INCORPORATION OF INORGANIC MATTER IN *CHONDROSIA RENIFORMIS* (PORIFERA: DEMOSPONGIAE): THE ROLE OF WATER TURBULENCE

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The role of sedimentation and sea conditions in relation to the amount of the foreign matter (sand grains and opaline sponge spicules) present in the body of the demosponge *Chondrosia reniformis* was evaluated monthly at two sites, each characterised by different sedimentary conditions along the rocky cliff of the Portofino Promontory (Ligurian Sea). Contrary to the process in keratose ('horny') sponges, the mineral particles incorporated by *Chondrosia* are subjected to an evident turnover probably linked to its unusual ability to dissolve quartz. The quantity and size of the particles taken up by the sponge are linked to environmental sedimentation and sea conditions. These data indicate that settlement of particles on the sponge is affected by the stickiness of the sponge's mucous surface. The large amount of quartz grains continuously incorporated and dissolved by *Chondrosia*, suggests a possible role played by the sponge in the local silica flux in shallow coastal waters. ❒ *Porifera, foreign matter, mineral selectivity, uptake, water turbulence, silica.*

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Sedimentation on rocky bottoms influences the distribution of organisms, impacting significantly on larval settlement and its further development, and compromising the filtering structures of filter feeders, even with the extreme result of total exclusion from their habitat (Loosanoff & Tommers, 1948; Wilber, 1971; Rogers, 1990). Porifera, living under high sedimentation regimes, might also be subjected to both abrasion by coarse sediment particles and occlusion of inhalant pores by fine ones (Sarà & Vacelet, 1973; Verdenal & Vacelet, 1985). The filtered water volume decreases proportionally to the amount of particulate matter present in the water column; e.g. in *Aplysina* (=*Verongia*) *lacunosa* (Gerodette & Flechsig, 1979). Sponges can live in oligotrophic waters owing to their high filtering efficiency, but cannot survive for long periods of reduced pumping (Reiswig, 1974). Some species have developed defense mechanisms against high sedimentation, as in the fresh-water species *Ephydatia fluviatilis,* where amoeboid cells of the exopinacoderm have endocytosis capabilities (Willenz & Van de Vyver, 1982) and can remove foreign particles (Harrison et al., 1985). Several species, such as the keratose sponge *Dysidea etheria,* can select sedimentary particles from their habitat, incorporating proper-sized ones in their primary fibers and removing others through the selective action of their external amoeboid cells (Teragawa, 1986a, 1986b).

Chondrosia reniformis does not produce its own spicules but engulfs foreign siliceous materials (i.e. siliceous sponge spicules present in the water column and sand grains), into its collagenous ectosome. Moreover, it recognises the mineralogical features of particulate material, dissolving quartz particles and reducing their original size (Bavestrello et al., 1995a, 1996, 1999).

The aim of this study is to explore the relationships between the amount of allochtonous matter engulfed by *C. reniformis* during an annual cycle, comparing this to the different sedimentation conditions, which are closely related to the local sea conditions in two different sites of the Portofino Promontory (Ligurian Sea, Tigullio Gulf, Italy): Punta del Faro and Paraggi Bay (Fig. 1).

These stations are well known from a biocoenotical (Tortonese 1961; Morri et al., 1986)

FIG. 1. Schematic figure showing the main current patterns in the studied area. At Punta del Faro, the current from the Golfo Tigullio meets the main cyclonic stream of the Ligurian Sea. Paraggi Bay represents a decantation area consequent to an eddy.

and a sedimentological point of view (Bavestrello et al., 1991; 1995b). The sedimentation rate is about seven times higher at Paraggi Bay than at Punta del Faro, owing to differences between their local hydrodynamic features (Esposito & Manzella 1982; Marullo et al., 1985). In fact, Paraggi represents a decantation area, while Punta del Faro is the meeting point of two currents, one from the Ligurian Sea and the other one flowing outwards from the Tigullio Gulf (Fig. 1).

