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Analysis of the impact of *Holothuria scabra* intensive farming on sediment

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Abstract: We analysed the impact of intensive *Holothuria scabra* farming by comparing various parameters of the sea cucumber food (i.e. the upper layer of the sediment) inside and outside the pens of two farms in the south-west of Madagascar. Our analyses showed that : (i) the proportion of the finest grain size fraction (< 250 µm) decreased from 5 to 14%, (ii) the global carbonate proportion decreased by 5%, (iii) aragonite was the carbonate type most affected by holothurians, followed by calcite and magnesian calcite, (iv) the total organic matter did not vary significantly, (v) the number of bacteria decreased by up to 50%, and (vi) the concentration of photosynthetic microorganisms fell by up to 22%, within sea farm pens. Based on these results we suggest that fallow practice should be considered in the future for holothuriculture.

Résumé : *Analyse de l'impact de l'élevage intensif d'*Holothuria scabra *sur le sédiment*. L'impact de l'élevage intensif d'*Holothuria scabra* sur le sédiment a été étudié par comparaison de plusieurs éléments alimentaires de cette espèce (i.e. provenant de la couche supérieure du sédiment) à l'intérieur et à l'extérieur des enclos d'élevage. Cette étude a été réalisée sur le sédiment de deux fermes dans le sud-ouest de Madagascar. Nos analyses démontrent que : (i) la proportion de grains de petite taille (< 250 µm) diminue de 5 à 14 % , (ii) le taux de carbonates diminue de 5 %, (iii) l'aragonite est le type de carbonates le plus affecté par l'activité des holothuries, suivie par la calcite et la calcite magnésienne, (iv) le taux de matière organique ne diffère pas significativement, (v) le nombre de bactéries diminue jusqu'à 50%, et (vi) la concentration de microorganismes photosynthétiques diminue jusqu'à 22%, à l'intérieur des enclos d'élevage. Au vu de ces résultats, la pratique de jachères devrait être développée à l'avenir en holothuriculture.

Keywords: Holothuria scabra ● Sea cucumber ● Aquaculture● Sediment ● Bacteria ● Organic matter

Introduction

World-wild stocks of sea cucumbers are depleting rapidly due to the high demand of these seafood organisms for the Chinese market (Purcell et al., 2011). The disappearance of wild stocks is not only a problem at the ecological level (these organisms being amongst the best bioturbators of the sediments in many marine ecosystems; Wolkenhauer et al., 2010) but it is also a huge social problem as the sea cucumber trade ensures a livelihood for many thousands of people in developing countries (Eriksson et al., 2012). One of the best solutions to this problem is the development of efficient aquaculture systems, such as farming methods where coastal villagers are involved in key aspects

(McVean et al., 2005; Robinson & Pascal, 2009; Eriksson et al., 2012). In tropical regions, *Holothuria scabra* Jaeger, 1833, commonly called sandfish, has become an ideal candidate for the development of sea cucumber farming because it has a high value on the Chinese market and because it can be reared in marine pens kept by coastal villagers (Robinson & Pascal, 2009). Farming sandfish requires lagoons for ranching or grow-out in sea pens which both offer a potential alternative economic activity for impoverished coastal communities and may also provide a base for replenishing depleted sandfish stocks (Bell et al., 2008). A community-based farming project using hatchery-reared sandfish is currently in place in Madagascar with the purpose of providing a livelihood alternative and establishing a trade company centered on sandfish aquaculture (Eeckhaut et al., 2008; Robinson & Pascal, 2009). Sea cucumber farming is currently done in Australia, Vietnam and Philippines and is extending to other countries (Toral-Granda et al., 2008).

It is well known that overfishing of sea cucumbers leads to indirect, deleterious effects on reefs and soft-bottom ecosystems (Purcell et al., 2011). The juveniles are important prey in food webs (So et al., 2010) and adults are important in ecosystem function (Birkeland, 1988; Uthicke, 2001b; Wolkenhauer et al., 2010). Many aspidochirote sea cucumbers feed on large quantities of sediments and convert organic detritus into animal tissue and nitrogenous wastes, which can be taken up by algae (Uthicke & Klumpp, 1998; Uthicke, 2001b). On coral reefs, where nutrients may be a limiting growth factor, their role in nutrient recycling is likely to be significant (Uthicke, 2001a). Through bioturbation, some species oxygenate sediments (Bakus, 1973; Massin, 1982; Uthicke, 1999 & 2001b) and increase the productivity of sea grasses (Wolkenhauer et al., 2010). Removal of sea cucumbers in some systems is believed to reduce primary production for the whole food chain (Purcell et al., 2011). In a healthy reef, the dissolution of $CaCO_3$ sediment by bioeroders appears to be an important component of the natural $CaCO_3$ turnover and a substantial source of alkalinity as well (Schneider et al., 2011).

