Assays for optimising the growth of *Holothuria scabra* juveniles during the nursery phase

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Abstract

The effect of sediment quality on the survival and growth of the sea cucumber *Holothuria scabra* reared in nursery ponds was previously studied by our team. Here, we tested the effects of three types of enrichment to optimise the growth of sea cucumbers in nursery ponds. The enrichments were: 1) marine sediment + provender based on fisheries byproducts; 2) marine sediment + green water, including tropical local diatoms; and 3) marine sediment + tilapia (*Oreochromis niloticus*) in co-culture. For this experimentation, eight outdoor ponds, each measuring 32 m^2, were used. The rearing density at the beginning of the experiment was 20 ind. m^{-2} (640 *H. scabra* juveniles in each pond). The results showed that at the end of the experiment, no significant differences could be detected between the survival rates, mean weights or biomasses obtained for each type of enrichment tested. However, marine sediment collected from a mangrove zone (control) and marine sediment + tilapia in co-culture produced good performance after seven weeks of rearing. The profitability of co-culture with tilapia should be further investigated.

Introduction

Juvenile *Holothuria scabra* pass from an epibenthic stage once they reach 1.0–1.5 cm in length, to an endobenthic stage, in which they bury in the substratum during the day (Eeckhaut et al. 2008). During aquaculture experiments run in southwest Madagascar, juveniles were transferred at the endobenthic stage from the hatchery to outdoor ponds filled with a layer of sediment. They remained in these ponds for 6–10 weeks until they reached an average size of 6 cm (approx. 15 g) (Lavitra 2008). At this point, they were able to cope with conditions in the natural environment (Battaglene and Bell 1999) and were then transferred to sea pens to allow them to grow to a marketable size (> 350 g).

The effect of sediment quality and stocking density on the survival and growth rate of *H. scabra* reared in nursery ponds and pens was previously studied by Lavitra et al. (2010). Three types of sediment (taken from a micro-atoll, and mangrove and seagrass beds) were tested for their food quality properties. Experiments were carried out in ponds at juvenile stocking densities of 10, 20, 30 and 40 ind. m^{-2}. The results showed that the nature of the sediment did not affect the survival or growth of *H. scabra*: high survival and good growth rates were observed during eight weeks of rearing in ponds (Lavitra et al. 2010). Yet, regardless of stocking density, juvenile growth ceased above a maximum biomass of 160 g m^{-2} in outdoor ponds (Lavitra et al. 2010).

The economic profitability of *H. scabra* aquaculture relies on the optimisation of each rearing phase and the two phases that are the most controllable are the hatchery and the nursery pond phases. Here, we show the effects of three assays for optimising the growth in nursery ponds: 1) the addition of green water including tropical local diatoms; 2) the addition of provender based on fisheries byproducts; and 3) co-rearing with tilapia *Oreochromis niloticus* together with the addition of provender. The three assays were compared with controls consisting of *H. scabra* reared on natural marine sediment collected from mangrove areas.

Materials and methodology

The experiments were run in the nursery ponds of a private company, Madagascar Holothurie S.A. Three types of sediment enrichments were tested:

- the addition of green water, including tropical local diatoms;
- the addition of provender based on fisheries byproducts; and
- co-rearing with the tilapia *Oreochromis niloticus,* with the addition of provender.

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Preparation of green water

First, 200 g of chicken droppings were dried and mashed to produce a powder. Then, the powder was placed in sealed bags at the four corners of the pond, 10 cm under the water surface, and left to "infuse" in the water for three days in order to produce green water (Fig. 1). These bags are wiped three times a day – in the morning, at noon and in the late afternoon.

Provender

The provender is manufactured by the laboratory PRADA (Projet de Recherche Appliqué au Développement de la Région Analanjirofo). It consists of granules made of fish flour as a source of animal protein (30%), soya powder as source of vegetable protein (26%), peanut pieces as a source of lipids (17%), and rice or cassava as source of carbohydrates (12%) (Rahoasa 2014). A quantity of 20 g of provender per pond was distributed two times per week after water replacement.

