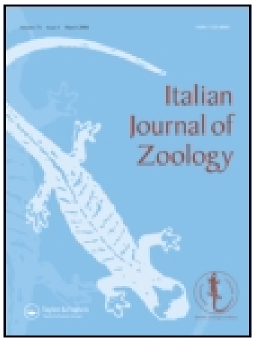

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Growth of the massive morph of *Cliona nigricans* (Schmidt 1862) (Porifera, Clionaidae) on different mineral substrata

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Abstract

Mineralogy of substratum plays several roles on benthic organisms, affecting both their distribution and abundance. Here we studied the long-term effect of biogenic limestone, travertine and quartz on the growth of the massive stage of the boring sponge *Cliona nigricans*. Experiments were performed in the field, on a detritic bottom 40 m depth, where a population of the massive *C. nigricans* lives. Experimental sets of sponges with the same dimensions were maintained for 500 days on the three different substrata and a comparison among relative growth was performed. At the end of the experiment all the sponges incorporated gravels but the specimens buried into calcareous particles, both biogenic and not, and increased in size while dimensions of the specimens buried into quartz sand decreased. Our results show a negative effect of quartz towards the growth of this species and highlight once again that mineral composition of substratum can affect morphogenetic processes.

Keywords: *Biomineralogy, quartz, carbonate, boring sponge, Ligurian Sea*

Introduction

Recent evidences have demonstrated the importance of biomineralogical interactions in the marine environment. Biological systems at different levels of complexity (cell, organism, species, and community), actively react towards the mineral substratum they encounter (Cattaneo-Vietti et al. 2004). In this way, several biosystems show the ability to recognize, select, react, and sometimes utilize the environmental mineral fraction.

At the community level, the importance of substrate mineral features appears sometimes more important than exposition in influencing the composition of benthic communities (Cerrano et al. 1999a, Bavestrello et al. 2000). Also, coastal fish assemblages can be affected by different mineral substrata (Guidetti et al. 2004). At the autoecological level there is evidence of the influence of substratum on the settling and growth of different animals, from Cnidaria to Chordates (Bavestrello et al. 2000, Groppelli et al. 2003, Soniat & Burton 2005).

Many data were obtained from sponges that are able to incorporate allochtonous materials, such as foreign spicules and sand grains. This phenomenon has been described particularly in species lacking a spicular skeleton such as several horny sponges, characterized by a network of spongine fibres (Sim & Lee 1999, 2004), and the collagenous sponge *Chondrosia reniformis* (Bavestrello et al. 1996, Cerrano et al. 1999a). The presence of active versus passive selection operated from the sponges on the foreign materials has been discussed for a long time (Teragawa 1986, Bavestrello et al. 1998a, 1998b). Recently we have submitted evidence that sponges that incorporate the largest amount of sediments are those living on soft bottoms, where the incorporated material is used to increase the anchoring in unstable substrata (Cerrano et al. 2002, 2004).

The selection and incorporation of foreign materials in sponges is a complex phenomenon that may be summarized considering two main modalities,

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based on different lifestyles: (i) hard-bottom species qualitatively select particles fallen on their ectosome by means of pinacocytes, on the base of size and mineralogy. The selected particles are then incorporated in the skeleton of the growing sponge (Teragawa 1986, Bavestrello et al. 1996, 1998a, Cerrano et al. 1999b); (ii) in soft-bottom species, particles present in the sediment are incorporated mainly from the basal portion of the sponge and selected on the base of their size. The incorporated particles improve the anchoring of the sponge both by increasing its weight (Cerrano et al. 2004, Ise et al. 2004) and by the so-called snow-shoes effect, given by semi-incorporated flattened particles like mollusc shells (Cerrano et al. 2002), which limits sponge rolling on unconsolidated sediments.

