VOL. 273 NO. 4, 20 DECEMBER 2007   ISSN 0044-8486

ELSEVIER

Aquaculture

Grow-out of sandfish *Holothuria scabra* in ponds shows that co-culture with shrimp *Litopenaeus stylirostris* is not viable

Johann D. Bell [a],*, Natacha N. Agudo [a], Steven W. Purcell [a], Pascal Blazer [a], Mateo Simutoga [a], Dominique Pham [b], Luc Della Patrona [b]

[a] *The WorldFish Center, c/-Secretariat of the Pacific Community, B.P. D5, 98848 Noumea Cedex, New Caledonia*
[b] *IFREMER, Noumea, New Caledonia*

Abstract

We examined the potential for producing the large numbers of sandfish (*Holothuria scabra*) needed for restocking programmes by co-culturing juveniles with the shrimp *Litopenaeus stylirostris* in earthen ponds. Our experiments in hapas within shrimp ponds were designed to detect any deleterious effects of sandfish on shrimp, and vice versa. These experiments showed that a high stocking density of juvenile sandfish had no significant effects on growth and survival of shrimp. However, survival and growth of sandfish reared with shrimp for 3 weeks were significantly lower than for sandfish reared alone. Increased stocking density of shrimp also had a significant negative effect on survival and/or growth of sandfish. A grow-out trial of juvenile sandfish in 0.2-ha earthen ponds stocked with 20 shrimp post-larvae m^{-2}, and densities of sandfish between 0.8 and 1.6 individuals m^{-2}, confirmed that co-culture is not viable. All sandfish reared in co-culture were dead or moribund after a month. However, sandfish stocked alone into 0.2-ha earthen ponds survived well and grew to mean weights of ~400 g within 12 months without addition of food. The grow-out trial demonstrated that there is potential for profitable pond farming of sandfish in monoculture. Further research is now needed to identify the optimal size of juveniles, stocking densities and pond management regimes.
© 2007 Elsevier B.V. All rights reserved.

1. Introduction

High demand for the sea cucumber, sandfish (*Holothuria scabra*), in China has resulted in over-fishing throughout the Indo-Pacific (Battaglene and Bell, 2004; Lovatelli et al., 2004). In locations where populations have been reduced to chronically low levels, and rebuilding spawning biomass using conventional management measures will take an unacceptably long time, release of cultured juveniles in restocking programmes promises to fast-track recovery of fisheries for sandfish. However, such interventions will depend on development of methods for producing young sandfish *en masse* at low cost, and for releasing them in the wild to achieve good rates of survival (Purcell et al., 2002). Once this technology is in place, the information on cost and survival of cultured juveniles can be used to model whether the restocking of sandfish will add value to other forms of management (Bell and Nash, 2004; Bell et al., 2005).

Basic methods for producing sandfish in hatcheries and rearing them to a size of ~1 g are well documented (Battaglene, 1999; James, 1999; Pitt, 2001; Pitt and Duy, 2004; Agudo, 2006). The bottleneck, however, is production of the large numbers needed for effective restocking

---

* Corresponding author. Tel.: +687 262000; fax: +687 263818.
E-mail address: johannb@spc.int (J.D. Bell).

(Bell et al., 2005). The problems arise because juvenile sandfish are deposit feeders and require large surface areas to forage for detritus and bacteria. Providing such large surface areas is expensive and threatens to make the cost of cultured juveniles unacceptably high.

One possible way of providing the necessary surface areas would be to co-culture sandfish with shrimp in earthen ponds (Battaglene and Bell, 2004). Preliminary experiments on combined culture of sandfish and *Penaeus monodon* in Vietnam (Pitt et al., 2004), and with *Litopenaeus stylirostris* in New Caledonia (Purcell et al., 2006), showed that sandfish had no effects on growth and survival of the shrimp. Sandfish also grew and survived well when reared with *P. monodon* during many of the trials; however, harassment and predation of sandfish occurred under some conditions (Pitt et al., 2004). Sandfish also survived well when reared with juvenile *L. stylirostris* but their growth was significantly slower due to increased levels of ammonia from shrimp (Purcell et al., 2006).

Although the preliminary experiments by Pitt et al. (2004) and Purcell et al. (2006) are encouraging, they were done mostly in tanks of 500–1000 L. There is a need to test whether similar results hold in ponds for the sizes of shrimp and sandfish likely to be reared together during commercial operations.

Here we examine survival and growth of sandfish and *L. stylirostris* under two scenarios likely to emerge if combined culture is implemented. First, co-culture of small shrimp (~1 g) and small sandfish (~1 g) at the start of the shrimp production cycle. Second, stocking of small sandfish (~1 g) and medium-size shrimp (10–15 g) to simulate situations where juvenile sandfish are not available at the start of the shrimp production cycle but added later. Our experiments covered the most important contrasts of body size for shrimp and sandfish throughout the production cycle of *L. stylirostris* and were designed to reveal any interactions between the species that may impede co-culture.

We found that sandfish did not affect shrimp but survival and growth of sandfish was significantly lower when stocked with shrimp. In contrast, survival and growth of sandfish in monoculture in earthen ponds was good, pointing to considerable potential for pond farming of this valuable species.

2. Materials and methods

2.1. Rearing of animals

The juvenile sandfish from New Caledonia used in this study came from two cohorts spawned during November and December 2004 and reared using methods for larval rearing described by Battaglene (1999), Pitt (2001) and Agudo (2006). They were then grown to ~1 g in hapas suspended 10 cm above the substratum in shrimp ponds using the techniques documented by Pitt and Duy (2004). Prior to the experiments, sub-samples of juveniles were examined for fungal or bacterial infection, discolouration and malformation as proposed by Purcell and Eeckhaut (2005) and were healthy and free of noticeable disease.

Juvenile shrimp, *L. stylirostris,* were produced from captive broodstock and reared using the methods described by Ottogalli et al. (1988) and Ottogalli (1992). Young post-larval shrimp were kept in the hatchery for 20 days (PL_{20}) before being transferred to earthen ponds, where they were reared on a local commercial diet. Shrimp from the same cohort were used for Experiments 1 and 2. Shrimp for Experiments 3 and 4 were from two different cohorts.