Rearing H. scabra *with tilapia*

Thirty tilapia individuals with a mean size of 4 g were introduced into nursery ponds and fed with the same provender used in the second experiment. A quantity of 40 g of provender per pond was distributed two times per week after water replacement.

Preparation of the nursery ponds

Eight outdoor ponds measuring 32 m^2 (8 m x 4 m) were used (Fig. 2). Marine sediment collected from the mangroves was placed in each pond at a thickness of about 1 cm. Before putting the juveniles in the ponds, the sediment was treated with tap water

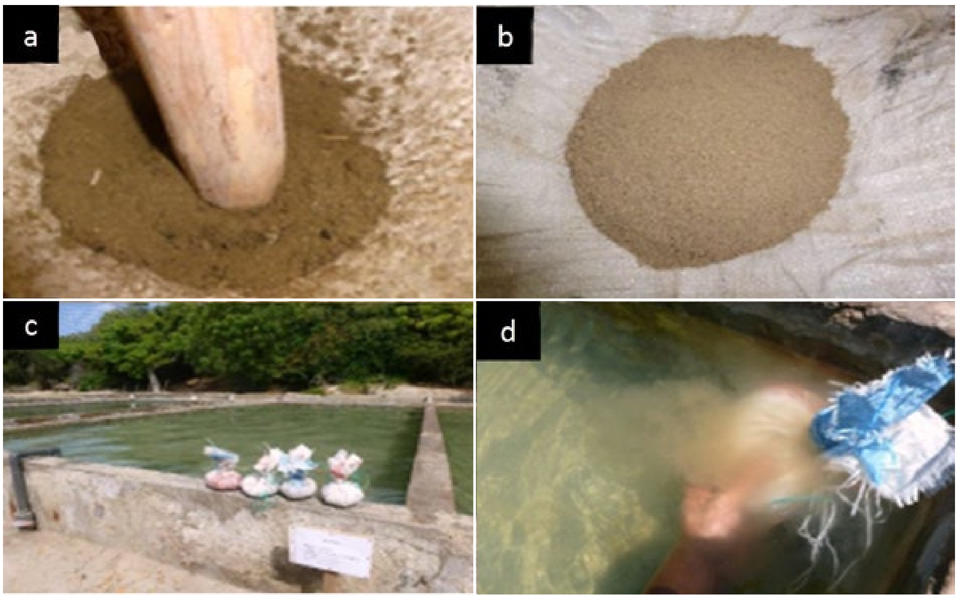

Figure 1. Preparation of green water.

a) dried and mashed chicken droppings;
b) powder;
c) powder packed in sealed bags;
d) bags placed in water at each corner of the pond.

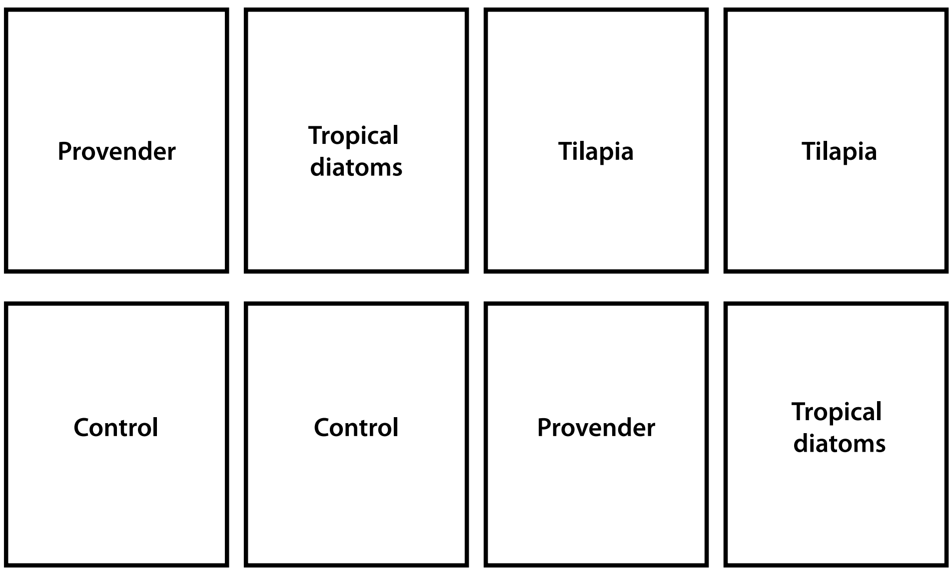

Figure 2. Design and disposition of the outdoor ponds.

