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RESEARCH ARTICLE

Effects of sediment on the survival of asexually produced sponge recruits

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Abstract Sediment deposition is known to affect the structure of marine rocky-bottom communities, but its specific effects on some key organisms, such as sponges, remain poorly investigated. In a 125-day field experiment involving different treatments of exposure to sediment deposition, we investigated survival of asexually produced recruits of the sublittoral demosponge *Scopalina lophyropoda*, a model organism suitable to understand similar processes in other sponges. A total of 660 explants obtained from 11 non-clonal sponges (explant donors) were distributed on 30 experimental plates. Each donor sponge contributed two clonal explants per plate, one settled under a roof at a silt-protected position and the other at a silt-exposed position. Plates were installed at the rocky walls of the natural community, also at the pillars of a local harbor where the sponge does not occur naturally. A 3-way ANOVA testing for differences in explant longevity as a function of explant donor, exposure to sediment, and habitat detected that longevity was affected by both an undetermined genetic condition of the explant donor and exposure to silt. Silt-protected explants lived longer than silt-exposed explants. A significant "Silt-exposure × Habitat" interaction detected that silt-exposed explants lived shorter within the harbor than in the natural community, suggesting that harbor silt, which was notably finer, is more deleterious. Inspection of daily mortality rates revealed that the detrimental effects of silt were very evident during the first 20 days in treatments and irrespective of habitat. Then, mortality rates progressively decreased, reaching negligible values in all 4 sponge groups by day 65. At this stage, an undetermined mortality factor other than purely sediment deposition reactivated mortality in all 4 sponge groups, but it affected more intensely the sponges in the harbor, irrespective of being protected from or exposed to sediment deposition. All together, the results of our field experiment suggest that sediment loads are a major mortality factor among small sponge individuals in sublittoral rocky communities. Because a significant "donor factor" suggests an unidentified "genetic component" to be involved in the ability to cope with sediment, natural or man-driven processes increasing coastal sediment deposition are susceptible to induce changes not only in the abundance but also the genetic structure of the sponge populations in the long term.

Introduction

One of the most obvious ecological patterns in sublittoral rocky-bottom communities of temperate latitudes is that horizontal and subhorizontal substrates are spatially dominated by macroalgae in detriment of sessile invertebrates, which proliferate more successfully on vertical walls, overhangs, cave entrances, and undersides of boulders (e.g., Péres and Picard 1964; Ballesteros 1989; Paine 1990; Baynes 1999; Bell and Barnes 2000; Preciado and Maldonado 2005). Although bright light itself may have negative effects on invertebrate survival (e.g., Jokiel 1980; Lesser 1996), scarcity of invertebrates on well-illuminated substrates is usually thought to result from macroalgae outcompeting them, with invertebrate abundance peaking at the low-irradiance microhabitats where algal growth is

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limited (e.g., Vacelet 1976; Harmelin 1985; Duggins et al. 1990; Paine 1990; Tanner 1995; Glasby 1999; Rützler et al. 2000). Yet the shaping of temperate, subtidal communities may be modulated by a third relevant factor, sediment deposition, which usually acts confounded with exposure to light and algal abundance.

A survey of the literature reveals that despite the potential role of sediment deposition to explain diverse aspects of the structure of hard-bottom invertebrate-dominated communities, the specific effects of this factor on some key marine invertebrates are still poorly investigated and understood (e.g., Maughan 2001; Irving and Connell 2002; Airoldi 2003). In this study, we investigated the effects of sediment deposition on the survival of small individuals of an encrusting, sublittoral sponge. Plenty of evidence supports that sponges are important space occupiers in many temperate and subtropical hard-bottom assemblages and that their abundance affect not only substratum availability for settlement of other sessile organisms, but also local levels of bacterioplankton, pikoplankton (Reiswig 1971; Peterson and Black 1987; Richter et al. 2001), and dissolved nutrients (Richter et al. 2001; Scheffers et al. 2004; Maldonado et al. 2005).

Sponge fauna in temperate, hard-bottom communities is mostly represented by members of Demospongiae, a class containing about 95% of the existing sponges. Nearly all reproduce sexually through a free-swimming larval stage that disperses in coastal waters for hours to days before attempting a more-or-less selective settlement at specific microhabitats (Maldonado 2006). Many of these demosponges also experience asexual propagation, which in many cases results from accidental fragmentation of their body, followed by re-attachment of the tissue pieces to the substratum and subsequent reorganization into small functional sponges (e.g., Wulff 1986; Maldonado and Uriz 1999a). Although swimming and sensorial abilities of sponge larvae are limited, they may be developed enough for settling larvae to avoid very unsuitable habitats where the survival of the juveniles will be compromised (e.g., Maldonado and Uriz 1998; Maldonado 2006). In contrast, sponge body fragments that may potentially act as asexual propagules are re-suspended and dispersed by hydrodynamic forces, sinking to the bottom whenever water movement attenuates below a threshold, with no possibility of accomplishing selective settlement. Rather, they often end on horizontal surfaces along with substantial sediment loads.

While sediment may be favorable to some sponges that are soft-bottom specialists (e.g., Werding and Sánchez 1991; Ilan and Abelson 1995), it is suspected to have detrimental effects on a vast majority of species (e.g., Sarà and Vacelet 1973; Wilkinson and Vacelet 1979; Zea 1993; Maldonado and Young 1996). Sediment loads may cause clogging of the sponge filter-feeding systems and, indeed, some anatomical structures appear to be aimed to protect them from silt (e.g., Maldonado 2002). Sediment loads may also hamper sponge recruitment by both lowering the chances of settling larvae to find available rocky surfaces for attachment and burying or scouring early settlers. Nevertheless, despite the theoretical relevance usually attributed to sediment effects, the idea that high exposure to silt has negative effects on the survival of most rocky-bottom sponges has been established from indirect field observations, with little direct evidence. The outcome of the only available study attempting experimental quantitation of the effects of exposure to sediment on a sponge (*Aaptos* spp.) revealed negative effects of terrigenous deposition on both the general condition of sponges (i.e., tissue weigh relative to body size) and oxygen consumptions rates (Lohrer et al. 2006).