At Gallinara Island (Ligurian Sea) the common boring sponge *Cliona nigricans*, a species that lives symbiotically with zooxanthellae, shows the three growing forms—α (endolithic), β (encrusting), and γ (massive)—known for clionaids. In the last case, specimens can grow on detritic bottoms, incorporating huge amounts of foreign bodies and selecting bigger granulometries (Calcinai et al. 1999). What is still to be clarified is if mineral composition of coarse sediment influences incorporation processes, growth and shaping. The aim of this paper is therefore to test, by means of field experiments, the influence of the mineralogical features of the foreign bodies on sponge morphogenesis.

Materials and methods

The species and its habitat

Cliona nigricans is a boring Atlanto-Mediterranean sponge that can grow with different shapes both endolithic, into coralligenous accretions, and massive, partially buried by detritic sediments. The symbiosis with zooxanthellae characterizes this species, which at Gallinara Island is distributed from the surface to 35 m depth on rocky cliff and from 35 to 45 m depth on detritic bottom. In this last habitat the size of specimens ranges from 200 to 1000 cm^2, oscular chimneys can reach 10 cm in height and the population shows a patchy distribution, related to the pattern of distribution of coarse sediments (Calcinai et al. 1999). Coarse sediments are spread on a muddy bottom and are constituted both by fragments of biogenic limestone and by quartzitic pebbles (the Gallinara Island is completely quartzitic) totally or partially covered by epilitic coralline algae (*Lithothamnion* cfr. *sonderi*).

The experiment

From the detritic bottom of the Gallinara Island (Ligurian Sea), 40 m depth, 24 massive specimens of *C. nigricans* were cored using a cylindrical sampler, 10 cm in diameter (Figure 1A). Ten cores were immediately collected while the remaining were left *in situ*, divided in different experimental sets.

The 10 cores which were immediately sampled (t_0) were dried and weighted in laboratory. The dried samples were then dissolved with H_2O_2 (120 vol.) and the obtained sediments dried again to evaluate the percentage of embedded sediments.

The remaining 14 samples were divided into 4 different experimental sets and collected after 500 days (t_{500}): 5 cores were placed, as control, directly on the detritic bottom, in the same sediment where the natural population of *C. nigricans* lives; 3 cores were placed in a square box containing a stratum 10 cm thick of freshly fractured travertine, 5 mm in size; 3 cores were placed in a square box containing a stratum 10 cm thick of freshly fractured quartzite, 5 mm in size; 3 cores were placed in a square box

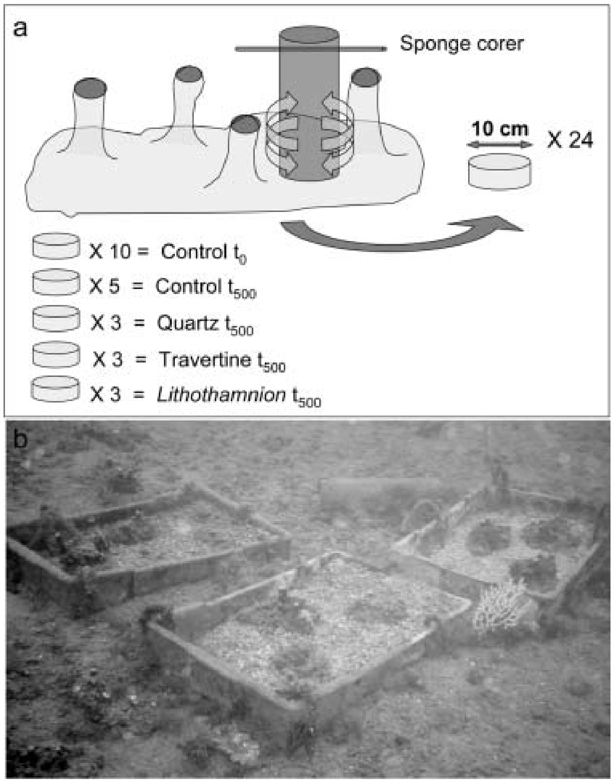

Figure 1. Scheme of the experimental set. **A**, drawing illustrating the coring of a massive specimen, the total number of replicas and the number of replicas for each experiment. **B**, photograph of the three experimental boxes with sand of quartz, travertive and *Lithothamnion*, placed on a detric bottom 40 m depth, where the natural population of the massive *Cliona nigricans* lives.

containing a stratum 10 cm thick of sand of a coralline alga, *Lithothamnion* spp., available exclusively 2 mm in size (Figure 1B).

