

The Effect of Sediment Quality and Stocking Density on Survival and Growth of the Sea Cucumber *Holothuria scabra* Reared in Nursery Ponds and Sea Pens

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Abstract—The effect of sediment quality and stocking density on the survival and growth of the sea cucumber *Holothuria scabra* reared in nursery ponds and in pens was studied at Aqua-Lab Farm in Toliara, south west Madagascar. Three types of sediment (micro-atoll, mangrove and seagrass bed) were tested for their food quality properties. Experiments were carried out in ponds at juvenile stocking densities of 10, 20, 30 and 40 individuals m⁻² (juveniles size: 0.24 to 15 g) and in sea pens at juvenile stocking densities of 3, 6, 9 and 12 individuals m⁻² (juveniles size: > 15 g). The results showed that the nature of the sediment did not affect the survival or growth of *H. scabra*. High survival (>95 %) and good growth rates (>0.22 g d⁻¹) were observed during eight weeks of rearing in ponds. However, stocking density clearly influenced its growth. Highest growth rates of 0.23 g d⁻¹ and 0.64 g d⁻¹ were respectively obtained at low stocking densities in the ponds (stocking density: 10 individuals m⁻²; rearing time: 10 weeks) and sea pens (stocking density: 3 individuals m⁻²; rearing time: 9 months). Regardless of stocking density, juvenile growth ceased above a maximum biomass of 160 g m⁻² in outdoor ponds and 692 g m⁻² in sea pens. In order to optimize the farming of *H. scabra*, we suggest (i) the use of sediment collected from seagrass beds for nursery ponds, although sediments from other sources may be used, and (ii) the stocking biomass should not exceed 160 g m⁻² in nursery ponds and 650 g m⁻² in sea pens or at natural ranching sites.

INTRODUCTION

Sea cucumbers constitute a traditional food in several Asian countries (Conand, 2004, 2006a, b). They are appreciated for their aphrodisiac properties and as a medicinal cure (Preston, 1993). Due to high demand on the Chinese market (Conand & Byrne, 1993; Rasolofonirina, 2005) and inadequate management of natural stocks (Bruckner, 2006), sea cucumbers are currently overexploited in several regions of the world (Conand, 2008).

Holothuria scabra is one of the edible sea cucumber species that has a high commercial value. It is also the most prized sea cucumber among the tropical species (Purcell, 2004) and, consequently, the most exploited in the Indo-Pacific region. However, *H. scabra* is considered the most promising for aquaculture because it has (i) a wide geographical distribution, (ii) a larval development easily managed in aquaculture, (iii) a large tolerance to variations in environmental conditions (Hamel *et al.*, 2001) and (iv) rapid growth under optimal conditions (James, 1999; Pitt & Duy, 2004). Thus, this species is used in research for aquaculture and/or stock

restoration in countries such as the Maldives (Manikandan, 2001), Solomon islands (Bell, 1997; Battaglione *et al.*, 1999; Mercier *et al.*, 2000), Philippines (Gamboa *et al.*, 2004), Vietnam (Pitt & Duy, 2004), New Caledonia (Purcell & Kirby, 2006; Purcell *et al.*, 2006; Agudo, 2006) and Madagascar (Rasolofonirina *et al.*, 2004, Lavitra, 2008; Lavitra *et al.*, 2010).

In the hatchery at Aqua-Lab Farm, in Toliara, south west Madagascar (Eeckhaut *et al.*, 2008), *H. scabra* juveniles pass from an epibenthic stage once they attain 1 to 1.5 cm in length, to an endobenthic stage in which they bury in the substratum during the day. At this stage, they are transferred from the hatchery to concrete outdoor ponds filled with a layer of sediment and reared for 6 to 10 weeks until they reach an average size of 6 cm (15 g). At this point, they are able to cope with conditions in the natural environment (Battaglione, 1999) and are transferred to sea pens until they reach a marketable size (>350 g).

The purpose of the present study was to assess the effect of sediment quality and stocking density on the survival and growth of *H. scabra* reared in ponds and in sea pens constructed in nearshore seagrass beds.

