Problems related to the farming of *Holothuria scabra* (Jaeger, 1833)

Thierry Lavitra[1,2], Richard Rasolofonirina[1], Michel Jangoux[1,2,3] and Igor Eeckhaut[2*]*

Abstract

Different problems related to *Holothuria scabra* farming were observed at the sea cucumber aquaculture project Madagascar Holothurie S.A. (Toliara, Madagascar). In this paper, those problems are presented, their impact on sea cucumber production is characterised and solutions permitting to avoid or to minimise their impact are proposed. A drastic salinity drop occurred during low tides in one period of the year and after cyclones. This provoked behaviour troubles in the *H. scabra*: individuals stayed burrowed in sediment even at night, a period when they are usually at the surface. However, that did not affect their growth. When possible, we suggest choosing a site far from areas where salinity is apt to drop.

Isopods *Cymodoce sp*. infested sea cucumbers in outdoor ponds during the hot season, provoking a high mortality rate (average 8% week^{-1}) in cultivated *H. scabra*. The introduction of the carnivorous fish *Terapon jarbua* in the ponds proved successful in preventing this problem. The fish eat isopods and can eliminate them within 10 days. Infections induced by isopods totally disappeared within two weeks.

The crabs *Thalamita crenata* were abnormally abundant in the pens during some periods of the year. They are the most redoubtable sea cucumber predators in the region and may provoke the mortality of the entire stock within one month. The crabs attack principally the newly transferred juveniles. The adults whose weight was more than 250 g were never affected. The elimination of the crabs in the surrounding area is to be carried out before the transfer of the juveniles and a daily watch of the pens is necessary in order to limit the impact of predation on production.

Introduction

The control of biotic and abiotic parameters of the sea cucumber rearing sites is primordial for sea cucumber farming (Purcell 2004; Wang et al. 2004). Juveniles of *H. scabra* are cultivated first in outdoor ponds for two months until they reach the size of 6–7 cm. They are transferred afterward to pens built in sea grass beds (Battaglene 1999; Pitt and Duy 2004). Thus, several factors are to be considered in order to choose the best site for the holothurian growth, including the physico-chemical parameters and the presence of predators (Chen 2004; Pitt and Duy 2004). The predators could be crabs, shrimps, gastropods and fishes of the family Siganidae which attack preferably the young holothurians that have been newly transferred (Pitt and Duy 2004).

Recurrent controls should be carried out to identify all diseases or parasitism in order to avoid the introduction of harmful organisms to the natural stock (Eeckhaut et al. 2004; Purcell and Eeckhaut 2005). Identified parasites of sea cucumbers include bacteria, protozoa and several metazoa (Jangoux 1990;

Eeckhaut et al. 2004). In some cases, they may provoke the death of diseased juveniles (Becker et al. 2004). However, as holothuriculture is relatively recent, pathogenic agents and their treatment are still widely unknown (Battaglene 1999; Xilin 2004). In Madagascar, a disease named skin ulceration was observed for the first time in September 2000 (Becker et al. 2004). The infection, which affects the integument of *H. scabra*, spreads very quickly in the ponds and may provoke the death of individuals within as little as three days after the appearance of the first symptoms. This disease results from infection by opportunistic bacteria. Nevertheless, the agent causing the ulcers was not identified (Becker et al. 2004). The research project Aqua-Lab/Belaza, which gave rise to the trade company Madagascar Holothurie S.A. in Toliara, has optimised the growth of *H. scabra* between 2004 and 2008. During this period, several problems related to biotic and abiotic parameters occurred and had to be solved. Three, in particular had an impact on the progress of the farming. They were: (*i*) a drastic salinity drop encountered during low tides at a certain period of the year and after cyclones, (*ii*) the abnormal abundance of isopod

parasites in outdoor ponds during the hot season and (*iii*) the overabundance of crabs in the pens at a certain period of the year. This article presents those problems, characterises their impact on the sea cucumber production and proposes solutions permitting to avoid or to minimise their impact.

Material and methods

Salinity drop

The salinity was measured daily between July 2006 and December 2007 in the ponds (Fig. 1) and in the pens (Fig. 2). When an important salinity change was observed, all changes in behaviour, nycthemeral cycle and anatomy of cultivated *H. scabra* were recorded. Twice (once in the cool season and once in the hot season) the salinity dropped to 20‰ (2%). These salinity drops always occurred during the low tide and were due to a freshwater resurgence that ran out to the pens. In order to analyse the effects of the salinity drop on the anatomy of specimens, four juveniles (7 cm length and 15 g weight) were transferred for four weeks into a 15 l tank at a low salinity of 20‰ and compared to four juveniles maintained at a normal salinity of 35‰. Oxygenation of each tank was assured by a diffuser linked to a compressor. Seawater was changed every two days and juveniles were fed ground *Sargassum* daily. Juveniles were photographed and their symptoms characterised. Also, at two times, tropical cyclones provoked a salinity drop in the lagoon to 10‰. To study the effects of this drastic salinity drop on the survival of the cultivated *H. scabra*, specimens (8 cm length and 41 g weight) were placed in tanks (five specimens per tank) at a salinity of 10‰ for 1, 2, 3, 6, 12, 18 and 24 hours (35 specimens in total) after which salinity was brought back to 35‰. Those specimens were kept afterward in the same tanks for four weeks in order to evaluate the eventual effects of the salinity drop on their behaviour

and their survival. Finally, the effects of the salinity drop on the growth of *H. scabra* were also studied. To do so, two ponds of 8 m² were used. Each pond was subdivided into 4 compartments of 2 m² each where 4 batches of 6 *H. scabra* of a respective average weight of 4 g, 24 g, 68 g and 117 g (48 specimens in total) were placed. Seawater (kept at 20‰ in one pond and 35‰ in the other) was changed weekly. Sediments (covering 10 cm depth) were unchanged during the experiment. This experiment lasted five weeks, after which specimens were weighed again and respective average weights calculated. The data analysis consisted of comparing the average weight of specimens placed at 20‰ with those kept at 35‰.

