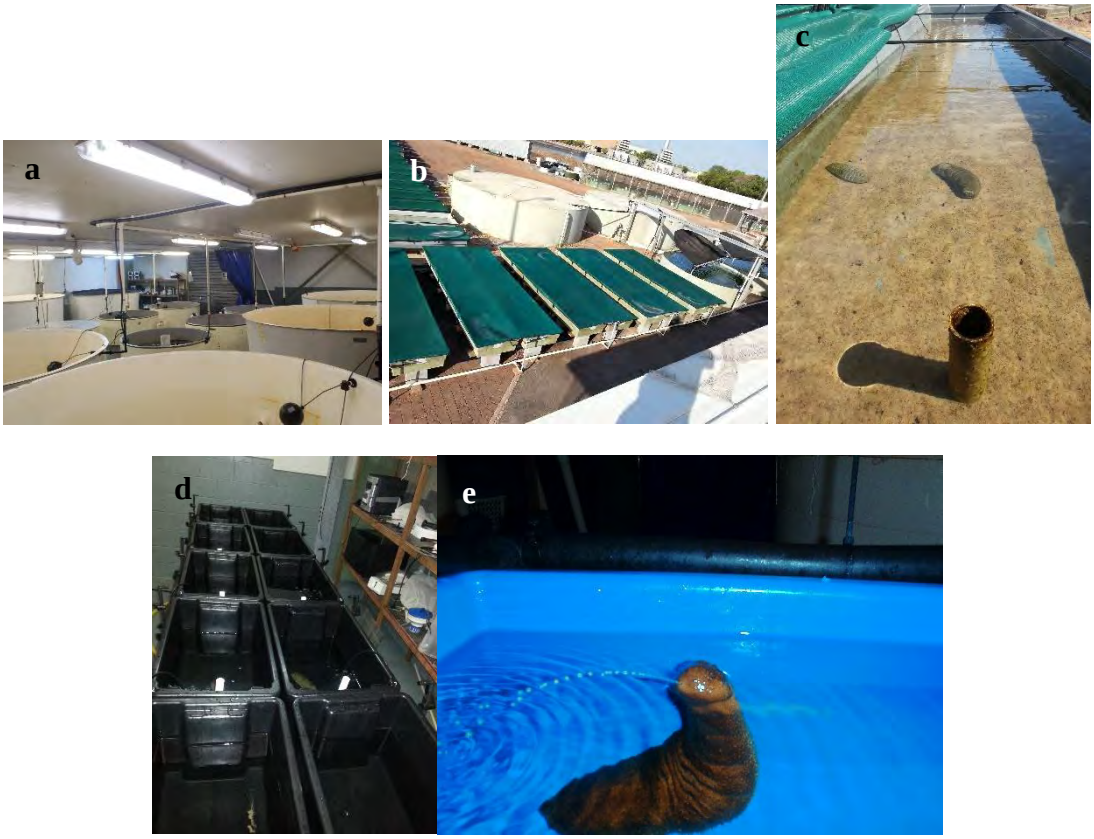



**Figure 46**: Increased capacity hatchery coststing of 6 x 5000l tanks and 6 x 1000l tanks (a). Broodstock conditioning system consisting of 12 tanks with the capacity to condition up to 72 broodstock (b). Conditioning tank with brood stock (c). New spawning system enabling high level control over breeding (d). Individual spawning of *H. scabra* broodstock female in spawning system (e).

# 5. Benefits and Adoption

Through this work the feasibility of sea ranching sandfish has been demonstrated with Tasmanian Seafoods P/L now well placed for the expansion of ranching activities across multiple sites in the NT. The project has led to major advancements in hatchery and nursery production. Implementation of the recommendations has increased production of juvenile sandfish. Up to 300 000 juveniles can be produced for ranching per year whilst minimising the labour requirements for stocking, maintaining, harvesting and transporting stock. This level of ranching production will potentially increase the catch of the fishery by c.a. 12t p.a (gutted, boiled and frozen) at an approximated value of $120,000 within two years. This equates to a 5% increase in the production of the NT fishery based on average landings from 2001-2011 landings of 229t.

