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Biofouling

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713454511>

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First published on: 27 May 2009

To cite this Article Guarnieri, Giuseppe, Terlizzi, Antonio, Bevilacqua, Stanislao and Frascchetti, Simonetta(2009) 'Local vs regional effects of substratum on early colonization stages of sessile assemblages', *Biofouling*, 25: 7, 593 – 604, First published on: 27 May 2009 (iFirst)

To link to this Article: DOI: 10.1080/08927010903013656

URL: <http://dx.doi.org/10.1080/08927010903013656>

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Local vs regional effects of substratum on early colonization stages of sessile assemblages

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(Received 12 January 2009; final version received 30 April 2009)

Substratum type and topographic complexity influence the settlement and persistence of benthic organisms. However, the combined effect of these two factors in affecting colonization patterns at different scales has rarely been investigated. A manipulative experiment was conducted to test the interplay of rock type and roughness in affecting the pattern of subtidal assemblages and to provide tests for the generality of effects across a range of spatial scales (centimetres to hundreds of metres). Replicate tiles of four different rock types, with two levels of surface roughness were deployed in rocky subtidal habitats (5 m depth) at two sites (separated by hundreds of metres) at each of three locations (separated by tens of kilometres). Spatial and temporal variation in the colonization patterns over 9 months differed among rock types. However, large-scale processes appeared to be far more important than substratum type or roughness in determining assemblage structure. Predicting the consequences of the introduction of artificial structures into the coastal marine environment is critical as increasingly parts of coastlines are being modified within the Mediterranean and other regions. The results suggest that further investment is needed to manage and mitigate the effects of the deployment of artificial structures in coastal areas.

Keywords: marine rocky habitats; substrate heterogeneity; colonization processes; spatial scale; subtidal; artificial substrata

Introduction

Spatial and temporal heterogeneity are widely recognized as general properties of natural systems, which are intrinsically dependent on the scale of measurement (Levin 1992; Wiens 2001). At small spatial scales, heterogeneity is affected by biological interactions and local physical processes, whereas biogeographic and climatic processes combine to affect patterns of variation in population and assemblages at large scales. As these processes operate simultaneously, quantification and prediction of potential changes in patterns of distribution in biodiversity require an investigation of the effects of local processes embedded within those at a larger-scale (Steele 1985; Halley 1996). From an applied point of view, investigating processes that shape populations and assemblages at different scales is crucial because human activities interact with ecological processes, and affect spatial and temporal variability at different scales (Peres et al. 2006; Angeler and Moreno 2007).

In both terrestrial and marine systems, the availability and the physico-chemical characteristics of substrata have been broadly acknowledged as crucial environmental drivers of variability in the distribution of organisms at multiple scales (Orwin et al. 2006; Nilsson et al. 2008). In the marine realm, settlement

and post-settlement events can be affected by the properties of the substratum, which act interactively to shape marine communities (Faimali et al. 2004; Herbert and Hawkins, 2006; Jenkins et al. Forthcoming 2009). Substratum availability, microtopography and surface smoothness (Schumacher et al. 2007; Prendergast et al. 2008; Scardino et al. 2008), color (Swain et al. 2006; Finlay et al. 2008) and surface contour (Wetthey 1986) can be limiting factors at local scale, and invertebrate larvae have developed complex behaviors and finely tuned discriminatory abilities to ensure successful settlement in the face of variations in substratum properties (Underwood and Keough 2001; Faimali et al. 2003; Frascchetti et al. 2003). On a larger scale, oceanographic processes affect benthic assemblages by influencing the rate of larval delivery to coastal habitats (Denny and Wetthey 2001). The mineralogical composition of substrata might also play a significant role in shaping benthic communities, affecting both primary colonization processes and later stages of community development (Cerrano et al. 1999).

Studies on the role of habitat structure (ie complexity and type of substratum) in affecting the heterogeneity of species and assemblages on rocky substrata have consistently revealed that species diversity can be

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regulated by habitat structure, and complex habitats are normally associated with greater species richness than simple ones (Bergeron and Bourget 1986; Johnson et al. 1998). However, quantifying the amount and variety of space available to organisms by topographically rough surfaces can be difficult (Johnson et al. 2003), and complexity *per se* depends on the spatial scale of examination (Kolasa and Pickett 1991).