Study of parasites

In 2007, during the warm season, an abnormal abundance of crustacean isopods was observed in outdoor ponds. This provoked a disease among the cultivated juveniles. A daily survey was conducted for six weeks from when the disease first appeared. The stages of disease were characterised and specimens presenting those stages were counted and weighed. The isopods responsible were identified. The numbers of isopods and diseased *H. scabra* were counted. At the laboratory, a stomach dissection of five isopods was carried out to see what they fed on and to check particularly if spicules of *H. scabra* might be found.

Integument of normal and diseased *H. scabra* (at different stages of infection) was fixed adequately to be characterised in histology and on a scanning electron microscope (SEM) (see below). Two experiments were conducted in order to find solutions to eradicate this disease: (*i*) the transfer of diseased juveniles (n = 189) to a new pond containing freshly collected sediment that was free of isopods and (*ii*) addition of carnivorous fish *Terapon jarbua* (Forskall, 1775), of the family of Teraponidae (average weight:

Figure 1. Outdoor ponds for juveniles of *H. scabra* from 1 to 7 cm.

Figure 2. Pens installed in sea grass bed for *H. scabra* growth (from 7 cm to marketable size: 22 cm).

44 g; average length: 12 cm; n = 15), to a pond containing diseased juveniles (n = 114). Each experiment lasted three weeks and the evolution of the disease was recorded daily.

Study of predators

An abundance of crabs in the pens, accompanied by an excessive mortality of *H. scabra*, was observed at certain periods of the year. After the identification of the crab, an experiment was carried out in an outdoor pond (with three replicates). It consisted of putting different batches of *H. scabra* in the presence of crabs. A pond of 8 m long and 4 m width was subdivided into four compartments, each containing (*i*) 20 *H. scabra* of an average weight of 17 g (7 cm) with 5 crabs and without any food supply; (*ii*) 10 *H. scabra* of an average weight of 54 g (10 cm) with 5 crabs and without any food supply; (*iii*) 20 *H. scabra* of an average weight of 17 g (7 cm) with 5 crabs and with a food supply; and (*iv*) 10 *H. scabra* of an average weight of 54 g (10 cm) with 5 crabs and with a food supply. The pond contained sediments collected from the sea grass bed covering 10 cm of depth. The sea water was changed two times a week. The crabs were of an average weight of 51 g. Their food consisted of hermit crab cut in small pieces (3 cm long). The food was distributed daily at the end of afternoon. The control group consisted of 20 *H. scabra* (17 g) and 10 *H. scabra* (54 g) in ponds without any crabs. The ponds were

daily observed during 30 days and dead juveniles were counted at each observation.

Histology and scanning electron microscopy

For histology analyses, samples were fixed for 48 hours in Bouin's fluid. Specimens were then dehydrated through a graded series of ethanol solutions (70%, 90% and 100%), placed in butanol (overnight at 60° C), embedded in paraplast, cut into 7 μm sections and stained with G orange aniline blue azocarmin. For SEM, specimens were fixed for 48 hours in Bouin's fluid (without acetic acid). They were then dehydrated through a graded series of ethanol solutions (50%, 70%, 90% and 100%), critical point dried, mounted on stubs, coated with gold and examined with a Jeol JSM-6100 electron microscope.

Results

Salinity drop

The salinity of the sea water observed near the pens varied over the year but also daily. The extremes observed in a year were 17‰ and 35‰. Specimens submitted to a low salinity of 20‰ or below changed their behaviour: they stayed burrowed into sediment even at night when they are usually on the surface. Their body swelled (Fig. 3 A and B). Placed in ponds at a normal salinity (35‰), diseased
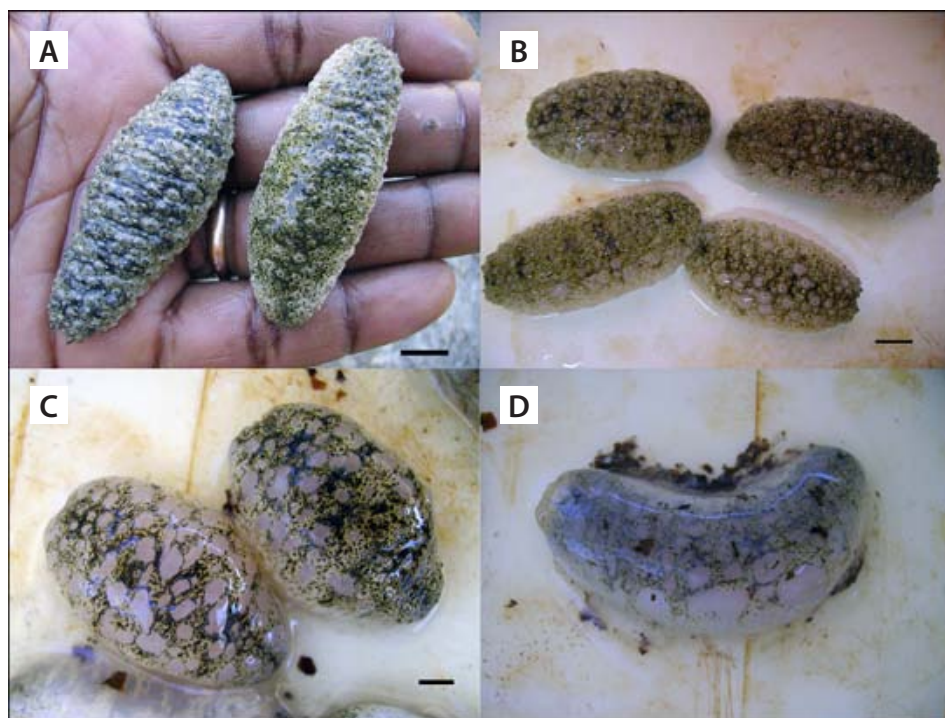