Even if the presence of sea cucumbers is beneficial for reefs and soft-bottom ecosystems, one could speculate that large-scale farming enterprises in lagoons may result in some alterations of benthic community structure. Until now, only the abnormal appearance of crab predators has occurred in sea cucumber farming (Lavitra et al., 2009) but this suggests a remodeling of benthic community structure. High levels of mortality have been experienced at some farming sites in Madagascar that has led to the development of a range of predator control techniques (Tsiresy et al., 2011; Robinson & Pascal, 2012). Such activities, particularly as farming area increases, may potentially impact the benthic faunal structure associated with adjacent sea grass beds. To date, there has been no study on the impact of intensive *H. scabra* farming on the sediment. It has, however, been proved recently that sea cucumbers process carbonate sand and dissolve $CaCO_3$ as part of their digestive process (Schneider et al., 2011; Vaucher, 2012) which raises the question as to whether high density sea cucumber farming could drastically change sediment components. The aim of the present paper is to analyse various mineral and organic components of the sediment from two farms in south-west Madagascar where intensive farming has been carried out since June 2009, in order to determine if the high density of sandfish has led to modifications.

Materials and Methods

Studied sites and sampling

H. scabra farming and monitoring began in June 2009 in two villages, Sarodrano and Tampolove, on the south-west coast of Madagascar (Fig. 1). The two villages are 150 km apart: Sarodrano is located to the south of Toliara, Tampolove other is in the north (Fig. 1). Farming is still ongoing in these villages. The farming began with the transfer of 6-cm long (approx. 15 g in weight) juveniles from the Madagascar Holothurie company, based in Toliara (Eeckhaut et al., 2008) to the villages. At that time, they were transferred into sea pens of 600 m^2 delineated by a plastic net and situated in the intertidal zone where sea grass beds occur. Sediments were muddy, always submerged even at low tides, and located several hundred meters from the villages (Robinson & Pascal, 2009). In total, 750 6-cm long *H. scabra* juveniles were transported by road in batches, according to the method of Purcell et al. (2006) and Purcell & Eeckhaut (2005). Juveniles were distributed in sea pens over the surface such that their density was at a maximum of 2 individuals per m^2 at the beginning of the trial, which is the density recommended in Lavitra et al. (2010). Farmers had to clean the pen nets every day and take care of their sandfish stocks (Robinson & Pascal, 2009).

Samples of sediments were taken from around farms in Sarodrano and Tampolove in September 2011. At that time, six cycles of farming had been completed in sea pens at each village. The upper layer of the surface sediment where *H. scabra* feeds (the top 2 cm) was sampled from inside sea pens of each village farm. To compare the impact of farming, we also collected samples of the upper sediment layer from outside the sea pens. Samples inside sea pens were taken at their center and near their borders. There was no cage control here but ’outside’ samples that were taken approximately 10 m distant from sea pens to the north, south, east and west. These samples were done because it