In this study, we investigated differences in the survival of small individuals of the demosponge *Scopalina lophyropoda* (Schmidt, 1862) as a function of exposure to sediment loads in field conditions. Previous laboratory experiments using small individuals of this sponge showed that sediment deposition stimulated sponges to crawl, probably in an attempt to find sites with lower sediment loads (Maldonado and Uriz 1999b). These observations, along with the fact that *S. lophyropoda* is fairly abundant on overhangs and rocky walls at the study site but extremely rare on sub-horizontal rocky surfaces, suggested that exposure to silt may be relevant to the spatial distribution pattern of this sponge. Nevertheless, the level at which sediment deposition is responsible for the spatial distribution and the processes through which it operates remain little investigated. Because many other encrusting demosponges show spatial distributions similar to that of *S. lophyropoda*, the results of the current study may help to improve our understanding of the spatial patterning of these common organisms. It is also noteworthy that the studied species is common on the vertical sides of large concrete blocks building the seawall of a small harbor located in the study area. However, despite its abundance on the external side of the seawall, it never occurs within the harbor. Therefore, this study is also an indirect test for the hypothesis that differences in sediment loads between the habitat of the harbor and that of the adjacent natural community may help to explain the inability of this sponge—and incidentally other sublittoral species—to colonize harbors.

Materials and methods

Study sites

This field study was conducted at 2 sites (Fig. 1). One site was the natural habitat of the sponge, a set of granitic

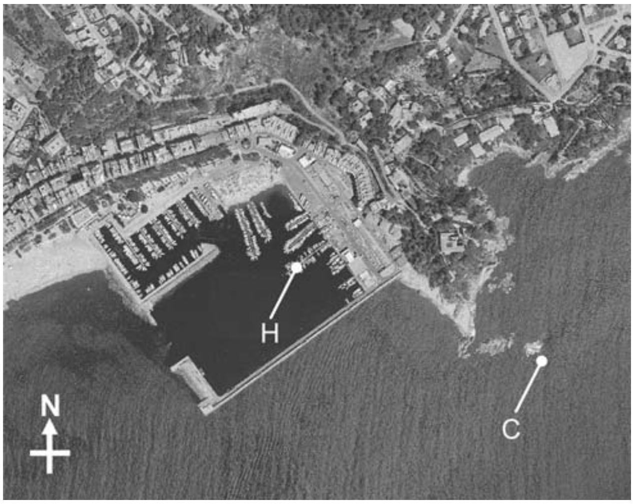


Fig. 1 Orthophotograph of the Blanes coast showing the position of the study sites within the harbor (*H*) and at the natural community (*C*) of the Sta. Ana Point. Image available from the Cartographic Institute of Catalunya (http://www.icc.es/)

outcrops with both northeast-facing and southwest-facing walls that extend from 5 to 12 m in depth, close to the Blanes coastline (2°48.12′N, 41°40.33′E; Sta. Ana Point, Spain, Western Mediterranean). The rocky walls host a typical western-Mediterranean sublittoral rocky-bottom community (e.g., Ros et al. 1985), dominated by a combination of invertebrates (sponges, cnidarians, bryozoans, sea urchins, tunicates, etc.) and semisciaphilic algae. The other study site, at which the sponge does not occur naturally, was the Blanes Marina. More specifically, we selected a set of concrete pillars supporting one of the central docks (Fig. 1). Pillars, which extend to a maximum depth of 8 m, were mostly covered by a typical fouling community: a short algal turf, including benthic cyanobacterial mats, and a combination of barnacles, serpulids, bryozoans, small colonial tunicates, and oysters. Individuals of four encrusting demosponge species (*Prosuberites* sp., *Crambe crambe*, *Hymedesmia* sp., and *Dysidea avara*) occurred here and there encrusting the pillars (personal observations). Of note, the individuals of these species were far smaller and thinner than their respective conspecific counterparts growing in the adjacent natural community outside the harbor.

Experimental setup testing for sponge survival

To obtain small individuals (approximately 1.5 mm in diameter) of *S. lophyropoda* on which assess survival as a function of the experimental treatment, we took body fragments (explants) of adult sponges (explant donors) and cultured them on experimental plates until attachment (see Maldonado and Uriz 1999a). We prepared a total of 30 plates, which consisted of 30 × 21 cm, 0.3 mm thick, translucent acetate sheets. Sheets were treated with sandpaper to create a coarse-grained surface appropriate for sponge attachment. Once coarsened, each acetate sheet was sewn onto a 0.1 mm thick, translucent sheet, on which labels for subsequent sponge identification and a grid with random-generated positions had been printed using a laser printer. These two-layer plates were immersed in running, unfiltered seawater for 2 weeks before being offered to explants for settlement in the laboratory. Then, plates were placed in 3 L plastic trays filled with 30-µm filtered seawater and explants laid with a Pasteur pipette at each of the 22 positions selected at random on each plate. Eleven positions (labeled as “A” to “K”) were provided with a 2 × 2 cm, protruding roof made of translucent acetate and aimed to protect settled sponges from sediment deposition (i.e., silt-protected; Fig. 2). Sponges at the 11 remaining positions (labeled as “a” to “k”) would be “unprotected” (i.e., silt-exposed).

Because we could not discard “a priori” whether different sponge individuals involved differences in the ability to cope with silt, we ran the experiment using a total of 660 explants obtained from 11 sponge individuals from the local population. An histological compatibility–incompatibility test assured the absence of clonality within the set of explant donors. Previous experiences on tissue fragmentation with this species (e.g., Maldonado and Uriz 1999b) and other sponges (Hildemann et al. 1979; Curtis et al. 1982) indicate that two sponge fragments placed into contact fuse each other whenever there are genetically identical (isogeneic) but reject each other whenever they are genetically different (allogeneic). Each of the 11 allogeneic explant donors contributed to each plate with two clonal (isogeneic) explants, one settled at a silt-protected position under a roof (i.e., “A” to “K” positions) and a clonemate settled at a silt-exposed position (i.e., “a” to “k” positions; Fig. 2a).