Figure 3. Juveniles of *H. scabra* placed in a tank at a low salinity (20‰). A: Juveniles at the beginning of the experiment (normal state); B: Juveniles under stress, the integument has become sluggish (stage 1); C: The integument is destroyed in different parts of the body wall (stage 2); D: The integument is totally destroyed (stage 3). Scale: bar = 1 cm.

Table 1. Variation of the average weight of *H. scabra* (n = 48 in total) after five weeks of farming in outdoor ponds at salinities of 20‰ and 35‰. The rearing density is 3 individuals m⁻². The statistical analysis compares final average sizes of specimens cultivated at 20‰ and 35‰.

Initial size	Final size (five weeks)		Statistical analysis
	Control (salinity: 35‰)	Experiment (salinity 20‰)	
3.80 +/- 1.49	28.04 ± 6.26	21.13 ± 8.67	$P_{T\,test}$ = 0.172
23.84 +/- 10.39	60.64 ± 17.03	70.70 ± 16.11	$P_{T\,test}$ = 0.318
67.79 +/- 5.06	128.53 ± 12.54	114.04 ± 22.64	$P_{U\,test}$ = 0.259
116.85 +/- 28.39	180.82 ± 50.22	188.36 ± 50.46	$P_{T\,test}$ = 0.801

specimens became normal within 96 hours. After 9 days at a salinity of 20‰ without the possibility to burrow into sediment, normal specimens (Fig. 3A) became weak, shrank in length, and developed a sluggish integument (Stage 1, Fig. 3 B). After 17 days, the epidermis was destroyed on various body parts (Stage 2, Fig. 3 C). Juveniles were eviscerated and their integument was strongly affected after 22 days (Stage 3, Fig. 3 D). They became translucent and died in the days that followed.

Table 1 shows the results of the experiment during which *H. scabra* of different sizes were kept on sediments at a low salinity of 20‰ during five weeks. During the experiment, they did not present any abnormal anatomic symptom. They stayed burrowed in the sediment even at night although their growth was not affected (Table 1). When *H. scabra* were placed in ponds at a very low salinity (10‰; equivalent to the conditions after a cyclone), they recovered from their stress if the exposure did not last more than 12 hours, after which they eviscerated (18 hours) or died (24 hours) (Table 2).

Table 2. Behaviour of *H. scabra* (n = 35 in total) at low salinity (10‰). Average weight and length of specimens: 41g, 8 cm.

Exposure time at low salinity of 10‰ (hours)	Specimen state
1	100% alive, normal
2	"
3	"
6	"
12	"
18	60% eviscerated, 40% dead
24	100% dead

Study of parasites

Epidemiology. The first signs of disease in the juveniles of *H. scabra* (7 cm, 15 g average) were observed in January 2007 in two outdoor ponds. In the first pond, there were 500 juveniles, of which 10% were infected. The second pond contained 480 juveniles, of which 8% were dead, 50% were highly infested and only 42% were healthy. The first symptoms of the disease always manifested near the cloacal opening: the integument became whitish over an area of few centimetres. This appeared afterward near the oral opening before covering the entire dorsal surface of the juvenile body wall. Once the whole surface was covered, juveniles became very weak, did not burrow into sediment anymore and died. We noted that the infection always appeared on the dorsal side of juveniles, never on the ventral side (Fig. 4). The same symptoms appeared again in February 2007 and reached three outdoor ponds: one pond for pre-growing and two ponds where larger individuals (> 6 cm long) were kept for growing. The two latter ponds contained respectively young sea cucumbers of 66 g (n = 82, of which 91% were still healthy) and 108 g (n = 114, which were all diseased). We note that this disease has never been observed in *H. scabra* cultivated in the pens set up in the sea grass bed.

Figure 4. Diseased juveniles of *H. scabra* (ulceration on dorsal side). Arrows indicate infected zone. Scale: bar = 1 cm.

Figure 5 (A, B, C) summarises the evolution of the disease in the affected ponds. On average, a mortality rate of 1% to 8% per week was recorded during the four weeks of the survey (Fig. 5 A). On the other hand no mortality was observed among the bigger specimens (Fig. 5 B and C). In general, whatever the specimen size, all specimens were totally infected and reached stage 3 in three to six weeks (Fig. 5 A–C).