TSF has developed a well-supported plan for ranching in the NT. This will lead to an expansion of ranching activity from pilot scale to commercial scale in 2015. Improved hatchery technology developed during this project allows juveniles to be produced economically to support the roll out of commercial ranching at a minimum of three

sites in the NT. This has direct benefit to the industry proponent and coastal communities; enabling TSF to pursue a commercial ranching venture.

The highest cost of producing juvenile sandfish is in labour and hatchery operational costs (lease). Pond, hapa net and tank based nursery systems were assessed for suitability during the early parts of this project. The increased labour requirements for pond based nurseries were a factor at time of harvest. Hapa net nurseries, commonly used for sandfish culture in other countries, had equivalent levels of productivity to tank based systems but required a great deal of maintenance and have a high associated level of risk of stock loss due to predation and severe weather events. Tank based nursery systems proved to be a better alternative with lower labour requirements, a greater level of control over abiotic conditions and reduced risk of stock loss due to severe weather. The high initial cost of setting up a tank based nursery system was more than offset by the reduced risk of significant mortality and low maintenance requirements.

Expansion beyond that stated above will involve a hatchery system with at least six 25,000l tanks along with a new standalone algae production facility. This will allow TSF to produce up to 4 million juveniles for ranching per year. Without a large enough land-based nursery system, these juveniles would need to be released after six weeks at 2-4mm in length. With a hatchery staff of two, minimising the labour requirements for stocking, and a dedicated vessel working to supply brood stock, transport juveniles to ranching sites and monitor released stock. This level of ranching production will potentially lead to a harvest of c.a. 80t p.a (gutted, boiled and frozen) at an approximated value of $960,000 within two years. These figures assume 10% survival rate and an average harvest mass of 400g per piece.

The modular design of the hatchery and nursery systems developed during this project enable the systems to be readily relocated and/or expanded. As demand for juveniles for ranching increases, satellite hatcher /nursery systems could be, with relative ease, set up closer to ranch sites. This would increase the production capacity as well as lower the cost of transporting juveniles.

Potential for modest economic opportunities for remote coastal communities has also been demonstrated. It is estimated that coastal ranching activities could provide harvesting, hatchery and grow out employment for local people for approximately 6 months per year with harvesting from June to august and hatchery and grow out from September through May. At Umbakumba, Aminjarrinja Enterprises Aboriginal Corporation played an important role in the ranching trials, this involved members of the corporation gaining training in survey work for stock assessments as well as in proper harvesting and processing techniques. In conjunction with TSF AAC built a seafood processing facility up to AQIS export standard at Umbakumba enabling the enterprise to process the sea cucumber to the same stage that TSF boats do (gutted, blanched and frozen). Therefore receiving the same price as TSF boats are paid for product. Being AQIS approved, this facility can be used for processing other seafood when not being used for sea cucumber. Estimates based on the training delivered by TSF during the trial is that harvesting and processing could provide work for about four to eight people and survey work for one to two for approximately 4-6 weeks per year. AAC is sufficiently diversified to support the employment of the staff through non harvest times.

If the commercialisation of the ranching technology proves successful in the first 1 to 2 years, the potential for hatchery infrastructure to be deployed to sites closer to ranching sites across the Northern Territory will be investigated. TSF estimate that 3-4 community members could be employed for remote hatchery operations for a

period of circa four months per annum. The establishment of mobile production facilities, currently being assessed, at coastal communities would alleviate some of the logistical constraints of transportation of stock and create further opportunity for employment within the industry in remote communities.

# 6. Further Development

Production of commercial scale quantities of juvenile sea cucumber and small scale ranching trials conducted during this project has generated knowledge on the biology, ecology and genetics of *H. scabra* in the Northern Territory. Tasmanian Seafoods P/L plans to work with NT Fisheries to integrate this knowledge with existing fisheries management frameworks to improve the productivity of the fishery. Initially this will mean the implementation of an approved commercial ranching program including the identification of appropriate ranching sites and development of a ranching monitoring plan. This body of work provides the basis for meeting these governmental requirements set out in the NT fisheries enhancement policy.