After 13 days in the laboratory, and once 70–100% of explants per sheet were attached, sheets were transported to the field. At all times, sponge-bearing sheets remained immersed in seawater. Landscape-oriented sheets were mounted onto 24 × 33 cm frames made of 2 cm-thick PVC pipe using SCUBA. The upper edge of each PVC frame had previously been drilled and a cable tie passed through the hole to tie plates from a climbing nail hammered into the substratum. The lower edge of each PVC frame had also been drilled and a 10 cm-long screw passed through this hole. Therefore, although plates were hanging from vertical substrata (i.e., rocky walls and pillars), they rested on the screw point and angled about 25° relative to the vertical. Such a sloping favored sheet surface to be moderately exposed to sediment deposition, stressing microhabitat differences between the sponges growing under the roofs and those unprotected. Fifteen plates were hung at the rocky walls in the natural habitat and other 15

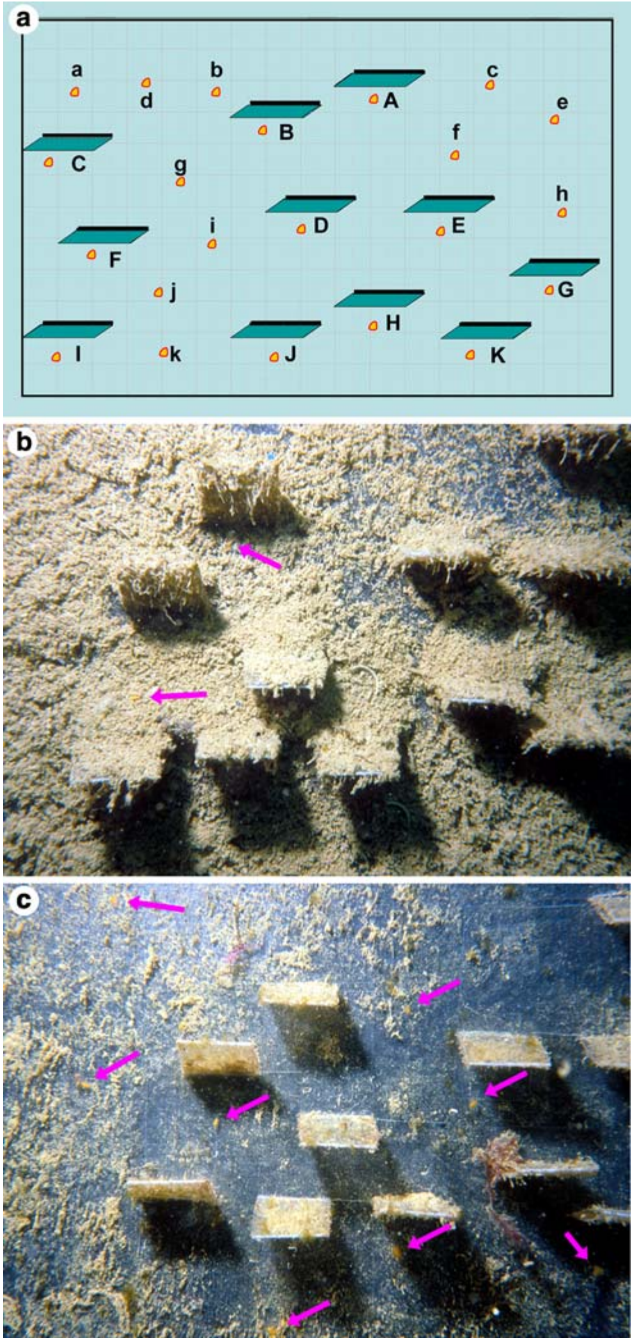


Fig. 2 Experimental panels. **a** Diagram of an acetate sheet illustrating the positions of explants to be protected from sediment deposition under the roofs (in *upper case*) and those of unprotected explants (in *lower case*). **b** Panel installed at the natural community before being cleaned and inspected for explants (*arrows*). **c** Panel installed at the natural community after being cleaned for inspection of explant survival. Note the presence of small, orange explants (*arrows*)

around 5 pillars of the harbor. To attenuate potential confounding effects of exposure to light, experimental plates at both the natural community and the harbor were randomly distributed with either northeastern or southwestern facing.

Upon completing plate installation at the two field sites (day "0" = March 27, 2001), we counted a total of 618 healthy sponges (18–22 per plate) out of the initial 660 sponge explants placed on the sheets in the laboratory. This pre-onset mortality (6.3%) was due to either explants failing attachment in the laboratory or accidental sponge damage during transportation and installation of plates in the field. To monitor sponge mortality after day "0", we visited plates at both sites using SCUBA, recording the number of sponges still alive between consecutive visits by both direct visual inspection and underwater photography (Nikonos V). Silt accumulated rapidly on the sheets because plates were sloping, so that we were forced to remove the sediment veneer at each visit to find out which sponges remained alive. Sediments were gently removed wafting water with one hand over the sheets until accumulated silt that had been re-suspended cleared away. As a result of this necessary sediment removal, we expect the detrimental effects of sediment on the sponges of our experimental approach to be less severe than those taking place in natural conditions, in which sediment loads are re-suspended from benthic organism in rare occasions only (i.e., by episodic storms).

Theoretically, daily versus weekly visits to the panels could provide a more accurate description of time dynamics of mortality, but it would also involve daily removal of silt from the plates, preventing sponges experiencing the potential detrimental effects of continuous exposure to silt. Because we could not decide whether a particular duration for the between-visits interval would have significant effects on sponge survival, we finally opted for between-visits intervals of aleatory duration, ranging from 5 to 19 days (averaging 11.2 ± 4.7 days). The experiment, which was initially planned as a 1-year monitoring, finished after 4 months (day 125), when a severe storm destroyed all experimental plates at the natural community. Because the plates located in the harbor were not affected by the storm, we decided not to uninstall them. Rather, they were left undisturbed for nearly 6 years and checked again for survival of *S. lophyropoda* explants on February 12, 2007.

Statistical analysis of sponge survival

We used a 3-way ANOVA to examine differences in sponge longevity (i.e., number of days surviving on the plates) as a function of explant donor ("a" to "k"), exposure to sediment (silt-protected vs. silt-exposed position), and habitat (natural community vs. harbor) because only about 10% of data were type-I censored (*sensu* Fox 1993), that is only 10% of sponges remained alive at the end of the experiment. Since sponges were not monitored every single day but at intervals, there was no reason to expect a normal distribution of time to death. Therefore, a rank transformation was applied to non-normally distributed data before running the 3-way ANOVA. A posteriori pairwise comparisons involving the main significant factors were made using the non-parametric Dunn's test.