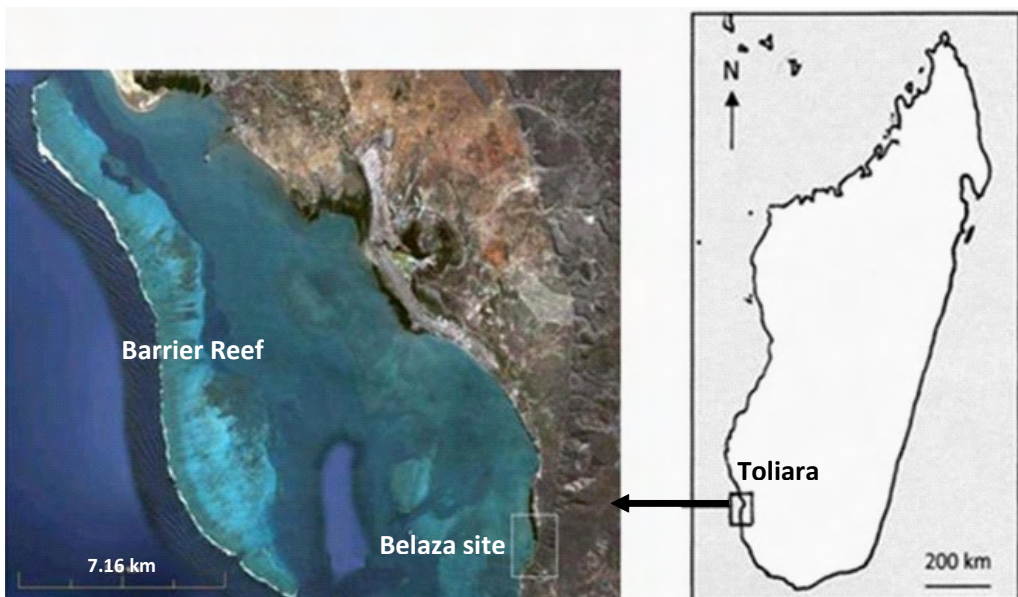


Figure 1. Map of Toliara and the Belaza study site.

MATERIAL AND METHODS

Study site

The hatchery of Aqua-Lab Farm is situated in Toliara, south west Madagascar, and the farm itself is located at Belaza, 23 km south of Toliara (Fig.1). The latter is located south of the great barrier reef of Toliara, inside a bay and adjacent to a littoral mangrove. Experiments were conducted in outdoor nursery ponds and sea pens respectively at these two sites between July 2006 and November 2007.

Rearing of individuals in nursery ponds: Pre-grow-out

Effect of sediment quality on survival and growth

Each concrete pond measuring 4 m x 2 m was subdivided into four equal compartments of 2 m² (2 m x 1 m) and filled with different types of sediment to a depth of 5 cm. The sea water (depth 0.5 m) was changed weekly but the sediment was not renewed. Three types of sediments were tested: (i) sandy sediments, collected from the micro-atoll on the reef, (ii) muddy sediments, collected from the mangrove at Belaza site and (iii) mixed sandy-muddy sediment, collected from the seagrass bed in front of Belaza. The control consisted of a pond without sediment where juveniles were fed daily with 5 g of *Sargassum* sp. extract.

An analysis of grain size and organic content was conducted on each type of sediment tested. For the grain size analysis, freshly collected sediment samples (4 replicates) were rinsed several times with distilled water, oven-dried for 48 h at 60°C and the total dry weight noted (Gillan *et al.*, 2005). The sediments were then sieved with distilled water through a 63 µm screen to determine the fine fraction of silt and clay, after which the coarse fraction was weighed and successively sieved through screens of mesh size 2 mm, 1 mm, 500 µm, 250 µm and 125 µm. The percentage weight of the different fractions was calculated. Debris of plant and animal origin was identified

in the sediments with a light microscope. Organic content of the sediment samples was determined by combusting weighed samples (4 replicates), oven dried for 48 h at 60°C, then fired at 450°C for 4 h (Gillan *et al.*, 2005). The organic content was using the formula: $OMR = (DwS - DwC) \times 100 / DwS$, where DwS and DwC represent the dry weight of the sediment before and after combustion.