- Control: Ponds where *H. scabra* juveniles were fed with only marine sediment.
- Provender: Ponds where *H. scabra* juveniles were fed with marine sediments + 20 g of provender per week per pond (20 g week^{-1} pond^{-1}).
- Tilapia: Ponds where *H. scabra* juveniles were reared with tilapia.
- Tropical diatoms: Ponds where *H. scabra* juveniles were fed with marine sediment + tropical diatoms (5 m^3 week^{-1} pond^{-1}).

for 24 hours to eliminate any possible predators and other organisms that would inhibit or infect the rearing of the individuals.

After the treatment of the sediment, tap water was replaced by seawater that was pumped directly from the bay of Toliara. The seawater flowed into the rearing ponds without any treatment or filtration.

Juvenile *H. scabra* were provided by the company Indian Ocean Trepang (IOT). At the beginning of the experiment, juveniles were 12 weeks old and had a mean weight of 0.03 g. After collection from the hatchery, juveniles were placed inside a bucket for counting. Counting was done manually, using no tools other than eyes and hands. After they were counted, the juveniles were put in a plastic bag full of filtered seawater for transportation. The transport took about 20 minutes; the distance between the IOT hatchery and Belaza, where the grow-out experiments took place, is about 10 km. Juveniles were then placed directly into the ponds. The rearing density was fixed at 20 individuals per m^2, equivalent to 640 juveniles per pond. In total, 5,120 juveniles were monitored during the experiment.

The temperature and salinity of the water were measured twice a day (in the morning at 07:00 and in the afternoon at 15:00). Because the experiment was conducted during the cool season (May–July 2014), each pond was covered with plastic sheeting to retain the heat, thereby producing a greenhouse effect.

For *H. scabra*, there is a tight correlation between length, width and weight (Lavitra 2008), with weight being the only parameter used to evaluate the growth of the studied specimens. The weight of all individuals in each pond was measured and recorded once a week. Weight was measured using an electronic balance.

The survival rate was only recorded at the end of the experiment by counting all of the remaining juveniles.

The biomass of the holothurians in each pond was calculated at the end of the experiment in order to evaluate the yield of each food type. The biomass is expressed in grams per square meter (g m^{-2}) and was calculated as:

(number of individuals x mean weight)/pond area

Statistical analysis of the data that were related to the survival and growth of the juveniles was done using the software SYSTAT V12.2. Analysis consisted of comparing the rate of survival and growth, and the mean biomass of the holothurians obtained for each type of enrichment at the end of the experiment. Therefore, a comparison test of proportions and an ANOVA test were done. It was noted that the data were homogenous and the distribution was normal after Ks and Leven tests.

Results

Physicochemical parameters of the water

The average temperature of the pond water during the experiment was 28°C, with an average minimum of 25.23°C and an average maximum of 30.74°C (Fig. 3). In general, no large variation in temperature was observed inside the rearing ponds during the experiment. The mean salinity value was about 34.5‰, and ranged from around 32‰ to 36‰ (Fig. 4).

Survival

At the beginning of the experiment, the number of juveniles in each pond was 640. After seven weeks, the survival rate varied from 42% to 66%, depending on the type of enrichment added (Fig. 5). However, a statistical analysis of the data did not show any significant difference between the values obtained ($p = 0.103$).

Growth

Juveniles had an average weight of 0.03 g at the beginning of the experiment. After seven weeks of rearing, the mean weight of juveniles was the highest (9.01 g ± 4.23) in the ponds where sea cucumber juveniles had been co-cultured with tilapia (Fig. 6). However, statistical analysis of the data revealed that the mean weights of *H. scabra* juveniles did not differ significantly ($p = 0.739$), no matter the enrichment type.