At the end of the experiment, after 500 days, each core was photographed before collection and, in the laboratory, the dry weight of cores was determined; incorporated materials were obtained as described above for the (t_0) cores and weighed.

The total growth of each core was evaluated by the program for image analysis ImageJ, considering the difference between the projection of the cores at end of the experiment (t_{500}) and at the beginning (t_0) (Figure 3) (Kruskal–Wallis test).

Moreover all the incorporated particles were examined, by a stereomicroscope, in order to study the erosion marks due to the boring activity of the sponge.

Results

The average ($\pm$SE) dry weight of the ten cores (t_0) was 132.97 ± 10.15 g while the weight of the sediments incorporated was 117.91 ± 10.03 g and represents the 89% of the dry weight of the cores (Figure 2).

At the end of the experiment, after 500 days, the average dry weight of the five control cores (t_{500}) was 172.97 ± 16.05 g while the weight of the sediments incorporated was 154.71 ± 16.07 g and represents the 89% of the dry weight of the cores. The weight of incorporated sediment in the samples, tested in the different kinds of gravels, was different according to the mineral kind. The average dry weight of samples maintained in *Lithothamnion* sand was 261.09 ± 19.48 g while the weight of the sediments incorporated was 258.27 ± 19.2 g and represents the 99% of the dry weight of the cores. The average dry weight of samples maintained in travertine gravel was 223 ± 35.56 g while the weight of the sediments incorporated was 220.99 ± 35.98 g and represents 99% of the dry weight of the cores. The average dry weight of samples maintained in quartzitic gravel was 157 ± 27.27 g while the weight of the sediments incorporated was 152.34 ± 27.68 g and represents the 97% of the dry weight of the cores (Figure 2).

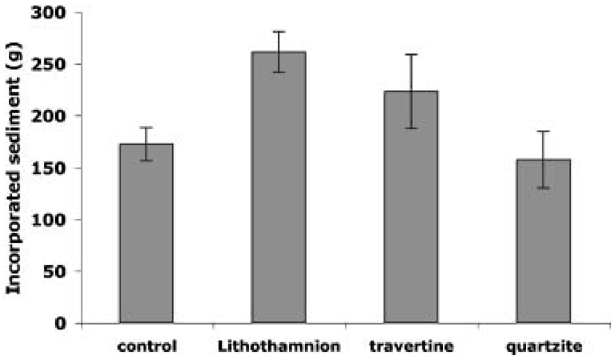

Figure 2. Average weights ($\pm$SE) of the incorporated sediments, after 500 days of experiments, in the different experimental sets.

All the explants were alive and all showed evident areas of sand grains incorporation, in the regenerative borderline of the core samples (Figure 3A–C). While the specimens tested in the quartzitic sediments show a general reduction of their size, samples tested in the carbonatic sediments (travertine and *Lithothamnion*) reveal an evident increase of their size producing more or less lobate forms that sometimes largely exceed initial core size (Figure 4). In the newly formed areas the sponges develop inhalant and exhalant papillae only when carbonatic gravels are incorporated, no papillae are evident in the specimens mantained in the quartz gravels (Figure 3A–C).

With respect to the controls, the specimens tested with carbonatic fragments have a significantly higher average growth of their surface ($44 \pm 20\ \text{cm}^2$ for *Lithothamnion*, $T=26.0$, $P<.06$ and $34.5 \pm 13.5\ \text{cm}^2$ for travertine, $T=26.0$, $P<.06$), while the specimens tested in the quartzitic fragments show a significantly negative average growth ($-11.33 \pm 6.35\ \text{cm}^2$; $T=34.0$, $P<.06$; Figure 5A). This is also true for weights (Figure 5B).

