Mineral and organic features of the sediment in the farming sea pens of *Holothuria scabra* (Holothuroidea, Echinodermata)

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Abstract

The mineral and organic features of the sediment in sea pens where *Holothuria scabra* is farmed in Madagascar are characterised here for the first time. The study was undertaken in four villages in southwest Madagascar where the growth rate of sea cucumbers was 0.5–2.1 g day^{-1}. The results show that the sediment from Tampolove, where the growth rate of *H. scabra* was the fastest among the four villages surveyed, had the highest proportion of fine sand, protein concentration, and bacterial count of the four villages. In contrast, the total organic carbon proportion and the value of $\delta^{15}N$ in the sediment from Tampolove were the lowest among the four villages.

Introduction

Holothuria scabra is a deposit feeder whose growth rate has been estimated to reach values of 0.2–0.9 g d^{-1} (Hamel et al. 2001). As with other sea cucumbers, *H. scabra* extracts nutrients required for its growth from sediments on the sea floor. Little is known, however, about the mineral and organic components of the sediment ingested by *H. scabra*. Knowing the features of the sediment is important for farming because the quality of the sediment could explain some variations in the growth rate of reared sea cucumbers. Recently, Schneider et al. (2011) showed that holothuroids influenced seawater alkalinity in the vicinity by digesting carbonates from the sediment, a point already recorded by Hammond (1981). Since 2008, holothuroid sea farming has been practiced in four villages that are spread across 200 km of coastline in southwestern Madagascar. Monitoring was done there in order to compare the growth rates of *H. scabra* in sea pens in four villages (Tsiresy et al. 2011). The aim of the present work was to determine the mineral and organic features of the sediment in the sea pens where *H. scabra* was farmed in southwestern Madagascar. Because this is the first time that the characteristics of the sediment ingested by *H. scabra* are detailed, this work is a reference for anyone who wishes to grow this species in sea pens.

Materials and methods

Growth monitoring was conducted in four villages in southwestern Madagascar near Toliara from May 2009 to October 2010. One of the villages, Sarodrano, is situated south of Toliara and the three others (Andrevo, Fiherenamasay and Tampolove) are in the north (Fig. 1).

Figure 1. Southwestern Madagascar and the location of the four villages where intensive sea cucumber farming has been monitored since 2009.

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Juvenile sea cucumbers were spread out in sea pens such that their density was 2 ind. m^{-2} maximum at the beginning of the trial (see Eeckhaut et al. 2009 and 2012 for the development of juveniles before farming), a density recommended by Lavitra et al. (2010). Results of the growth monitoring were detailed in Tsiresy et al. (2011) except for that in Tampolove. The daily growth rate was 2.1 g in Tampolove, 1.7 g in Andrevo, 1.5 g in Sarodrano and 0.5 g in Fiherenamasay.

Sampling

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 in October 2010. Sediment samples were either directly stored at 4°C or placed in 4% paraformaldehyde (in 0.2 µm filtered sea water) 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 analyses at the Biology of Marine Organisms and Biomimetics Laboratory. These dried samples were used to determine the grain size, organic matter content, carbonate abundance, and nature of the minerals. Samples fixed with 4% paraformaldehyde were used to quantify bacteria.

Organic matter and mineral composition analyses

The methods used here are explained in Plotieau et al. (2013a). For each trial, the average values (given in the Results section) were calculated from five replicates.

The percentage of organic matter content was determined by carbonisation. The quantity of bacteria was estimated by using diamidino-2-phenylindole (DAPI) colouration. The quantity of photosynthetic microorganisms in sediments was estimated by chlorophyll *a* concentration. Stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) were conducted on sediment samples and sea cucumbers.

