Aquaculture Reports 3 (2016) 198–208

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Optimising methods for community-based sea cucumber ranching: Experimental releases of cultured juvenile *Holothuria scabra* into seagrass meadows in Papua New Guinea

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ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form 10 March 2016
Accepted 13 March 2016

Keywords:
Holothuria scabra
Mariculture
Sea pens
Predator exclusion
Principal components analysis
Biophysical variables

ABSTRACT

Hatchery-cultured juveniles of the commercial holothurian, sandfish (*Holothuria scabra*), were used for release experiments in a variety of marine habitats under traditional marine tenure near Kavieng, Papua New Guinea (PNG). Juveniles of approximately 4 g mean weight were released inside 100 m^2 sea pens installed within seagrass meadows nearby partner communities, under the care of local 'wardens'. Within each sea pen, varying levels of protection (free release, 1-day cage and 7-day cage) were provided at release in order to determine if short-term predator exclusion improved survival. Ossicles of juvenile sandfish were tagged with different fluorochromes for each treatment and sandfish survival and growth was recorded after release. A range of biophysical parameters were recorded at the four sites. Contrary to expectations, short-term cage protection did not lead to higher survival at three sites, while a fourth site, despite meeting all considered criteria for suitable release habitat, experienced total loss of juveniles. There were significant differences in mean weight of juveniles between sites after four months. Multivariate analysis of biophysical factors clearly separated the sea pen habitats, strongly differentiating the best-performing site from the others. However, further research is needed to elucidate which biophysical or human factors are most useful in predicting the quality of potential sea ranch sites. Methods developed or refined through these trials could be used to establish pilot test plots at potential ranching sites to assess site suitability and provide guidance on the level of animal husbandry required before commencing community sea ranching operations in New Ireland Province, PNG.

1. Introduction

A promising commodity for mariculture in the Indo-Pacific region is the tropical holothurian, sandfish (*Holothuria scabra*) (Battaglene, 1999; Bell et al., 2005; Hamel et al., 2001). This valuable sea cucumber has been collected and processed into beche-de-mer for a predominantly Chinese market for more than two centuries. Aquaculture techniques were pioneered in India in the early 1980s (James, 1996) and subsequent hatchery advancements have been made by researchers in other parts of the world (see Raison 2008; Mills et al., 2012; Purcell et al., 2012). Sustained overfishing in the last three decades has prompted a surge in research into mariculture opportunities for stock restoration and sustainable livelihood activities based on cultured *H. scabra* (Hair et al., 2012; Purcell et al., 2012; Robinson, 2013).

Following the hatchery stage, juvenile sea cucumbers can be grown to commercial-size in seawater ponds (Duy, 2012), the sea (Robinson and Pascal, 2012; Juinio-Meñez et al., 2013; Tsiresy et al., 2011) and potentially in land-based recirculating systems (Robinson, 2013). Where cultured juveniles are released into the sea, semi-intensive culture, or sea farming, involves release into enclosures attended with basic husbandry (Robinson and Pascal, 2009; Rougier et al., 2013). Extensive culture options include restocking, stock enhancement and sea ranching (Bell et al., 2008). Of these, sea ranching using low-technology methods (i.e. cultured juveniles are released into marine environments under traditional

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http://dx.doi.org/10.1016/j.aqrep.2016.03.004

marine tenure in a 'put, grow, and take' operation) in Papua New Guinea (PNG) has potential as a sustainable livelihood opportunity (Hair et al., 2016). However, its successful development depends on resolving a number of technical and social constraints (Eriksson et al., 2012; Mills et al., 2012; Purcell et al., 2012; Robinson, 2013), not least of which is maximising the number of small juveniles that survive to commercial harvest size. Highest mortality in experimental release of juveniles occurs in the period immediately following release of small juveniles (Dance et al., 2003; Purcell and Simutoga, 2008). Mortality is inversely related to size of the released juvenile (Purcell and Simutoga, 2008) but small juveniles are cheaper to produce and transport (Raison, 2008). Case studies report highly variable and usually low survival of juvenile sandfish after release into the sea (Purcell et al., 2012): 20-30% in sea ranches in the Philippines (Juinio-Meñez and Dumalan, 2012; Juinio-Meñez et al., 2013); 0-80% in pens and sea farms in Madagascar (Robinson and Pascal, 2012; Rougier et al., 2013); 20-40% in small sea pens in Fiji (Hair et al., 2011); 14% from field releases in northern Australia (Andrea Taylor, Pers. comm.); and less than 15% in lagoonal sea ranches in the Maldives (James, 2012). Purcell (2012) suggested 10-20% survival among 3-10 g juveniles as a suitable benchmark in sea ranching operations.

Reasons for poor recovery of juveniles in sea-based mariculture include predation, transport stress, freshwater inundation, being washed away by strong currents, escape from enclosures and extreme weather (Purcell, 2004; Robinson and Pascal, 2012). Of these, predation is the major cause of juvenile sandfish mortality (Bell et al., 2005; Robinson and Pascal, 2012). Predators of holothurians include fish, crustaceans, sea stars and gastropods (Knopp, 1982; Francour, 1997; Dance et al., 2003; Zamora and Jeffs, 2013). Of these, fish and crustaceans have been most problematic to aquaculture activities. Measures adopted to minimise predation commonly fall into four categories: (i) maximising the size of juveniles at release; (ii) improved methods of release; (iii) removal of predators; and (iv) protection from predators. Size at release is inversely related to the risk of predation (Bell et al., 2005; Purcell and Simutoga, 2008). The minimum recommended release size of 3 g for sandfish was made after observing total mortality among 1-g juveniles in an experimental release (Purcell and Simutoga, 2008) and this standard has been adopted in several subsequent studies (e.g. Juinio-Meñez et al., 2013, this study). Large-size sea cucumbers are released in some mariculture operations to reduce predation; e.g. >5 cm juvenile *Apostichopus japonicus* (Chen, 2004), 15 g sandfish (Rougier et al., 2013). However, survival of 0-5 g juveniles was not significantly different to that of 15-20 g juveniles in a Madagascan sea farm where predators were not abundant (Lavitra et al., 2015). The time and manner of release (i.e. handling and transport to the release site), in addition to adequate on-site acclimation, can also improve survival (Purcell, 2004; Rougier et al., 2013). For example, sand conditioning (acclimation to sand prior to release) has been identified as an important process for hatchery-bred juveniles, leading to increased burying activity in the first hour after release (Juinio-Meñez et al., 2012). Dance et al. (2003) suggested releasing juveniles at night when fish predators are less active, however, Robinson and Pascal (2012) reported heavy predation by crabs at night. Removal of predators may also improve survival. Consistently high survival of juvenile sea cucumbers (40–85%) has been reported from ponds where predators are removed prior to stocking with 2 g sandfish juveniles (e.g. Agudo, 2012; Duy, 2012). However, in larger, open sea ranches, predators will be less easily controlled by active hunting.