The effect of sediment type was studied at different times of the year (in May 2005 for the first trial, December 2005 for the second and third trial and March 2006 for the fourth and fifth trials). Juvenile *Holothuria scabra* at the end of their epibenthic stage were used for each trial, 12 weeks old (including the larval stage), approximately 1.5 cm long and 0.24 g in weight. At the beginning of each trial, the juveniles were transferred from the Aqua-lab hatchery into the outdoor nursery ponds (one control, without any sediment and three filled with the sediments under test). The stocking density was 20 individuals per pond (viz. 10 individuals m⁻²) throughout the 8-week trials.

Effect of density on the survival and growth.

The effect of stocking density was studied on the survival and growth of endobenthic *H. scabra* juveniles in outdoor nursery ponds. Four different densities were tested (10, 20, 30 and 40 individuals m⁻²) at different times of the year (January 2007 for the two first trials, May 2007 for the third and fourth and August 2007 for the fifth). Juveniles at the beginning of the experiment were 10 weeks old (larval stage included) and measured on average 1 cm in length. The sea water was changed weekly but a 5 cm layer of sediment (collected from the sea grass bed) was not renewed during the experiments.

Rearing of juveniles in sea pens: Grow-out

When the *H. scabra* juveniles reached 6 cm in length (15 g), they were transferred to sea pens constructed in nearshore seagrass beds (their natural environment) without any food.

In this study, only the effect of the rearing density was examined. The same experiment was repeated three times: June 2006 (first trial) and August 2006 (second and third trial). The experimental enclosures were constructed from plastic net fixed to wooden stakes at 50 cm intervals and buried to a depth of 30 cm to prevent escape of the juveniles. Each pen had a surface area of 9 m² (3 m x 3 m) and was 50 cm high. Four different *H. scabra* densities were tested: 3, 6, 9 and 12 individuals m⁻², corresponding to 27, 54, 81 and 108 individuals pen⁻¹ respectively.

Measurements

Sea water temperature and salinity were measured daily in both the ponds and sea pens. Wet weight of *H. scabra* was used as the measure of its growth as the length is influenced by stress. This was obtained 2 mins after removal of the sea cucumbers from the water to achieve maximal expulsion of water from the coelom. As *H. scabra* is endobenthic and buries underneath the sediment during the day, measurements were taken at night. Juveniles (<15 g) were weighed fortnightly and adults (>15 g) monthly. Survival was recorded as the number of live specimens at the end of each experiment.

Data analysis

As the trials were conducted at different times of the year, two-way ANOVA and Friedman tests were used to analyze the data. The normality of distribution and the homogeneity

of variances of the samples were assessed using Levene's and Kolmogorov-Smirnov's tests, respectively. All statistic analyses were performed using SYSTAT v9, SPSS v13 and R.2.6.1 software.

RESULTS

Temperature and salinity

In general, the sea water temperature was relatively high (>23°C) during the study period in the outdoor ponds as well as in the sea pens (see also Lavitra *et al.*, 2010). During the hot season (from November to April), the sea water temperature was above 30°C and reached 36°C (January, March and April 2007). In the cold season, the water temperature remained below 30°C and was always lower in the ponds than the sea pens (Fig. 2A). The salinity in the ponds was stable (35‰), except in February 2007 due to the passage of a cyclone. On the other hand, the salinity in the sea pens was variable and fell below 25‰ during some periods (Fig. 2B).

Rearing of individuals in nursery ponds: Pre-grow-out

Sediment characteristics

Results of the grain size analyses are presented in Table 1. Microscopic examination of animal and plant debris in the sediment revealed that it comprised shells (composed of bivalves and gastropods), coral debris (particularly in sediment collected from the micro-atolls) and