Sediment samples of 100 g from each village were sieved and weighed to determine their grain size composition. Carbonates were recorded after HCl dissolution and x-ray diffractometry was carried out to determine the abundance of four minerals: quartz, which consisted of almost pure SiO_2; calcite, the most stable polymorph of calcium carbonate ($CaCO_3$); aragonite, a thermodynamically metastable form of $CaCO_3$ at standard temperature and pressure; and 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 found in echinoderm skeletons.

For direct DAPI counts, total organic content, carbonate proportions and chlorophyll *a* content, the non-parametric Kruskal Wallis test was performed ($\alpha < 0.05$) in order to compare the sea pens with the arcsin transformation used for percentages.

Results

Table 1 summarises the results obtained. Sand fractions < 500 µm represent 85% of the dry weight of the sediment from Tampolove, 73–50% from Sarodrano and Andrevo, and less than 50% from Fiherenamasay. Fiherenamasay is the only village where the sediment had more than 25% of sand grains greater than 1 mm in size. In comparison, the sediment from Tampolove had less than 2% of these particles.

The total organic carbon (TOC) in the sediments constituted 0.4–2.0% of the total dry weight of the sediment sampled in the four villages (Table 1). The TOC in the sediments from Fiherenamasay and Tampolove differ significantly, and also differ from the two other villages ($P < 0.05$). The highest TOC observed was in Sarodrano, followed by Andrevo

Table 1. Mineral and organic features of the sediments in the sea pens. Higher values are highlighted in bold, lower values are in italics.

	Sarodrano	Andrevo	Fiherenamasay	Tampolove	Average
Growth rate	1.5	1.7	*0.5*	**2.1**	1.45
Proportion of particles < 500 µm (%)	73	50	*43*	**85**	62.7
Total organic carbon (%)	**2.0 ± 0.24**	1.8 ± 0.26	1.1 ± 0.13	*0.4 ± 0.13*	1.325
Protein concentration (µg μl^{-1})	*0.015 ± 0.001*	0.024 ± 0.01	0.018 ± 0.003	**0.049 ± 0.002**	0.0265
$\delta^{15}N$ (‰)	3.1 ± 0.2	2.2 ± 0.5	**4.2 ± 0.2**	*0.9 ± 0.9*	2.6
Bacterial count (DAPI) (x 10^9)	0.9 ± 0.4	*0.7 ± 0.07*	0.8 ± 0.1	**2.0 ± 0.9**	1.1
Chlorophyll *a* concentration (mg g^{-1})	**6.9 ± 1.1**	*1.4 ± 0.3*	3.3 ± 0.8	4.5 ± 3.7	4
Carbonate proportion (%)	*35 ± 1*	48 ± 5	**94 ± 2.5**	74 ± 0,5	62.75
Magnesium calcite (%)	9	7	**35**	16	16.7

and Fiherenamasay. Surprisingly, Tampolove, the village with the highest *H. scabra* growth rate, had the lowest TOC. The protein content of sediments varied from 0.015 µg µl^{-1} to 0.049 µg µl^{-1} (Table 1). Only the protein content of the sediment from Tampolove differed significantly from the other three villages ($P < 0.05$): it included three times as many proteins as the other villages. The three other villages have protein contents less than 0.025 µg µl^{-1}. The mean number of bacteria found with the DAPI colouration varied between 7 x 10^{8} and 2 x 10^{9}, and was highest in the sediment from Tampolove (Table 1). The number of bacteria in the sediment from Tampolove is quite different from the number in the sediments from Andrevo and Fiherenamasay ($P < 0.05$). The number of bacteria from Tampolove does not, however, differ significantly from that of Sarodrano because the standard deviations in both locations are important, although the mean is two times higher in Tampolove. Chlorophyll *a* mean concentrations varied between 1.4 mg g^{-1} and 6.9 mg g^{-1} of sediment (wet weight) (Table 1), and was highest in the sediment from Sarodrano and lowest in the sediment from Andrevo.