Some success in reducing predation of juvenile sea cucumbers released into the sea has been demonstrated with the use of cages to exclude large predators (Dance et al., 2003; Purcell, 2004; Rougier et al., 2013). In Madagascan sea farms, predation can be so intense that 15 g cultured juveniles are reared in covered nursery pens until they reach about 50 g in size and crabs are culled from the farm area (Robinson and Pascal, 2009; Rougier et al., 2013). The benefit of caging newly released juveniles may extend beyond simple predator exclusion if cages provide naïve, hatchery-produced juveniles a greater chance to acclimate to the wild and normalise behaviours such as seeking shelter, predator avoidance and feeding (Purcell, 2004, 2010). Protection from predators until the normal diel burrowing habit is established may be worthwhile. These practices may improve survivorship but the trade-off between animal size and cost of production for the resultant productivity gain must be considered (Raison, 2008), and there are logistical constraints to using cages for large numbers of juveniles over long periods (Purcell, 2004).

Observations and field studies of juvenile sandfish in the wild and in captivity indicate that relatively sparse seagrass habitats with muddy-sandy sediment of moderate penetrability, sediment low in organic matter, more than 20 cm water depth, minimal freshwater input and populated by a range of invertebrate fauna are favourable for juvenile sandfish release (Mercier et al., 1999, 2000; Purcell, 2004; Schiell, 2004; Purcell and Simutoga, 2008; Lavitra et al., 2010). There is also a growing body of literature relating to mineral and organic characteristics of the sediment which may promote juvenile sea cucumber growth. The roles of benthic microalgae, organic matter, microorganisms (e.g. bacteria), grain size and so on, are now being examined more carefully (Hamel et al., 2001; Slater and Jeffs, 2010; Lavitra et al., 2010; Plotieau et al., 2014a,b). However, the exact nature of ideal habitat is still unknown.

In this study we investigated whether short-term protection from predation improved survival of cultured juvenile sandfish released into a range of seagrass habitats located within potential community sea ranching sites. It also describes the biophysical properties of the habitats and relates these to sandfish growth. The results will assist project managers and community farmers in optimising methods for releases and in selecting suitable sites for sea ranching operations in New Ireland Province (NIP), PNG.

2. Materials and methods

2.1. Study area

Our study sites were adjacent to island communities collaborating in sandfish sea ranching trials. These were Limanak, Enuk and Ungakum, located near Kavieng, NIP (Fig. 1). All communities had previously productive sandfish fisheries in their marine tenure areas. The Limanak and Enuk sites were 20 min from the hatchery by boat, and Ungakum 1 h.

Cage release experiments were conducted within round 100 m^2 sea pens, which were installed as part of a longer-term study to monitor survival and growth of cultured sandfish. Sea pen location was chosen on the basis of the optimum release microhabitat criteria of Purcell and Simutoga (2008) with respect to depth, sediment and seagrass type, in conjunction with traditional knowledge of sandfish abundance and our observations of conspecifics in the area. Local issues such as boat traffic and community amenity were also considered. Two sea pens were installed at Limanak, one site was characterised by patchy *Cymodocea rotundata* and *Enhalus acoroides* seagrass (Limanak 1), and the other in an area dominated by bare sand with patches of *C. rotundata*, *Thalassia hemprichii* and *Halodule uninervis* complex (Limanak 2). The Enuk sea pen enclosed sparse but homogeneous *E. acoroides* and the Ungakum sea pen was part *E. acoroides* and part bare sand.

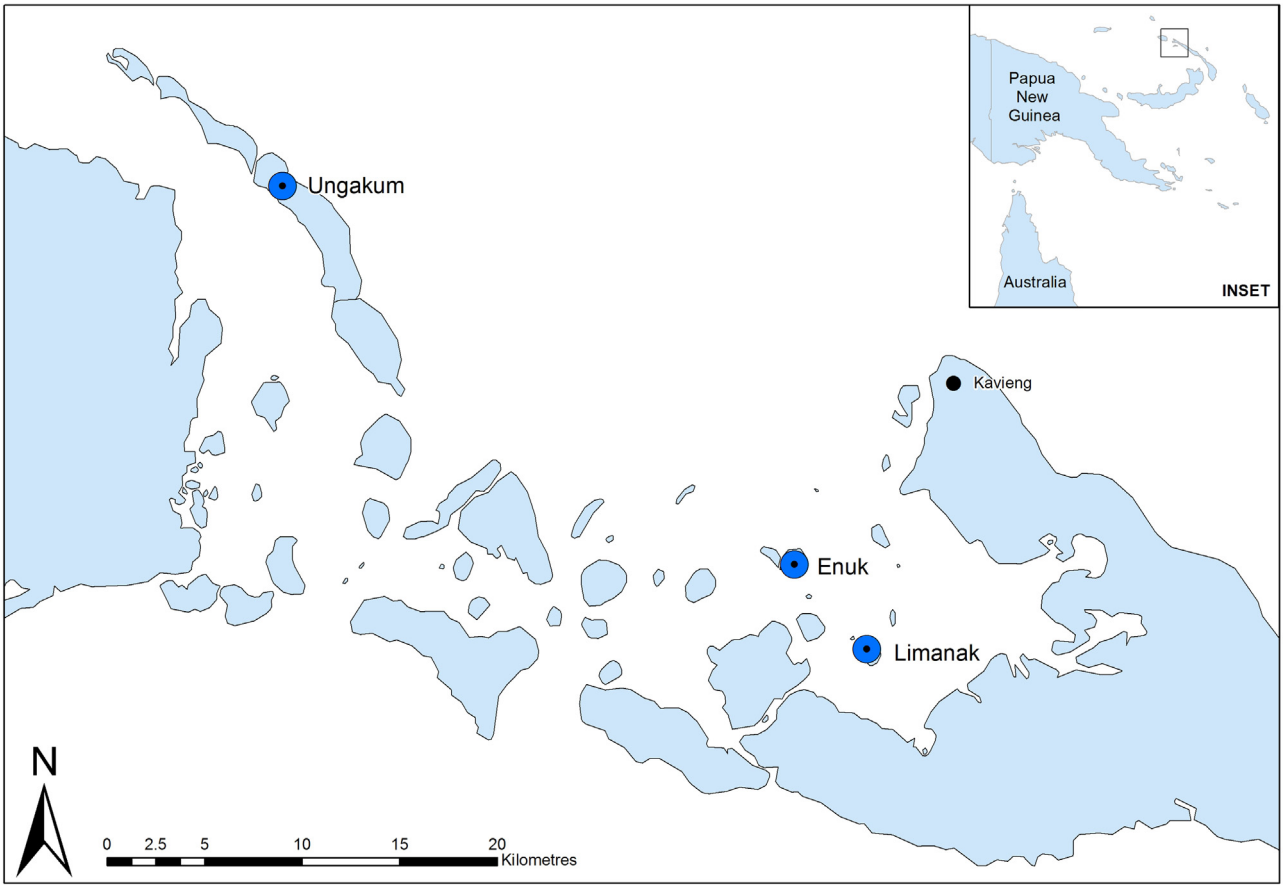

Fig. 1. Map of study area showing the location of collaborating island communities at Limanak, Enuk and Ungakum, near Kavieng, New Ireland Province, Papua New Guinea.