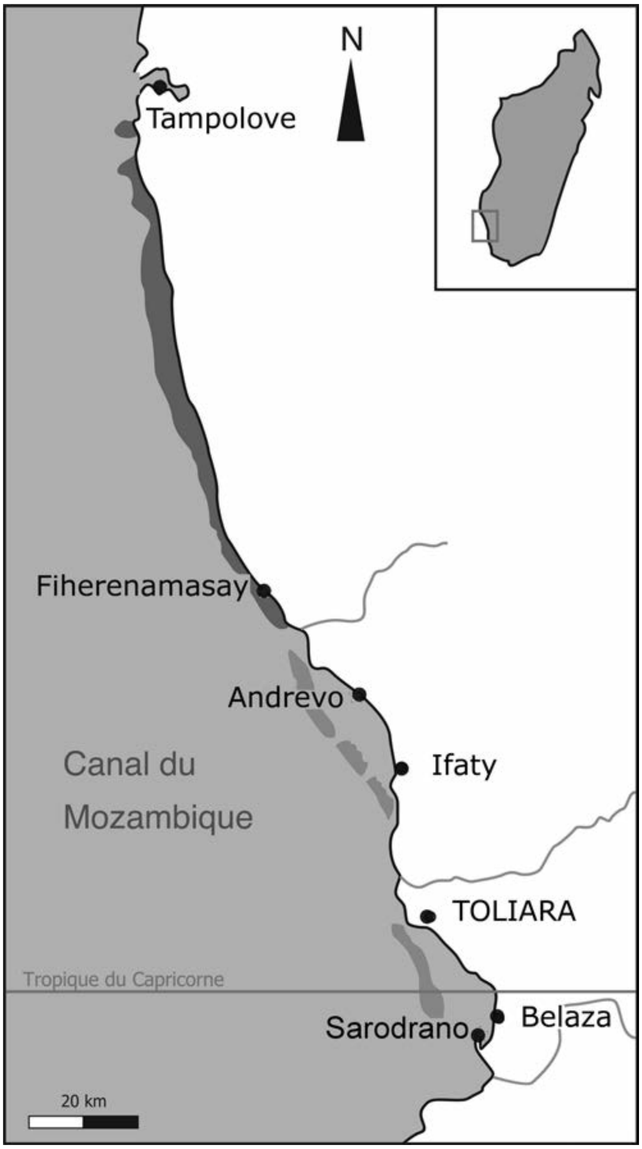

Figure 1. Map of South-West Madagascar showing the location of the two villages where intensive sea cucumber farming was practised during two years.

was just impossible to make understand the villagers-seafarmers to maintain cages without sea cucumbers during two years. Sediment samples were either directly stored at 4°C or placed in 4% paraformaldehyde (in 0.2 mm filtered seawater) before being stored at 4°C. Once in Toliara, a fraction of the non-fixed samples was used to quantify the chlorophyll a concentration arising from photosynthetic microorganisms. Another fraction was put in a drying kiln at 60°C for 48 hours, for later in analysis in the Biology of Marine Organisms and Biomimetics Laboratory. These dried samples were used for the determination of: (i) the grain-size, (ii) the organic matter content, (iii) the carbonate abundance, and (iv) the nature of the minerals. Samples fixed with 4% paraformaldehyde were used to quantify bacteria.

Organic compounds

Total organic matter (TOM). The organic matter content was determined by carbonization ($n = 5$). Because marine sediments close to coral reefs are carbonate-rich, sediment samples (150 g) were first decarbonated by dissolution in 37% HCl until effervescence disappeared. The decarbonated sediments were then rinsed with distilled water, centrifuged and dried for 48 hours at 60°C. Samples were weighed, carbonized at 450°C for 4 hours and reweighed. The organic matter content was calculated as the weight lost during carbonization (Gillan et al., 2005).

Bacterial count and chlorophyll a quantification. The quantity of bacteria and photosynthetic microorganisms in sediments were estimated using diamidino-2-phenylindole (DAPI) coloration and chlorophyll a quantification, respectively.

Sediment samples ($n = 5$ for each village) fixed with paraformaldehyde were rinsed three times with 0.2 µm filtered seawater and stored at -20°C in a 1:1 mix of filtered seawater and 100% ethanol. Tubes containing 3 g (wet weight, ww) of each sediment sample and 750 µl of the mix were treated three times by sonication (Ika, Labortechnik, Germany) for 30 seconds using pulse mode, with a rest of 30 seconds between two runs with a sterilized sonic probe (50 W, cycle 0.5 and 80% amplitude). This treatment detaches bacteria from the particles (Epstein & Rossel, 1995). After the last run of sonication, samples were rested for 30 sec to allow large particles to settle. A volume of the supernatant (75 µl) was put in a tube containing 10 mL of filtered seawater, then this mixture was filtered using an isopore membrane filter (0.2 µm, Millipore GTTP) placed above a 0.45 µm filter (Millipore HAWP). Filters were stained with DAPI (1 µg mL^{-1}) for 3 minutes, rinsed with MilliQ water and 70% ethanol, air dried and mounted between a microscope plate and glass slide in Vectashield (Vector Lab., Burlingame, USA). Pictures of every filter were taken with a Carl Zeiss Axio Scope.A1 microscope fitted for epifluorescence microscopy, and connected with a Carl Zeiss AxioCam Icc3 camera controlled by Carl Zeiss AxioVision software. In each filter 14 pictures were taken randomly along two transects, a vertical and a horizontal which crossed at the center of the filter, forming a right angle. The bacteria in each picture were manually recognized and counted with Jeol SamAfore 3.0 pro software. This counting scheme guarantees the lowest amount of errors (Kirchman et al., 1982; Montagna, 1982). The number of bacteria from 14 pictures was multiplied by 1,468 in order to obtain the number of bacteria in a filter and again multiplied by 10 to obtain the total number of bacteria in the supernatant, expressed as number per gram of sediment (dry weight, dw).

Sediment samples (n = 5 for each village) crushed with a mortar and pestle. Three grams of the crushed samples were put in a tube with 9 ml of 90% acetone and kept in the dark for 12 h at 4°C in order to extract pigments (Plante-Cuny, 1974). Tubes were then centrifuged at 3,000 rpm for 1 min. The optical density of the supernatant was measured at 665 and 750 nm before and after acidification with 1N HCl (Lorenzen, 1967).

Mineral composition

Grain size. Ten sediment samples (n = 5 inside and n = 5 outside pens) of 100 g from each village were sieved through meshes of 2000, 1000, 500 and 250 µm and weighed to determine their grain size distribution.