MATERIALS AND METHODS

At Punta del Faro, where the cliff ends at 55m depth, specimens of *C. reniformis* were sampled monthly by SCUBA diving during March 1994-June 1995, at depths of 3m, 12m and 25m. At these last two depths two sediment traps, as described by Bavestrello et al. (1991), were installed to collect the fraction of sediments available for sponges. At Paraggi Bay, where the cliff ends at 25m depth, sponges were collected from 3m and 15m depths, and a sediment trap was installed at this last depth only. At both localities, a superficial (3m depth) sediment trap was also installed, but strong wave action prevented a sufficient continuity in data collection at this station.

At each station, 1cm^2 fragments of the sponge ectosome were collected monthly from six specimens. To analyze quantity and granulometry each fragment was dissolved in boiling hydrogen peroxide (39% weight/volume; about 130 vol.). The dissociated foreign material was centrifuged at 5000G for 5mins, washed twice in 95% ethanol, resuspended in 0.5ml of 100% ethanol, and finally two subsamples of 0.1ml were mounted on two slides. All particles (sand grains and spicules) were counted on each slide. The main axis of 100 sand grains per slide from Punta del Faro specimens was measured using a GRAPHTEX KD 4300 digitiser connected to an IBM PC. The area of incorporated particles was expressed per square centimeter of sponge surface.

Sediments collected from traps were evaluated monthly as dry-weight after combustion at 550°C for 4hrs in order to reach the inorganic fraction. Three slides were prepared from each sediment sample to collect the granulometric data.

Wave height data (cm above the free sea surface) were kindly provided by the Meteorological Observatory of Chiavari. Measurements of wave height commenced 10 days prior to each sampling date in order to compare trends. This period was chosen after initial trials of 7, 10 and 15 days prior to sampling, as it provided the best comparison between environmental conditions and collected sediments.

Additionally, investigation of the sponge ectosome was conducted by SEM analyses to evaluate morphological relationships between sponges and settled sediments. Samples were collected and fixed underwater in 2.5% glutaraldehyde. After rinsing in artificial seawater, samples were dehydrated in an ethanol gradient, followed by critical-point drying in a CO_2 Pabish CPD apparatus. They were mounted on stubs with

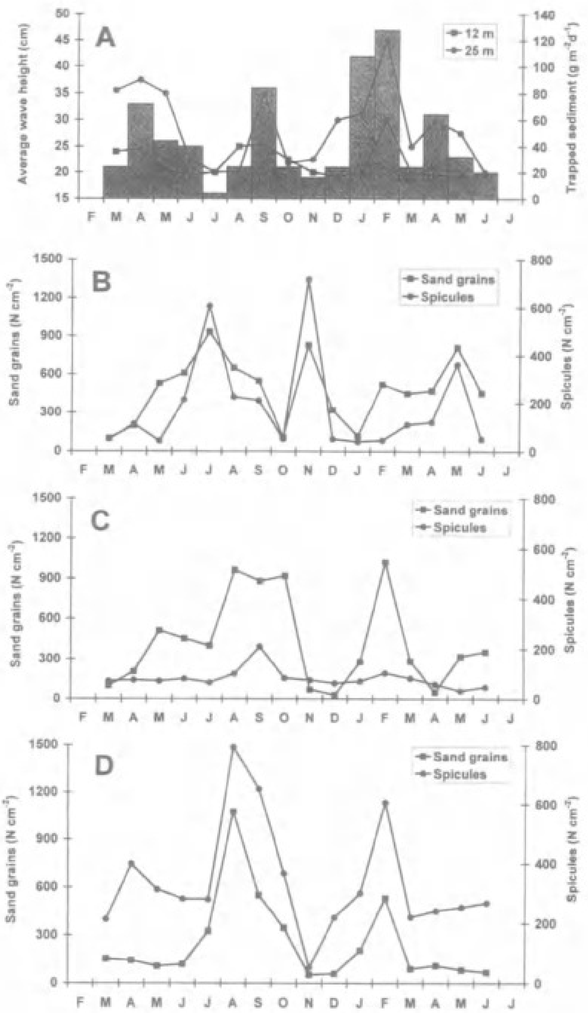

FIG. 2. Punta del Faro. A, Relationship between annual trend in sea conditions (histogram) and sediments collected by traps at 12 and 25m depths. B-D, Foreign matter (spicules and sand grains) incorporated by *Chondrosia reniformis* at 3, 12 and 25m depths, respectively.

silver conducting paint, sputter-coated with gold-palladium in a Balzer Union Evaporator, and observed using a Philips EM 515 electroprobe microscope.