2.2. Description of ponds

We used two earthen ponds at the IFREMER aquaculture research station, St Vincent, New Caledonia for this study. Both ponds had been used previously to rear shrimp to market size, or maintain shrimp broodstock. The main characteristics of these ponds are summarized in Table 1. Pond sediments were predominantly siliceous clay. Pond 1 was smaller than Pond 2 and, because it was filled much earlier, had a different

Table 1
Attributes of the two earthen seawater ponds used for Experiments 1–4

Attribute	Pond 1			Pond 2		
	Mean	SD	n	Mean	SD	n
Sediment grain size (%)						
>2 µm<50 µm	58	18	4	35	10	4
>50 µm<2000 µm	40	10	4	59	10	4
Meiofauna (10-cm^{-2})						
-Ciliophora	52	37	3	128	131	3
-Rotifers	0	0	3	248	231	3
-Nematodes	208	61	3	659	348	3
-Unidentified eggs	613	436	3	2042	773	3
Turbidity - Secchi depth (cm)	69	7	8	104	6	8
Salinity (ppt)	35.7	1.6	7	35	1	7
Temperature (°C)*	24.3	1.6	408	24.1	1.5	312
DO (ppm)*	5.9	1.3	408	6.9	3.3	309
pH sediment*	7.6	0.2	165	7.3	0.3	129
Redox potential*	126	36	117	118	25	81
Area (m^2)	1310			11400		
Days filled prior to Experiment 1	210			10		

*Measurements taken in hapas in the ponds (see text for details of hapas). Salinity, temperature, DO, pH and Redox potential were measured 10 cm above the surface of the sediment.

meiofauna. Pond 1 also had finer sediments than Pond 2. The water quality of the ponds, and in the hapas used for this study within the ponds (Table 1), was within the range considered to be suitable for rearing both shrimp and sandfish (Avalle et al., 2003; Purcell, 2004).

2.3. Design of experiments

Experiment 1 was designed to test the scenario of rearing small sandfish and shrimp at the start of the shrimp production cycle. The null hypothesis was that small (∼1 g) sandfish do not affect growth or survival of small (∼1 g) shrimp. The reason for placing the emphasis on shrimp was to determine whether sandfish posed any risk to this commercially important species. Experiment 1 was done in 12 hapas in each pond. All hapas were 1 m^2, made of ∼0.7 mm mesh, and covered on the top with 3 mm mesh to prevent shrimp from escaping and to provide uniform shading for all experimental treatments (see below). The floor of each hapa was covered with a 10–20 mm layer of the upper 50 mm of pond sediment. Hapas were installed so that the walls extended ∼20 cm above the surface of the water and the base rested on the substratum.

Four combinations of shrimp and sandfish ($n = 3$) were allocated at random to the 12 hapas in each pond: 1) 16 shrimp with 40 sandfish; 2) 16 shrimp without sandfish; 3) 32 shrimp with 40 sandfish; and 4) 32 shrimp without sandfish. The stocking densities of 16 and 32 shrimp m^{-2} represented the local semi-intensive and intensive farming practices. The stocking density for sandfish (40 sandfish m^{-2}) was greater than needed if survival during co-culture is high. However, in the absence of data on survival of sandfish stocked with *L. stylirostris* in ponds, or the best size to harvest them from ponds for release to the wild, the high stocking density provided a good test for any effects of sandfish on shrimp. Thus, the experiment tested for the effects of pond, stocking density, and a high biomass of sandfish on growth and survival of shrimp, and the effects of pond and shrimp stocking density on growth and survival of sandfish. Due to limits on availability of hapas, there was no control for effects of shrimp *per se* on growth and survival of sandfish.

Shrimp were placed into hapas 3 h before introduction of sandfish and fed the 'crevette croissance hiver' diet (SICA, Boulouparis, New Caledonia), consisting of 40% protein, 6% lipid, 11% ash, 3.6% fibre, and vitamins. Shrimp were then fed twice a day at 5% body weight day^{-1}; a rate designed to maximize growth without overfeeding. Due to the relatively turbid water (Table 1), survival of shrimp and sandfish could not be monitored easily, so animals that died were not replaced.

This was also in keeping with our aim to simulate conditions expected under any commercial co-culture of the two species. The duration of the experiment was 3 weeks, commencing 13 April 2005.

Experiment 2 simulated the scenario where small sandfish were placed in ponds halfway through the shrimp production cycle. The null hypothesis was that small (∼1 g) sandfish have no affect on growth or survival of shrimp of 10–15 g. In particular, we were interested to determine whether larger shrimp preyed on small sandfish and, if so, whether sandfish proved toxic to shrimp - holothurians produce saponins (Chen, 1999), and some of them affect fish and invertebrates (S. Sotheeswaran, personal communication).

The design and set up of this experiment was the same as for Experiment 1 except that it was done in hapas of 4 m^2 with mesh of 1 mm and covers of 10 mm mesh, and the larger shrimp were fed at 2.5% body weight day^{-1}. The semi-intensive and intensive stocking densities for the larger shrimp were intended to be 12 and 24 individuals m^{-2}, respectively. However, sorting and weighing procedures exacerbated the vulnerability of the larger shrimp to 'winter mortality'(Syndrome 93) (Mermound et al., 1998), and many of them died within a day. The individuals used to replace the dead shrimp were not weighed. Even so, some of them also died and the eventual initial semi-intensive and intensive stocking densities were ∼10 and 21 shrimp m^{-2}, respectively. The duration of the experiment was 3 weeks, commencing 18 April 2005.

Experiment 3 was designed mainly to test the null hypothesis that shrimp of ∼15 g had no effect on survival and growth of small (∼1 g) sandfish. However, it also provided another test for the null hypothesis that small sandfish had no effect on survival and growth of large shrimp. This experiment was done in 1 m^2 hapas in Pond 2 installed in the same way as described for Experiment 1. Three combinations ($n = 4$) of sandfish and/or shrimp were used: 1) 40 sandfish without shrimp; 2) 40 sandfish and 12 shrimp; and 3) 12 shrimp without sandfish. These combinations were allocated at random to the 12 hapas. We limited the 'shrimp' treatment in this experiment to larger individuals at semi-intensive stocking density for two reasons: 1) there was greater potential for aggressive behaviour by shrimp towards sandfish, and 2) the semi-intensive stocking density reduced the risk of 'winter mortality syndrome'. The shrimp were fed at 2.5% body weight day^{-1}. Sandfish kept on their own were not fed because they grow well by ingesting sediments in ponds used previously for shrimp (Purcell, 2004). The duration of the experiment was 3 weeks, commencing 29 April 2005.