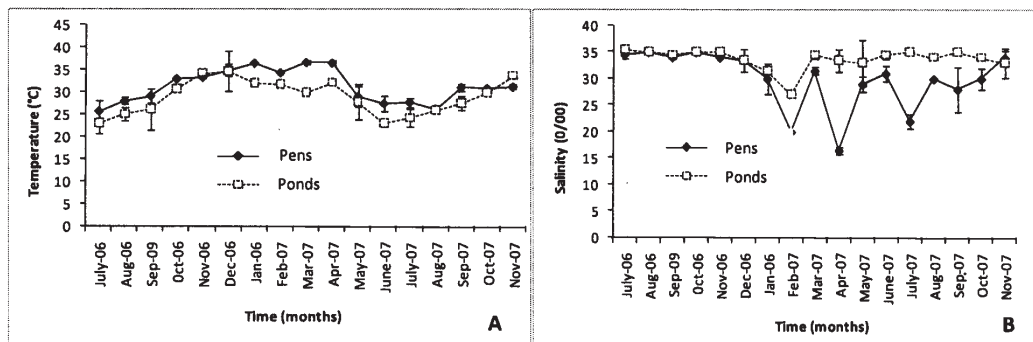


Figure 2. Monthly variation of (A) temperature and (B) salinity recorded in sea pens and ponds during the study. Vertical lines represent standard deviations.

Table 1. Grain size of sediment (% ± sd) used in *Holothuria scabra* nursery ponds.

Particle diameter (µm)	Component	Seagrass bed	Mangrove zone	Micro-atolls
0 - 63	Clay and silt	0.71 +/- 0.30	2.72 +/- 1.75	0.04 +/- 0.02
63 - 125	Very fine sand	6.10 +/- 0.91	6.76 +/- 1.80	0.71 +/- 0.77
125 - 250	Fine sand	49.72 +/- 4.52	37.84 +/- 2.43	14.63 +/- 9.87
250 - 500	Medium sand	29.82 +/- 6.24	43.44 +/- 1.33	36.22 +/- 9.23
500 - 1000	Coarse sand	7.65 +/- 1.87	7.44 +/- 1.03	26.31 +/- 9.03
1000 - 2000	Very coarse sand	3.13 +/- 2.35	1.20 +/- 0.24	13.91 +/- 5.63
> 2000	Gravel	2.85 +/- 3.66	0.59 +/- 0.20	8.18 +/- 3.71

algae. In general, the sediment collected from the seagrass bed and from the mangrove had the same grain size structure. Sediment collected from the micro-atoll was coarser and contained less silt, clay and fine sand and comprised coarse or very coarse sand and gravel.

The Belaza site was poor in organic matter (less than 3%) (Fig. 3), and the organic content of the seagrass sediment was lower ($p = 0.022$) than that of the mangrove or the micro-atolls.

Effect of sediment quality

Sediment type did not affect the survival of *H. scabra* juveniles ($p = 0.247$). Regardless of the sediment used, a very high survival rate (>95 %) was recorded in all the experiments. In addition, sediment type did not affect *H. scabra* growth ($p = 0.115$). Juveniles at the start weighed 0.24 g and, at 8 weeks, their weight averaged 13, 17 and 18 g respectively in ponds with a layer of sediment collected from micro-atolls, the mangrove zone or seagrass beds (Fig. 4). On the other hand, juveniles

reared in the control pond (without sediment) manifested poor growth and weighed only 0.63 g at the end of the experiment.

Effect of density

Stocking density did not influence the survival of *H. scabra* juveniles ($p = 0.182$). After 10 weeks of rearing, the survival rates were 79, 86, 88 and 87% at stocking densities of 40, 30, 20 and 10 individuals m^{-2} respectively. However, growth was affected ($p = 0.001$). The average weight of juveniles after 10 weeks was 5, 6, 10 and 15 g at rearing densities of 40, 30, 20 and 10 individuals m^{-2} respectively (Fig. 5), the highest growth rate being obtained at the last-mentioned stocking density.

Regardless of stocking density, the biomass peaked and did not increase after ten weeks of rearing ($p = 0.688$) in the indoor ponds and averaged 160 g m^{-2} (Fig. 6).

Rearing of individuals in the natural environment: Grow-out.

Regardless of the density tested, the survival rate of *H. scabra* in sea pens was always high (>75 % after nine months; $p = 0.272$). Their growth appeared to peak at seven months when they attained sizes that were density dependent: 73, 84, 126 and 198 g at densities of 12, 9, 6 and 3 individuals m^{-2} respectively (Fig. 7). Thus, stocking density significantly affected the growth of *H. scabra* in sea pens ($p = 0.001$).