Although the importance of the nature of the substratum is broadly recognized in experimental deployments of colonization substrata, there is still scant information on the effects of the material used to produce artificial substrata. Specifically, it is often not known whether introduced substrata can mimic surrounding natural rocky substrata (Connell 2000; Bulleri 2005). Most studies have used artificial substrata in experiments that examine the effects of habitat complexity (eg topographic complexity) on recruitment and assemblage development by manipulating different kinds of structural components that are assumed to mimic features of natural substrata (Lapointe and Bourget 1999; Pech et al. 2002). Because artificial substrata are usually different from natural ones, the use of the former can limit the ability to quantify the influence of substratum characteristics on marine assemblages (Connell 2000; Bulleri 2005). Furthermore, the effects of habitat structure have been traditionally analyzed at local scales (Keough 1983; Connell and Glasby 1999; Downes et al. 2000), thereby limiting the potential to understand the relative importance of local *vs* regional processes in shaping the distribution patterns of populations and assemblages.

In this study, a manipulative experiment was carried out at scales ranging from centimeters to hundred of meters to test whether the effects of habitat structure, in terms of substratum type and topographic complexity, are scale dependent. It was hypothesized that local factors may determine idiosyncratic patterns of colonization of species and assemblages, eg high variability in species number, identity and relative abundance. The spatial patterns of populations and assemblages on natural rocky subtidal substrata were also quantified and compared with those observed in assemblages colonizing artificial substrata. As far as is known, this is the first study exploring the interactive effect of the nature of the substratum and its topographic complexity of rocky subtidal habitat at a range of spatial scales. It is anticipated that the results will provide baseline information on the interactive effects of processes operating at different spatial scales on the settlement and on post-settlement events of sessile marine assemblages. The outcomes of this study are therefore relevant in the event of introduction of artificial substrata in the rocky subtidal and highlight

the role of artificial structures in affecting the biodiversity of the marine landscape.

Materials and methods

Study site

The study was conducted in the SE of Apulia (Italy) between February and December 2005. Three locations on exposed rocky shores without clear signs of human disturbance were chosen, *viz* P.to Cesareo (Location 1; 40°12'10"N–17°55'06"E); Otranto (Location 2; 40°08'21"N–18°30'30"E); T.re Guaceto (Location 3; 40°43'02"N–17°47'58"E) (Figure 1). The adoption of multiple locations was not intended to provide contrasting physical conditions, which would result in non-replicated location for the level of exposure, but was aimed at testing for the consistency of patterns across variable ecological settings. Previous studies (Fraschetti et al. 2001, 2005, 2009; Terlizzi et al. 2007) and a preliminary survey allowed comparable sites to be identified at each location. All sites were selected within wave-exposed headlands, with the same slope (about 30% in each site) and substratum (limestone). Two sites, 100 m apart, were randomly selected and at each location tiles were deployed and sampled on two dates at each site. The quantification of the spatial patterns of populations and assemblages was carried out on the same dates and sites where tiles were deployed, which allowed the pattern of variability of local populations and assemblages with those colonizing artificial substrata to be compared.

Effect of the substratum

Tiles, 15 cm × 10 cm × 3 cm, of four different types, namely limestone, sandstone, granite and concrete were used in the experiment. Limestone occurs naturally along rocky shores in Apulia. Sandstone and granite are common along other Mediterranean

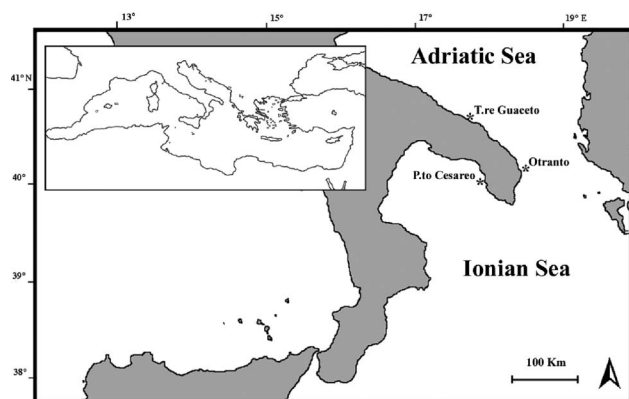


Figure 1. Study area and position of the three locations (*).