2.2. Experimental sea pens

Sea pens were constructed from rigid plastic mesh (3 mm pore size) held in place with wooden stakes and not covered (Purcell et al., 2012). They were designed to retain sea cucumbers within natural habitat while predation, water exchange and food supply occurred as they would outside the pen (Purcell and Simutoga, 2008). Escape by 3 g juveniles was minimised by digging the base of the pen mesh 15 cm into the sediment to prevent burying underneath, while 30 cm of mesh wall extended above the sediment, the upper inside edge of which was painted with a 10 cm strip of antifoul to discourage climbing (Purcell and Simutoga, 2008; Robinson and Pascal, 2012). Community 'wardens' at each site cleaned biofouling from the mesh walls and reported any problems.

2.3. Experimental juvenile sandfish

All sandfish juveniles were produced at the Nago Island Mariculture and Research Facility, near Kavieng, using the hatchery protocols of Duy (2010) and reared to at least 3 g weight in ocean hapa nets (sensu Juinio-Meñez et al., 2012). Prior to being released into pens for experiments, juveniles were batch-marked using fluorochromes (Purcell et al., 2006). Batches of juveniles of 3–20 g weight were fluorochrome tagged with either tetracycline, calcein or calcein-blue, then transferred to raceways with sediment in the base (<1 mm grain size) for recovery for 10 days before release.

2.4. Cage release experiments

The protective cages were constructed from rigid plastic mesh sewn onto a metal frame (Fig. 2, length = 90 cm, width = 90 cm, height = 20 cm with no floor, 6 mm pore-size). The cages were positioned within sea pens with the bottom cage edge pushed into the sediment to a depth of 1-2 cm, any spaces at the base of the cage were buried with sediment (Fig. 2). Metal stakes held the cages in place. The purpose of cages was not to prevent escape of juveniles as they could bury underneath or squeeze through the mesh, but rather to exclude predators. Potential predators were removed from the cage interior if seen during deployment. Each sea pen

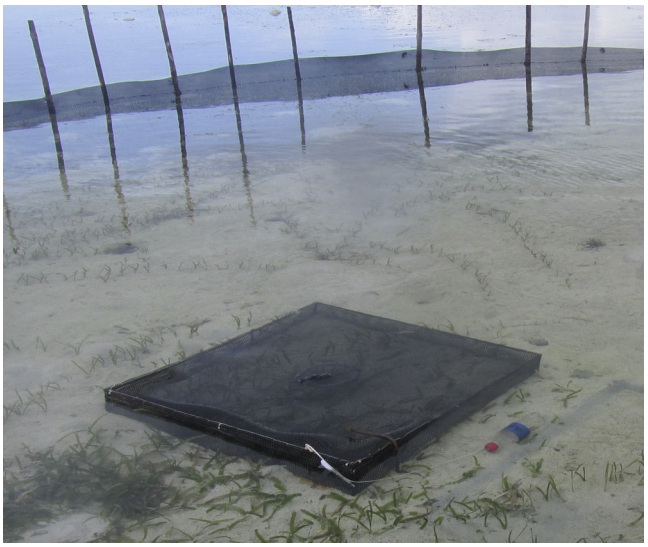

Fig. 2. Release cage within a sea pen at the Limanak 2 site.

Table 1
Experimental treatment and associated fluorochrome stain for releases within each 100 m^2 sea pen.

Treatment	Description	Stain	n
Free release	Juveniles released loose onto the sediment	Tetracycline	67
1-day cage	Protective cage left in place for 24 h	Calcein	67
7-day cage	Protective cage left in place for 7 days	Calcein blue	67

Table 2
Sampling schedule for releases and monitoring.

Event	Limanak 1	Limanak 2	Enuk	Ungakum
Release (Time 0)	22 March 2014	15 May 2014	15 May 2014	15 July 2014
Time 1 sample	20 May 2014	11 July 2014	10 July 2014	11 Sept 2014
Time 2 sample	10 July 2014	4 Sept 2014	5 Sept 2014	na
Follow up trial 1				22 Dec 2014
Follow up trial 2				5 Feb 2015

received 201 juveniles: n = 67 per treatment, each tagged with a different fluorochrome (Table 1).

To ensure release habitat was not a confounding factor, similar microhabitat was used for every release treatment in each sea pen; the release microhabitat in Limanak 1 and Limanak 2 pens was sand with patchy, short seagrass; Enuk was *E. acoroides* (the long-bladed seagrass was bunched inside the cage for the protective treatments); and Ungakum was bare sand.

Experiment releases were staggered because of reliance on hatchery production of juveniles in the desired size range. The Limanak 1 release was in March 2014, Limanak 2 and Enuk in May 2014, and Ungakum in July 2014 (Table 2). Fluorochrome-tagged juveniles were packed into separate, labelled plastic bags with seawater and oxygen, and transported in an insulated container to each site ($n = 67$ tetracycline tagged, $n = 67$ calcein tagged, $n = 67$ calcein blue tagged). After a 20 min acclimation period, juveniles were released either through the trapdoor of a cage (marked for removal either 1-day or 7-days later) or loose into the sediment ('free release'). The free-release juveniles were observed until they started to move or bury. Sandfish interaction with potential predators was noted but not prevented.

2.5. Survival and growth data collection

Sea cucumbers within each pen were sampled twice after their release (Time 0): after approximately two (Time 1) and four (Time 2) months (Table 1). Sampling commenced in the late afternoon when juvenile sandfish were most likely to be on the surface (Mercier et al., 1999; Purcell, 2010). Pens were searched by a snorkel diver and searching was discontinued when no new individuals were found within a 30 min period. Sandfish were handled gently, collected in small numbers and kept submerged until they reached the sampling station. They were then removed from the water, left to drain for several minutes and weighed to the nearest 0.1 g. After measurement, about 5 mm^2 of skin was shaved from the ventral surface of each individual, preserved in 70% ethanol, and stored in a cool, dark place until processing to check for the presence of fluorochrome-tagged ossicles (Purcell et al., 2006). All sea cucumbers were returned to the pen after sampling was completed.