Biomass

The average biomass per pond at the beginning of the experiment was 0.6 g m^{-2}. After seven weeks of rearing, biomass varied, depending on the type of enrichment provided. It was higher (more than 110 g m^{-2}) in the control ponds and in the ponds in co-culture with tilapia than in the ponds using the two other types of enrichment (less than 72 g m^{-2} for juveniles fed with marine sediment enriched with tropical diatoms and for those fed with marine sediment enriched with provender). However, statistical analysis of data showed that there was no significant difference between the various enrichments ($p = 0.198$) (Fig. 7).

Discussion

The various enrichments did not produce significant differences in survival rates, mean weights or biomass of *H. scabra* juveniles at the end of the

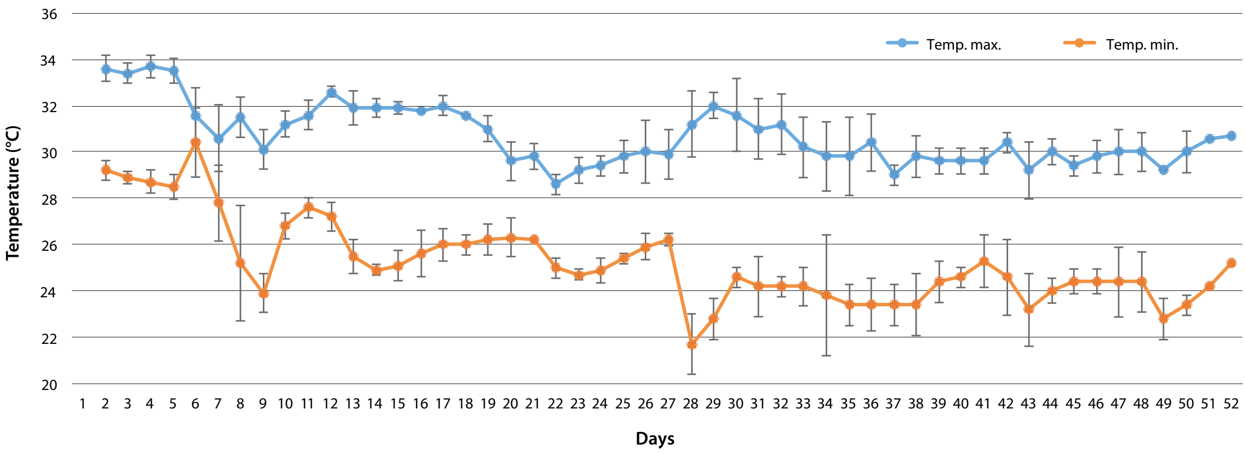

Figure 3. Maximum and minimum temperatures recorded each day in the rearing ponds (May–July 2014).