However, whatever the stocking density, the biomass yield per square meter at the end of the rearing remained statistically similar ($p = 0.232$). Maximum biomasses of 771 and 746 g

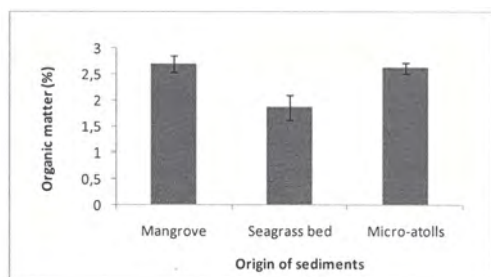


Figure 3. Differences in organic matter content (expressed as a percentage of the dry weight of the sediment) in the experimental sediments. Vertical lines represent standard deviations ($n = 4$).

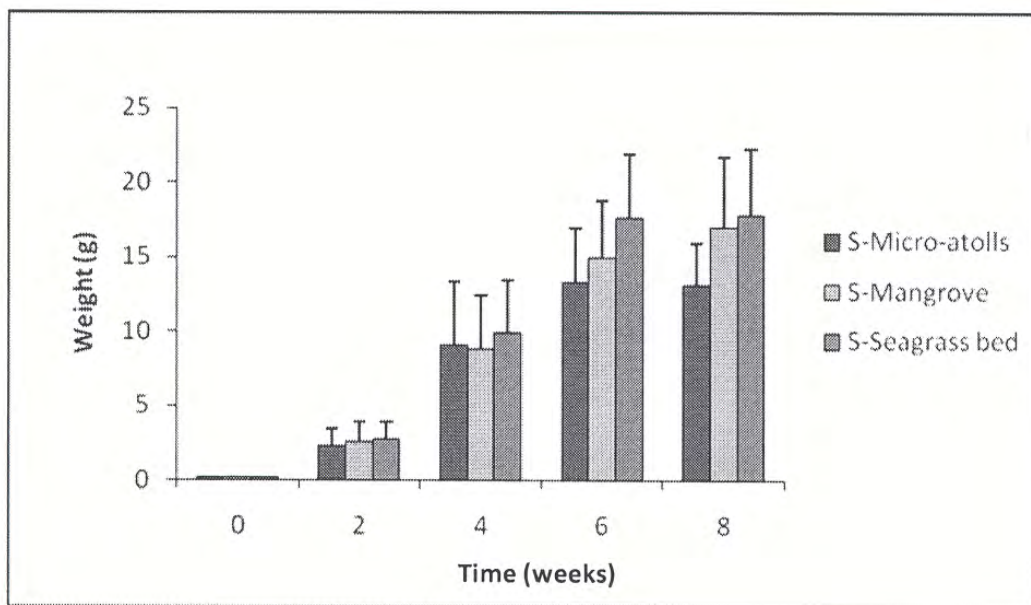


Figure 4. Mean weight of *H. scabra* juveniles during grow-out on different sediments. Vertical lines represent standard deviations. Abbreviations: S-Micro-atolls: sediment collected from the micro-atolls; S-Mangrove: sediment collected from the mangrove zone; S-Sea grass bed: sediment collected from the sea grass bed.