Experiment 4 addressed the possible significance of differences in sediments between ponds. During Experiment 1, survival and growth of sandfish were significantly greater in Pond 1, raising the possibility that sediment type affected sandfish. This was also supported by the variability of growth and survival within ponds. In particular, there was high survival and growth of sandfish in the hapa from Pond 2 located furthest from the edge, where the sediment is typically finer. There was also poor survival and growth in the one hapa from Pond 1 with conspicuously coarser sediment.

To test the null hypothesis that pond sediment does not have a greater effect on survival and growth of sandfish than other pond attributes, we set up an experiment in 24 hapas of 1 m^2 that included a treatment in which sediment from Pond 1 was placed within hapas in Pond 2, and vice versa. This experiment also tested the effects of relatively small ($\sim$6 g) shrimp stocked at a density of 25 individuals m^{-2} on growth and survival of sandfish. The experimental design was: pond $\times$ presence/absence of shrimp $\times$ sediment type. In both Ponds 1 and 2, for both levels of the shrimp treatment, there were 3 hapas with sediment from Pond 1, and 3 hapas with sediment from Pond 2. The 12 hapas in each pond were positioned haphazardly within the suitable depth range and 40 small ($\sim$1 g) sandfish were placed in each hapa. In all other aspects, hapas were set up as described

for Experiment 1. The duration of this experiment was 3 weeks, commencing 10 May 2005.

2.4. Collection of data

At the beginning of each experiment, the average wet weight of sandfish and/or shrimp placed in each hapa was calculated by dividing the total drained group weight by the number of individuals. Shrimp were gently blotted dry and sandfish were drained on a saturated towel for 1 min before weighing to the nearest 0.1 g. Total weights of shrimp and sandfish placed in each hapa were inspected to ensure that the coefficient of variation (CV) was <0.1 (Table 2).

At the end of each experiment, shrimp and sandfish surviving in each hapa were counted and their drained weights estimated as described above. Growth of shrimp and sandfish was calculated by subtracting average starting weight from average finishing weight for each hapa. Due to high initial mortality of large shrimp in Experiment 2, a different method was used to estimate growth. For each hapa, 10 individuals were marked with fluorescent dye and weighed to the nearest 0.1 g; those surviving were then reweighed to calculate average growth. Some mortality of shrimp also occurred at the beginning of Experiment 3 but these shrimp were not replaced. The mean ($\pm$SD) wet weights of random sub-samples

Table 2
The numbers of shrimp and sandfish per hapa; coefficient of variation (CV) for total starting weights of shrimp and sandfish among hapas; and mean ($\pm$SD) individual weights of shrimp and sandfish, for each experiment

Experiment	Hapa size (m^2)	Number of shrimp		Number of sandfish	Initial total shrimp wt (g) Range CV n		Initial total sandfish wt (g) Range CV n	Initial individual shrimp wt (g) Mean$\pm$SD $n=30$	Initial individual sandfish wt (g) Mean$\pm$SD $n=30$
		Semi-intense	Intense		Semi-intense	Intense			
1	1	16	32	40	23.0–25.1 0.026 12	44.9–48.5 0.020 12	36.5–39.4 0.022 12	1.28$\pm$0.32	1.0$\pm$0.06
2	4	48*	96*	160	586–593 0.003 12	1179–1192 0.003 12	162–164 0.003 12	11.5$\pm$2.94	1.0$\pm$0.05
3	1	12		40	150–182 0.063 8		46.7–47.0 0.002 8	14.6$\pm$1.81	1.0$\pm$0.07
4	1		25	40		138–158 0.040 12	36.1–37.6 0.009 24	5.77$\pm$0.57	0.8$\pm$0.03

*Eventually reduced to 14 and 26 due to 'winter mortality syndrome'.

$(n = 30)$ of shrimp and sandfish used for each experiment are given in Table 2.

2.5. Analysis of data

We used a variety of ANOVA models to test for differences in mean survival of shrimp and sandfish. For Experiments 1 and 2, we used a 3-way ANOVA to test the effects of pond, presence/absence of sandfish and stocking density on survival of shrimp, and a 2-way ANOVA for effects of pond and shrimp stocking density on survival of sandfish. For Experiment 3, we used 1-way ANOVA to compare survival and growth of sandfish with and without shrimp, and survival and growth of shrimp with and without sandfish. For Experiment 4, we used a 3-way ANOVA to test the effects of pond, presence/absence of shrimp and sediment type on survival of sandfish, and a 2-way ANOVA to examine the effects of pond and sediment type on survival of shrimp in the presence of sandfish.

Data for percentage survival were transformed to arcsin square root when error estimates were constrained (Underwood, 1981). In Experiment 3, there was no

variance (100% survival of sandfish) for one treatment so we used the Mann–Whitney U Test to compare differences in survival of sandfish with and without shrimp.

We used ANCOVA to analyse differences in mean growth rate of shrimp, with average starting weight as the covariate. There was no significant relationship between initial weight and mean growth for any experiment. Consequently, we simply report the main factors and interactions affecting growth of shrimp, as per the ANOVA models used for survival above. We used only ANOVA to analyse growth of sandfish because variation in mean weights among hapas at the start of each experiment was usually trivial (Table 2). Data on growth of shrimp and sandfish were checked for homogeneity of variance using Cochran's text and transformed to $\log_{10}(x)$ where necessary.

Pearson's correlation was used to evaluate relationships between survival and growth of sandfish in co-culture in Experiments 1–4, and when reared on their own in Experiments 3 and 4.