Observed on SEM, the integument at the level of the wounds presented a disorderly structure (Fig. 6 B and C) compared to that from healthy areas (Fig. 6 A). The spicules were exposed in some areas (Fig. 6 D). In highly infected zones, the integument

was totally destroyed and spicules were entirely exposed (Fig. 6 E and F). The observation of the wounds did not show any high concentrations of bacteria (Fig. 6 C and F).

The histological study showed that the integument of healthy juveniles of *H. scabra* measured 1 mm thick and included an epidermis and a cuticle (0.24 mm), a connective tissue layer (0.5 mm), a circular muscle layer (0.24 mm) and a coelomic epithelium (0.02 mm) (Fig. 6 G). In the infected zone, the cuticle and the skin were totally destroyed as well as the upper part of the connective tissue; only the coelomic epithelium was intact (Fig. 6 H).
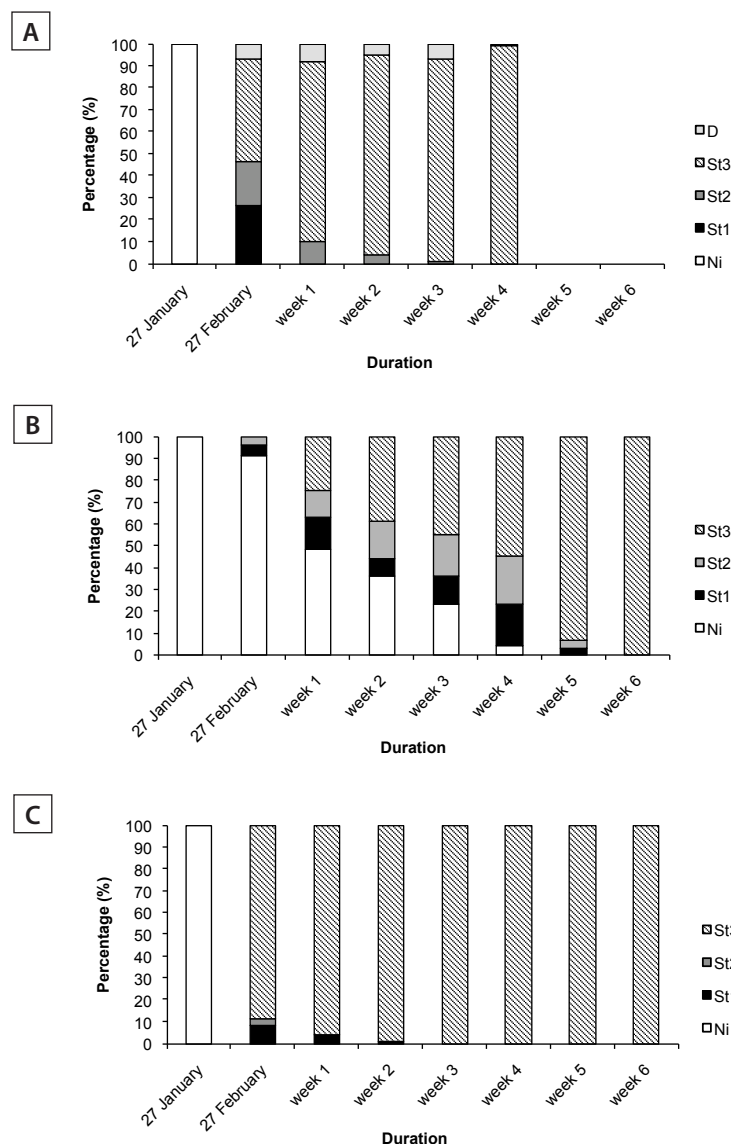