Further development of hatchery capacity beyond 300 000 juveniles p.a. will require larger or additional nursery and hatchery facilities. The modular design of the hatchery and nursery systems developed during this project enable the systems to be relocated and/or expanded. As demand for juveniles for ranching increases, satellite nursery systems could be, with relative ease, set up closer to ranch sites. This would increase the production capacity as well as lower the cost of transporting juveniles. Alternatively, due to the difficulties of finding suitable land for the development of larger scale broodstock and nursery systems, TSF is investigating the potential of building larger scale hatchery facilities and releasing greater numbers of juveniles at a smaller size. There by reducing the amount of land required to produce economically viable numbers for ranching (see Benefits and Adoption).

There are a number of areas in which further research could improve the successful commercialisation of ranching and stock augmentation of sea cucumber. These areas are listed below.

1. Nursery production

Improvements have been made in addressing the requirements of juvenile sea cucumber in nursery systems. However, knowledge is lacking on the nutritional requirements and optimal substrates for tank based culture systems. Following on from the advancements made in this area during this project, fine tuning the nursery shade regime, substrate type and development of a formulated diet have the greatest potential for improving productivity in the hatchery.

2. Live transportation techniques

Small and large juveniles can be transported by both land and sea. Transportation of large numbers of juvenile sea cucumbers is required for the commercialisation of ranching and stock enhancement. As the size of the transport increases logistical constraints such as cost of air freight and deck space required for sea transports begin affect productivity. Efficient transport technology such as on-board live holding

systems, that maximise the payload of juveniles which can be transported, is a key researchable constraint to commercialisation.

3. Habitat productivity

Variation in growth of released sea cucumbers reported in this work supports reports from studies elsewhere which show different release sites support different levels of productivity in terms of growth. This has implications for the selection criteria used when selecting specific locations for ranching and enhancement. Areas with high carrying capacity may be favourable to those with lower carrying capacity for stocking operations. Understanding the factors which promote or indicate productivity would be a valuable asset when selecting stocking sites.

4. Migration

The dispersive nature of *H. scabra* movements during the ranching trial components of this project highlight the importance of migration as a factor to consider when designing stocking programs. Due to the inherent difficulties with tagging sea cucumbers there is a lack of data on the patterns of migration. Whilst it is generally accepted that there is a migration of juveniles from intertidal sea grass beds to slightly deeper mud and sand habitat the factors influencing the migration are unknown. The clustered patterns of the distribution of *H. scabra* could suggest that migration is not random and further research is required to understand the movements of released animals and the impact of migration patterns on harvest strategies.

5. Larval recruitment

A Seafood CRC supported survey of the genetic diversity across the top end of the Northern Territory has suggested wide dispersal of larvae. Understanding finer scale larval flow could improve stock enhancement outcomes given that dispersal of released animals means some level of contribution to spawning biomass from released stock is likely.

# 7. Planned Outcomes

## 7.1. Public Benefit Outcomes

Evidence from this work and similar studies (Juinio-Meñez *et al*, 2013) suggest that release of hatchery produced sea cucumber will not affect existing populations and could be effectively integrated with existing management frameworks to improve the sea cucumber resource of the Northern Territory.

An indirect result of this work has been the development of government policy on fisheries enhancement for the NT, providing the legal basis for the approval of enhancement and ranching operations in the NT.

Potential for modest economic opportunities for remote coastal communities has also been demonstrated. Interest has been expressed from the communities of Warruwi

(South Goulburn Island) and Umbakumba (Aminjarrinja Aboriginal Corporation, Groote Eylandt) in sea cucumber ranching. Aminjarrinja Corporation played an important role in the success of the ranching trials in this work with members of the corporation gaining training in survey work for stock assessments as well as harvesting and processing techniques.

### 7.2. Private Benefit Outcomes

On the back of this research TSF will develop a well-supported plan for ranching in the NT. Once approved, this will lead to an expansion of ranching activity from pilot scale to commercial scale over the next 2-5 years. Increased hatchery capacity and the hatchery manual developed during this project will allow juveniles to be produced to support the roll out of commercial ranching at a minimum of three sites in the NT. This has direct benefit to the industry proponent; enabling TSF to pursue a commercial ranching venture. The predicted 12t of additional product produced through the first stage ranching would have a value of approximately $260000 (processed product).
Breeding SOPs and the GMP will allow the management of genetics out of the hatchery respecting wild populations and meeting the government requirements for fisheries enhancements.