Carbonate fraction. The sea cucumber skeleton is composed of magnesian calcite. Carbonates, originating from inorganic and organic sources that could be important for the development of these organisms were investigated. To achieve this, dried sediment samples (n = 5 for each village) were weighed, decarbonated by dissolution in 37% HCl until effervescence disappeared. The decarbonated sediments were then rinsed with distilled water, centrifuged, oven-dried for 48 h at 60°C and finally reweighed. The carbonate content was calculated as the weight lost during dissolution.

X-ray diffractometry (XRD). XRD was used to identify specific crystalline compounds, both mineral and organic, based on their crystal structure. XRD was carried out to identify the minerals in sediments from both villages. Only mineral phases that are crystallized are detectable with this technique and the detection limits are about 3-10%, depending on the mineral. The sediment samples were dried at 60°C for 48 h and sieved through a 500 µm mesh to separate coarse grains (fraction > 500 µm) from the fine grains (fraction < 500 µm). Each fraction was ground in an agate jar down to an average grain size of about 50 µm. The resulting powder was pressed into sample holders and X-rayed using a Bruker-Siemens D5000 diffractometer. Operating conditions were set at 40 kV and 30 mA (CuKα radiation). XRD patterns were recorded from 4 to 70° 2θ with a 0.05° 2θ step length and 3 sec counting time per step. A longer counting time (10 sec) was set for the measurement of particular diffraction peaks. A graphite monochromator was mounted in front of the X-ray detector to filter out undesirable radiations, particularly X-ray excited fluorescence from iron atoms. The intensity of the diffraction peaks was used to determine the abundance of four minerals: (i) quartz, constituted of almost pure SiO_2, (ii) calcite, the most stable polymorph of calcium carbonate ($CaCO_3$), (iii) aragonite, a thermodynamically metastable form of $CaCO_3$ at standard temperature and pressure, and (iv) magnesian calcite $(Ca,Mg)CO_3$, a variety of calcite consisting of randomly substituted magnesian carbonate in a disordered calcite lattice. Magnesian calcite is the typical biomineral in echinoderm skeleton.

Statistical analyses

For direct DAPI counts, total organic content, carbonates proportions and chlorophyll a content, non-parametric Mann-Whitney *U*-tests were performed (Statistica 7.0) in order to compare inside and outside sea pens. The arcsin transformation was used for percentages ($x'=\arcsin \sqrt{x}$).

Results

Granulometry and minerals

The grain-size distribution of the sediment was different in Sarodrano and Tampolove (Fig. 2). The finest grain class (< 250 µm) accounted for more than 79% in Tampolove but no more than 34% in Sarodrano, both inside and outside the pens. Comparison of grain sizes between the villages indicated that the grain class < 500 µm was substantially more important in Tampolove than in Sarodrano. Very coarse grains (> 2 mm) were uncommon in Tampolove. However, even though the grain size distribution observed in the two villages were different, comparison of sand grain classes between sediments from inside and outside pens within each village showed a similar trend. The average loss of fine grains (< 250 µm) inside pens was 14 and 5% in Sarodrano and Tampolove, respectively (the loss of fine grains inside pens was significant in Sarodrano but not in Tampolove, $p < 0.05$). Each of the other four grain classes in Sarodrano gained 3% on average. The uniformity of the gain suggests that the decrease in the proportion of fine grains (< 250 µm) was due to their loss and not an absolute gain in coarser grains.

The carbonate proportion inside the pens, as determined by acid dissolution of the sediments, was significantly lower than those recorded outside ($p < 0.05$) (Fig. 3). The carbonate contents were 32 and 37% inside and outside the pens, respectively, in Sarodrano, and 78 and 84%, in Tampolove. The loss of carbonates in the pens was about 5% since the beginning of the farming.

Figure 4 illustrates the abundance of quartz, calcite, magnesian calcite and aragonite in Tampolove and Sarodrano for the two grain classes (> 500 µm and < 500 µm). In Sarodrano, the quartz abundance inside the pens increased by 32% for sand grains > 500 µm and by 35% for those < 500 µm. Magnesian calcite, calcite and aragonite