Although the above ANOVA approach allowed examination of differences in average cumulative mortality after a 125-day monitoring, it neglected a global comparative analysis of time course of mortality in the different treatments. Therefore, we subsequently run a survival analysis for the four sponge groups resulting from the significant interaction between the two major environmental factors (i.e., "Silt-exposure" and "Habitat") revealed by the above 3-way ANOVA. We constructed a life table for the 618 sponges that entered the experiment, assuming that two sponges in the same habitat and under the same level of the "Silt-exposure" factor provided independent observations for survival (= failure time), irrespective of co-occurrence on a same experimental plate or not. By neglecting the potential effects of "Explant-donor" factor, we estimated the survival function for each of the four sponge groups. Differences among the survival functions of all four groups were examined using a multiple sample test, implemented in the Statistica 5.1 software package as an extension of the Gehan's generalized Wilcoxon test. We used the Cox–Mantel test (Lee 1980) to test for differences between pairs of sponge groups. The few sponges still alive at the end of the experiment (125 days) were treated as type-I censored data (Fox 1993) in both two-sample and multiple-sample tests.

Finally, we estimated rate of mortality (MR) per day and panel during each of the 11 time intervals (t1 = day 0–5, t2 = day 5–13, t3 = day 13–26, t4 = day 26–37, t5 = day 37–51, t6 = day 51–58, t7 = day 58–65, t8 = day 65–79, t9 = day 79–98, t10 = day 98–107, t11 = day 107–125) for the four sponge groups of interest. A strong exponential structure in these MR data (i.e., much higher values in the initial intervals) prevented a 3-way ANOVA analysis of differences in MR as a function of "Time", "Silt-exposure", and "Habitat" factors and their interactions. Nevertheless, because daily mortalities per plate for a given time interval are theoretically decoupled from the remaining ones, we examined differences in mean MR within each interval as a function of exposure to silt and habitat using a 2-way ANOVA on rank-transformed data. A posteriori pairwise comparisons involving the main significant factors were made using the non-parametric Dunn's test.

In the above ANOVA and Survival analyses, the possibility existed that the two levels of the "Silt-exposure" factor (i.e., silt-exposed vs. silt-protected positions) yielded non-independent data of mortality, since the treatments for both factor levels were physically associated on a same plate. We used 2 different methods to test whether or not "Plate" factor had a significant effect. First, we run a Kruskal–Wallis analysis of variance on ranks to test for between-plate (plate 1–15) differences in average individual longevity. The outcome revealed a non-significant "Plate" effect ($H = 20.359$; $df = 14$; $P = 0.119$). Additionally, we performed a randomization approach, re-distributing (permuting) at random the positions of silt-exposed and silt-protected sponges among the 15 plates of each habitat to re-calculate percent mortality for silt-exposed and silt-protected treatments in each randomized plate. By using a home-made routine running through the "macro" utility of Microsoft® Office Excel, we repeated this randomization process 500 times for each monitoring time. Mortality values for each set of randomized plates were then submitted to a 2-way ANOVA, examining differences in percentage of sponge mortality per plate as a function of "Silt-exposure" and "Habitat" factors. Whenever more than 95% of the 500 randomized ANOVAs at each monitoring time yielded significance values (i.e., $P > 0.05$, $0.001 < P < 0.05$, or $P < 0.001$) similar to those yielded by the ANOVA based on the observed data, silt-exposed and silt-protected can be considered to produce independent data. This requirement was fulfilled at all times for both the main factors and their interaction, indicating no "Plate effect" and complete independence of the levels of the "Silt-exposure" factor within our experimental setup.

Sediment deposition

Determination of sediment deposition at each of the 30 settlement plates over the study period was logistically unrealistic given our available field equipment and marked differences in sediment loads between microhabitats and over time. Therefore, our sediment sampling was addressed to assess just gross between-habitat differences (harbor vs. natural community) in sediment loads and grain-size structure. Sediment deposition was assessed by placing simple sediment traps (N = 5 per habitat) at the base of both pillars and rocky walls. Traps consisted of 33 cm-high, hollow PVC cylinders, with a 1:3 height-diameter ratio to avoid sediment re-suspension (Blomqvist and Häkanson 1981). Starting on May 1, 2001, traps were deployed five times during the experiment, being left in the field for periods ranging from 6 to 14 days, which were decoupled from sponge monitoring intervals. Upon trap retrieval, sediment samples were decanted, dried at 60°C to constant weight after seawater removal, then combusted to ash at 450°C for 5 h. Dry weight of sediment samples served to estimate average (±SD) sedimentation rate (g dry sediment per m^2 and day), while weight difference between dried and combusted samples served to estimate mean (±SD) organic content of the sediment entering the traps (i.e., % of organic matter weight relative to dry sediment weight). We examined differences in mean sedimentation rates and organic content as a function of habitat at each sampling event, also for data pooled across the five samplings. Between-habitat differences were addressed using the *t* test, except in two cases in which the non-parametric Mann–Withney rank sum test was used to deal with non-normally distributed data.

We also conducted two complementary granulometric analyses to assess between-habitat differences in grain-size structure of the sediment veneer collected on both plates and natural horizontal substrates. After gently sucking the sediment veneer with diver-operated, 60 ml plastic syringes, the silt re-suspended in the syringes was collected by decantation, dried at 60°C for 24 h, oxidized in 6% hydrogen peroxide for 48 h, and dried again at 60°C to constant weight. Subsequently, we processed sediment samples in two different ways. First, we run a gross analysis using an electrical CISA sieve (A model), which separated eight grain-size fractions (i.e., $x < 0.063$ mm, $0.063 \text{ mm} < x < 0.125$ mm, $0.125 \text{ mm} < x < 0.250$ mm, $0.250 \text{ mm} < x < 0.350$ mm, $0.350 \text{ mm} < x < 0.5$ mm, $0.5 \text{ mm} < x < 0.75$ mm, $0.75 \text{ mm} < x < 1$ mm, $x \geq 1$ mm). Granulometric structure was expressed as percentage of dry weight of the various grain-size fractions, and fractions named according to the Udden–Wentworth scale (Wentworth 1972). We performed a second analysis to examine grain-size distribution in the sediment component smaller than 100 µm. Grains in such a size range may theoretically be quite harmful to sponges, since they are susceptible to clog the filtering system by entering the narrow inhalant channels. For this analysis we used a Mastersizer 2000 laser-diffraction particle analyzer, following instructions by the manufacturer (Malvern Instruments). Between-habitat differences in the structure of this sediment component were assessed by depicting and comparing the relative abundances (volume %) of 10 µm-size grain classes (i.e., $0 \text{ µm} < x < 10$ µm, $10 \text{ µm} < x < 20$ µm, … , $90 \text{ µm} < x < 100$ µm).