Figure 2 illustrates the means of δ^{15}N and the δ^{13}C obtained for the sediments and the holothuroids reared in the four villages. The mean values of δ^{15}N obtained for holothuroids are significantly different in the four villages meaning that their diets are different. Yet, the sediments of the four villages have similar mean values of δ^{13}C but have significantly different mean values of δ^{15}N, suggesting that the organic matter content coming from primary producers is different in the sediments.

The mean proportion of carbonates determined by acid attack is 35 ± 1% of the wet weight of sediment from Sarodrano, 48 ± 5% of the wet weight of sediment from Andrevo, 94 ± 2.5% of the wet weight of sediment from Fiherenamasay and 74 ± 0.5% of the wet weight of sediment from Tampolove. Carbonate fraction is surprisingly most important at the location where holothuroid growth is the lowest.

The relative abundance of the main identified minerals (quartz, magnesian calcite, aragonite and calcite) of the two grain-size fractions (> and < 500 µm) in the four villages is illustrated in Figure 3, which shows the mineralogical profile of each farm. The most important mineral at Sarodrano, Andrevo and Tampolove is quartz, no matter the analysed fractions. However, the most important mineral at Fiherenamasay, the site with the lowest holothuroid growth, is magnesian calcite. For all sites, there is more magnesian calcite than calcite (except in the fraction < 500 µm at Fiherenamasay where the proportion is the same). At Fiherenamasay, the pattern is very different from the one observed in the other villages, with the most important mineral in the two fractions being magnesian calcite followed by aragonite, quartz and finally calcite.

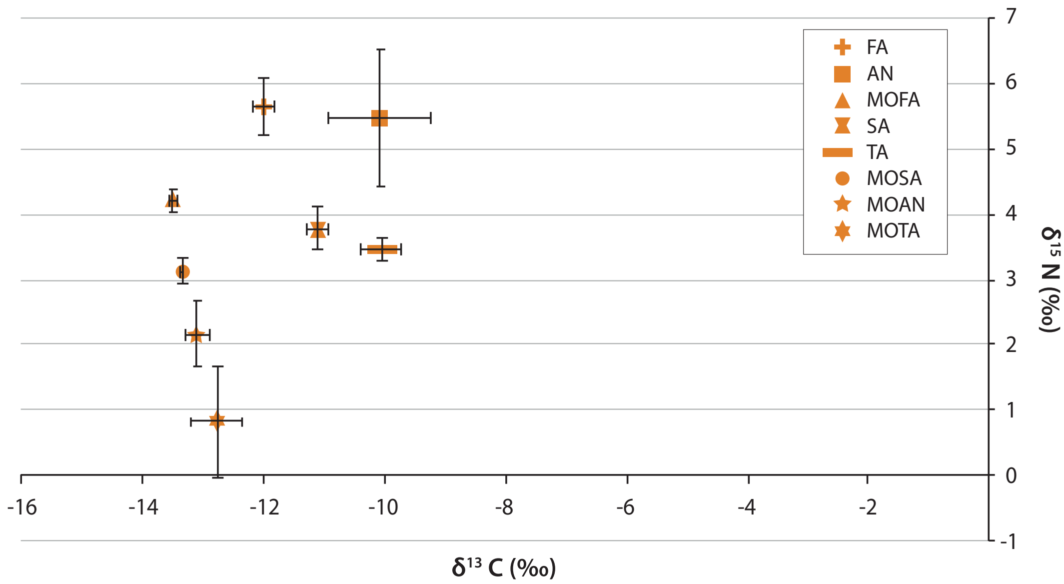

Figure 2. Mean δ^{13}C and δ^{15}N values of sea cucumber and organic matter of each studied villages. TA = sea cucumbers of Tampolove; AN = sea cucumbers of Andrevo; SA = sea cucumbers of Sarodrano; FA = sea cucumbers of Fiherenamasay; MOTA = organic matter of Tampolove; MOAN = organic matter of Andrevo; MOSA = organic matter of Sarodrano; MOFA = organic matter of Fiherenamasay.