Figure 5. Evolution of the disease observed in outdoor ponds infested by isopod *Cymodoce* sp. The percentage expresses the number of specimens at different stages of the disease in relation to the total number of stock.
A: Average weight 15 g (n = 189); B: Average weight 66 g (n = 82);
C: Average weight 108 g (n = 114).
NI: non-infested specimen; St1: cloacal opening infested; St2: cloacal and oral openings infested; St3: the entire body wall infested; D: dead specimen.
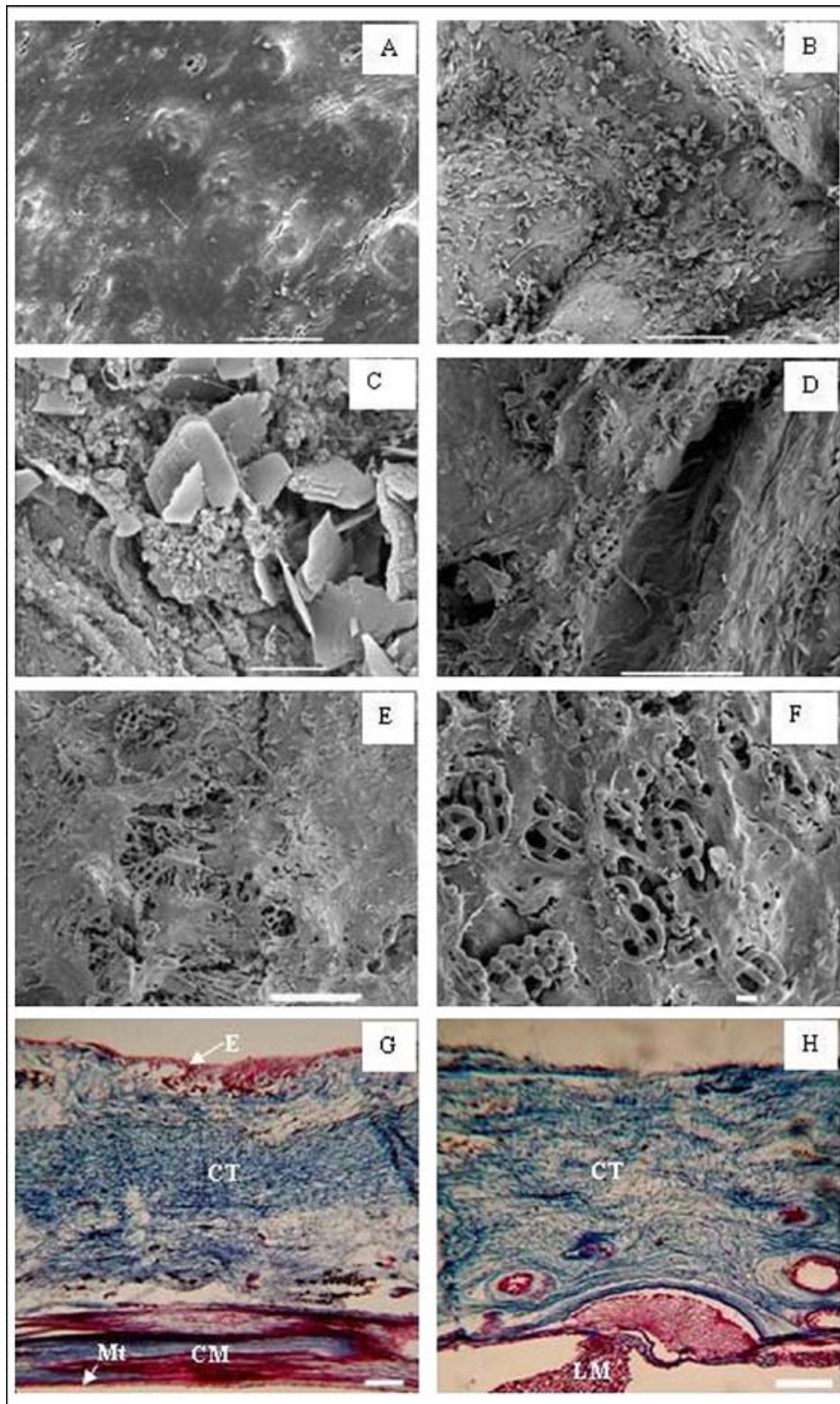
Figure 6. Integuments of *H. scabra* (A–F: observed on SEM; G–H: transversal section).
A and G: Healthy integuments; B–F and H: Infected integuments. B: disorderly structure;
C: disorderly structure (observed at 2000 x); D: spicules are exposed; E: spicules are totally
uncovered. F: E observed at 500 x; H: cuticle and epidermis totally destroyed.
E: epidermis; CM: circular muscle; LM: longitudinal muscle;
Mt: mesothelium; CT: connective tissue layer.
Scale: Bars = 100 µm for A, B, D, E, G and H; 10 µm for C and F.

Etiology

Field observations and laboratory experiments showed that the isopod *Cymodoce sp.* of the family of Sphaeromatidae (Order: Flabellifera) (Fig. 7) was responsible for the disease. They were observed in abundance on diseased *H. scabra* (15 to 30 isopods per juvenile, see Fig. 8), as well as on the substrate and in the water column. Outside of the period of the disease, we found only 10 isopods m^{-2} in the outdoor pond. On the other hand, during the period of the disease, we recorded 520 isopods m^{-2} on average. The density of the isopods was not uniform in the ponds: they were found in greater concentration near the sides close to the concrete wall. Stomach dissection of isopods showed presence in abundance of holothurian spicules.

Symptoms of diseased juveniles (n = 189) placed in ponds without isopods disappeared within the days following the transfer. Only two days after their transfer, the integument lesion closed up and juveniles became very active and began once again to follow the burrowing circadian cycle. After a week, 95% of juveniles were cured; after two weeks, they recovered totally from the disease (Fig. 9 A). The transfer of *T. jarbua* fish also provided very good results in curing the infection. The fish ate and eliminated the isopods in less than 10 days. After two weeks, the infection totally disappeared (Fig. 9B) as well as the isopods.

Study of predator

In 2007, an abnormal mortality of newly transferred juveniles of *H. scabra* in pens (in sea grass bed) was observed while the adults (average weight > 250 g) were never affected. A disappearance of 70% of 400 juveniles transferred in March 2007 was observed one month after the transfer; by the following month they had all disappeared. In August 2007, 800 juveniles disappeared one month after their transfer, followed by 500 more at the end of the same month. Only a few juveniles were found dead in the pens during the observation. Several day and night surveys helped to identify the causes of these abnormal disappearances. Crabs *Thalamita crenata* (Rüppell, 1830) (Fig. 10) of the Portunidae family were observed in abundance near and inside the pens. After several observations, we often found these
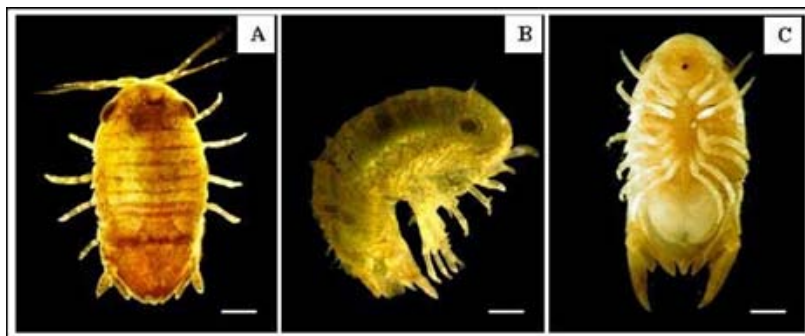