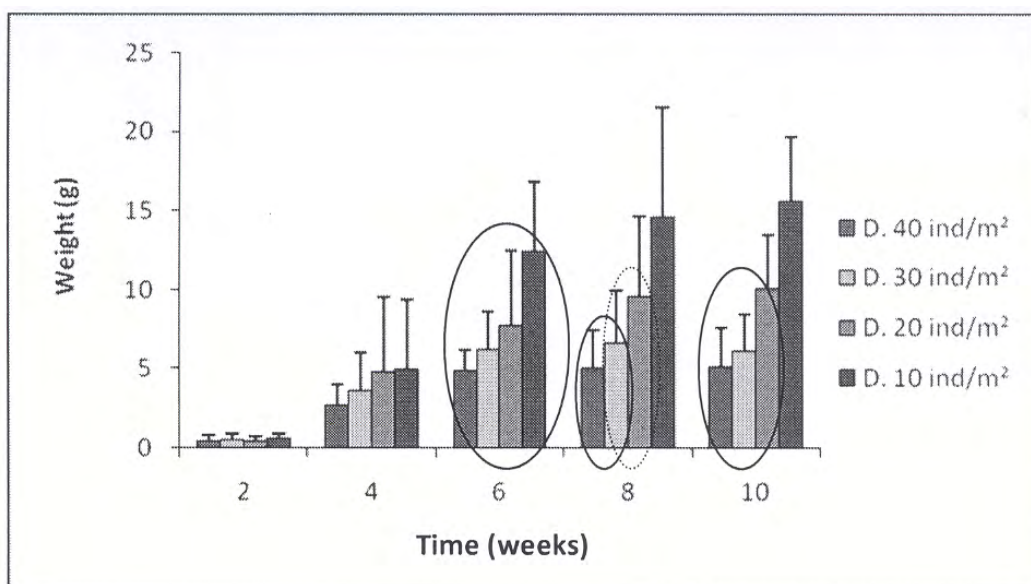


Figure 5. Mean weight of endobenthic juveniles of *H. scabra* reared in outdoor ponds at different stocking densities (D individuals m⁻²). Vertical lines represent standard deviations. Differences in circled bars were not significant.

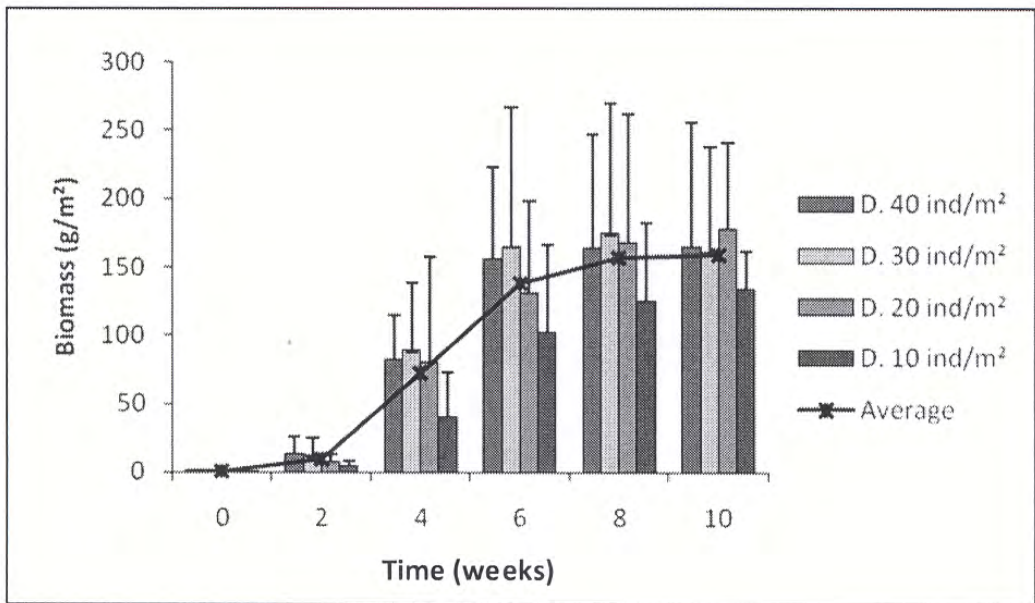


Figure 6. Biomass production per m² of *H. scabra* endobenthic juveniles in outdoor ponds at different stocking densities (D individuals m⁻²). Vertical lines represent standard deviations.