Wet weight was used as an indicator of growth. Weight measurements from one sample time to the next were taken of the same group of sandfish but individual sandfish could not be differentiated. The specific growth rate (SGR, %) was calculated using the mean individual size of the group from the previous sample time as follows: $SGR = 100^* [\ln(WW_t\text{-}MnWW_0)]/t$, where WW_t is individual wet weight in grams after t days and $MnWW_0$ is mean initial wet weight in grams. The co-efficient of variation for sandfish weight (CV, %) was calculated as: $CV = 100^* (SD/MnWW)$, where SD is the standard deviation in weight and MnWW is the mean weight (g).

A number of tagged juveniles were maintained at the hatchery to monitor the persistence of the fluorochrome tags throughout the experiment duration. These individuals were checked at each sampling time to verify that fluorescent ossicles remained visible.

2.6. Additional Ungakum trials

At Time 1, no sandfish were found within the Ungakum sea pen. Possible causes were speculated as a seasonal effect due to release later in the year, transport stress due to greater distance to this site, heavy rain leading to low salinity, predation, a quicksand effect (the site had a higher proportion of fine-grain sand) or displacement (the site experienced tidal flow). Two short-term trials were carried out to investigate these factors. In the first of these, slightly larger juveniles were used (mean weight 4.1 ± 0.1 g) and community wardens checked the pen every night to observe emergent juveniles, presence of potential predators, report heavy rain episodes and note any unusual events. After careful packing and transport, two batches of 100 juveniles were released inside the sea pen, one free release and one with a 1-day cage protection. When the juveniles again disappeared completely within 2 weeks, a second trial was devised where, in addition to free releases, juveniles were also kept within fully enclosed net (length = 2 m, width = 1 m, height = 0.4 m, with floor and roof, pore size 1 mm) with sand in the base. The closed system totally excluded predators and prevented escape of sandfish for the duration of the trial. A total of 84 juveniles were released freely into the pen and 28 juveniles into each of two enclosed nets. The sea pen was checked nightly for signs of emergent juveniles and predators. The nets were checked periodically to ensure that there were still live sandfish inside. Number and weights of retrieved juveniles were recorded after 40 days.

2.7. Biophysical description

At the start of the experiment, the habitat within each pen was characterised using descriptive factors and a number of biophysical properties related to seagrass and sediment variables were measured. Data were collected from within five haphazardly thrown quadrats of 0.5×0.5 m in each 100 m^2 sea pen, and comprised: seagrass species present; percentage total cover of seagrass to the nearest 5%; average canopy height of the dominant seagrass species (discounting the tallest 20% of leaves); epiphyte index (estimated from proportion of the leaf surface covered with epiphytes, and proportion of leaves with epiphytes, after McKenzie and Campbell, 2002); penetrability (cm, measured with a pointed metal rod dropped from a standard height); and presence, depth and strength of an anoxic layer. Sediment samples were collected from within each quadrat using a corer (internal diameter 29 mm). Sediment from surficial 10 mm sediment cores ($n = 2$ cores combined) were analysed for chlorophyll-*a* content as a measure of benthic microalgae. Sediment from surficial 20 mm sediment cores ($n = 5$ cores combined) were analysed for organic matter (OM) and grain size.

Sediment for chlorophyll-*a* analysis was kept in the dark and on ice in the field, transferred to a freezer, and processed within 30 days (ISO, 1992). Approximately 2 g of each sediment sample was weighed into a tube, 5 mL of 95% ethanol added, mixed on a vortex stirrer, stood in a 60 °C water bath for 1 h, and left to extract for 12 h at room temperature. After 12 h the sample was inverted to remove any gradient, centrifuged (8 min at 4000 rpm) and the supernatant measured in a spectrophotometer at 665 and 750 nm against a 95% ethanol blank. After measurement, the sample was oven-dried at 60 °C to obtain the dry weight. Chlorophyll-*a* con-

centration ($\mu g\, g^{-1}{}_{dw}$) was calculated using the formula of Nusch (1980).

Sediment for OM and granulometric analyses was kept on ice in the field and then dried in a 60 °C oven to a constant weight. For OM determination, the loss on ignition (LOI) method was employed at two temperatures: 280 °C for the labile OM component, and 500 °C for the refractory OM including the loss of carbon due to the biogenic carbonate particles of the sediment (Loh et al., 2008; Kristensen, 1990). Approximately 3 g of dried sediment (DW) were transferred to a labelled foil envelope, heated in a muffle furnace to 280 °C for 6 h, cooled and reweighed to obtain the ash weight (AW_{280}), then heated to 500 °C for a further 6 h, cooled and reweighed to obtain AW_{500}. Percentage OM fractions were calculated as: % Labile OM = 100*(DW-$AFDW_{280}$); % Total OM = 100*(DW-$AFDW_{500}$); and % Refractory OM = (% Total OM)-(% Labile OM).

Grain size distribution was determined by dry sieving samples through a series of mesh sizes (2000, 1000, 500, 250, 125 and 63 μm) with a mechanical sieve shaker for 10 min, then weighing the fraction retained by each sieve. For analysis, grain-size classes were combined into three broad categories: coarse-grained (> 1000 μm); medium-grained (≥250–1000 μm); and fine-grained (<250 μm) sediments.

Other descriptive factors comprised proximity to mangrove forests and the village, exposure to tidal currents and wave action.

Water temperature (°C) near the seafloor at all sites was recorded at 4-hourly intervals by Hobo™ data loggers. Salinity (ppt) was recorded at 4-hourly intervals by a Star Oddi™ DST logger at the Ungakum and Enuk sea pens.

2.8. Statistical analyses

Prior to analysis all data were tested for normality and homogeneity of variance with Levene's test at $P = 0.05$ (IBM SPSS Statistics 22).

Survival was analysed with one-way ANOVA with the cage protection treatment ($C = 3$) at 2 months only, as it is the critical early period where mortality occurs. Growth of juveniles was analysed with two-way ANOVA with the cage protection treatment ($C = 3$) and sea pen site ($S = 3$) at Time 1 and Time 2. A Tukey's post-hoc test ($P = 0.05$) was used to compare significant differences between survival and growth variables between protection treatments and sea pen sites.