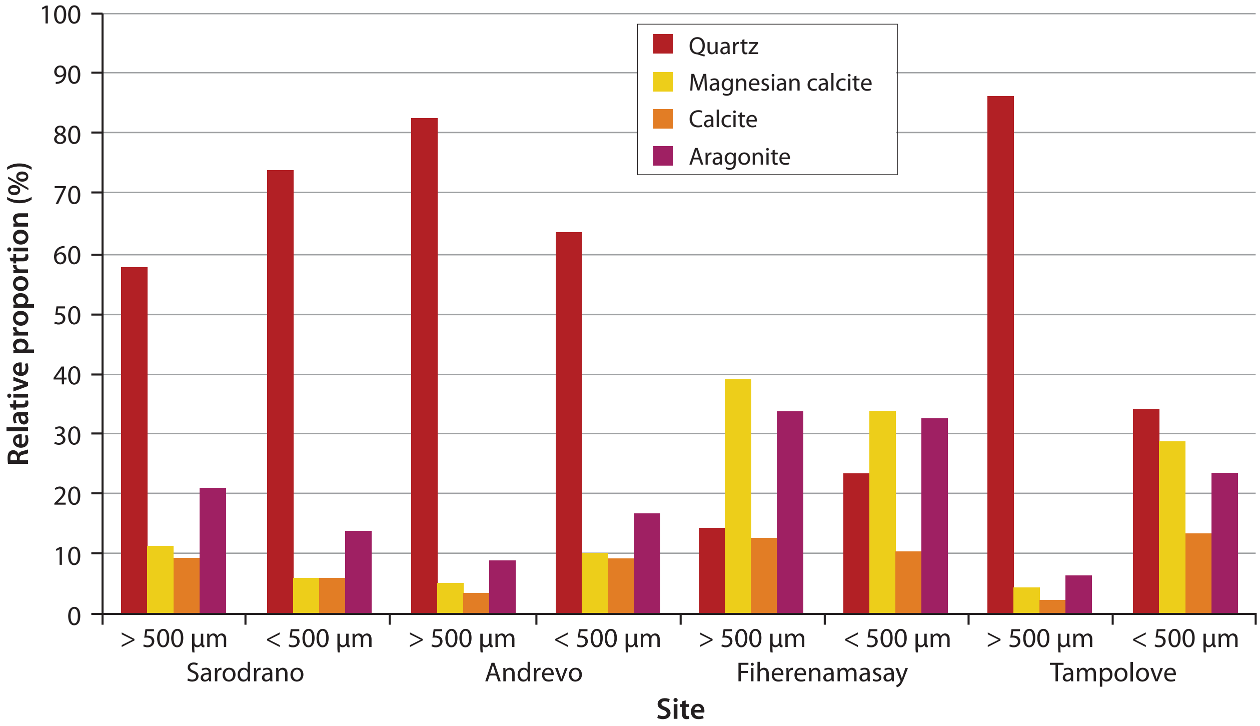

Figure 3. The abundance of principal minerals identified by x-ray diffractometry in the two grain-size classes of sediments from the four villages.

Discussion

In a recent paper (Plotieau et al. 2013a), we compared the sediment features inside (where holothuroids fed) and outside the sea pens of two villages after the completion of six cycles of farming. The samplings for this study were done one year after the present analyses (Plotieau et al. 2013a). We observed that the component of the sediment was sometimes greatly influenced by holothuroid feeding: the proportion of the finest grain-size fraction (< 250 µm) decreased from 5% to 14%, the global carbonate proportion decreased by 5% and the number of bacteria decreased by up to 50% (Plotieau et al. 2013a).