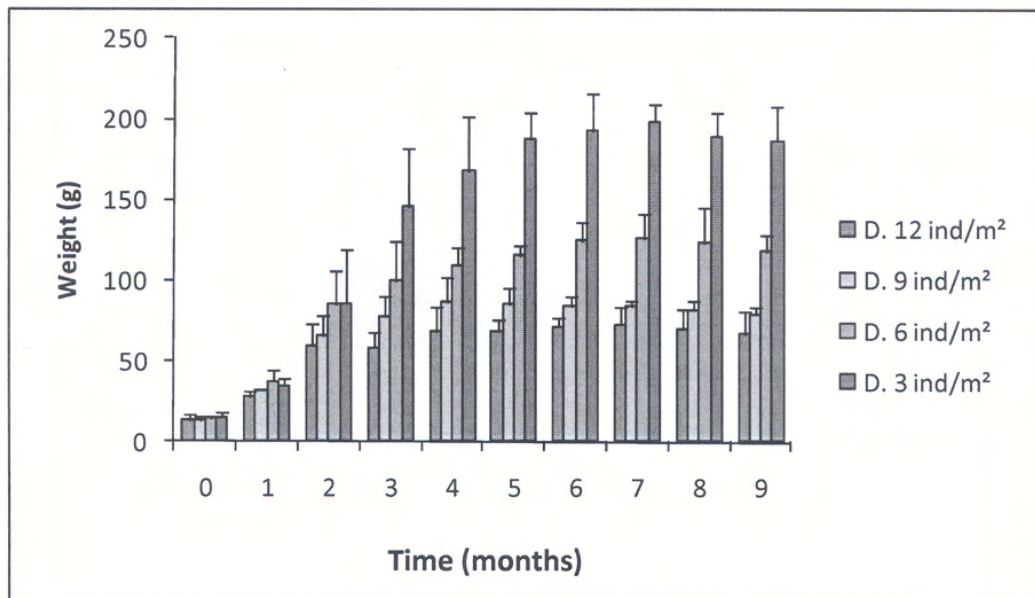


Figure 7. Mean weight of *H. scabra* reared in sea pens at different stocking densities (D individuals m⁻²). Vertical lines represent standard deviations.

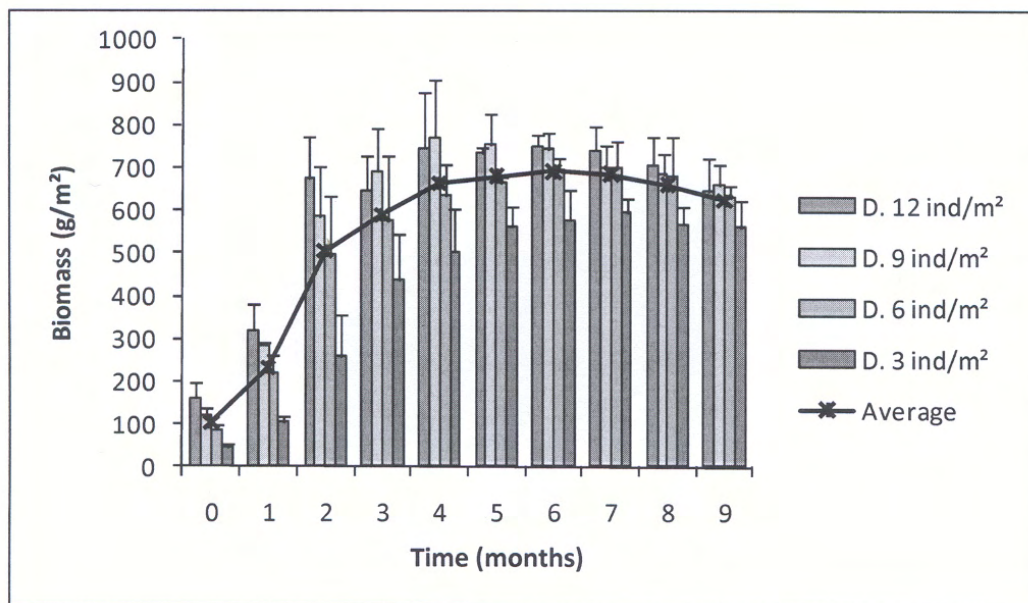


Figure 8. Biomass production of *H. scabra* produced in sea pens at different stocking densities (D individuals m^{-2}). Vertical lines represent standard deviations.

m^{-2} were obtained after four months of rearing at densities of 9 and 12 individuals m^{-2} respectively and, after seven months, the maximum values were 599 and 699 $g m^{-2}$ at stocking densities of 3 and 6 individuals m^{-2} (Fig. 8). On average, after attaining a biomass of 692 $g m^{-2}$ following six months of grow-out in the natural environment, the *H. scabra* stopped growing.