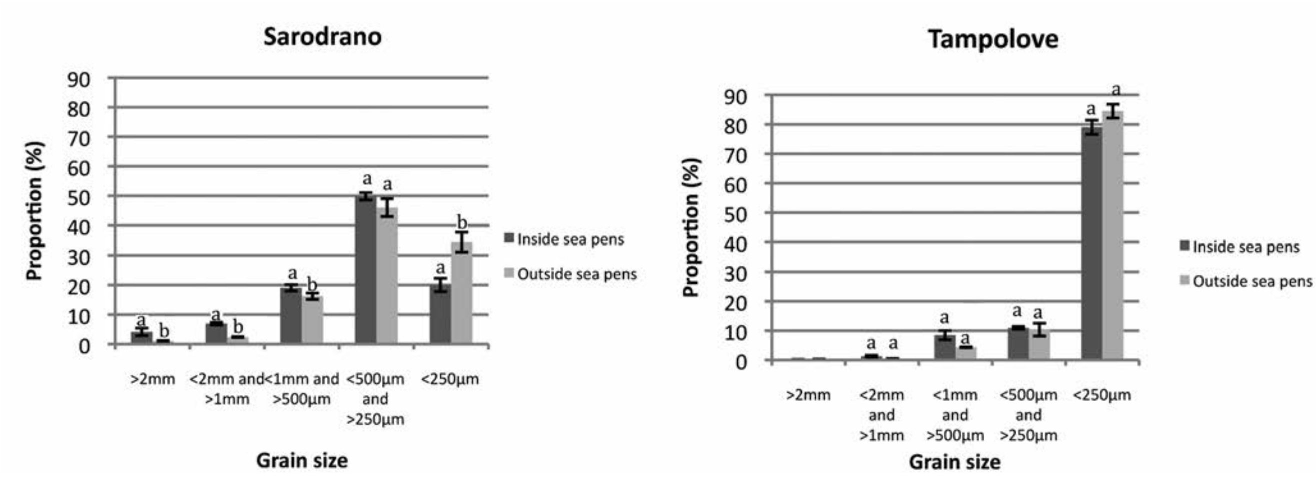

Figure 2. Grain size of sediment (mean ± SD, $n = 5$) from Sarodrano and Tampolove, comparing inside and outside sea pens where intensive *H. scabra* farming was practiced. Different symbols (a, b) in a given grain size class indicate significant difference between inside and outside pens (U-test, $\alpha = 0.05$).

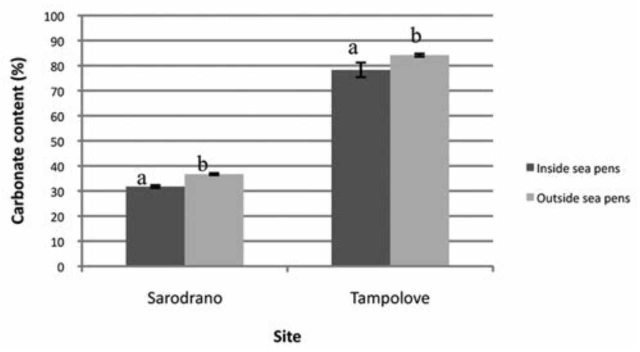

Figure 3. Carbonate abundance (mean ± SD, $n = 5$) in sediments from inside and outside sea pens in the two villages. Different symbols (a, b) in a site indicate significant difference between inside and outside pens (U-test, $\alpha = 0.05$).

concentration decreased by 4, 9 and 19%, respectively, in grain class > 500 µm and by 8, 10 and 17% in grain class < 500 µm. A similar trend is observed for small grains in Tampolove, where grain class < 500 µm represented 90 and 95% inside and outside the pens, respectively. This grain class showed an increase in quartz abundance of 11% in relation to a decrease in magnesian calcite, calcite and aragonite abundance of 5, 1 and 5%, respectively. Sand grains > 500 µm comprised only 10% of the total sand dry weight in Tampolove and variations in mineral content were not observed. In both villages, aragonite was the carbonate type most affected by farming.

Organic components

The total organic matter (TOM) values recorded from sediments inside and outside sea pens were not significantly different (U-test, $p < 0.05$) in either village. TOM represented 1.07 and 0.56% of the total dry weight of sediments from inside and outside sea pens in Sarodrano, respectively, and 0.64% (inside) and 0.74% (outside) in Tampolove (Fig. 5). The average number of bacteria revealed by the DAPI counts was different in Sarodrano and Tampolove: 9×10^8 g^{-1} and 4×10^8 g^{-1}, respectively. However, the trend observed in the two villages was similar: the average number of bacteria was significantly lower inside the pens than outside (U-test, $p < 0.05$). The difference was 1.34×10^8 cells g^{-1} in Sarodrano and 2.3×10^8 cells g^{-1} in Tampolove (Fig. 6). Likewise, the lowest concentrations of chlorophyll a were found in Tampolove which should indicate that photosynthetic microorganisms were less concentrated in the sediments of Tampolove (Fig. 7). Further, the concentration of chlorophyll a in sediments was significantly lower inside the pens than outside for both villages (U-test, $p < 0.05$): 26 mg g^{-1} (inside) and 39 mg g^{-1} (outside) in Sarodrano; and 4 mg g^{-1} (inside) and 18 mg g^{-1} (outside) in Tampolove.