Figure 7. *Cymodoce* sp. A. Dorsal view; B: lateral view; and C: ventral view. Scale: Bar = 1 mm.
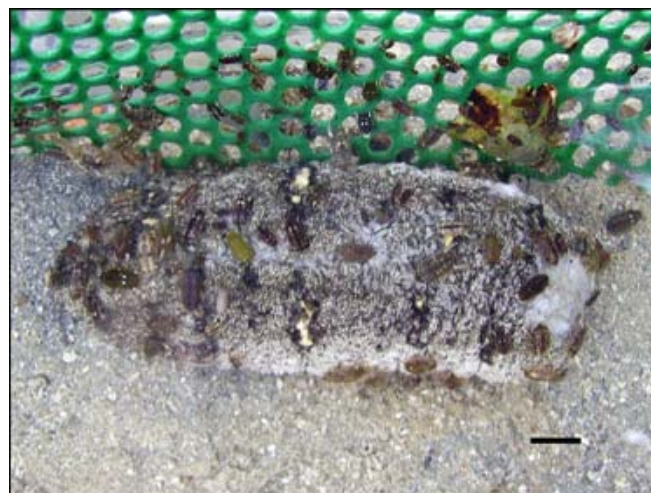

Figure 8. Young *H. scabra* covered by isopods.
Scale: Bar = 1 cm

crabs eating the newly transferred juveniles of *H. scabra*. Experiments in outdoor ponds showed that the crabs did not eat *H. scabra* when they were fed daily. On the other hand, five crabs were enough to kill and eat 20 juveniles of an average weight of 17 g within five days and 10 juveniles of an average weight of 54 g within 10 days when they were kept in external ponds without any food supply (Table 3).
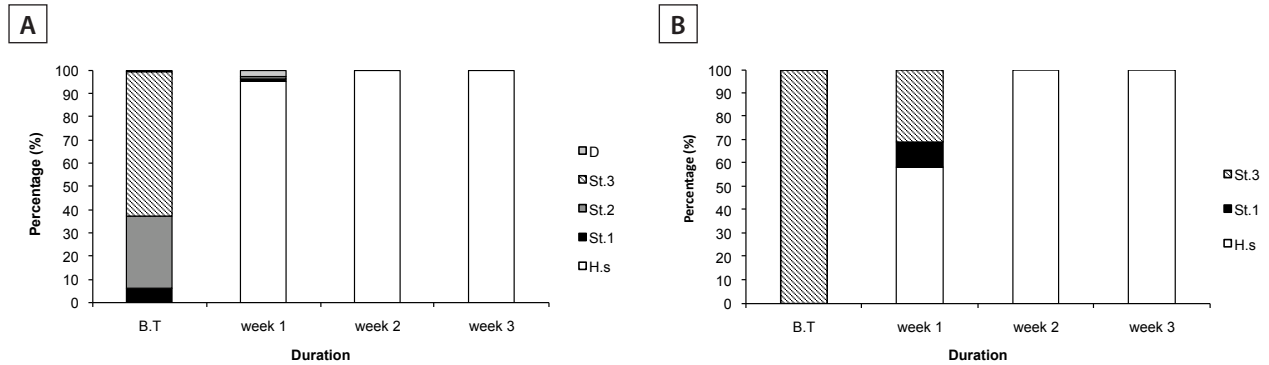

Figure 9. Evolution of the disease in farming ponds after the treatment. The numbers express the percentage of specimens at different stages of disease in relation to the total number of stock.

H.s: healthy specimen (or recovered from disease); St.1: infestation near the cloacal opening; St.2: cloacal and oral opening are infested; St.3: the entire body wall is infested; D: dead specimen; B.T: beginning of the treatment;

A: Treatment with newly collected sediments from the sea grass bed. Average size of *H. scabra*: 15 g (n = 189). B: Treatment with transfer of carnivorous fish *T. jarbua*. Average size of *H. scabra*: 108 g (n = 114).

Figure 10. Dorsal view of crab *Thalamita crenata* (Rüppell, 1830). Scale: Bar = 1 cm.

Table 3.   Average mortality (expressed in percentage) of *H. scabra* reared in outdoor ponds in presence of crabs *T. crenata*. Control: *H. scabra* farmed without crab. Batch A: 20 juveniles (average weight: 17 g; average length: 7 cm) + 5 crabs, without any food supply. Batch B: 10 adults (average weight: 54 g; average length: 10 cm) + 5 crabs, without any food supply.