Patterns in habitat of sea pens were generated by extraction of principal components axes (PCA) using Primer software (Clarke and Gorley, 2006). PCAs whose eigenvalues were greater than 1.0 were used to plot the habitats according to seagrass and sediment characteristics. Significant component loading factors were evaluated using $r < -0.05$ and $r > 0.5$ as cut-off values.

3. Results

3.1. Fluorochrome marking of juveniles' ossicles

After 1 week of post-tagging recovery at the hatchery, all juveniles were visibly healthy, and showed normal burying and feeding behaviour. The juveniles retained at the hatchery displayed an acceptable proportion of brightly-fluorescing ossicles after 4 months. A separate experiment to determine if there were differential mortality or growth from the staining treatments resulted in zero mortality due to any fluorochrome, and found no significant difference in growth rates among the different tagging treatments after 1 month (one-way anova $F = 1.492$, $P = 0.221$).

3.2. Effects of site and cage protection on survival of cultured sea cucumbers released into the wild

At Time 1, sandfish were retrieved from sea pens at Limanak 1, Limanak 2 and Enuk, but there were no sandfish found at Ungakum. The free-release treatment had the highest survival at all sites (97%, 96% and 66% for Limanak 1, Limanak 2 and Enuk, respectively) (Fig. 3), followed by the 7-day protective cage treatment at Limanak 1 and Enuk (95.5 and 52.2%, respectively) and the 1-day protective cage treatment at Limanak 2 (76.1%). There were significant differences in overall survival between sites (one-way anova, $F = 11.88$, $P = 0.008$): Limanak 1 had the highest survival (93.5%), followed by Limanak 2 (84.6%) and Enuk (52.7%). Overall survival at Limanak 1 was not significantly different to that at Limanak 2 and both were greater than that at Enuk (Tukey's post-hoc means comparison). Estimated survival at Time 2 varied little from Time 1 (92.5%, 86.6% and 52.7% for Limanak 1, Limanak 2 and Enuk, respectively).

3.3. Growth of juvenile cultured sandfish in the wild

Growth results refer to the three sea pens where animals were retrieved. There was a significant difference in mean individual weight due to protection treatment at Time 1 but not at Time 2 (Table 3, Fig. 4). The Time 1 difference arose from variability in growth of sandfish at Enuk only.

In terms of overall mean individual sandfish weights between sites, means at Limanak 1 and 2 were not significantly different but both were greater than that at Enuk at Time 1 (Tukey's means comparisons). By Time 2, mean weight at Limanak 1 was greater than that at Limanak 2 and both were greater than that at Enuk (Table 3, Fig. 4).

SGR (%) showed similar patterns to individual sandfish weight. Sandfish at Limanak 1 and 2 showed significantly higher rates of growth than those at Enuk at Time 1. At Time 1, there were also differences due to protection treatment. At Time 2, there were no significant differences in growth rates due to protection treatment. At Time 2, sandfish at Limanak 1 had significantly higher growth rates than those at Limanak 2 or Enuk.

3.4. Coefficient of variation for sandfish weight

The co-efficient of variation (CV) for sandfish weight was highest at Time 0 stocking and then decreased with time at all sites (Table 4). CV varied inversely to survival and growth: sandfish at Limanak 1 had the lowest CV with the highest survival and growth; sandfish at Enuk had the highest CV with the lowest survival and growth.

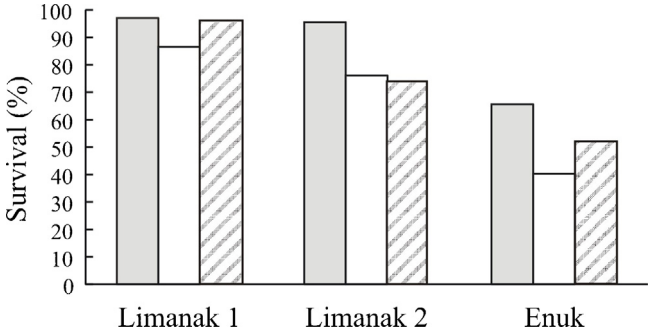

Fig. 3. Survival (%) of juveniles from each protection treatment at each site at Time 1. Solid bars denote free (uncaged) release; white bars denote 1-day cage protection; striped bars denote 7-day caged protection.

Table 3
Two-way ANOVA results and Tukey's post-hoc means comparisons of mean individual sandfish weight (g) at times 1 and 2.

Sampling time	Levene's test	Site	Protection treatment	Interaction	After pooling	Tukey's means comparisons
Time 1	Ns (P = 0.215)	F = 6.173, P = 0.002**	F = 4.74, P = 0.009**	F = 2.043, P = 0.087 ns	Site** Protection*	Lim1 = Lim2 > Enuk; free > 1-day = 7-day
Time 2	Ns (P = 0.508)	F = 49.646, P = 0.000***	F = 0.160, P = 0.852 ns	F = 1.281, P = 0.276 ns	Site***Protection ns	Lim1 > Lim2 > Enuk

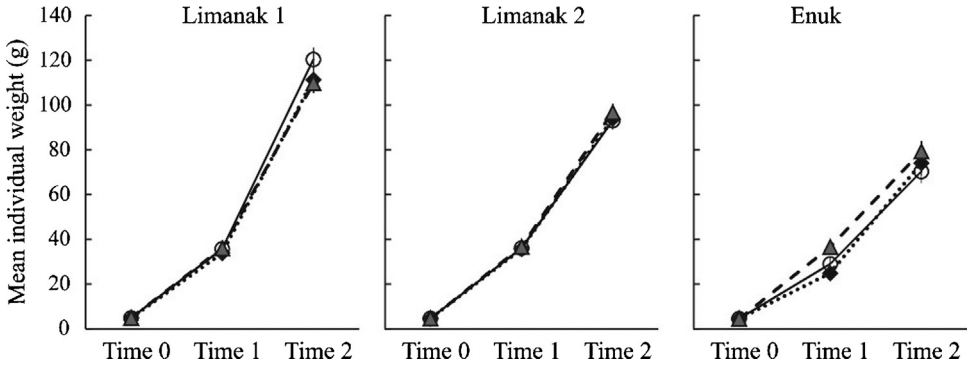

Fig. 4. Changes in mean individual weight (g ± se) of juveniles from each protection treatment within each sea pen during the study. Dashed lines denote free (uncaged) release; solid lines denote 1-day cage protection; dotted lines denote 7-day caged protection.