Linear regression was used to examine the relationship between the percentage survival of shrimp (independent variable) and sandfish (dependent variable) for each
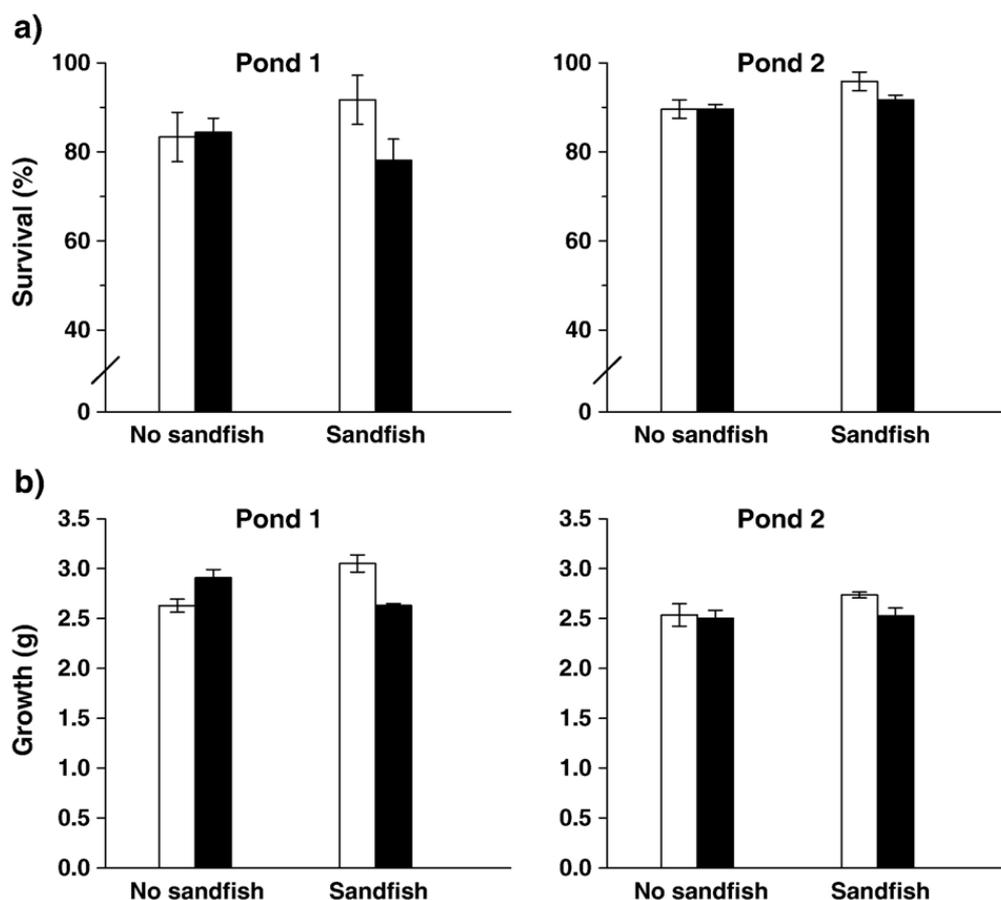

Fig. 1. Survival (a) and growth (b) of small shrimp in 1 m² hapas in two earthen ponds at densities of 16 (open) and 32 (solid) individuals m⁻², with and without small sandfish. Data are means (±SE).
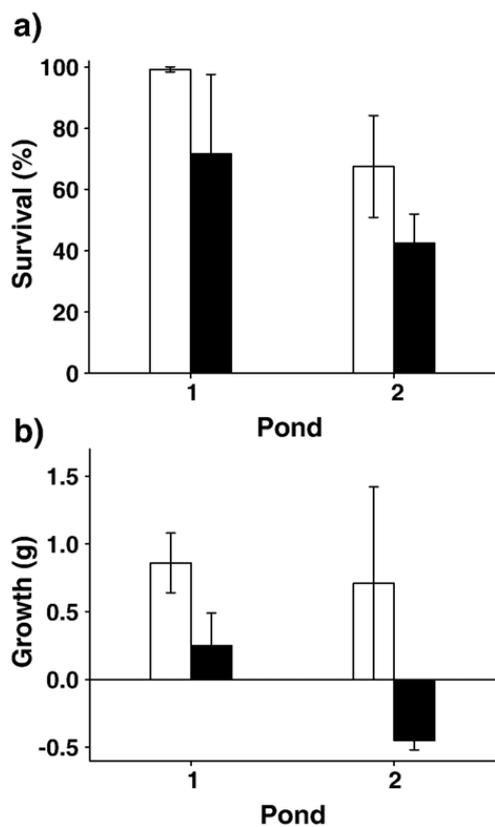
Fig. 2. Survival (a) and growth (b) of small sandfish in 1 m^2 hapas in two earthen ponds stocked with shrimp at densities of 16 (open) and 32 (solid) small individuals m^{-2}. Data are adjusted means (±SE) (see text).

experiment, except Experiment 3 where the sample size was considered to be too low. Separate regressions were done for the semi-intensive and intensive densities of shrimp in Experiments 1 and 2.

2.6. Pond grow-out trial

To test the outcome of co-culture of shrimp and sandfish at the scale of ponds, we used four 0.2-ha earthen ponds at a commercial shrimp farm at Tontouta, New Caledonia. Two sizes of juvenile sandfish (small: 0.9 g±0.6 SD, and large: 11.7 g±17.8 SD) were reared with and without post-larval shrimp (PL$_{30}$) in four ponds in an unreplicated grow-out trial. The trial was designed to simulate rearing the two species together at the start of the shrimp production cycle. However, stocking densities of sandfish were lowered to levels likely to be used for pond grow-out of sandfish to market size. The stocking density of small sandfish was 1.6 individuals m^{-2} and 0.8 individuals m^{-2} for the larger size class. The density of shrimp post-larvae was 20 m^{-2}. The ponds were filled with seawater 2 weeks before sandfish were released on 6 June 2005 and shrimp were released one day later. Shrimp were fed as per local commercial

practices for semi-intensive culture. No food was added to the two ponds that contained sandfish only. Growth of sandfish was estimated each month from the mean wet weight of a random sample of 30 individuals. Survival of sandfish was estimated by counting all sandfish exposed by draining the ponds to harvest the shrimp, and by draining the pond containing only the larger sandfish 6 months after stocking.