In the present paper, we provide information on the values of some important parameters of the sediment inside the range of which holothurians were observed to grow from 0.5 g d^{-1} to 2.1 g d^{-1}. Table 1 summarises the results obtained in this study. Although no direct correlation can be made between the growth rate of *H. scabra* and the features of the sediment, a general trend can be suggested when comparing the farms. In Tampolove, where the growth rate is fast, fine sand, protein concentration, and bacterial count had the highest values among the four villages. Also in Tampolove, the TOC proportion and the value of $\delta^{15}N$ are lowest. A low TOC value recorded in Tampolove indicates that the growth of *H. scabra* is not better if sediments are charged in organic matter. The TOC concentration seems particularly low, with an average of 1.325% when pooling all farms, the rest being minerals.

A low $\delta^{15}N$ suggests that organic matter includes mainly primary producers (dead and alive). Isotopic analyses reveal that there are differences in the global nature of organic matter eaten by holothuroids: $\delta^{13}C$ is similar in the four villages but $\delta^{15}N$ differs. Variations in $\delta^{15}N$ suggest that dead organisms in organic matter were not on the same level of the trophic chain when they were alive. The results further suggest that the organic matter in the sediment from Tampolove includes a high proportion of primary producers in contrast to Fiherenamasay where organic matter included fragments of organisms coming from a higher level in the trophic chain. This could be explained by the occurrence of a very dense population of ophiuroids (*Ophiocoma scolopendrina*) with a density of > 3 ind. m^{-2} in Fiherenamasay (pers. obs.) but not in the three other villages. When dying, these organisms would contribute greatly to the formation of organic matter, a result that is supported by the high proportion of magnesium calcite found in the sediment, magnesium calcite composing the skeleton of echinoderms.

We sometimes observed poor coherence in some of the obtained results. Chlorophyll *a* concentration does not match perfectly with what was found with the isotopic analyses. This could be due to the fact that chlorophyll *a* concentration is correlated with live primary producers while a low $\delta^{15}N$ value would suggest that the organic matter includes primary producers that are dead or alive. Protein concentrations also differ from both the bacterial counts and chlorophyll *a* concentrations when the four farms are compared. We suppose that this is because a protein concentration sums all micro- and macro-organisms (such as nematodes) of a sample and can thus differ from the concentration of one particular category of organisms, here bacteria or primary producer. We observed, however, that protein concentration was the highest in Tampolove where the highest growth rate and the highest bacterial concentration were recorded (see Plotieau et al. 2013b for the bacterial composition of the gut of *H. scabra*).

Results obtained with the mineral fraction show that the mineral profile varies greatly from one farm to another but that the changes do not seem to significantly affect the growth of holothuroids. The quartz proportion varies greatly from 6% to 65%, indicating that mineral composition is dependent on water runoff from rivers. Andrevo and Sarodrano are influenced by runoff from the Manombo and Onilahy rivers, respectively, while Fiherenamasay and Tampolove do not receive direct runoff from rivers. Fiherenamasay in particular has a totally different mineral composition from the other villages, having a very weak proportion of quartz and much more calcite and aragonite. It is also the only farm that lies on a fringing reef; the farms at Sarodrano and Andrevo are located in lagoons behind a barrier reef, while the farm at Tampolove is in a bay. Recently, Schneider et al. (2011) investigated the potential role of holothurians on the $CaCO_3$ balance of a coral reef. These deposit feeders process carbonate sand and rubble through their digestive tract and dissolve $CaCO_3$ as part of their digestive process. The holothuroid population was estimated to be responsible for nearly 50% of night-time $CaCO_3$ dissolution. We have shown that the growth of *H. scabra* was good even in low concentrations of $CaCO_3$ and did not improve when holothuroids were farmed on sediments that had a higher concentration of carbonate compounds. It is known that the presence of quartz is often accompanied by feldspar in which K, Na and Ca ions are more bioavailable but, at present, it is impossible to say that the growth of *H. scabra* is facilitated by the presence of either quartz or calcite. Laboratory experiments should be designed with laboratory-made sediments to explore the real impact of minerals and organic compounds on the holothuroid growth.

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