Discussion

Good survival and the fastest possible growth in sea pens are two of the main conditions for insuring the economic sustainability of sea cucumber farming. In sea cucumber farming, survival is more a matter of predation and theft: high survival can be obtained by cleaning the fences properly and having adequate security (Tsiresy et al., 2011). Sea cucumber growth relies primarily on the correct stocking density. Lavitra et al. (2010) showed that, in Madagascar, there is a critical biomass value of 692 g m^{-2} above which individuals do not grow anymore. As the

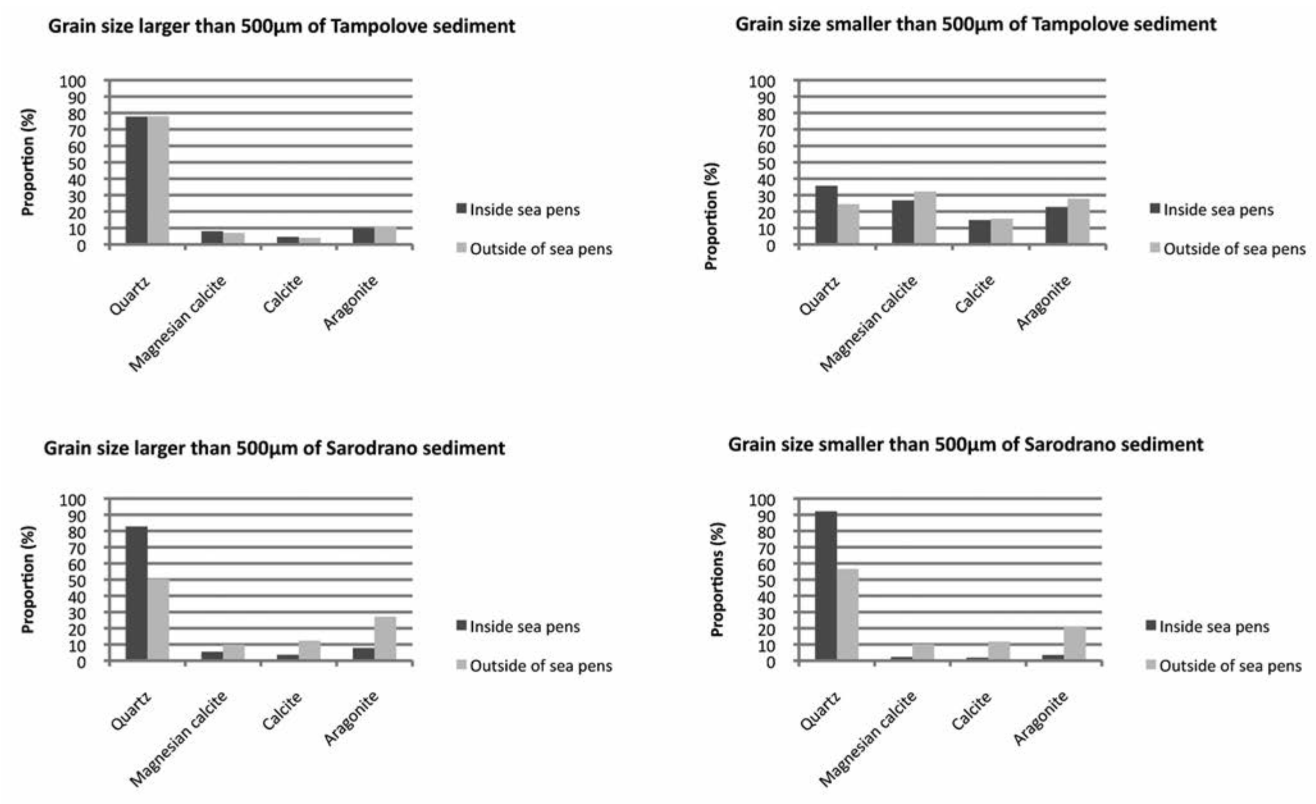

Figure 4. Mineral diversity and semi-quantification of minerals by X-ray diffraction from sediments of (**a & c**) grain size larger than 500 µm (*n* = 1) and (**b & d**) grain size smaller than 500 µm (*n* = 1) in the two villages, Tampolove (**a & b**) and Sarodrano (**c & d**).