DISCUSSION

Our experiments highlight, firstly, that there appears to be a critical biomass value (CBV) for *H. scabra* above which it no longer grows in nursery ponds as well as in sea pens. In the Toliara region south-west of Madagascar, these values are, on average, 160 $g m^{-2}$ in outdoor ponds and 692 $g m^{-2}$ in sea pens. Thus, in order to reach marketable size (>350 g), the stocking density in sea pens must be lower than 2 individuals m^{-2} . This CBV in ponds has already observed in other rearing experiments; it varies from one author to another but remains in general less than 300 $g m^{-2}$, regardless of species. For *H. scabra*, it is 220-225 $g m^{-2}$ in the Solomon Islands (Battaglione, 1999), 250-350 $g m^{-2}$ in

Vietnam (Pitt & Duy, 2004) and 225 $g m^{-2}$ in New-Calédonia (Purcell, 2005; Purcell & Simutoga, 2008). For *A. mauritiana*, it proved to be 260 $g m^{-2}$ (Ramofafia *et al.*, 1997). This CBV in sea cucumber farming may be related to food availability in ponds. As *H. scabra* is a deposit feeder (Moriarty, 1982; Hamel *et al.*, 2001; Conand, 2006a), it ingests organic matter from the sediment which is depleted throughout rearing. Some authors think that sea cucumbers feed principally on photosynthetic organisms (periphytic algae) (Uthicke, 1999; Uthicke, 2001; Pitt & Duy, 2004; Taddei, 2006) and also bacteria (Moriarty, 1982; Hammond, 1983; Moriarty *et al.*, 1985; Taddei, 2006; Lavitra, 2008); the former may explain their rapid growth in the presence of light (Pitt & Duy, 2004).

During the nursery phase, several factors may influence the growth of *H. scabra* juveniles: (i) artificial food (Rasolofonirina, 2004; James, 1994; James, 1999), (ii) photoperiod (Pitt & Duy, 2004), (iii) season, (iv) the addition or renewal of sediment (James, 1994) and (v) rearing density (Battaglione, 1999; Pitt & Duy, 2004). Survival rates in ponds were always high, >79%. The value

of sediment was demonstrated in the present study and the best growth rate recorded was 0.53 g d⁻¹, obtained between the fourth and the sixth week of rearing at a stocking density of 10 individuals m⁻². In comparison, the best growth rates recorded in the literature were: 0.7 g d⁻¹ at a low stocking density and 0.3 g d⁻¹ at a high density (Battaglène, 1999); 0.28 g d⁻¹ at a rearing density of 20 individuals m⁻² (fed with shrimp food; Pitt & Duy, 2004); and 0.05 g d⁻¹, at a rearing density of 85 individuals m⁻² (fed with *Sargassum* with 3 % *Spirulina*; Rasolofonirina, 2004).

After 6 to 10 weeks of rearing in a nursery, juveniles reach 15 g and must be transferred into pens (Battaglène, 1999; present work). In fact, higher survival rates are obtained when releasing sea cucumbers of a larger size (Battaglène, 1999; Pitt & Duy, 2004), with the best growth rates at a low stocking density (James, 1999; Purcell & Simutoga, 2008; present work). Under such favorable conditions, *H. scabra* are capable of rapid growth of 1 to 3 g d⁻¹ (Pitt & Duy, 2004). In the present work, the survival rate of sea cucumbers in the sea pens was >75% and the highest growth rate was 0.64 g d⁻¹ at a stocking density of 3 individuals m⁻². Growth rates of *H. scabra* in the literature are: 1.2 g d⁻¹ at a rearing density of 3 individual m⁻² (James, 1999); 0.57 to 1.16 g d⁻¹, density not stated (Purcell & Kirby, 2006); 0.49 g d⁻¹ at a rearing density less than 3 individuals m⁻² (Pitt & Duy, 2004); and 0.46 g d⁻¹, density not stated (Shelley, 1985).

In conclusion, in order to optimize the rearing of *H. scabra*, a nursery phase in external ponds seems advisable. For that, our experiments suggest that juveniles of 1.5 cm (0.24 g) should be transferred into ponds filled with a layer of sediment. When the juveniles reach 6 cm (15 g) and a stocking biomass of 160 g m⁻², they should be transferred to pens at a projected stocking density that will not exceed the CBV after grow-out. For the seagrass beds of Belaza, a stocking density lower than 2 individuals m⁻² is recommended to attain a marketable size of >350 g within 6 months.

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