3.5. Additional short-term trials in the Ungakum sea pen

The first follow-on trial at Ungakum resulted in total loss of juvenile sandfish within about 2 weeks. Observations by the community wardens indicate that some individuals were seen on the surface up to 10 days after release when heavy rain resulted in brown-coloured water being discharged from nearby mangroves (although the logger did not record a noticeable drop in salinity). Thereafter, very few sandfish were seen and none were found after 1 month. Small bream (*Scolopsis trilineata*) were observed pecking at the juvenile sandfish at the time of release and mangrove crabs were observed inside the pens at night.

In the second follow-up trial, none of the free release individuals were recovered. However, 93% survival of juveniles in the fully enclosed systems was recorded after 40 days.

3.6. Description of sea pen habitat

All four sea pens were installed in sandy areas with some seagrass and resident wild sandfish populations, but they differed in other aspects (Table 5). Many features were common to two of the four pens although the combinations of features varied. However, a number of features were unique to a single pen. For example, sea pens at Enuk and Ungakum were located in protected areas of tidal channels, near to mangrove forest, while sea pens at Limanak were in an open bay environment. The granulometry of the Limanak 2 and Enuk sites was quite similar while that of Limanak 1 and Ungakum were both different and unique, being composed of more coarse and more fine sediment, respectively. Limanak 1 sediment had the highest chlorophyll-*a* content, in addition to a more obvious anoxic layer close to the surface. Limanak 2 was the shallowest site and also experienced very low daytime tides during the study, with water temperatures reaching 40 °C. Juveniles at this site were observed to remain buried during low tide periods and emerged during night-time high tides. Predators were noted only at Ungakum, however, the lack of predator sightings at other sites does not confirm their absence; the wardens did not check all sites at night. Ungakum and Enuk experienced occasional heavy rainfall during the first 2 months of the experiment, and salinity dropped as low as 19.9 and 23.0 ppt, respectively, for several hours. Wardens reported that brown water discharged from the nearby mangroves at Ungakum during heavy rain. Only temperature was logged at the Limanak sites, which were not located near any freshwater sources.

3.7. Principal component analysis of sea pen habitat

Four principal components (PCs) had eigenvalues greater than 1 and together contributed about 85% of the variation between sea pen habitats (Table 6). The first two PCs accounted for about 50% of the variation (Fig. 5).

The first PC explained 30% of observed variation in the dataset, and separated Limanak 1 from the Enuk and Ungakum sites, and Limanak 2 from Ungakum (ANOVA: df = 3, F = 18.84, $p < 0.0001$). PC 1 was dominated by the proportion of coarse grain in the sediment (note that sediment chlorophyll-*a* content was also relatively high but fell just short of the commonly recommended 0.5 cut-off value). The second PC (22% of variation) separated Limanak 2 from Limanak 1 and Ungakum, and Limanak 1 from Enuk (ANOVA: df = 3, F = 16.41, $p < 0.0001$). This PC had higher loadings for labile organic matter content, and proportion of fine sediment grain size. PC 3 (19%) had highest loading for refractory and total organic matter content, while PC 4 had high loadings for seagrass cover and epiphyte cover. Sediment penetrability became an important loading in PC 5, which explained less than 10% of the variation in pen habitats.

4. Discussion

Key outcomes from the study relate to the utility of protective release systems for juvenile sandfish and the comparative quality of the microhabitat they are released into. The cage protection exper-

Table 4
Coefficient of variation for juvenile sandfish weight (CV) for each sea pen site at each time and overall survival (%) and mean (±se) individual weight (g), at Time 2.

Site	CV (Time 0)	CV (Time 1)	CV (Time 2)	Survival (Time 2)	Mean weight (Time 2)
Limanak 1	56.4%	38.4%	31.2%	92.5%	113.6 (±2.6) g
Limanak 2	53.0%	38.4%	33.3%	86.6%	94.7 (±2.4) g
Enuk	53.0%	53.5%	40.8%	52.7%	74.8 (±2.9) g

Table 5
Habitat characteristics for the four pens. Variables marked with an asterisk were used in the principal component analysis.

Feature	Limanak 1	Limanak 2	Enuk	Ungakum
Patchiness (seagrass)	Very patchy	Very patchy	Uniform	Very patchy
Anoxic layer (depth/strength)	0.3 cm/medium	none	1.6 cm/light	none
Abundance of conspecifics	Few	Many	Few	Many
Other invertebrate fauna	Yes, abundant and varied	Yes, abundant and varied	Yes, not abundant, mostly sea stars	Yes, abundant, mostly sea cucumbers
Presence of predators	Not observed	Not observed	Not observed	Fish and crabs
Depth range (m)	~0.3–1.0 m	~0.05–1.0 m	~0.5–1.3 m	~0.5–1.3 m
Water flow	Normal tidal	Normal tidal	Moderate: one-way tidal	Mild; one-way tidal
Wave exposure	Moderate	High	Low	Low
Distance from shore	30 m	100 m	15 m	10 m
Proximity to mangroves	100 m	Very distant	3 m	3 m
Proximity to houses	In view	In view	Out of view	Out of view
Dominant seagrass sp (+other seagrass)	*C. rotundata* (*E. acoroides*)	*C. rotundata* (*T. hemprichii*)	*E. acoroides* (*T. hemprichii*)	*E. acoroides*
Mean seagrass cover	16.4%	16.4%	7.0%	1.8%
Mean canopy height	11 cm	11.2 cm	59.6 cm	55.3
Benthic algae	Nil	Low	Nil	Nil
Mean SG epiphyte index*	75	20	30	63
Mean chlorophyll*	7.4 $\mu g\, g^{-1}$	3.9 $\mu g\, g^{-1}$	2.2 $\mu g\, g^{-1}$	2.1 $\mu g\, g^{-1}$
Mean labile OM*	1.3%	0.9%	1.0%	1.4%
Mean refractory OM*	1.9%	2.3%	2.2%	1.6%
Mean total OM*	3.2%	3.2%	3.1%	2.9%
Grain size* ratio (coarse:medium:fine)	36: 58: 6	19: 79: 2	18: 77: 5	6: 77: 17
Penetrability*	5.8 cm	3.4 cm	6.7 cm	5.8 cm
Water temp				
Time 0–1 (mn ± se)	31.9 (±0.08) °C	32.2 (±0.10) °C	30.9 (±0.05) °C	30.1 (±0.10) °C
Time 1–2 (mn ± se)	32.4 (±0.09) °C	32.6 °C (est.)	30.5 (±0.05) °C	–
Range	26.8–39.8 °C	27.9–40.3 °C	26.8–34.6 °C	26.2–35.5 °C
Salinity				
Time 0–1 (mn ± se)	na	na	30.5 (±0.05) psu	34.6 (±0.07) psu
Range			19.9–32.4 psu	23.0–36.8 psu

Table 6
Important principal components (eigenvalues > 1) from variables describing habitat, with high component loadings (r < −0.05 or r > 0.05) shown in bold. PC 5, with an eigenvalue approaching 1, is also shown.