3. Results

3.1. Experiment 1

Mean rates of survival of small shrimp among the four combinations of stocking density and presence/absence of sandfish ranged from 78 to 92% in Pond 1, and from 90 to 96% in Pond 2 (Fig. 1a). Mean growth of small shrimp ranged from around 2.5 to 3.2 g in Pond 1 and from 2.3 to 2.8 g in Pond 2 (Fig. 1b). These results are well within the expected rates of survival and growth for small *L. stylirostris* in commercial shrimp ponds in New Caledonia (D. Pham, unpublished data). Nevertheless,
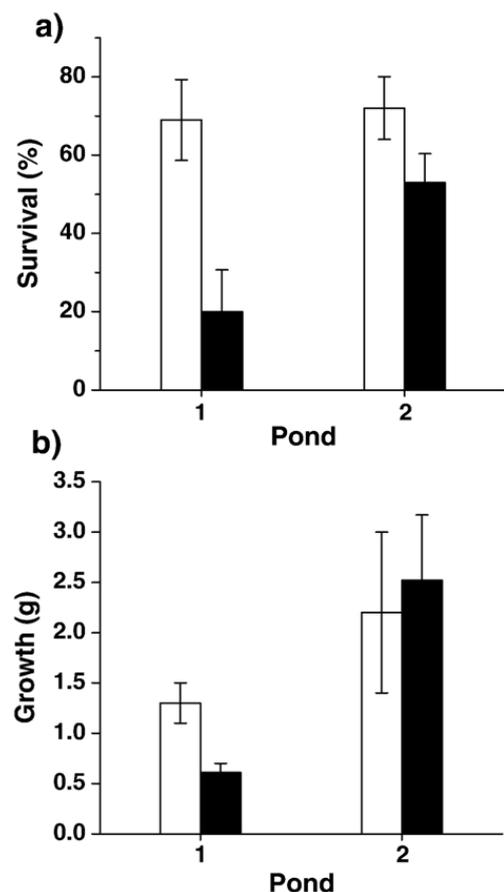

Fig. 3. Survival (a) and growth (b) of small sandfish in 4 m^2 hapas in two earthen ponds stocked with shrimp at final densities of 3.5 large (open) and 6.5 large (solid) individuals m^{-2} (see text). Data are means (±SE).

there were some significant differences among experimental treatments. The pond environment had a significant effect on shrimp - survival was greater ($p=0.049$) in Pond 2 and growth was faster ($p<0.001$) in Pond 1 (Fig. 1). The presence of sandfish did not affect survival of shrimp ($p=0.16$). There was also a significant interaction for the effects of stocking density and presence/absence of sandfish on growth of shrimp ($p<0.001$). Growth of shrimp was faster at the semi-intensive stocking density, but only in co-culture with sandfish (Fig. 1b).

There was unusual variation in survival of sandfish: survival in one replicate from Pond 1 was far lower than for the other 5 replicates and, conversely, survival in one replicate in Pond 2 was far greater than for other replicates. The sediments placed in hapas for these 'outlying' replicates appeared to be atypical for each pond. When these hapas were removed from the analysis, and replaced by the mean of the other replicates for the relevant level of treatment, survival of sandfish in Pond 1 was significantly greater than in Pond 2 ($p<0.001$) (Fig. 2a). There was no significant effect ($p=0.29$) of shrimp stocking density on survival of sandfish.

Growth of sandfish was highly variable and generally much slower in Pond 2 than in Pond 1. Individuals in 5 of the 6 hapas in Pond 2 lost weight over the 3-week period, whereas in the remaining replicate, there was a >3-fold increase in mean weight. In Pond 1, there was positive growth in all replicates, except the one with low survival. When the two hapas described above were removed from the analysis, and replaced by the mean of the other replicates, growth of sandfish was significantly greater in Pond 1 than in Pond 2 ($p<0.001$) (Fig. 2b). The effect of shrimp stocking density on growth of sandfish was marginally non-significant ($p=0.055$); growth of sandfish was slower at the intensive shrimp stocking density (Fig. 2b).

3.2. Experiment 2

Survival of shrimp was poor due to the 'winter mortality syndrome'. Survival ranged from 34 to 36% in Pond 1, and from 23 to 34% in Pond 2, among the four combinations of stocking density and presence/absence of sandfish. As a result, the two original stocking densities of shrimp (~ 10 and 21 individuals m^{-2}) were
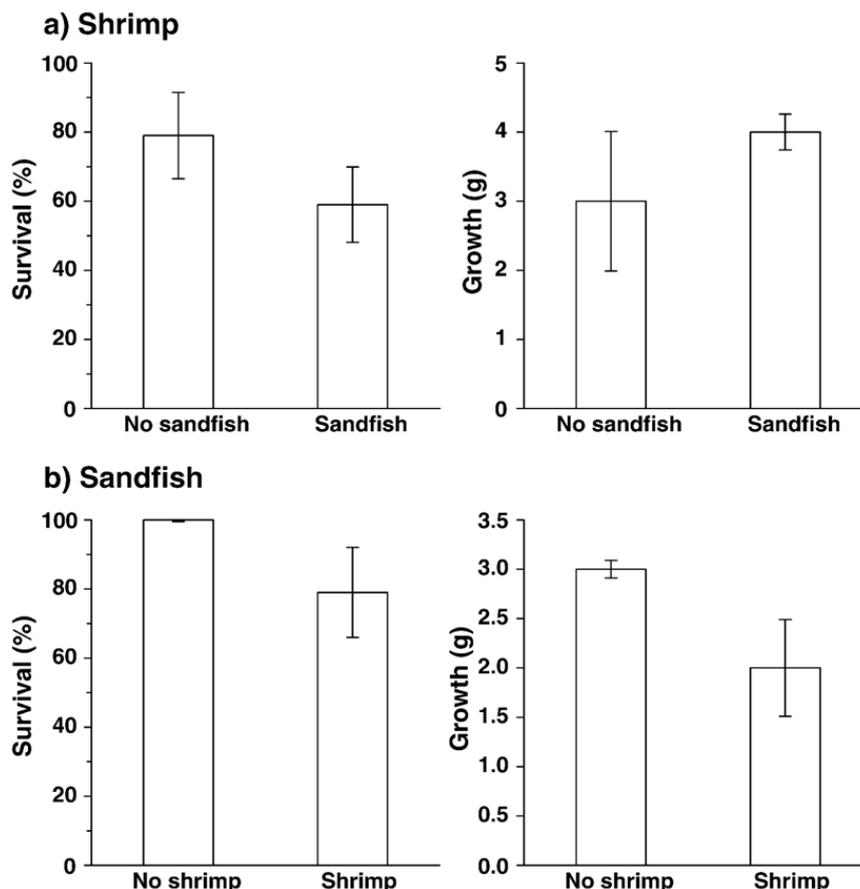