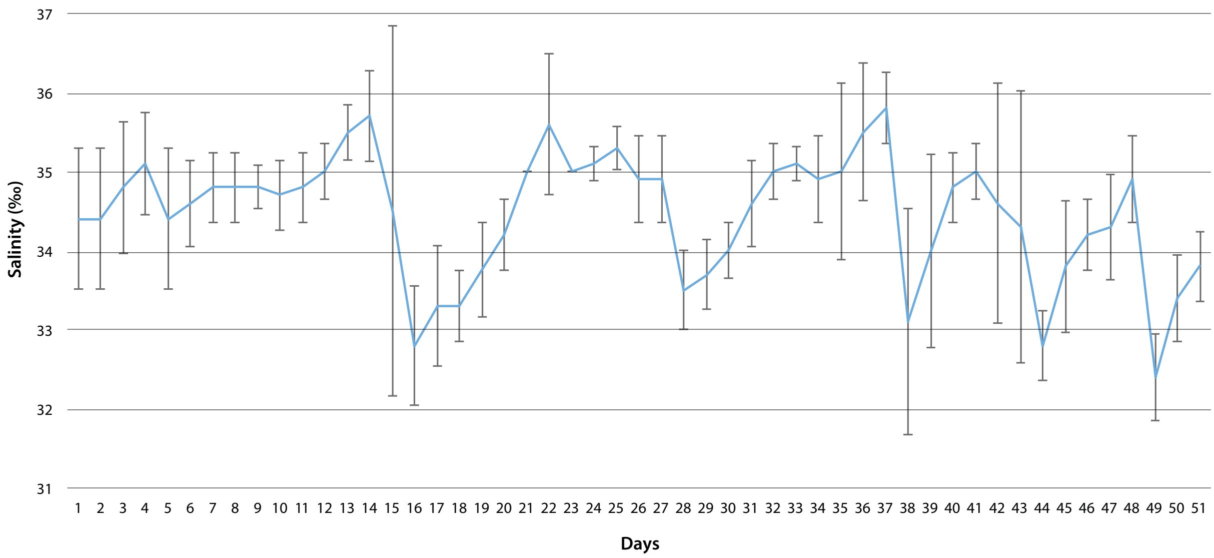

Figure 4. Salinity within the rearing ponds (May–July 2014).

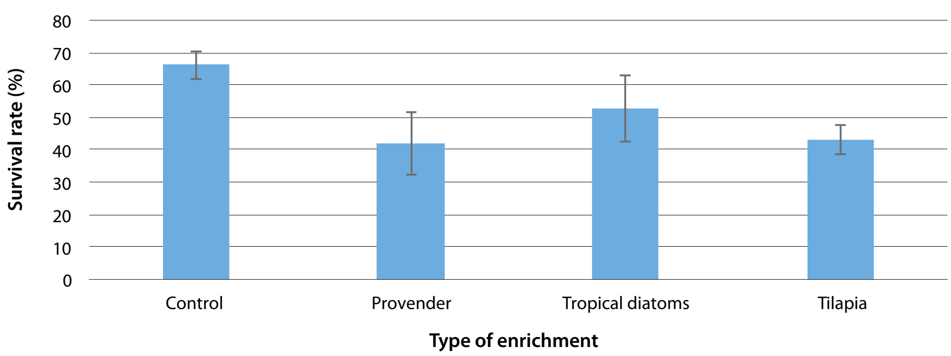

Figure 5. Survival rate of *H. scabra* juveniles after seven weeks of rearing.

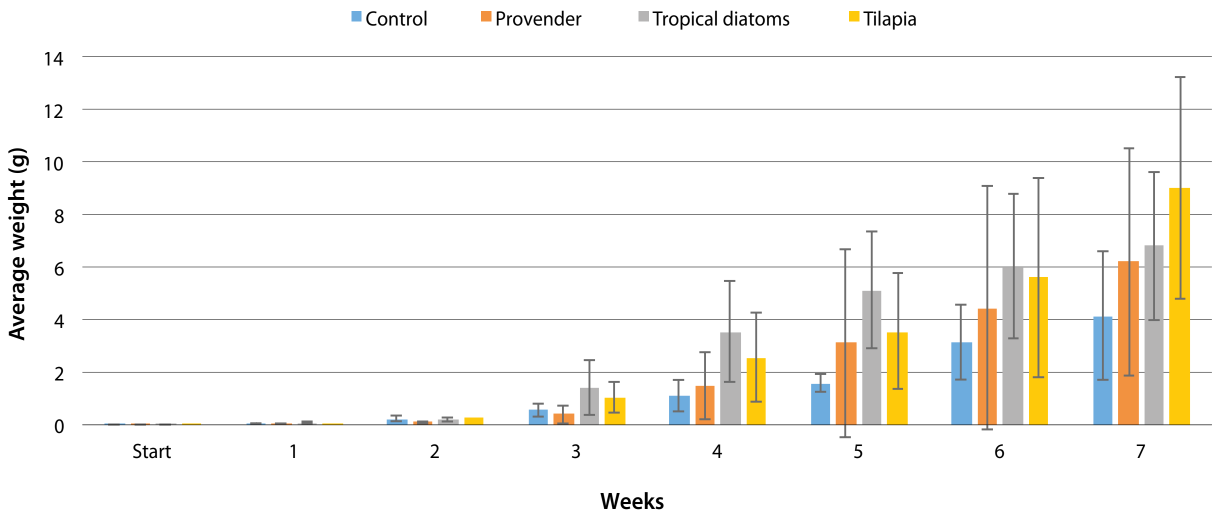

Figure 6. Average weight of *H. scabra* juveniles after seven weeks of rearing.