The bioeconomic fishery enhancement model will give insight to managers and Tasmanina Seafoods on the economic potential of expanding ranching operations and provide a valuable resource for planning levels of enhancement and expected economic yields into the future.

### 7.3. Linkages with CRC Milestone Outcomes

This project links with the Seafood CRCs program 1 outcome to increase production and profitability of wild harvest fisheries and specifically contributes to Output 1.2 “Enhanced yields from wild-harvest innovations” and milestone 1.2.2 “Production interventions implemented in at least one fishery”.

# 8. Conclusion

During this project TSF successfully established a large scale production system for juvenile sandfish with the capacity to produce 300,000/year. Over the course of the expansion, significant improvements were made in hatchery production and broodstock conditioning (Luke Turner PhD), providing continuity to hatchery operation. It was also revealed that there is potential to make further improvements to the adopted nursery raceway system. Hatchery protocols for larval rearing were confirmed as being optimal during larval rearing studies. Existing water exchange protocols and feeding regimens for larvae could not be improved during larval experiments. Peak mortality during the hatchery production of sandfish occurs at settlement where survival can drop from 80% down to 5%. During these trials,

experiments on settlement achieved higher rates of survival and juvenile metamorphosis than observed in the hatchery using biofilms and settlement substrates conditioned with various substances. Survivals of well over the target of 30% were achieved in research scale systems. These results have not yet been confirmed at the full hatchery scale. Work on scaling up these techniques and adapting to the commercial hatchery system is ongoing.

The highest cost of producing juvenile sandfish is in labour and leasing hatchery space. Pond, hapa net and tank based nursery systems were assessed for suitability during the early parts of this project. The increased labour requirements for pond based nurseries were a factor at time of harvest. Hapa net nurseries, commonly used for sandfish in other countries, had equivalent levels of productivity to tank based systems but required a great deal of maintenance and have a high associated level of risk of stock loss due to predation and severe weather events. Tank based nursery systems proved to be a better alternative with low labour requirements, a greater level of control over abiotic conditions and reduced risk of stock loss due to severe weather. The high initial cost of setting up a tank based nursery system was more than offset by the reduced risk of significant mortality and low maintenance requirements.

During the sea ranching trials we found hatchery produced juvenile sandfish released into the wild, after suffering an initial mortality, could then grow at a comparative rate to their wild recruit counterparts, reaching maturity 12-18 months after release. Sea ranching trials on Groote Eylandt achieved 20% survival and good growth rates. The observed survival and growth rates support data on ranched *H. scabra* from elsewhere (Juinio-Meñez et al. 2013). Migration of the stock was a factor. It seems that seasonal migration needs to be considered in designing ranching operations. Using divers to survey surrounding areas will limit stock loss due to natural migration away from seagrass beds. Both caged release and dispersed release strategies appeared to work for the release of sandfish. It is likely that the most important factor is low tidal currents at the time of release to enable the released animals time to settle in and attach to the substrate. Particular release methods are likely to depend on site specific factors such as sea grass coverage, predator density, seabed topography and tidal currents. Suitability of release sites and acknowledgement of sandfish behaviour was identified as an important consideration when undertaking sea ranching programs. Sandfish densities on seagrass beds appear to fluctuate depending on larval recruitment and sandfish movement. Migration is not necessarily in response to density effects. The natural movement of sandfish on the study sites was to disperse and keep the densities low.

A bioecconomic model was generated using available data. Given the fishery is relatively data poor there are some doubts to the accuracy of the assumptions underlying the model. The model suggests the economic gain of sea ranching *H. scabra* is will be directly proportional to the number of animals released. This model is currently being reviewed and an updated analysis will be included in the final report.

While further research is required, it does appear that optimal sea ranching of sandfish in the Northern Territory is likely to require utilising large areas releasing at low densities rather than attempting to contain animals for high production / high stock turnover. In the present study, released sandfish successfully integrated into wild populations without any apparent adverse effects, and it seems likely an inevitable outcome of sea ranching will be some degree of augmentation of wild stocks. Genetic analysis of hatchery produced juveniles showed that careful control of spawning is required to meet the necessary levels of diversity to mitigate

augmentation of the wild stocks of *H. scabra*. Using the data collected from three hatchery spawning events, Dr Graham Mair developed a Hatchery Genetics Management Plan for Tasmanian Seafoods. The plan sets out the minimum requirements for responsible production of hatchery bred sea cucumber for release in the NT.