Fig. 4. Survival and growth of (a) shrimp reared in 1 m^2 hapas in an earthen pond with and without sandfish, and (b) sandfish reared in 1 m^2 hapas with and without large shrimp at an initial density of 12 individuals m^{-2}. Data are means ($\pm$SE).
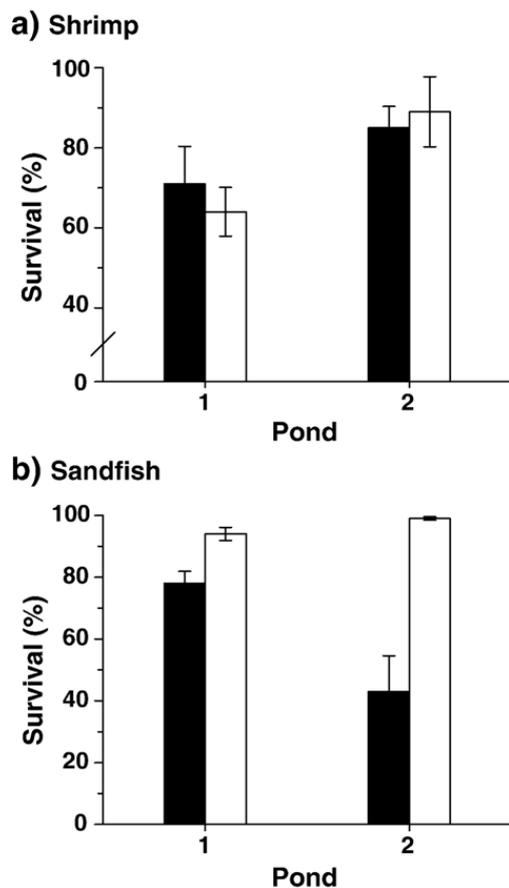
a) Shrimp

b) Sandfish

Fig. 5. Survival of (a) shrimp in 1 m^2 hapas in two earthen ponds with sediment from Pond 1 (solid) and Pond 2 (open), and (b) sandfish in 1 m^2 hapas in two earthen ponds with (solid) and without (open) shrimp at a density of 25 individuals m^{-2}. Data are means (±SE).

reduced to 3.5 and 6.5 individuals m^{-2}, respectively. In addition, only 16 of the 240 individually tagged shrimp survived; too few individuals to calculate mean growth for each treatment level. Consequently, we have not reported the effects of the treatments on survival and growth of shrimp.

Survival of sandfish did not vary significantly ($p = 0.14$) between ponds but was significantly ($p < 0.01$) lower at the higher shrimp stocking density (Fig. 3a). Growth of sandfish was significantly greater ($p = 0.03$) in Pond 2 than in Pond 1, but was not affected by density of shrimp ($p = 0.49$) (Fig. 3b).

3.3. Experiment 3

Survival of the larger shrimp used in this experiment was not as good as expected due to 'winter mortality syndrome' (Fig. 4a). However, there was no significant difference in survival ($p = 0.28$) or growth ($p = 0.34$) of shrimp due to the presence of sandfish (Fig. 4a).

Survival of sandfish was significantly greater ($p < 0.01$) in the absence of shrimp, although survival was also relatively high with shrimp (Fig. 4b). The effects of shrimp on growth of sandfish were marginally non-significant ($p = 0.07$); sandfish grew faster without shrimp (Fig. 4b).

3.4. Experiment 4

Survival of shrimp was significantly lower ($p < 0.05$) in Pond 1 than in Pond 2, but was not affected significantly ($p = 0.86$) by sediment type (Fig. 5a). There was no significant difference in growth of shrimp due to sediment type ($p = 0.21$) or pond ($p = 0.77$).

Sediment type had no significant effect ($p = 0.14$) on survival of sandfish. However, survival of sandfish was lower in the presence of shrimp. This effect was much stronger in Pond 2, causing a significant interaction ($p < 0.01$) between the effects of presence/absence of shrimp and pond for survival of sandfish (Fig. 5b).

There was no significant difference in growth of sandfish between ponds ($p = 0.80$), but there was a significant interaction ($p < 0.01$) between effects of sediment type and presence/absence of shrimp. Sandfish grew less in co-culture with shrimp and, contrary to the outcome of Experiment 1, growth was much slower on sediments from Pond 1 (Fig. 6).

3.5. Correlations between survival and growth

There was a significant positive correlation between survival and growth of sandfish when reared with shrimp ($p < 0.05$ for Experiment 2 and $p < 0.01$ for Experiments
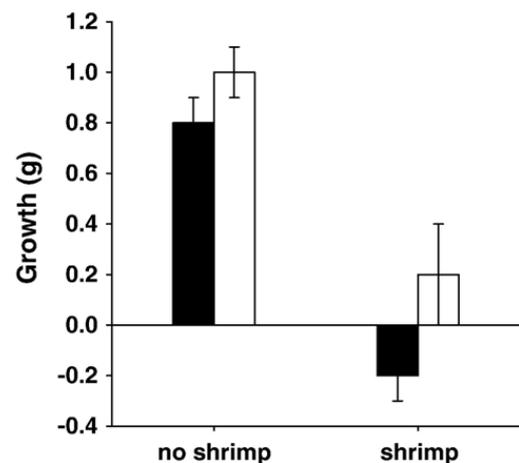

Fig. 6. Growth of sandfish in 1 m^2 hapas in earthen ponds with and without shrimp stocked at a density of 25 individuals m^{-2} on sediments from Pond 1 (solid) and Pond 2 (open). Data are means (±SE).

Table 3
Summary statistics for regressions of sandfish survival vs shrimp survival

Experiment	n	β	r^2	P
1 (SI)	6	−0.42	0.17	0.41
1 (I)	6	0.12	0.01	0.83
2 (SI)	6	−0.06	0.00	0.91
2 (I)	6	−0.89	0.79	0.02
4	12	−0.60	0.36	0.04

SI = semi-intensive shrimp density (see text); I = intensive shrimp density.