To estimate the silica production by *C. reniformis* in the studied area, the sponge abundance and its surface were evaluated by visual census along 10 vertical 1m belt transects from the base of the cliff of the Promontory (50m depth) to the sea surface, following Hiscock's (1987) method.

RESULTS

At Punta del Faro, a high energy site, the amount of sediments collected by traps was directly related to the sea conditions at both depths (Fig. 2A). Sand grains and spicules (number cm^{-2}) incorporated by *Chondrosia reniformis* at 3m depth, peaked during periods of calm sea and declined during rough seas (Fig. 2B). Conversely, at intermediate (12m) and deep (25m depth) stations, higher quantities of sand and spicules were incorporated by the sponge during periods of rough seas, when sediment availability was higher (Fig. 2C-D).

Similarly, at Paraggi Bay, a decantation site, the amount of sediments collected by traps was strongly related to the sea conditions (Fig. 3A). Both sponge stations (at 3m and 15m depths) showed the same phenomenon as did the most shallow station at Punta del Faro: high values of incorporated particles were recorded during calm periods (Fig. 3B-C), even if the available sedimentary material was greater during periods of rough seas, as shown by our data on the trapped matter (Fig. 3A).

The granulometries of the incorporated sand grains by *C. reniformis* at Punta del Faro showed a similar trend for all depths sampled (Fig. 4). Average values ranged between 18-51µm diameter, with maxima occurring in July and November-December and minima occurring mainly from August to October and during winter. Comparison between these granulometries and sea conditions reveals an inverse relationship: large particles were present exclusively following periods of calm water.

SEM observations on the intact sponge surface showed that numerous crystals, organised in spherical-like balls (of about 5-15µm), and enveloped by a thin mucus web, emerge from the sponge ectosome (Fig. 5). Electroprobe analysis of crystals (indicating silica as the major constituent) and their shape, allowed us to conclude that these are quartz crystals.

DISCUSSION

Many demosponges are able to incorporate allocthonous inorganic material into their skeletons, a mechanism that is generally considered to provide additional strength to their organic fibrous skeleton. This phenomenon occurs most widely in the 'horny' keratose sponges (Lendenfeld, 1889; Teragawa, 1986a; Pronzato et al., 1998), comprising the orders Dictyoceratida, Dendroceratida and Verongida. In keratose sponges the uptake seems to be irreversible, since foreign matter is cemented into primary fibers. Conversely, *Chondrosia reniformis* shows an evident turnover

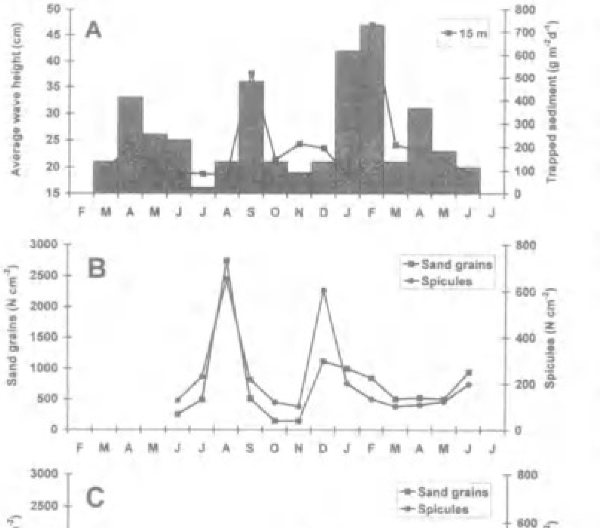

FIG. 3. Paraggi Bay. A, Relationship between annual trend in sea conditions (histogram) and sediments collected by the trap at 15m depth. B-C, Foreign matter (spicules and sand grains) incorporated by *Chondrosia reniformis* at 3 and 12m depths.