By definition (Bell *et al.* 2008) this approach does move toward stock enhancement, however compared to other countries working to develop sea cucumber ranching, the Northern Territory is in a unique position having a strong management regime and a sustainable wild catch fishery (Fleming, 2012). It is now possible for Tasmanian Seafoods to develop a sea ranching program which can operate within the management arrangements of the well regulated fishery; it seems pertinent that release strategies and management arrangements should be designed to accommodate an optimal approach.

# 9. List of Publications

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# 11. Appendices

## 11.1. Hatchery Manual

### Hatchery protocols for Sandfish production

**2013**

## ***Introduction***

Tasmanian Seafoods P/L (TSF) set up a pilot scale sea cucumber hatchery and nursery facility at the Darwin Aquaculture Centre (DAC) in 2004 to establish sea ranching of the highly valuable sandfish, *Holothuria scabra*. In 2010 TSF was awarded a research grant by the Australian Seafood Cooperative Research Centre to assess the propagation and sea-based growout of sea cucumber in the Northern Territory. This manual summarises the current hatchery protocols for culturing *H. scabra* larvae which are based on the research conducted under Seafood CRC Project 2009/744.

## ***Broodstock***

*Broodstock Collection*

Broodstock should be collected from the same area where the release is planned to avoid genetic contamination. The main criteria for selecting specimens are their size/weight and general health. As per the hatchery genetics management plan; broodstock must be collected from at least three sites within one of the populations in the NT. The three sites should be at least 50km apart and representatives from each site collected in roughly equal proportions (appendix 3).

## ***Spawning***

*Spawning Induction*

1. Jet spray for 30 - 45min
2. Thermal stimulation for 1h

Broodstock are held in round, flat-bottom 300L tanks and stocked at a density of between 15 - 30 broodstock tank$^{-1}$. Broodstock are subject to a 1µm- and UV-filtered seawater (FSW) jet spray in the tanks for 30 to 45min. Following that, heated FSW is circulated through the spawning tanks to raise the temperature to a maximum of 34°C. Expose the broodstock to the elevated temperatures for 1h before the FSW in the spawning tanks is slowly replaced by FSW with ambient temperature.

The broodstock are then left for spawning to start anytime during the evening or throughout the night. Spawning tanks require flow-through FSW, with all water from the spawning tanks going through 1000Ltr egg collectors with 63 µm screen. Throughout this period, faeces periodically siphoned from the tanks with minimal

disturbance to the broodstock. In response to the spawning stimuli, some individuals may raise their anterior end or roll from side to side. It is typical for many male sandfish spawn first. Spawning males will raise their anterior end and sway from side to side releasing sperm in a continuous stream from the gonopore and can spawn without interruption for hours (Fig 1). Females may also raise their anterior ends but will not display the swaying motion as males do. Between 1- 2 $x10^6$ eggs can be released in a series of quick bursts.

**Figure 1.** Broodstock raising its anterior head prior to spawning.

*Fertilisation and Hatchery Stocking*

The eggs are fertilised in the spawning tanks and captured in the egg collectors. Eggs are then stocked into hatchery tanks by draining the egg collector through a 63µm screen and taking subsamples for counting to estimate total number. Stocking density of eggs should be approximately 0.1 $mL^{-1}$ and definitely not exceed 0.5 $mL^{-1}$.

Hatchery tanks should be treated with an antibiotic such as tetracycline or oxytetracycline at 50ppm. Gentle aeration should be provided and the larvae will generally hatch after approximately 12 to 18h at the gastrula stage. Therefore, the hatching rate should be determined approximately 24h post-fertilisation.