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalue	2.93	2.21	1.87	1.54	0.93
% variation explained	29.1	22.1	18.7	15.4	9.3
(cum.% variation explained)	(29.1)	(51.4)	(70.1)	(85.5)	(94.8)
Component loading					
Seagrass cover (%)	−0.335	0.068	0.062	−0.559	−0.271
Penetrability (cm)	0.124	−0.247	−0.155	0.362	**−0.773**
Seagrass epiphyte cover (%)	−0.138	−0.314	−0.043	**−0.595**	−0.289
Labile OM (%)	0.064	**−0.508**	−0.283	−0.175	0.442
Refractory OM (%)	−0.141	0.338	**−0.595**	0.057	−0.111
Total OM (%)	−0.104	0.059	**−0.711**	−0.02	0.113
Chlorophyll-a (mg cm^{-1})	−0.465	−0.153	0.131	0.315	0.156
% Coarse sediment	**−0.554**	−0.039	0.027	0.173	−0.031
% Medium sediment	0.42	0.424	0.02	−0.184	−0.002
% Fine sediment	0.345	**−0.505**	−0.11	0.065	−0.032

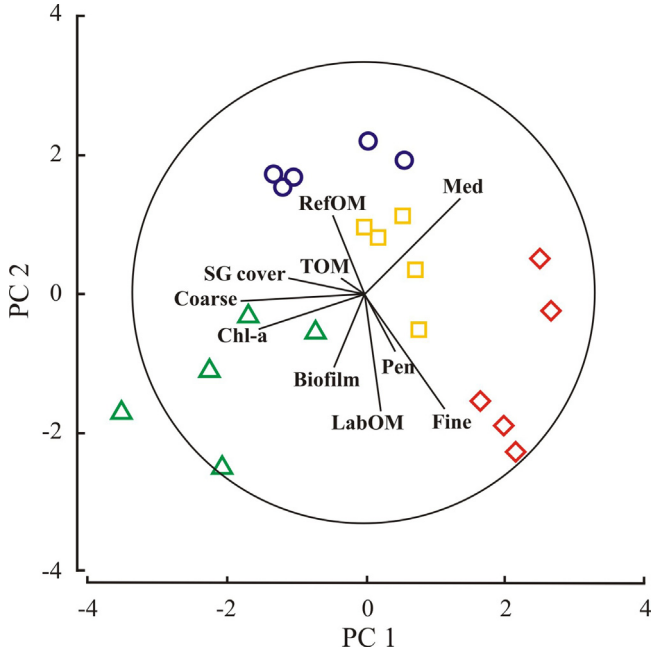

Fig. 5. Ordination of the four sea pen habitats ($n = 5$ reps per pen) based on the PCA values in Table 6 for the habitat variables: % fine grain sediment (Fine); % medium grain sediment (Med); % coarse grain sediment (Coarse); % labile OM (LabOM); % refractory OM (RefOM); % total OM (TOM); % seagrass cover (SG cover); concentration of chlorophyll-a (Chl-a); % seagrass epiphyte cover (Biofilm); and sediment penetrability (Pen). Green triangles represent Limanak 1; blue circles represent Limanak 2; yellow squares represent Enuk; and red diamonds represent Ungakum. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

iment showed that short-term protection from predation made no significant difference to survival of juvenile cultured sandfish, regardless of the release habitat. In fact, the free release had the highest survival at all sites where animals were recovered. While further research would be needed to elucidate the drivers of this outcome, it is notable that free release did not lead to immediate predation and mortality, nor did protection prevent loss of juvenile sandfish. Subsequent grow-out of juvenile sandfish for four months also showed significant differences in growth between the sea pen sites, independent of release protection treatment. At three sites, there was a direct relationship between survival and growth, and these were inversely related to CV, i.e. high survival sites had high growth and low CV and vice versa. At the fourth site, no juveniles were recovered. Sea pen site biophysical features were examined in an attempt to explain the observed variation in sandfish growth between sites. Shortcomings of the study, due largely to the pragmatics of working with communities in traditional marine tenure areas in a remote location, include the lack of sea pen replication. However, the results contribute to baseline knowledge for cultured sandfish requirements and indicate worthwhile areas for further sea ranching site selection research.

Small juvenile sandfish are associated with seagrass meadows and begin burying behaviour at around 10 mm in length (approximately 1 g in weight), later migrating away from dense seagrass to nearby mud-sand substrata when they are greater than 40 mm or around 3 g (Mercier et al., 2000). They bury during the day or when conditions are adverse (e.g. low salinity) but emerge and feed at night, possibly as predator avoidance behaviour (Hamel et al., 2001; Mercier et al., 1999). We theorised that cage protection would improve survival by enabling juveniles to acclimate and recover from transport and handling stress, while allowing natural burying and feeding patterns to develop before exposure to predators, as recommended by Dance et al. (2003). Our expectation was that juveniles released with no protection would be more vulnerable to predation; those with the 1-day cage protection would partially recover from release stress; and those with the 7-day cage protection would recover totally from stress and fully develop a natural diel burying and feeding cycle. Instead, we found that predator exclusion conferred no advantage for these short periods of time. Furthermore, juveniles released with no protection had both absolute and comparatively high survival within pens and between sites. In a similar study, Lavitra et al. (2015) found that protection of juveniles at a site with low predator density in Madagascar did not lead to significantly higher survival, suggesting that protective nurseries will not always be necessary. Conversely, at the fourth site, where predators appeared to be abundant and active, the cages did not convey any survival advantage, suggesting that one week of protection was not sufficient to improve survival in areas where predation is likely.

We found strongly differing results for cultured sandfish released into four differing, but 'suitable', seagrass habitat (Purcell and Simutoga, 2008; Tsiresy et al., 2011; Plotieau et al., 2013). At the two extremes, one site (Limanak 1) supported one of the highest recorded survival rates for cultured juvenile sandfish and excellent growth compared to published studies (Purcell et al., 2012), while another (Ungakum) supported zero survival. Based on the checklist of variables implicated in contributing to well-being of juvenile sandfish (Table 5), no single site stands out as being particularly good or bad. However, Limanak 1, supported the best survival and growth and was clearly differentiated from the other sites using principal component analysis. The main variable responsible for this separation was a higher proportion of coarse-grained sediment, but this site was also characterised by high chlorophyll-*a* content, seagrass cover and epiphyte growth

on seagrass blades. These components present an interesting mix because sediment chlorophyll content and the seagrass factors may be associated with higher nutrient availability for a deposit-feeding sea cucumber (Moriarty, 1982) but most sources suggest that sandfish prefer fine- to medium-grain sediment (Mercier et al., 2000; Plotieau et al., 2013). Another site supporting high survival and relatively high growth, Limanak 2, separated in the PCA along different component axes. These results highlight the complexity of the biophysical mechanisms controlling growth of small sandfish. Other researchers investigating survival and growth of juvenile sea cucumbers in the wild have also reported highly variable (and sometimes contradictory) results. For example, Slater and Jeffs (2010) found that wild-caught *Australostichopus mollis* juveniles thrived in non-natural habitats that differed in biophysical sediment characteristics to natural habitat, including OM content and grain size. Tsiresy et al. (2011) highlighted the interplay between combinations of factors such as OM content and sediment grain size and compactness. Both of these studies suggested that the quality as well as the quantity of OM needs to be considered, as did Plotieau et al. (2014a).