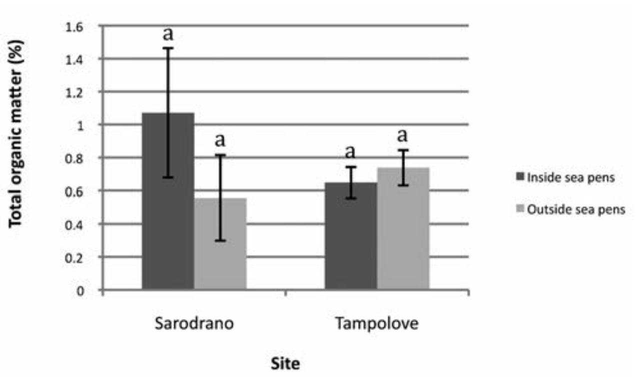

Figure 5. Total organic matter (mean ± SD, *n* = 5) of sediment determined by carbonization after acid dissolution for sediment from inside and outside sea pens in the two villages. Symbol (a) in a site indicates no significant difference between inside and outside pens (*U*-test, α = 0.05).

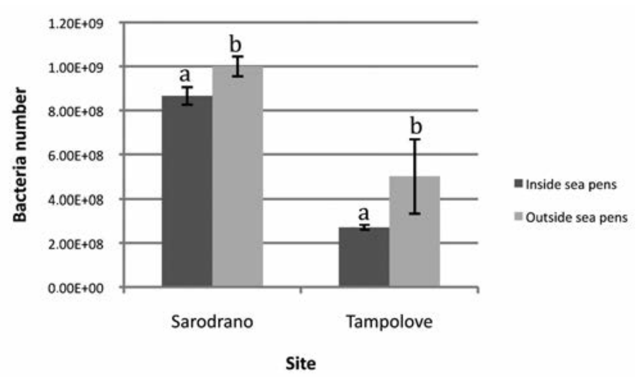

Figure 6. Bacterial biomass (No/g of sediment in dw) as revealed by DAPI staining (mean ± SD, *n* = 5) in the sediment collected *in situ*, from inside and outside sea pens Different symbols (a, b) in a site indicate significant difference between inside and outside pens for a site (*U*-test, α = 0.05).

weight of commercial *H. scabra* must be at least 350 g (22 cm long), the density in sea pens should not be more than 2 individuals per m^{-2}. This optimal density has been applied in the farming of *H. scabra* in the two monitored villages. We analysed the impact of farming at this density by comparing various parameters of the sea cucumber food, i.e. the upper layer of the sediment, inside and outside sea pens after two years of intensive farming. Our analyses showed that: (i) the proportion of the finest grain size class (< 250 um) decreased from 5 to 14%, (ii) carbonate abundance decreased by 5%, (iii) aragonite was the carbonate type most affected by holothurians farming,

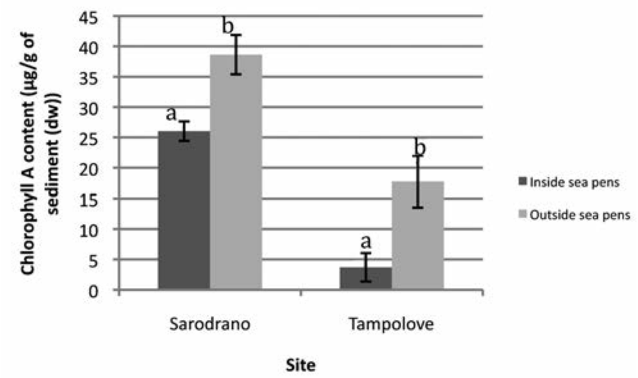

Figure 7. Chlorophyll a content (µg.g^{-1} dry weight) (mean ± SD, *n* = 5) from inside and outside sea pens in the two villages Different symbols (a, b) in a site indicate significant difference between inside and outside pens (*U*-test, α = 0.05).

followed by calcite and magnesian calcite, (iv) total organic matter did not vary significantly inside or outside sea pens, (v) the number of bacteria decreased by up to 50%, and (vi) the concentration of photosynthetic microorganisms fell by up to 22%.