	Starting	D1	D2	D 3	D4	D5	D6	D7	D8	D9	D10	D30
Control	0	0	0	0	0	0	0	0	0	0	0	0
Batch A	0	15	37.5	47.5	55	100	-	-	-	-	-	-
Batch B	0	0	0	0	5	5	5	55	65	80	100	-

Discussion

The rapid expansion of holothuriculture may favor the appearance of several diseases that could compromise the production of sea cucumbers on a large scale (Wang et al. 2004; Purcell and Eeckhaut 2005). The diseases could reach the larvae, juveniles, adults and even brood stock (Table 4). They are caused by various pathogenic agents such as bacteria (Morgan 2000; Becker et al. 2004; Eeckhaut et al. 2004; Wang et al. 2004), fungi (Wang et al. 2004), protozoa (Eeckhaut et al. 2004; Mercier et al. 2004), copepods (Wang et al. 2004), platyhelminthes (Eeckhaut et al. 2004; Wang et al. 2004), gastropods (Jangoux 1990), crabs (Jangoux 1990; Mohan and James 2005), isopods and viruses (Wang et al. 2007). Pathogenic agents and their treatment are still largely unknown (Xilin 2004). Within the diseases of sea cucumbers, skin ulceration is a widespread symptom (Table 4). It can be induced by various pathogenic agents and affect various holothurian species such as *A. japonicus* in China, *Isostichopus fuscus* in Ecuador and *H. scabra* in Australia, New Caledonia and Madagascar (Becker et al. 2004). Skin ulcerations are often accompanied by mucus secretions on the body, discoloration of the skin and behaviour changes (Purcell and Eeckhaut 2005). Other diseases without skin ulceration do not generally cause high mortality. Diseased specimens got thinner and became weak and lethargic. Some eviscerated in cases of severe infection, however, the integument do not present any suspicious lesion (Wang et al. 2004).

Besides pathogenic parasites, holothurians in farming are also victims of predators (Hamel et al. 2001). In the hatchery, copepods and ciliates are the most redoubtable predators of auricularia larvae (James et al. 1994). They may also attack newly metamorphosed juveniles (Tanaka 2000; Wang et al. 2004). Tectibranches (gastropods) and some amphipod species feed on pentactula larvae and newly metamorphosed holothurian juveniles (Mercier et al. 2000). In the natural environment, newly released juveniles are attacked and eaten by (*i*) different species of fishes (Hamel et al. 2001; Pitt and Duy 2004), (*ii*) crabs (Pitt and Duy 2004), (*iii*) shrimps (Pitt and Duy 2004) and (*iv*) sea stars (Hatanaka et al. 1994). This predation may lead to disappearance of the entire stock in a very short period of time (Mercier et al. 2000; Tanaka 2000).

In the natural environment, predators constitute one of main risks to be considered for sea cucumber aquaculture using pens. Careful observation of sites must be carried out in order to avoid them. In this work, the crab *T. crenata* was found to be the most redoubtable predator in the Toliara region. Aside from the control of biotic parameters, the choice of adequate sites for building sea pens is one of the key parameters for insuring the success of sea cucumber

farming. The sites should be sea grass bed zones, protected from winds and waves, with tides such that sea cucumbers are not out of the water for too long. Sandy muddy substrates, rich in organic matter, are also a favorable habitat. Even *H. scabra* could support a low salinity of 20‰ (Mercier et al. 1999a, 1999b; Pitt and Duy 2004). It is preferable to have the farming site far away from estuaries and fresh water flow. The ideal salinity for sea cucumbers is between 28‰ and 31‰ (Chen 2004; Xilin 2004).

References

Battaglene S.C. 1999. Progress in the production of tropical sea cucumbers *Holothuria scabra* and *Holothuria fuscogilva* for stock enhancement. SPC Beche-de-mer Information Bulletin 12:32.

Becker P., Gillan D., Lanterbecq D., Jangoux M., Rasolofonirina R., Rakotovao J. and Eeckhaut I. 2004. The skin ulceration disease in cultivated juveniles of *Holothuria scabra* (Holothuroidea, Echinodermata). Aquaculture 242:13–30.

Chen J. 2004. Present status and prospects of sea cucumber industry in China. p. 25–38. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. Fisheries Technical Paper No. 463. Rome: Food and Agriculture Organization of the United Nations.

Eeckhaut I., Parmentier E., Becker P., Da Silva S.G. and Jangoux M. 2004. Parasites and biotic diseases in field and cultivated sea cucumbers. p. 311–325. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. Fisheries Technical Paper No. 463. Rome: Food and Agriculture Organization of the United Nations.

Hamel J. F., Conand C., Pawson D. L., Mercier A. 2001. The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its biology and exploitation as Bêche-de-Mer. Advances in Marine Biology 41:129–223.

Hatanaka H., Uwaoku H., Yasuda T. 1994. Experimental studies on the predation of juvenile sea cucumber, *Stichopus japonicus* by sea star, *Asterina pectinifera*. Suisanzoshoku 42:563–566.

Table 4.   Diseases of cultivated sea cucumbers recorded between 2000 and 2007. The bar indicates that the responsible agent was not identified.