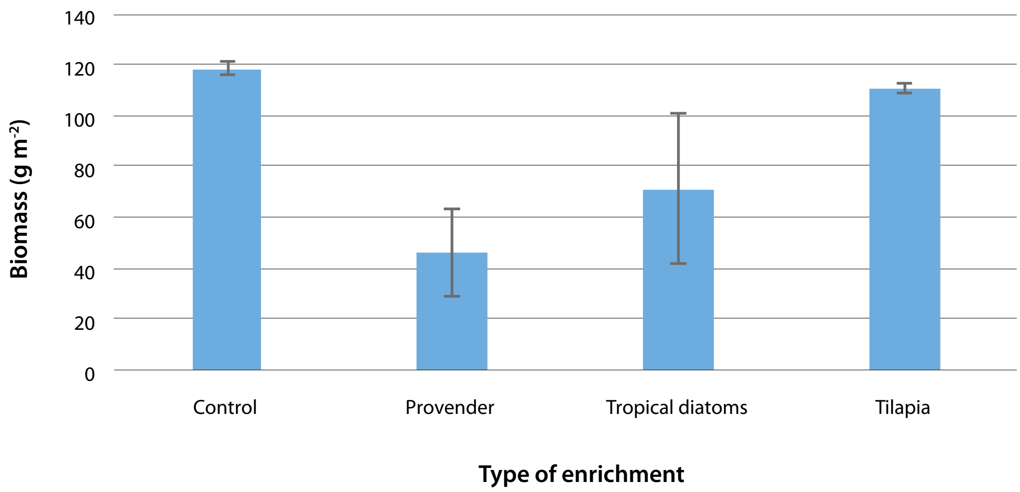

Figure 7. Biomass of *H. scabra* juveniles after seven weeks of rearing.

experiment. These results suggest that the organic matter present in the natural marine sediment collected from mangrove areas is sufficient for *H. scabra* growth during the nursery phases, and that the enrichments tested during the experiment were not necessary.

Much research has been conducted with marine sediment enrichments in order to improve *H. scabra* growth during the nursery phase; for example, marine sediment + *Thalassia hemprichii*; marine sediment + *T. hemprichii* + spirulina; marine sediment + *Sargassum latifolium*; marine sediment + *S. latifolium* + spirulina; marine sediment + *Thalassodendron ciliatum*; marine sediment + *Siryngodium isoetifolium*; and marine sediment + organic biofilm (Lavitra 2008). The results were the same as those obtained here: the enrichments had no significant impact on the biomass of reared *H. scabra*. All of these experiments suggest that the use of provender and tropical diatoms as sediment enrichment do not give good results in terms of growth, and they are not promising.

Before the end of the experiment, we found the co-culture with tilapia to be promising. The rearing of tilapia in seawater has been reported by multiple authors, some of whom reported the possibility of obtaining good growths (Persand and Bhikajee 1997; Watanabe et al. 1990). We originally thought that rearing sea cucumbers with tilapia would provide advantages for both the holothurians and the fish, with the first eating the waste material of the latter. However, our study showed that the average survival rate of juvenile *H. scabra* in the co-rearing experiment was very low (43.3%). This could be explained by the fact that either the juveniles were too small at the beginning of the experiment and could not consume all of the wastes of the tilapia, or they were prey for tilapia. Our experiment with the mixed rearing of holothurians and tilapia was the first of its kind. Nonetheless, many authors have tested the mixed rearing of holothurians with shrimp, and most authors affirmed that they obtained a good yield of holothurians and shrimp, while a few authors highlighted the uselessness of the mixed rearing (Bell et al. 2007; Pitt and Duy 2004). Further experiments are necessary to test the efficacy of sediment enrichments, especially those involving tilapia.

Acknowledgements

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