Hammond (1981) observed through laboratory experiments that *Holothuria Mexicana* Ludwig, 1875*, Holothuria arenicola* Semper, 1868*,* and *Isostichopus badionotus* (Selenka, 1867) dissolve about 0.065-0.256% of the calcium carbonate sediments ingested. He estimated that the process of dissolution by holothurians has insignificant implications for the mass carbonate balance of coral reefs, as only 2% or less of the annual carbonate fixation in reef flats or lagoons may be lost. Sedimentological effects would also be inappreciable, as experimental dissolution of sediment resulted in only minor changes in their grain size distributions (Hammond, 1981). However, Schneider et al. (2011) suggested that nearly 50% of the observed reef nighttime $CaCO_3$ dissolution could be explained by the sea cucumber digestive process. Their results indicate that the digestive physiology of sea cucumbers may play an important role in the life of a coral reef. The aspidochirotes would be involved in reef ecology through two contrasting processes: reef dissolution (Schneider et al., 2011) and enhanced productivity due to nutrient cycling (Uthicke & Klumpp, 1998; Uthicke, 2001a & b). Schneider et al. (2011) claimed that there is an urgent need to understand the impact of removal of sea cucumbers through commercial harvest on reef health and resilience. Conversely, in the present study, we have a clear example that increasing the numbers of sea cucumbers induces modifications on the sediment composition and grain size: the fine fraction of the sediment and its carbonate proportion were reduced after two years of intensive farming. In Tampolove, as 80% of the sediment was composed of grains < 250 µm, the decrease was less important. In a recent work (Plotieau, 2013), we suggested that rapid growth of *H. scabra* was favored when individuals live on sediment where the grain size class < 250 µm was substantial. For a given volume, fine sediments would be nutritionally superior to coarse sediments because of their greater surface area and consequent higher number of nutritive microorganisms. The fact that the proportion of fine sediments < 250 µm in sea pens decreased by up to 14% (dw) in the current study means that fine sediments are digested if they are made of organic matter and/or dissolved if they are carbonaceous elements. We demonstrated that the global carbonate proportion decreased by 5%, and that the quartz proportions increased by up to 34% in the coarse sediment fractions (> 500 µm) and by 30% in the fine fractions (< 500 µm). The difference observed between the results obtained by the two methods is not surprising: acid dissolution relates to dry weights of sediments while the use of DRX is a semi-quantitative method for which it is known that quartz abundance is overestimated due to the higher diffraction yield of this mineral. The increase of quartz abundance is coupled with the decrease of calcite, magnesian calcite and aragonite concentration. Except in samples with a grain size > 500 mm from Tampolove, the three other samples showed a decrease in the proportion of carbonates. The decrease in aragonite was the greatest, which is logical as it is the less thermodynamically stable form of $CaCO_3$ at standard temperature and pressure. The decrease of calcite abundance, which is the most stable polymorph of calcium carbonate, and of magnesian calcite $(Ca,Mg)CO_3$, which is present in the echinoderm skeleton, were lower albeit substantial. Similarly, Vaucher (2012) found that microscopic preparations of sediments (i.e. thin sections and acid dissolution) showed a decrease in the proportion of carbonate inside pens.

H. scabra individuals choose sediments with a medium grain size, then the fine and finally the coarse size, even if the sediment with the medium grain size is not the richest in organic matter (Mercier et al., 1999). A balance between burrowing energetics and feeding efficiency might be occurring: medium-sized grains may constitute an ideal substrate because it retains a sufficient load of organic material, and is easy to ingest and to burrow into. Coarser grains may be harder to process through the gut, especially for small individuals, while organic matter content is probably less readily available due to the lower surface:volume ratio. Yet, Mercier et al. (2000) observed *in situ* that *H. scabra* avoided substrata of fine silt or shell and coral pebbles, and sediment with an organic content > 30%. Adults > 250 mm body length were found mainly on sand, with < 5% organic matter and individuals 10-250 mm were found mostly on mud and muddy sand with OM content between 5 and 10% (Mercier et al., 2000). In the region of Toliara (SW Madagascar), the quantity of organic matter that we recorded was always very low, no more than 5% (Lavitra et al., 2010; Tsiresy et al., 2011). In the present study, we did not observe any difference between TOM content in sediments taken inside and outside the pens after two years of intensive farming. This suggests that the natural input is sufficient to compensate the TOM uptake by sea cucumbers. On the other hand, the number of bacteria and the concentration of photosynthetic microorganisms decreased significantly in the pens after 2 years of farming. It has been well demonstrated by Yingst (1976) that *Parastichopus parvimensis* (H.L. Clark, 1913) ingests and assimilates compounds from bacteria and diatoms. We also found that *H. scabra* assimilates ^{15}N labeled compounds from *Vibrio* bacteria (Plotieau, 2013). Uthicke & Karez (1999), in investigating the patch selectivity of holothurians, observed that *Stichopus* species selected sediments with the highest content of microalgae. Moreover, Slater & Jeffs (2010) recorded the highest growth rate of *Australostichopus mollis* (Hutton, 1842) when microphytobenthic activity was the highest. It is therefore not surprising that the concentrations of bacteria and photosynthetic microorganisms decreased in the pens, even if the TOM remained stable after 2 years of farming.

The present results show that 2 years of intensive farming of *H. scabra* had an impact on the composition and grain size of the sediments. We suggest that an approach whereby sea pens are left fallow for periods of time between farming cycles (i.e. adopt a set-aside practice) should be considered in the future in holothuriculture. The renewal of bacteria and microorganisms in farmed sea pens should be analyzed in detail in order to determine the time necessary for the sediments to reacquire their natural levels. In addition, the loss of carbonates in the sediment due to sea cucumber digestive process should also be followed to ensure the development of optimal farming.

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