1, 3 and 4). However, there was no significant correlation between growth and survival of sandfish reared on their own in Experiment 3 ($p = 0.81$) and Experiment 4 ($p = 0.26$).

3.6. Relationships between survival of shrimp and sandfish

There was a significant negative relationship between survival of sandfish and shrimp in two of the five regression analyses (Table 3). There were no significant positive relationships between survival of the two species.

3.7. Pond grow-out trial

Sandfish survived poorly in both ponds stocked with 20 shrimp post-larvae m^{-2}; few individuals were found alive after one month. The remainder were moribund and had severe damage to the body around the mouth and anus. When the ponds containing shrimp were harvested after 6 months, yields of shrimp were within the normal range for commercial operations, but no sandfish were present. In contrast, survival of sandfish in monoculture exceeded 70% for the larger size class after 6 months. Growth of sandfish in both ponds was rapid initially, with the smaller and larger size classes attaining mean wet weights of ∼290 and ∼400 g, respectively, after 12 months (Fig. 7). However, growth slowed from months 12 to 18, when water temperatures in the ponds were lower (Fig. 7) and total biomass approached carrying capacity for the larger size class.

4. Discussion

Our experiments demonstrate that sandfish do not have an adverse effect on survival and growth of *L. stylirostris*. Indeed, Experiments 1 and 3 showed that survival and growth of shrimp reared with an usually high density of juvenile sandfish did not differ significantly from those reared without sandfish. Rather, the main factors affecting survival and growth of shrimp were stocking density, and the pond environment. This is consistent with other research on *L. stylirostris* in New Caledonia (Anon., 2006).

Although co-culture has no effect on *L. stylirostris*, the same cannot be said for sandfish. Experiments 3 and 4 showed that survival and growth of the sea cucumbers were significantly lower when reared with shrimp. The
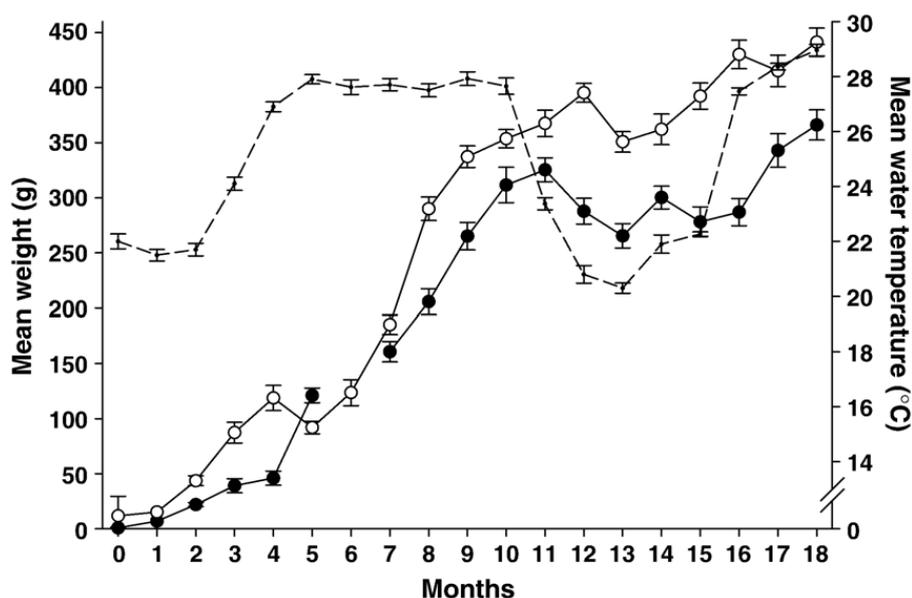

Fig. 7. Growth of two groups of sandfish with initial mean sizes of 0.9 g (solid dots) and 11.7 g (open dots) in 0.2-ha earthen seawater ponds at Tontouta, New Caledonia between June 2005 and November 2006. Monthly water temperatures for earthen ponds at Tontouta and/or Saint Vincent are also shown. Data are means (±SE) ($n = 30$ for sandfish and $n = 60$ for water temperature).

effects of shrimp on sandfish were also evident when sandfish were reared at two densities of shrimp during Experiments 1 and 2. Growth of sandfish was significantly slower at the high shrimp stocking density in Experiment 1, and survival was significantly lower at high shrimp density in Experiment 2.

The impact of shrimp on sandfish was most pronounced in the pond grow-out trial, where all sandfish died in co-culture. Damage to the bodies of sandfish indicated that shrimp much smaller than those placed in hapas harassed and eventually killed the sandfish. The dramatic differences in results from experiments in hapas and the pond grow-out trial can probably be attributed to differences in the ratios of shrimp to sandfish. In the experiments, these ratios were always lower than 1:1, and as low as 0.4:1, whereas in the pond grow-out trial the ratio of shrimp to sandfish was ∼20:1, albeit with smaller shrimp. We were not able to make direct observations of shrimp in the hapas due to the turbid water but aggressive behaviour by shrimp during the 3-week experiments in hapas would have been spread among the relatively large number of sandfish. The result was significantly reduced survival and growth, not total mortality. The fact that a significant, positive correlation between survival and growth occurred only when sandfish were reared with shrimp also shows that sandfish were stressed by shrimp.

Our experiments did not include a test for the effects of shrimp feed on sandfish in the absence of shrimp. Therefore, we cannot say unequivocally that increased mortality of sandfish reared with shrimp was due to harassment and predation by shrimp. It may have been due to poor water quality caused by uneaten food and decaying shrimp in hapas where some shrimp died. However, the two significant negative relationships between survival of sandfish and shrimp, and lack of any significant positive regressions, provide reasonable evidence that mortality of sandfish reared with shrimp was due to predation, not water quality.