Diseases	Symptoms	Infected stages	Responsible agent	Country	References
I	Darkening of the body edges; diseased specimens undergo autolysis and the body completely disintegrates within two days.	Auricularia	Bacteria	China	Wang et al. 2004
II	The stomach and/or the intestine walls of larvae are atrophic.	Auricularia	Bacteria	China	Wang et al. 2004
			/	China	Liu et al. 2004
			Protozoa	Ecuador	Mercier et al. 2004
III	Presence of gas bubbles inside the body of the larvae, which results in anorexia.	Auricularia	/	China	Wang et al. 2004
IV	Diseased specimens present oedema near the peristome. The juveniles' tentacles cannot retract; they lose the ability to remain attached to available substrate. The juveniles may eviscerate. The body wall becomes covered of mucus; the epidermis disappears and the whole body can dissolve with autolysing process.	Juveniles	Bacteria	China	Wang et al. 2004
			Virus	China	Wang et al. 2007
V	The wound appears near the cloacal orifice and extends over the whole body surface. The integument becomes whitish and spicules may be exposed. The highly infested specimens become weak and died in the days that followed. The disease is highly contagious and expands quickly in farmed populations.	Juveniles	Copepods	China	Wang et al. 2004
		Juveniles	Bacteria	Madagascar	Becker et al. 2004; Eeckhaut et al. 2004
		Juveniles	/	Solomon Islands	Hamel et al. 2001
		Juveniles	/	/	Purcell and Eeckhaut 2005
		Juveniles and adults	Bacteria	China	Wang et al. 2004
		Broodstock	Bacteria	Australia	Morgan 2000
		Juveniles and adults	Isopods	Madagascar	Present work
VI	Infected individuals are weak and anorexic. The body becomes stiff and is covered in excessive mucus. As the infection progresses, the entire viscera is usually expelled and eventually the infected specimen dies.	Juveniles and adults	Platyhelminth	China	Wang et al. 2004
VII	The papillae of diseased specimens become white. The body wall appears bluish white with the development of the infection. The body wall becomes thinner and the affected individuals develop oedema.	Juveniles and adults	Fungi	China	Wang et al. 2004
VIII	The protozoa live in the digestive tract and in the respiratory trees of holothuroids and may provoke internal wounds. The infected animals tend to be weak and sluggish. The body usually shows no conspicuous lesions, however the intestine, respiratory tree, etc. can be eviscerated in severe infections.	Juveniles and adults	Protozoan	China	Wang et al. 2004
			Protozoan	/	Eeckhaut et al. 2004

James D.B., Rajapandian M.E., Gopinathan C.P. and Bascar B.K. 1994. Breakthrough in induced breeding and rearing of the larvae and juveniles of *Holothuria (Metriatyla) scabra* Jaeger at Tuticorin. p. 66–70. In: Rangarajan, K. and James D.B. (eds). Proceedings of the National Workshop on Beche-de-mer. Central Marine Fisheries Research Institute, Cochin, India. 46.

Jangoux M. 1990. Diseases of Echinodermata. p. 439–567. In: Kine, O. (ed). Disease of marine animals. Vol. 3. Hamburg, Germany: Biologische Anstalt Helgoland.

Mercier A., Battaglene S.C. and Hamel J.F. 1999a. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Journal of Experimental Marine Biology and Ecology 239:125–156.

Mercier A., Battaglene S.C. and Hamel J. F. 1999b. Daily activities of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Abstracts 34th European Marine Biology Symposium, Ponta Delgada, 13–17 September 1999, Azores, Portugal, p. 83.

Mercier A., Battaglene S.C. and Hamel J.F. 2000. Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*. Journal of Experimental Marine Biology and Ecology 249:89–110.

Mercier A., Hidalgo R.Y. and Hamel J.F. 2004. Aquaculture of the sea cucumber, *Isostichopus fuscus*. p. 347–358. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. Fisheries Technical Paper No. 463. Rome: Food and Agriculture Organization of the United Nations.

Mohan R.M.K. and James D.B. (2005). Incidence d'une infestation parasitaire dans *Holothuria atra* Jaeger. SPC Beche-de-mer Information Bulletin 22:38.

Morgan A.D. 2000. Aspects of sea cucumber broodstock management (Echinodermata: Holothuroidea). SPC Beche-de-mer Information Bulletin 13:2–8.

Pitt R. and Duy N.D.Q. 2004. Breeding and rearing of the sea cucumber *Holothuria scabra* in Viet Nam. p. 333–346. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. Fisheries Technical Paper No. 463. Rome: Food and Agriculture Organization of the United Nations.

Purcell S.W. 2004. Criteria for release strategies and evaluating the restocking of sea cucumber. p. 181–192. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. Fisheries Technical Paper No. 463. Rome: Food and Agriculture Organization of the United Nations.

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

Tanaka M. 2000. Diminution of sea cucumber *Stichopus japonicus* juveniles released on artificial reefs. Bulletin of the Ishikawa Prefecture Fisheries Research Center 2:19–29.

Wang Y.G., Zhang C., Rong X., Chen J. and Shi C. 2004. Disease of cultured sea cucumber, *Apostichopus japonicus*, in China. p. 297–310. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. Fisheries Technical Paper No. 463. Rome: Food and Agriculture Organization of the United Nations.

Wang P., Chang Y., Yu J., Li C. and Xu C. 2007. Acute peristome edema disease in juvenile and adult sea cucumbers *Apostichopus japonicus* (Selenka) reared in North China. Journal of Invertebrate Pathology 96:11–17.

Xilin S. 2004. The progress and prospects of studies on artificial propagation and culture of the sea cucumber, *Apostichopus japonicus*. p. 273–276. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. Fisheries Technical Paper No. 463. Rome: Food and Agriculture Organization of the United Nations.