The particles obtained for this experiment were checked in order to study the boring aptitude of this species of *Cliona*. Obviously all the incorporated quartzitic particles remained not etched (Figure 3A′) while the carbonatic ones show signs of the sponge erosion. Nevertheless, between the two kinds of carbonatic particles strong differences arose: while a small fraction (about 5%) of *Lithothamnion* particles are little bored (Figure 3B′,B″), about 70% of the travertine particles are generally severely bored (Figure 3C′,C″).

Discussion

When *C. nigricans* specimens live in the sediment in the γ massive form incorporate particles of large size to increase their anchoring in the sediments. There are evidences indicating that, for this species, the growth in the soft bottoms is related to the availability of coarse sediments (Calcinai et al. 1999). Here, the growth of sponge transplants shows wide differences among specimens but this is in accordance with the growth variability recorded for other sponge species when cuttings are used (Verdenal & Vacelet 1990, Corriero et al. 2004).

The experimental data presented here demonstrate some remarkable and unknown aspect of the

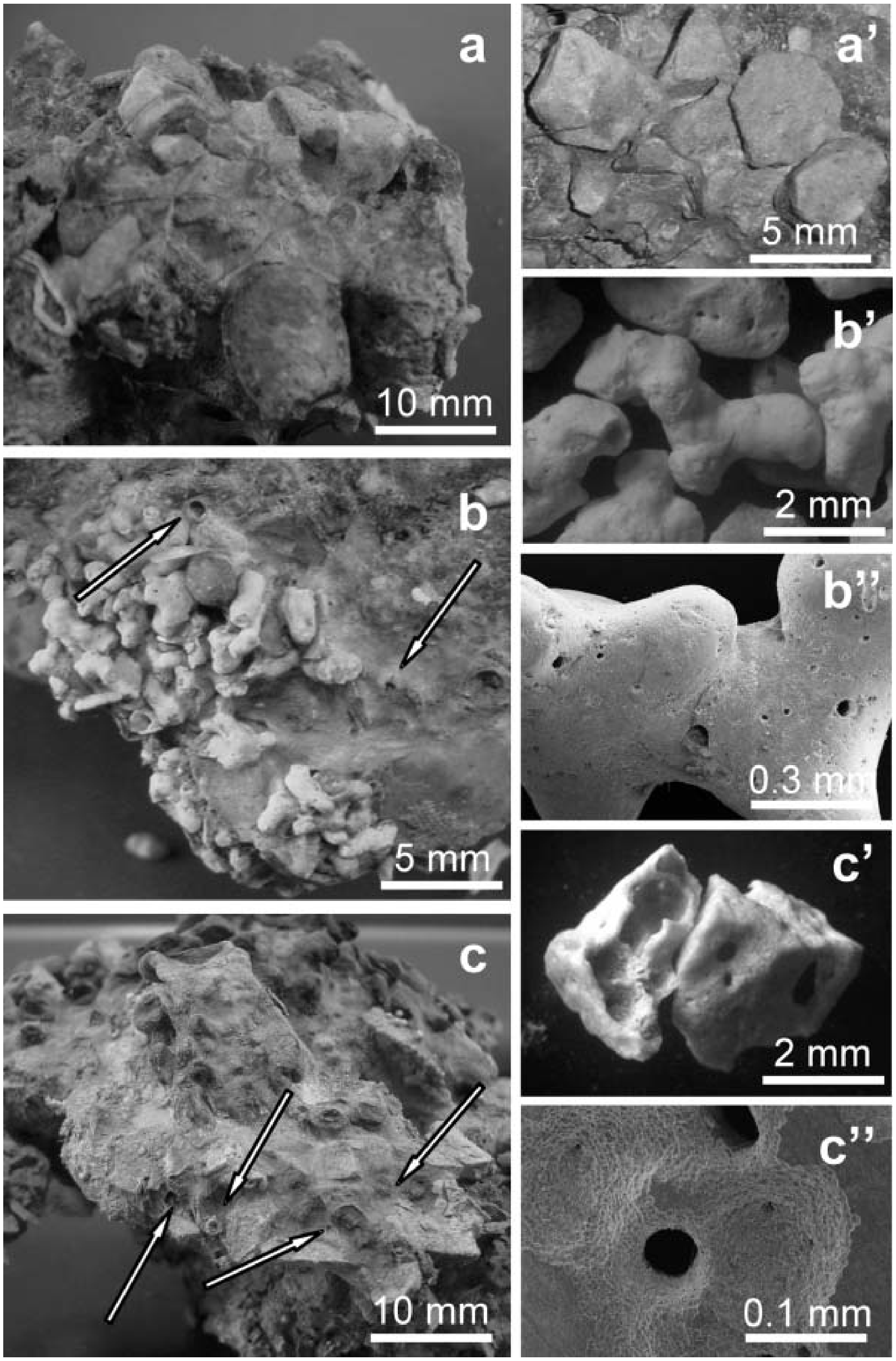