Results

Sediment deposition

In the natural community, estimated sediment deposition rates ranged from 3.1 to 247.7 g of dry sediment per m^2 and day, with organic content varying from 5.5 to 18.6%, depending on trap and sampling event (Fig. 3). In the

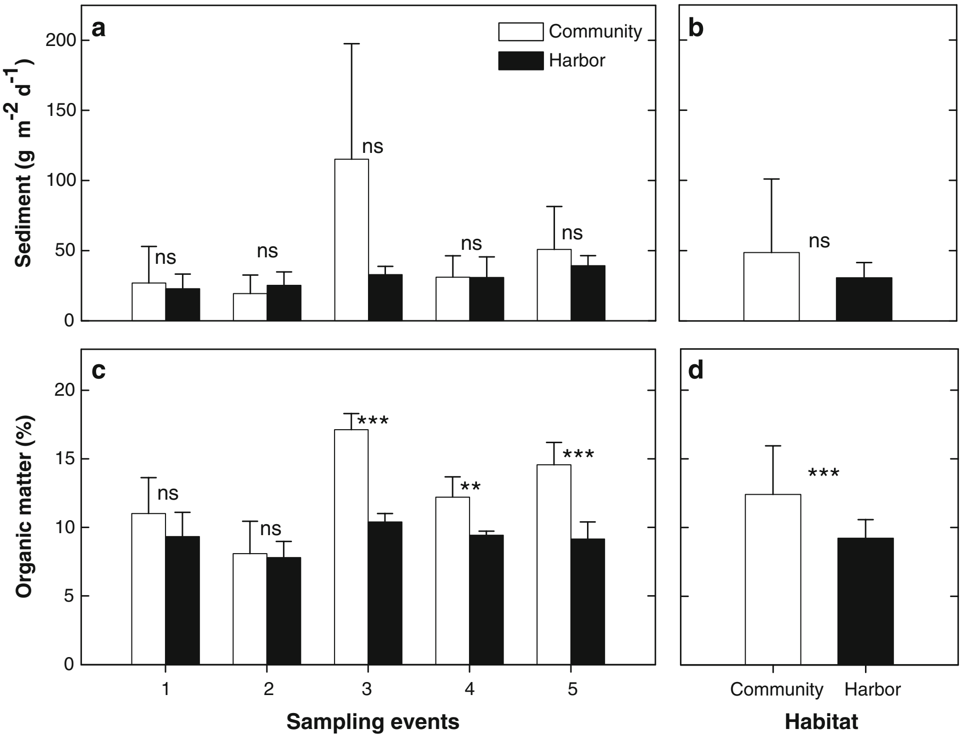


Fig. 3 Sedimentary characterization of habitats. **a** Mean (±SD) sediment deposition rates (dry weigh of sediment per m^2 and day) estimated in the two studied habitats (harbor vs. natural community) during each of the five samplings ($N = 5$ per habitat). **b** Mean (±SD) sediment deposition rates estimated in the two studied habitats after pooling data from five samplings ($N = 25$ per habitat). **c** Mean (±SD) organic content of sediment entering the traps in the two studied habitats during each of the five samplings ($N = 5$ per habitat). **d** Mean (±SD) organic content of sediment entering the traps in the two studied habitats after pooling data from five samplings ($N = 25$ per habitat). "ns" refers to non-statistical significance of either the *t* test or Mann–Withney statistic used to examine mean differences between habitats, while asterisks refer to statistically significant differences (*$0.05 > P \geq 0.01$; **$0.01 > P \geq 0.001$; ***$P < 0.001$)

harbor, sediment deposition rates showed smaller variability, ranging from 12.1 to 51.3 g m^2 day^{-1}, with organic content varying from 6.3 to 11% (Fig. 3). Despite between-trap variability, the statistical analyses detected no significant between-habitat difference in sediment weight. This pattern was confirmed by the analysis of each of the five sampling events (Fig. 3a), as well as by an analysis of pooled data ($N = 25$, per habitat; Fig. 3b). In two of the sampling events there was no significant between-habitat difference in the organic content (%) of the sediment collected in the traps, whereas the remaining three samplings showed significant differences (Fig. 3c). The analysis of pooled data indicated that mean organic content was significantly higher at the natural community than in the harbor (Fig. 3d).

The granulometric analyses revealed that the sediment veneer accumulating on horizontal and subhorizontal subtrates consisted of finer material in the harbor than in the natural community (Fig. 4a, b). The sediment in the harbor was mostly mud (68.6%), with moderate amounts of fine (10.1%) and very fine (7.9%) sand. In contrast, the sediment in the natural community was dominated by fine sands (50.6%), with only moderated contributions by very fine sand (15%) and mud (7.5%). A small percentage (3–5%) of skeletal fragments, consisting mostly of calcareous pieces (>1 mm) of bryozoans, bivalves, gastropods, and sea urchins occurred at both habitats. The granulometric analyses of the finest fraction (<100 µm) using a laser analyzer revealed that particles in the 0–30 µm range were about four times more abundant in harbor sediment than in the sediment veneer of the natural community (Fig. 4b).