Whereas our experiments showed that co-culture with *L. stylirostris* is not an option for mass-producing sandfish for restocking projects, the grow-out trial in ponds revealed considerable potential for monoculture of sandfish. The larger size class of sandfish grew from ∼12 g to ∼400 g in 12 months, without the addition of food, in a locality near the edge of their natural distribution (Conand, 1989). Faster growth in ponds has been observed in Indonesia and Vietnam (R. Pitt, T. Nguyen and K. Sugama, personal communication). In addition, survival in ponds was high (>70%) after 6 months. Assuming that sandfish can attain >700 g within 18 months in ponds in New Caledonia under optimum conditions, which is predicted by Purcell

(2004) and the growth model of Purcell and Kirby (2006), current prices for A-grade beche-de-mer (Lovatelli et al., 2004) indicate that farming sandfish could well be profitable if juveniles can be produced *en masse* at reasonable cost (N. Agudo, unpublished data).

Questions that need to be answered to fully assess the potential, and possible environmental benefits, of farming sandfish in ponds are: What are the optimal sizes and densities for stocking sandfish into ponds, including those used previously to farm shrimp? Do sandfish have a beneficial effect on sediments in recently harvested and refilled shrimp ponds? Do sandfish need to be thinned out, or does food have to be added, later in the production cycle to maintain good growth rates? If enrichment of sediments is needed, what is the best method? Are some sediments or pond types better than others for rearing sandfish? Regarding the last question, the differences in survival and growth of sandfish between ponds with different sediments in Experiment 1 were not repeated in Experiments 2 and 4. Therefore, a range of pond sediments may be suitable for rearing sandfish.

Acknowledgements

References

Agudo, N., 2006. Sandfish Hatchery Techniques. Australian Centre for International Agricultural Research, Secretariat of the Pacific Community and the WorldFish Center, Noumea. 44 pp.

Avalle, O., Millous, O., Virmaux, J.-F., 2003. L'Elevage de la Crevette en Zone Tropicale. Centre Pour le Developpement de l'Entreprise, Bruxelles. 98 pp.

Anon, 2006. DEfi SANte *Stylirostris* 2002-2006. In: IFREMER (Ed.), Rapport final DESANS. Centre du Pacifique, Département Aquaculture en Calédonie, Nouvelle-Calédonie. 200 pp.

Battaglene, S.C., 1999. Culture of tropical sea cucumbers for stock restoration and enhancement. Naga–The ICLARM Quarterly, vol. 22(4), pp. 4–11.

Battaglene, S.C., Bell, J.D., 2004. The restocking of sea cucumbers in the Pacific Islands. In: Bartley, D.M., Leber, K.L. (Eds.), Case Studies in Marine Ranching. FAO Fisheries Technical Paper, vol. 429, pp. 109–132.

Bell, J., Nash, W., 2004. When should restocking and stock enhancement be used to manage sea cucumber fisheries? In: Lovatelli, A., Conand,

C., Purcell, S., Uthicke, S., Hamel, J.-F., Mercier, A. (Eds.), Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Paper, no. 463, pp. 173–180.

Bell, J.D., Rothlisberg, P.C., Munro, J.L., Loneragan, N.R., Nash, W.J., Ward, R.D., Andrew, N.L., 2005. Restocking and stock enhancement of marine invertebrate fisheries. Adv. Mar. Biol. 49, 1–370.

Chen, J., 1999. Overview of sea cucumber farming and sea ranching practices in China. SPC Beche-de-mer Inf. Bull. 18, 1–6.

Conand, C., 1989. Les Holothuries Aspidochirotes du Lagon de Nouvelle-Caledonie: Biologie, Ecologie et Exploitation. Etudes et Theses ORSTOM, Paris. 393 pp.

James, D.B., 1999. Hatchery and culture technology for the sea cucumber, *Holothuria scabra* Jaeger, in India. Naga - The ICLARM Quarterly, vol. 22(4), pp. 12–16.

Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J.F., Mercier, A., 2004. Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Paper, no. 463. FAO, Rome. 425 pp.

Mermound, I., Costa, R., Ferre, O., Goarant, C., Haffner, P., 1998. Syndrome 93 in New Caledonia outdoor rearing ponds of *Penaeus stylirostris*: history and description of the three major outbreaks. Aquaculture 164, 323–335.

Ottogalli, L., 1992. New water management in penaeid larval rearing in New Caledonia. Memorias 1er Congreso Ecuatoriano de Acuicultura, pp. 87–91.

Ottogalli, L., Galinie, C., Goxe, D., 1988. Reproduction in captivity of *Penaeus stylirostris* over ten generations in New Caledonia. J. Aquac.Trop. 3, 111–125.

Pitt, R., 2001. Preliminary sandfish growth trials in tanks, ponds and pens in Vietnam. SPC Beche-de-mer Inf. Bull. 15, 17–27.

Pitt, R., Duy, N.D.Q., 2004. Breeding and rearing of the sea cucumber *Holothuria scabra* in Viet Nam. In: Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J.-F., Mercier, A. (Eds.), Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Paper, no. 463, pp. 333–346.

Pitt, R., Duy, N.D.Q., Duy, T.V., Long, H.T.C., 2004. Sandfish (*Holothuria scabra*) with shrimp (*Penaeus mondon*) co-culture in tanks. SPC Beche-de-mer Inf. Bull. 20, 12–22.

Purcell, S., 2004. Rapid growth and bioturbation activity of the sea cucumber *Holothuria scabra* in earthen ponds. Proc. Australas. Aquac. 2004, p. 244.

Purcell, S., Eeckhaut, I., 2005. An external check for disease and health of hatchery-produced sea cucumbers. SPC Beche-de-mer Inf. Bull. 22, 34–38.

Purcell, S.W., Kirby, D.S., 2006. Restocking the sea cucumber *Holothuria scabra*: sizing no-take zones through individual-based movement modeling. Fish. Res. 80, 53–61.

Purcell, S., Gardner, D., Bell, J.D., 2002. Developing optimal strategies for restocking sandfish: a collaborative project in New Caledonia. SPC Beche-de-mer Inf. Bull. 16, 2–4.

Purcell, S.W., Patrois, J., Fraisse, N., 2006. Experimental evaluation of co-culture of juvenile sea cucumbers, *Holothuria scabra* (Jaeger), with juvenile blue shrimp, *Litopenaeus stylirostris* (Stimpson). Aquac. Res. 37, 515–522.

Underwood, A., 1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr. Mar. Biol. Ann. Rev. 19, 513–605.