of incorporated foreign material and a capability to discriminate amongst the incorporated particles. This finding opens new perspectives on sponge behaviour. Although influenced by environmental parameters, these phenomena suggest a continuous utilisation of the incorporated matter as evidenced by the quartz dissolution ability (Bavestrello et al., 1995a), and the production of quartz 'pellets' on the ectosome.

Annual trends in the amount and size of sedimentary matter incorporated by *C. reniformis* appear to depend mainly on the local sea conditions and on the sponge etching. During calm periods, mainly in shallow waters, the sponge also uptakes large particles, as suggested by the inverse relationship between particle size and sea conditions. These phenomena are most evident in the shallow stations, where the highest amounts and largest sizes of incorporated foreign materials are present, corresponding to periods of calm waters. Conversely, during rough periods, the sponge surface is not sticky enough to retain large particles and consequently the quantity and size of engulfed matter decrease. In deeper water, where wave disturbance is reduced and resuspension processes are higher, populations of *C. reniformis* respond to these environmental conditions, incorporating higher amounts of siliceous matter. This is evident at Paraggi Bay, a more protected site than Punta del Faro, where swell conditions are frequent. In this way, it is possible to assume that sea conditions influence this phenomenon in two ways: on one hand, rough sea conditions can limit the uptake of particles, where the effect of waves action is strong, but on the other hand, the same conditions increase the availability of sediment material, owing to resuspension processes. This causes a higher amount of incorporated sediments in sponges living in deeper waters, where wave action is not strong enough to detach particles from the sponge surface.

In *C. reniformis* the mechanism of incorporation of inorganic matter involves different physical, mineralogical, and biological aspects: the settled particles are transferred, at variable speeds, to special areas of the sponge ectosome where they are quickly engulfed and, after incorporation, the collected material remains scattered in the fibrous ectosome, where particle sizes are re-elaborated (Bavestrello et al., 1995a, 1996, 1999).

Selectivity in the incorporation of foreign bodies in sponges has long been debated (Haeckel, 1872; Schulze, 1879; Lendenfeld, 1889; Sollas, 1908; Shaw, 1927; Teragawa 1986a). The uptake of particles in *C. reniformis* seems to be determined by an active selection of the minerals (Bavestrello et al., 1998b), and a passive one regarding their size. In agreement with Schulze's hypothesis (1879), it is possible that the uptake

FIG. 4. Relationship between annual trends in sea conditions (histogram) and granulometries of sand grains incorporated by *Chondrosia reniformis* at 3m (circles), 12m (squares) and 25m (triangles) depths at Punta del Faro (Portofino Promontory).