Figure 3. Details of the newly formed areas of the sponges with the magnification of incorporated sediments. **A**, embedded quartz; **A′**, detail of grains. **B**, embedded *Lithothamnion*. Arrows indicate some newly formed papillae; **B′**, detail of grains; **B″**, scanning electron microscope image with a detail of the perforations carried out by the sponge. **C**, embedded travertine, arrows indicate some newly formed papillae; **C′**, detail of a broken grain with visible wide perforating chambers; **C″**, scanning electron microscope image with a detail of the perforations carried out by the sponge.

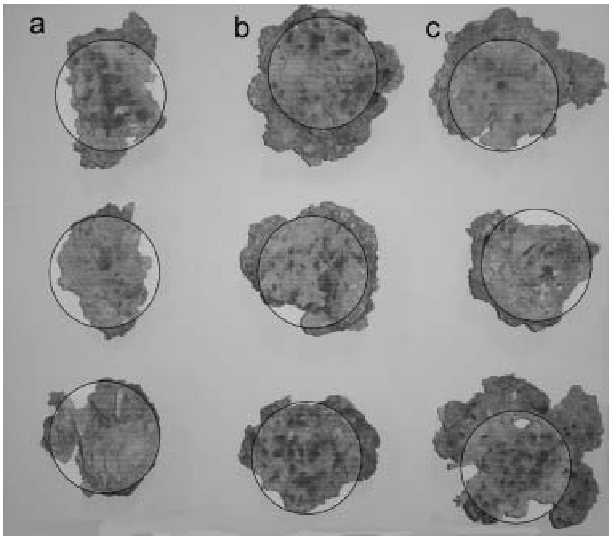

Figure 4. Comparison between the surface of the cores at the beginning of the experiment (t_0—circle, 10 cm in diameter) and at the end (t_{500}—dried sponge), in **A** quartz, **B** *Lithothamnion*, and **C** travertine grains.

incorporation of foreign materials in sponges. In natural conditions, the fraction of mineral detritus incorporated by massive *C. nigricans* specimens represents about 89% of the sponge dry weight, a value that corresponds to the percentage of sediment found in the controls specimens at the end of the experiment. After 500 days of burial in the experimental particles, the incorporated fraction represents 99% in the case of travertine and *Lithothamnion*, and 97% in the case of quartz.