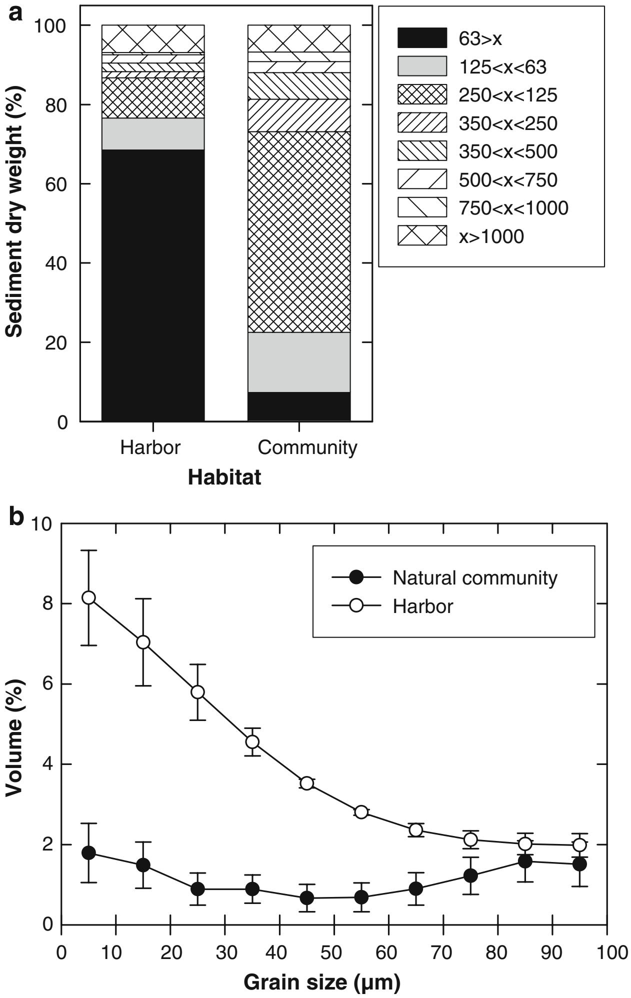


Fig. 4 **a** Grain-size structure of the sediment deposited on the experimental plates and other horizontal and subhorizontal substrates at the natural community and the harbor. **b** Between-habitat comparison of mean abundances (volume percentage ± SD) of the 10 µm, grain-size classes in the sediment fraction smaller than 100 µm, as determined by a Mastersizer 2000 laser-diffraction particle analyzer. Data were calculated on five samples per habitat

Sponge survival

Only 70 sponges (i.e., 11.3%) out of the 618 entering the experiment survived on the plates after 125 days. Average longevity of sponges was 45.9 ± 45.3 days. Surprisingly, a 3-way ANOVA revealed that sponge longevity was unaffected by "Habitat" factor (Table 1). In contrasts, it was significantly affected by both an undetermined genetic/physiological condition inherent to the explant donor and the level of exposure to silt. Although the main term of the ANOVA reported that explants originated from different donor sponges had different chances of survival on the plates (Table. 1), subsequent a posteriori comparisons revealed that differences were subtle (Fig. 5a). The complex pattern of differences between donors can be summarized by two major trends: (1) a significantly decreased survival of explants obtained from "j" donor relative to that of most other donors (i.e., "b", "d", "i", "c", "e", "a") and (2) an increased survival of explants from "b" donor relative to that of most other donors (Fig. 5a). The 3-way ANOVA also revealed that explants protected from silt under the experimental roofs had significantly increased longevity (54 days) when compared to that of silt-exposed explants (37 days; Fig. 5b). Interestingly, a significant interaction term in the ANOVA (Table 1) indicated that the magnitude of the "Silt-exposure" effect on explant longevity significantly varied with the level of "Habitat" factor. A posteriori comparisons revealed that mean longevity of silt-exposed explants was significantly shorter within the harbor (31 days) than in the natural community (43 days; Fig. 5c), suggesting that silt is more deleterious within the harbor than at the natural community.

A multiple-sample test (Chi-square = 29.83, $N = 618$, $df = 3$, $P < 0.001$) detected significant differences among the survival distributions of the four explant groups resulting from the interaction between the "Silt-exposure" and "Habitat" factors. The survival chances of silt-exposed sponges placed within the harbor were estimated to be

Table 1 Results of a 3-way ANOVA on *Scopalina lophyropoda* explant longevity (days; ranked data) as a function of explant donor (“a” to “k”), exposure to silt (silt-exposed vs. silt-protected) and habitat (harbor vs. natural community)

Source of variation	*df*	MS	*F*	*P*
Donor	10	221415.454	8.255	<0.001***
Silt	1	774201.144	28.865	<0.001***
Habitat	1	4445.990	0.166	0.684
Donor × Silt	10	21867.983	0.815	0.614
Donor × Habitat	10	19842.578	0.740	0.687
Silt × Habitat	1	162284.215	6.051	0.014*
Donor × Silt × Habitat	10	13749.674	0.513	0.882
Residual	574	26821.558		
Total	617	30973.462		

Asterisks indicate significant effects (*0.05 > $P \geq 0.01$; **0.01 > $P \geq 0.001$; ***$P < 0.001$)

lower than those of the remaining groups. Likewise, silt-exposed explants placed in the natural community had lower chances of survival than their silt-protected partners. By pooling through levels of the “Habitat” factor, we also estimated survival distributions for silt-exposed and silt-protected explants and corroborated higher chances of survivorship for silt-protected sponges using a Cox–Mantel test (statistic = 0.476, $P < 0.001$). Conversely, by pooling data through levels of the “Silt-exposure” factor, we estimated survival distributions for the explants located in the harbor and in the natural community, and found no significant between-habitat differences in survival distributions (statistic = 4.951, $P < 0.633$).

Because of the significant interaction between “Silt-exposure” and “Habitat” factors in the 3-way ANOVA, we examined in greater detail the course of mortality in each of the four explant groups resulting from the interaction of these factors (Figs. 6, 7). Mortality rates in all four groups showed a marked exponential distribution (Fig. 7), with high values during the first time intervals and subsequent gradual attenuation over time, becoming virtually zero by the middle of the experiment (t6). The set of 2-way ANOVAs revealed that exposure to silt significantly increased mortality rates mostly in the beginning of the experiment (t1 and t3). Mortality reactivated after day 65 (t6) in all four sponge groups (Fig. 6, 7), but peaked more abruptly in the two sponge groups located within the harbor, irrespective of being exposed to or protected from sediment deposition (Fig. 7). Such a reactivation of mortality among silt-protected sponges in the harbor (87.9%) lead them to be the second group in cumulative mortality at the end of the experiment (Fig. 6), ranking just behind the silt-exposed sponges in the harbor (98.1%). At this final stage, cumulative mortality per panel was lower in the natural community, averaging 85.6 and 83.5% for the silt-exposed and silt-protected explants, respectively. Interestingly, when all 15 panels remaining immersed in the harbor for 6 years were re-visited, they were completely (100%) covered by an assemblage of typical fouling organisms, such as oysters, barnacles, bryozoans, hydrozoans, ascidians. Among these dominant invertebrates, small individuals of the five sponge species that are able to survive “naturally” on the adjacent pillars (i.e., *C. crambe*, *D. avara*, *Prosuberites* sp., *Hymedesmia* sp., and *Clathrina coriacea*) also occurred. Nevertheless, no *S. lophyropoda* explant survived on the experimental plates.