For the progeny of hatchery spawning to be released into ranching sites in the wild, an effective population size of contributing parents of at least 10 is required. In order

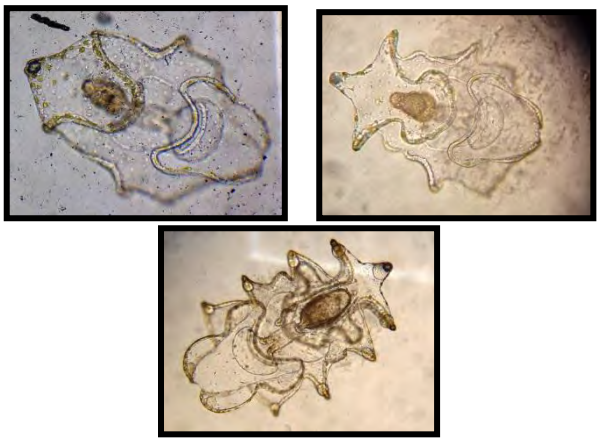
to achieve this, a minimum of 3 separately pooled spawnings or 5 single paired matings will be required. Fertilised embryos from the pooled spawnings or paired matings should be reared separately until post settlement (see genetic management plan).

## ***Larval Rearing***

*Auricularia Larvae*

Larval rearing tanks are supplied with FSW and light aeration throughout larval rearing. The larvae are pelagic for the first 10 to 14 days (auriculariae), and during this stage, larvae continuously deposit lipids in hyaline spheres which become larger and darker as development progresses.

During the pelagic phase, 30% of the FSW should be exchanged daily larvae fed on the diatom *Chaetoceros muelleri*. A suggested feeding schedule is shown in Table 1.



(a) (b) (c)

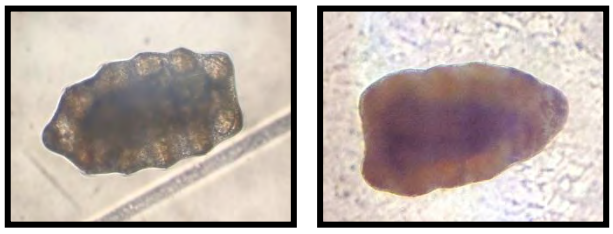
**Figure 2**. The three stages of auricularial development are (a) early auricularia (350 to 440µm), (b) mid auricularia (500 to 800µm) and (c) late auricularia (850 to 1,150µm). Note the development of hyaline spheres in (c).

**Table 1.** Feeding rate for live *Chaetoceros muelleri* fed to larval sandfish (*Holothuria scabra*).

| Day | Feeding rate (cells $mL^{-1}$) |
|---|---|
| 2 | 10,000 |
| 3 | 12,500 |
| 4 | 15,000 |
| 5 | 17,500 |
| 6 | 20,000 |
| 7 | 22,500 |
| 8 | 25,000 |
| 9 | 27,500 |
| 10-12 | 30,000 |

*Doliolaria Larvae*

Once fully grown with hyaline spheres, auriculariae reduce their body size and eventually form a barrel-shaped doliolaria (Fig. 3). Initially, doliolariae remain in the water column but towards the later stages have a tendency to swim to the bottom. Once a significant number of doliolariae is observed, settlement substrates (mussel rope) can be added to the larval tanks.



(a) (b)

**Figure 3**. Early (a) and advanced (b) doliolaria. Early stages tend to be bigger (550 to 650µm) than the advanced stage (450 to 550µm).

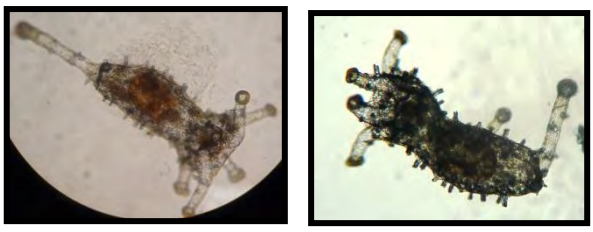
## ***Settlement***

Once larvae have reached the doliolaria stage, the vast majority will settle within three to four days. Before adding settlement substrate, care should be taken not to introduce unwanted organisms such as copepods and protozoans. These organisms affect the survival of *H. scabra* either as competitors for food or as predators. Should contamination occur, it is necessary to incubate tanks (and substrates) with a pesticide such as 'Lepidex 500' (Nufarm Australia P/L) at a concentration of 1ppm for 2 to 4h.