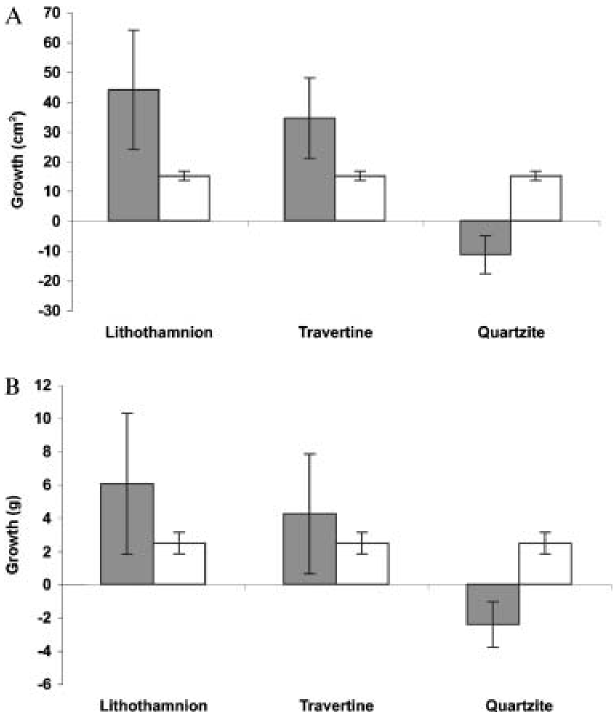

Figure 5. Comparison among the different average growths (core surface t_{500} – core surface t_0) recorded on the three kinds of grains, in relation to the control (±SE), considering **A** area and **B** weight.

At the end of the experiment, all cores showed a higher amount of sediment with respect to the t_0 controls, and this is likely related to regenerative processes that, producing new biomass, facilitate the embedding of particles. Anyway, the low percentage of incorporated material in the control specimens and in the natural population may be due to the low percentage of coarse elements (gravel >5 mm is about 10–15% of the ambient sediment (Calcinai et al. 1999)) in the detritic bottom respect to the experimental conditions, where the coarse fraction is 100%. Slight differences also arise among the specimens maintained in the three different gravels: the explants tested in the quartzitic gravel incorporate less material than those tested in the two calcareous substrata.

The most interesting finding of this experiment is the different growth of sponge explants buried in different substrata. Although all the explants tested both in quartzitic and carbonatic materials incorporated a higher percentage of sediments respect to the controls, the growth of specimens tested in carbonatic sediments is significantly higher than the controls while those tested in the quartzite show a decreasing of their original size. This fact may be related to the toxic properties of quartz that can act at different biological levels negatively affecting the aquiferous system development in the newly formed portions (Figure 3A), and limiting, consequently, the feeding efficiency of these areas. The negative effect of quartz involves chemical processes taking place at the surface of particles in contact with the cells (Giovine et al. 2002). Several factors have been identified to contribute to the onset of a chronic inflammation process in vertebrates, among which is production of ROS (reactive oxygen species). Lipid peroxidation is activated during this process, with subsequent interference with arachidonic acid metabolism and damage to DNA (Fubini 1998), followed by the release of cytokines (Lardot et al. 1998), and nitrogen oxide (Blackford et al. 1997).

In conclusion, *C. nigricans* appears unable to distinguish the mineralogical features of sediments. The particles present in the sediments are actively incorporated only in relation with their size and their amount in the sponge is influenced by the availability in the sediments of coarse grains (Calcinai et al. 1999). When incorporated, the mineralogical features of the particles affect the growth of the sponge that is positive into carbonatic sediments while negative into quartzitic ones.

The case of *C. nigricans* is different from that of *Chondrosia reniformis* that actively selects and incorporates quartz. In this species, characterised by a thick collagenous cortex, an high silica concentration could stimulate the collagen gene expression as demonstrated by Krasko et al. (2000) for primmorphs of *Suberites domuncula*.

The data here presented also put in evidence for some intriguing differences in the erosion rate of carbonatic materials of different origins. In fact, while the travertine gravels are widely bored (Figure 3C′,C″), only a few pieces of *Lithothamnion* show signs of erosion (Figure 3B′,B″). This evidence may be related to the known preference of boring sponges for compact substrata (Schönberg 2002; Calcinai et al. 2004) or with the size of *Lithothamnion* particles, which are probably too small to be strongly bored. Both hypotheses need to be elucidated by further more focused studies.

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