Discussion

The various statistical tests indicated that exposure to silt was a significant cause of sponge mortality. Inspection of

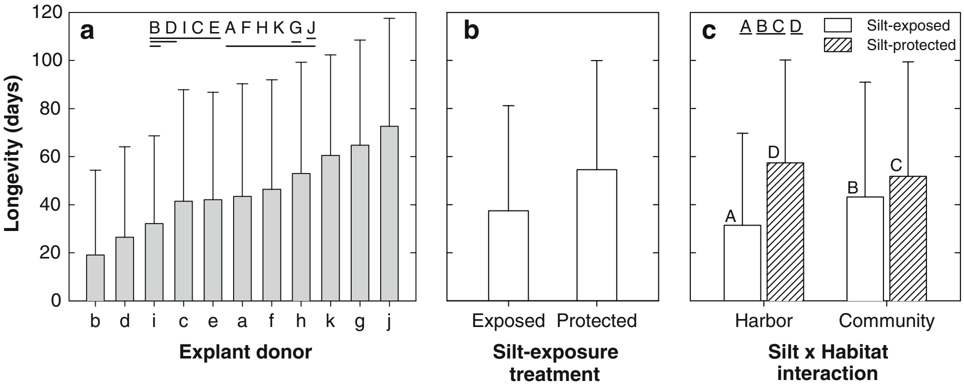


Fig. 5 Illustration of the significant factors of 3-way ANOVA (see Table 1) that tested for differences in mean explant longevity (±SD) as a function of **a** explant donor (“a” to “k”), **b** exposure to silt (silt-protected vs. silt-exposed sites), and **c** habitat (harbor vs. natural community). Letters in **a** (“A” to “J”) and in c (“A” to “D) indicate mean values arranged in ascending order of magnitude. *Groups of underlined letters* refer to non-significant differences ($P > 0.05$) between pairs of means according to a posteriori Dunn’s tests following a 3-way ANOVA (Table 1)

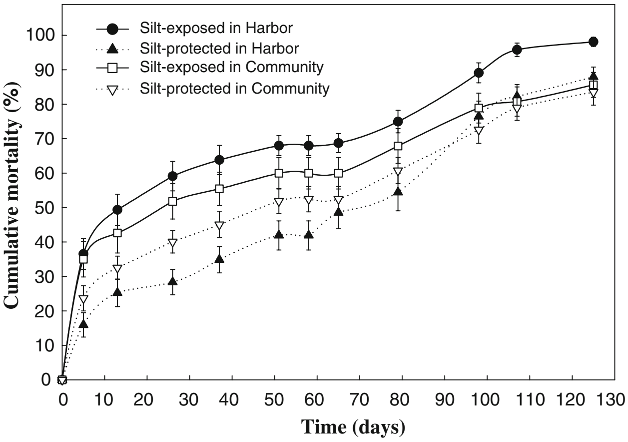
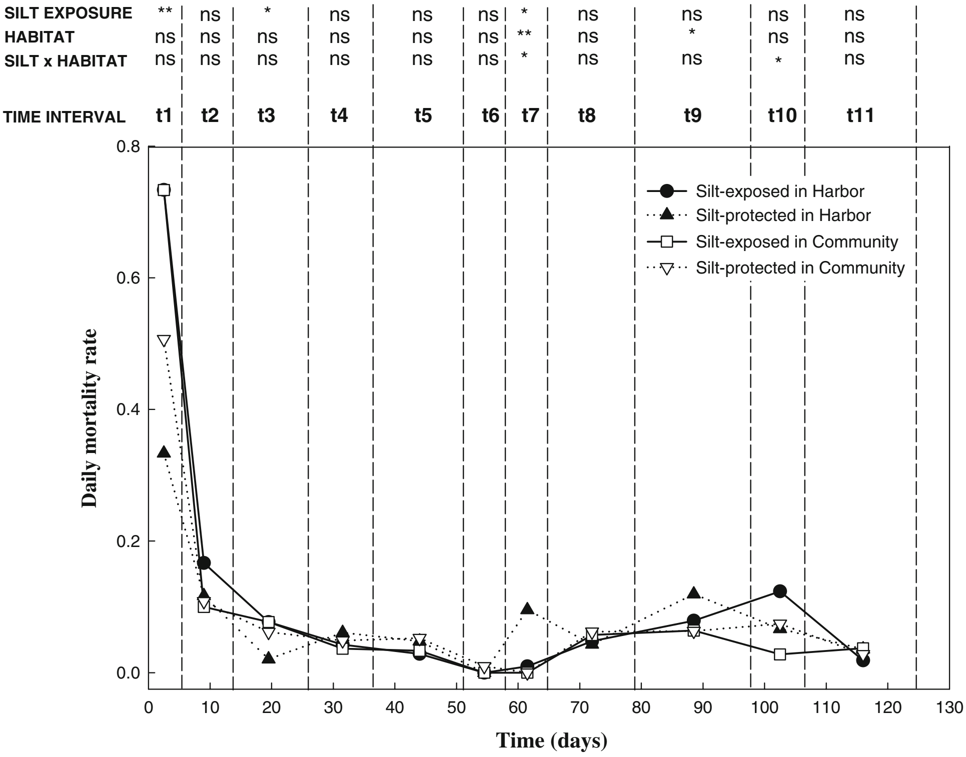