The PCA also differentiated the worst performing site, Ungakum, although we have no data on growth of sandfish juveniles since none of those released in the experiment survived. Fully enclosed juveniles did survive at this site, indicating that escape or predation were responsible for the mortality, not water or sediment quality. Of these, predation seems most plausible since juveniles did not escape from any other sea pens. Proximity to mangroves may have played a part in these results because the other site close to mangrove (Enuk) had the lowest survival after Ungakum. Human factors may have also played a role because lower survival of juveniles was recorded at the two sites most distant from wardens and the village. Incidence of human interference may have been greater in pens farther from the village. Interference does not always imply poaching of large sea cucumbers; vandalism and mischief can also affect survival of sandfish in experimental pens. In addition, sites closer to people are exposed to more intense fishing pressure on predators; gleaning was common nearby both Limanak pens. The role that these variables or combinations of variables play in driving survival and growth require longer-term, replicated studies with greater community involvement to tease out the key drivers. Optimal site selection is a crucial component of the mariculture model and research should focus on learning how to identify such sites.

A sustainable and economically viable community-based sea cucumber sea ranching model rests upon minimising the following variables: (i) size at release of cultured sandfish (i.e. lower cost of production), (ii) post-release mortality and other losses (e.g. animals leaving the sea ranch area, poaching), (iii) time taken to reach harvest size (i.e. shorter cropping cycle), and (iv) material and labour costs. Concurrently, the following must be optimised: (i) number of released sandfish, (ii) sandfish size at harvest, (iii) value of the beche-de-mer product; and (iv) community harmony and system management. Key to achieving several of these aims is knowing if husbandry is required and the appropriate level of effort to reduce predation of small juveniles, and selection of sea ranching sites that will promote fast growth. While the benefit of an intensive approach can increase survival, it is an expensive and time-consuming option and should be adopted only if clearly warranted, since production of larger juveniles, materials to build cages and time spent clearing predators will reduce profit margins (Raison, 2008). Community members are most likely to undertake sandfish ranching as a part-time activity, also devoting effort to other subsistence activities and customary duties. Furthermore, there is evidence from small-scale agricultural livelihoods suggesting that labour- and time-intensive methods may be culturally incompatible and have less chance of uptake in PNG rural communities (Curry et al., 2015).

There is substantial advantage in identifying key parameters prior to large-scale releases. PCA could be a useful tool for future identification of suitable habitat as it provides a repeatable and objective protocol for classifying habitats (e.g. Verfaillie et al., 2009). However, this one-off study did not permit the development of a simple classifications based on individual PCs as some proponents of this method have done (e.g. Salita et al., 2003). Nor will all important variables be effectively incorporated within this framework (e.g. human factors, predator presence, occasional but critical water quality problems etc.), although efforts to include as many variables as possible should improve its utility. Various authors have also advocated pilot studies or test plots to assess site suitability and estimate approximate carrying capacity (Purcell, 2004; Robinson and Pascal, 2012). Preliminary site checks might include a test for predation risk with caged and uncaged juveniles, since our study showed that more than one week may be needed to allow juveniles to avoid predation. Protective cages, if employed, should have sufficiently small mesh to omit predators but retain sandfish juveniles. Results can then be compared to baseline data such as that presented here and from other studies (see Purcell et al., 2012). If predation is deemed a threat to sea ranching success, a worst case scenario is that the site will be abandoned, or further investigation can be made into the level of protection and husbandry needed (see Lavitra et al., 2015). Initial growth rates will also indicate if the site supports a growth rate appropriate for sea ranching. Sea ranch managers must remain vigilant though, as site suitability can change due to external events (Purcell and Simutoga, 2008; Juinio-Meñez et al., 2013; Hair et al., 2011), and can fail to hold when release experiments are scaled-up (Robinson and Pascal, 2012). Predators can also learn that abundant, vulnerable prey are available when releases are made (Robinson and Pascal, 2012). Since releases into good habitat are not guaranteed to succeed, the use of open pens as a monitoring tool after stocking is still advisable (Purcell, 2012).

5. Conclusions

With respect to community-based sea ranching, practicality and equality dictate that some sub-optimal sites will be utilised since benefits will need to be shared amongst a range of communities. However, improved knowledge of key parameters will enable managers to predict how a site will perform and fine-tune selection criteria to avoid investing effort at biophysically unsuitable sites, and better manage community expectations regarding the outcomes of aquaculture ventures (Eriksson et al., 2012). Our results provide a basis for more specific studies towards developing a technically and socially acceptable protocol for best-practice sea cucumber mariculture. Juvenile survival and growth rates obtained in this study compare well to those reported in similar studies in other countries indicating a promising future for sandfish ranching in PNG. Methods refined through this and similar trials could be used to establish and assess pilot test plots at potential ranching sites and provide guidance on the level of animal husbandry required in order to optimise community sea ranching operations in New Ireland Province, PNG.

Acknowledgements

This study was supported by the Australian Centre for International Agricultural Research (ACIAR) and National Fisheries Authority (NFA) in Papua New Guinea through ACIAR projects FIS/2010/054 “Mariculture Development in New Ireland, Papua New Guinea” and FIS/2010/042 “Expansion and Diversification of Production and Management Systems for Sea Cucumbers in the Philippines, Vietnam and northern Australia” for which the Uni-

versity of the Sunshine Coast is the commissioned organisation. Hatchery staff of the Nago Island Mariculture and Research Facility produced the juvenile sandfish. We thank B. Peni, P. Kanawi, P. Minimulu, D. Kershler, E. Leini, B. Saesaria and C. Dako for assistance in the field and hatchery. I also extend my appreciation to the leaders and community members of Limanak, Ungakum and Enuk for their cooperation, hospitality and support of research activities.

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