After settlement substrate has been added, *C. muelleri* is still added daily at a rate of approximately 30,000 cells $mL^{-1}$ to account for slow developing larvae. Once larvae transform into the final larval stage, the pentactula (Fig. 4a), and all larvae have disappeared from the water column, they are considered settled at this stage and water can be on flow-through with no screens at 200%/day.

*Post-settlement or First Stage Juveniles*

Newly settled juveniles (Fig. 4b) are supplied with supplementary feed including benthic diatoms (*Navicula* spp., *Nitzschia* spp.), Algamac-Protein Plus, and Spirulina, In the absence of recommended daily feeding rates for early juveniles, it is advisable to observe tanks on a daily basis as it is important to realise that even if the observed juveniles continue to grow and appear healthy, many small juveniles may be dying.



(a) (b)

**Figure 4.** Pentactula (a) and early juvenile sandfish (b). The former are 600 to 700µm in body length and early juveniles range from 700 to 800µm.

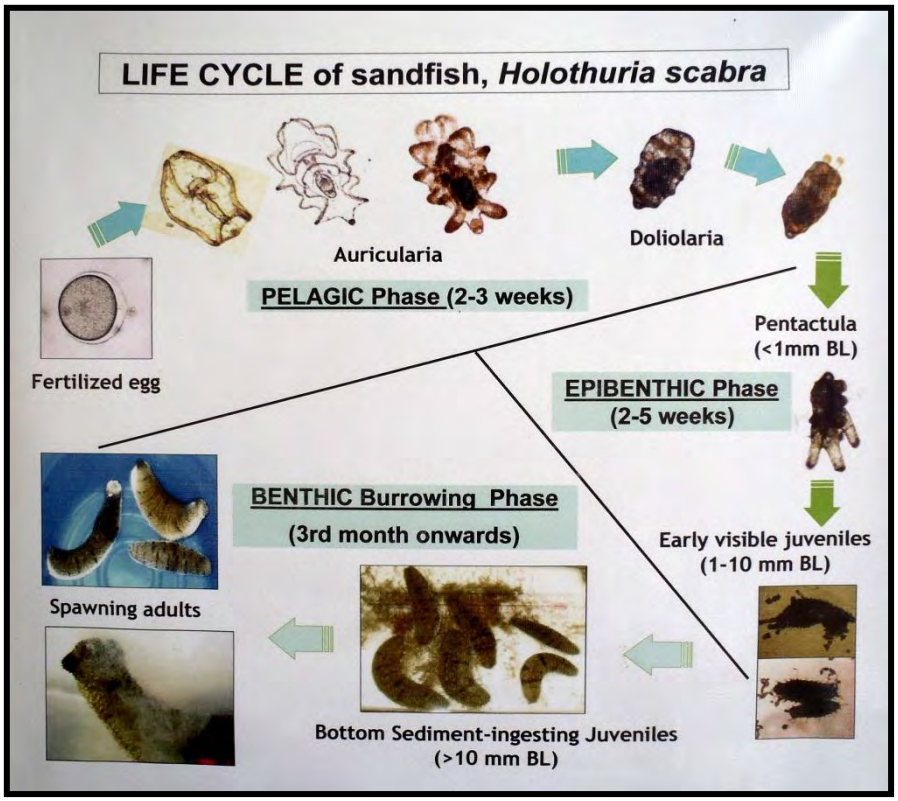
## ***Nursery***

Once juveniles are approximately 500µm in length they are ready to be moved to nursery tanks. These tanks have been conditioned prior with a natural biofilm and may contain a thin (<1mm) layer of fine sediment for the juveniles to feed on and bury in. The sediment is either introduced with the seawater or can be collected from the shore.

Juveniles are harvested from the hatchery tanks by draining through a 500µm sieve, during this process it is routine to use 1%KCL to detach juveniles from tanks walls with a spry gun. Juveniles are stocked at 150-200/$m^2$ into the nursery tanks and water is be exchanged continuously and an occasional supplementary feed such as Spirulina, Algamac-Protein Plus or manure may be applied to enrich the sediment.

***Appendix 1.***

Life cycle of sandfish, *Holothuria scabra* (photo courtesy of SEAFDEC, Iloilo City, Philippines).



## 11.2 Genetic Management Plan