Fig. 6 Average (±SD) cumulative mortality (%) per panel for the four sponge groups resulting from the interaction of "Silt-exposure" and "Habitat" factors over the entire experiment

mortality rates revealed that the negative effects of silt were mostly operating on early settlers at the beginning of the experiment (Fig. 7). Nevertheless, the differences caused by such initial withdrawal in the number of silt-exposed explants relative to silt-protected explants persisted in the plates during nearly the entire duration of the experiment (Fig. 6). We also obtained evidence that the effects of silt were modulated by habitat, being more deleterious within the harbor than in the natural community. Given that there were no significant between-habitat differences in sedimentation rate and that the organic content of the sediment was higher in the natural community than in the harbor, the increased harmfulness of harbor silt is thought to derive from its finer grain-size structure (Fig. 4b). Our granulometric analysis revealed that particles smaller than 30 μm are about four times more abundant in the sediment veneer laid on horizontal substrates in the harbor than in the natural community. In addition to enhanced chances of clogging, the more deleterious effect of harbor silt may also derive from adsorption of toxicans (oils, heavy metals from antifouling paints, etc.) on the small particles that enter the aquiferous system of the sponges or end on the delicate external epithelium. It is also worth noting that episodic wavy or stormy conditions were noticed to wash the sediment veneer out of the sponges and other sessile organisms in the natural community during the experiment, a periodical "natural" cleaning that may facilitate survival of early recruits. Such facilitation never happened in the studied harbor and it is unlikely to happen in other harbors and similar wave-protected environments.

Global survival of *S. lophyropoda* explants on the experimental plates after 4 months was about 11%, a modest value contrasting with nearly 100% survival found for these explants in laboratory conditions (e.g., Maldonado and Uriz 1999a, b). Initial mortality (t1), i.e., that measured upon the onset of the experiment, ranged from 15% for silt-protected sponges in the harbor to 35% for silt-protected sponges in the harbor, showing subsequent progressive attenuation for all 4 explant groups (Figs. 6, 7). This exponential pattern is consistent with a variety of studies on marine invertebrates

Fig. 7 Mean mortality rates (MR), calculated as number of individuals dying per panel and day in each treatment group within each time interval ("t1" to "t11"), are plotted versus time. Error bars for mortality rates have been omitted because of uninformative overlapping. On top, the results of 2-way ANOVAs testing for differences in MR as a function of habitat and exposure to silt for each time interval are summarized. "*ns*" indicates non-statistical significance, while *asterisks* refer to statistically significant differences (*0.05 > $P \geq 0.01$; **0.01 > $P \geq 0.001$; ***$P < 0.001$)



other than sponges, in which mortality rates of newly settled individuals are reported to be slightly higher than that of subsequent stages (e.g., gastropods: Underwood 1975; barnacles: Gaines and Roughgarden 1985; ascidians: Hurlbut 1991). Nevertheless, values of initial mortality (first week) among our explants were twice (15–35% on average) those detected for juveniles of *S. lophyropoda* developed from swimming larvae that settled on experimental settlement plates offered in the field (6–12% on average; Maldonado and Uriz 1998). This difference may derive from the fact that sexually produced larvae are provided with substantial amounts of special nutritional storage (i.e., yolk) aimed to cover energy demands during the process of settlement and until a functional aquiferous systems for initiating filter feeding is formed in the settlers. In contrast, explants are just tissue fragments that experienced no special process of energy accumulation prior to detachment, a clear disadvantage to face the non-feeding transition towards functional settlers and the first days as recruits.

It is noteworthy that mortality in all four sponge groups leveled down close to zero by the mid of the experiment. Although this was a transitory situation, a study ending after just a 2-month monitoring would have erroneously concluded that the success of early recruitment was about 30–60% and that it was unaffected by the "Habitat" factor (Fig. 6). Likewise, a study lasting only 2 months would easily have concluded that the chance of survival within the harbor were higher than in the natural community whenever the explants were protected from silt (Fig. 6). Nevertheless, our monitoring detected that about 2.5 months after the onset of the experiment, an undetermined factor of mortality came into action, being particularly deleterious for the sponges within the harbor, irrespective of exposure to silt (Figs. 6, 7). Identification of such mortality factor falls beyond the scope of our experimental approach. It is possible that heavy metals or other toxicans gradually accumulating within the body of the sponges in the harbor surpassed the threshold of sublethal toxicity after 2 months and became lethal for these small individuals. Accumulation of toxic compounds by sponges that are artificially transplanted to harbors has been demonstrated (e.g., Cebrian et al. 2003, 2006). Alternatively, we cannot discard that mortality rates were re-activated by seasonal agents, such as recruitment of selective predators (e.g., Mariani and Uriz 2001), outbreak of harmful bacteria or fungi that became more deleterious for the stressed sponges installed in the polluted environment of the harbor, etc. Whatever the mortality agent, the fact is that sediment deposition itself cannot be blamed for the inability of this sponge to establish successful populations within the harbor, since, although the survival of the explants in the harbor was initially modulated by their exposure to silt, soon other mortality factors came into action, causing serious damage even among the silt-protected explants. Furthermore, inspection of the panels placed in the harbor 6 years after their initial deployment corroborated the inability of *S. lophyropoda* for a long-term survival in this unnatural habitat.

The detrimental effects of sediment on the small individuals of *S. lophyropoda* revealed in this study suggest that natural recruitment in this species, either from sexual or asexual origin, is expected to be negligible on horizontal substrata but enhanced on vertical walls, overhangs, and in silt-protected crevices and microrefuges. Such a prediction agrees well with the field distribution of the studied local population, in which sponges never occur on subhorizontal substrata. Occasional occurrence of individuals of *S. lophyropoda* growing on subhorizontal substrata is known from some sites at Port-Cros (France) and the Croatian coast (J. Vacelet, pers. comm.), but, unfortunately, the potential differences in the sedimentological regime between those sites and the community monitored in this study remain unknown. Our results also support the idea that asexual propagules, which are unable to swim, would rarely have the chance to recruit on silt-protected sites and survive in the field. Consequently, low levels of clonality are predicted to occur in populations of both this species and other sublittoral sponges being similarly sensitive to silt. Likewise, it cannot be ruled out that particular regimes of sediment deposition may have potential to modify the genetic structure of natural populations in the long run by both reducing the impact of clonality and favoring recruitment of those genotypes with better performance when exposed to silt. Rates of sediment deposition have kept increasing over the past few decades in most coastal areas as the result of deforestation and augmented erosion (Valiella 1995; Airoldi 2003). Therefore, such a direct relationship between sediment deposition and aspects of population genetics for invertebrates that are key member in many marine communities cannot be regarded as trivial and may deserve further attention.

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