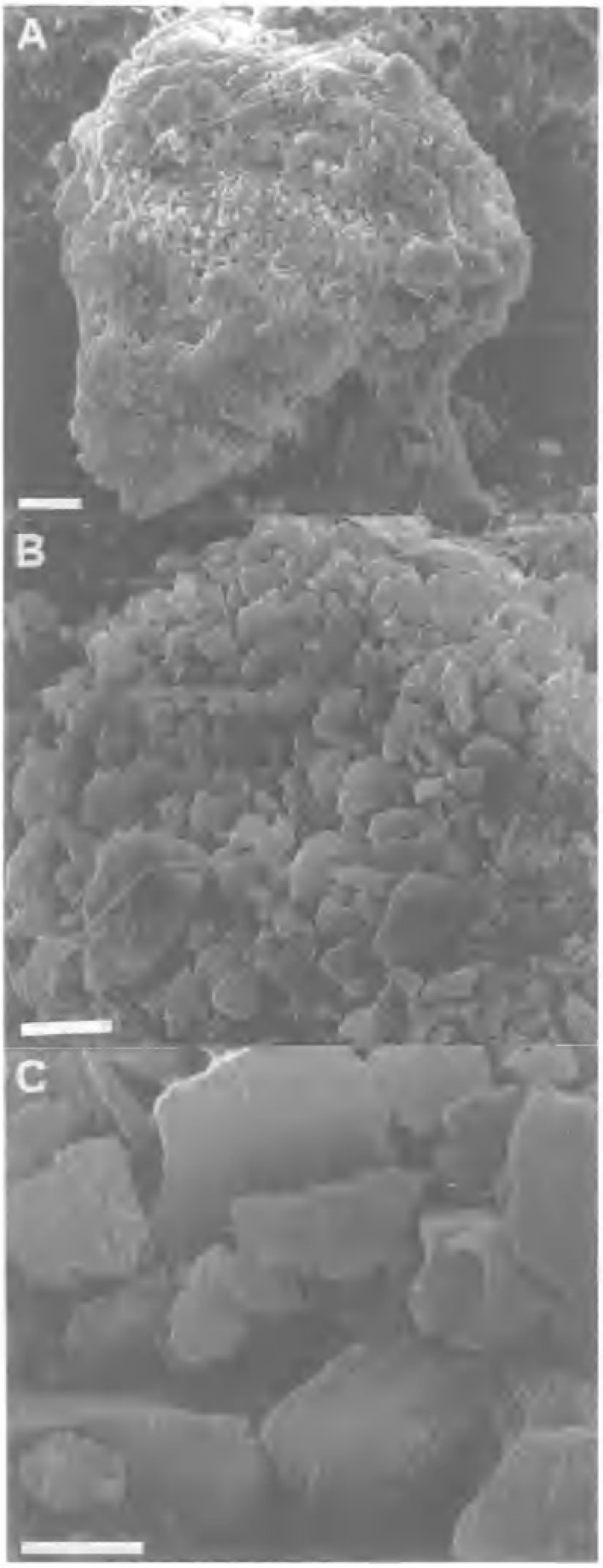

FIG. 5. SEM photographs, at three different magnifications (A-C), of spherical-like structures showing quartz particles expelled by the ectosome and enveloped by a thin mucus web. Scale bars: A = 11mm; B = 9mm; C = 2 mm.

mechanism is determined by the interaction between the stickiness of the sponge surface and the intensity of water movement, and that the biological activity of the sponge towards the quartz particles affects the granulometric trend and the amount of incorporated sediments.

An important consequence of this unusual behaviour is the output of dissolved silica, thus biologically available to other organisms. Under experimental conditions (Bavestrello et al., 1995a; 1996), with excess quartz grains available on its ectosome, *C. reniformis* engulfs about 0.2mg cm^{-2} day^{-1} of quartz and produces 0.1mg cm^{-2} day^{-1} of dissolved silica. On the Portofino Promontory cliff, the average daily quartz availability, evaluated with sediment traps, is 0.4mg cm^{-2}. This suggests that quartz availability is not a limiting factor, allowing us to hypothesise that the sponge maintains the same ratio of incorporation and dissolution shown in laboratory experiments.

Considering that the population density of *C. reniformis* along the Portofino Promontory is about 5,000cm^{-2} per meter of coast, and that the Promontory coast is about 13km long, it is possible to estimate a production of dissolved biologically available silica of about 2106g yr^{-1}.

Even if the most important contribution of silica to the Mediterranean Sea comes from the Gibraltar Strait (De Master, 1981), input from rivers into the Mediterranean, although generally modest, may also be locally important. In the Tigullio Gulf (Ligurian Sea), the Entella River, with an average annual flow rate of 14.8m^3 sec^{-1}, carries about 214x106g yr^{-1} of dissolved silica. However, the production by populations of *Chondrosia* at the Portofino Promontory, of about 2106g yr^{-1}, suggests that this species has a significant role in silicate turn-over, in rocky littoral areas, far removed from river input.

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