rocky shores, while concrete is utilized for man-made artificial structures such as seawalls. An industrial company (www.italcementigroup.com) provided tiles at two levels of topographic complexity (ie completely smooth and rough). To guarantee experimental units with comparable complexity, the surface area (SA) ratio (Dahl 1973) was used to estimate the topographic complexity obtained after the treatment of tiles. For a sub-sample of 40 tiles, seven parallel-profiles were measured across the length of the tiles. Each profile consisted of 15 data points with an *x*-axis spacing of 1.63 cm (105 readings per tile). The data were organized in an XYZ coordinate worksheet and interpolated by a GIS software (Arcview 8.1) in order to estimate the true surface of the tiles (Digital Elevation Model). The estimated true surface area was divided by the planar surface area of the tile to obtain the corresponding value of the SA ratio (Dahl 1973). The estimated topography of the different substrata showed the experimental units as characterized by a very similar level of complexity (SA ratio = 1.005 ± 0.002). The level obtained, as recorded by replicate readings of a profile gauge at the same scales of the tiles (Frost et al. 2005), was roughly comparable to the complexity of the natural rocky shore at the experimental sites. Differences in surface topography at a scale smaller than that considered in this study, ie a micron, are known to affect colonization processes of benthic organisms (eg Berntsson et al. 2000; Herbert and Hawkins 2006).

At each site, 80 tiles (five replicates for each combination of substratum, roughness and time) were randomly deployed and fixed by plastic ties on plastic nets (mesh 3 cm), which were anchored to the bottom at 5 m depth. All tiles were deployed in February and sampled after 3 and 9 months from the beginning of the experiment. After collection, each tile was stored in 4% formalin and seawater solution and transported to the laboratory for analysis. Throughout the experiment, the presence of sea urchin grazers, including *Paracentrotus lividus* and *Arbacia lixula*, was only occasionally recorded at the three locations. However, in order to avoid confounding the grazing effect with those of the substratum, they were removed from the experimental sites, when found to be present. Removal of sea urchins was effective and they were never found on any of the experimental tiles.

To avoid edge effects only an area 9 cm \times 14 cm was sampled for each tile, leaving a margin of 0.5 cm for each side. The percentage cover of sessile organisms on tiles was estimated under magnification by superimposing a grid of 24 sub-quadrats on each tile (sub-quadrat surface was 2.3 cm \times 2.25 cm). The total number of taxa and their relative abundance were assessed on a scale from 0 (absence) to 4 (totally

covered) for every taxon present on each sub-quadrat; these values were then added up for the 24 sub-quadrats (Meese and Tomich 1992; Dethier et al. 1993). Final values were expressed as a percentage. Organisms that were not easily identifiable at species level were collapsed into higher taxonomic groups or into morphological groups (Steneck and Dethier 1994, see Appendix A in supplementary information for more details [Supplementary material is available *via* a multimedia link on the online article webpage.]). Motile animals such as gastropods, polychaetes and peracarid crustaceans were not counted.

Assemblages of local rocky subtidal: quantification of natural spatial patterns

Assemblages at a depth of 5 m were sampled photographically using a Nikonos V underwater camera, 28 mm focal length, close-up macrosystem and two SB105-Nikon strobes. In order to prevent the potential loss of observation units, 13 randomly located surfaces of 16 cm \times 23 cm were photographed in each site at each sampling time. Randomization in sampling was achieved through the use of random digits. Random subsets of 10 out of the 13 pictures were used in the analysis, yielding a total of 120 units of observation.

The slides were analyzed under a binocular microscope by superimposing a transparent sub-dividing grid of 24 equally sized squares on the entire photographed surface, following the same rationale described above for the analysis of tiles.

Destructive samples were collected for later identification of organisms present on the slides. Organisms not identified at species level were collapsed into higher taxonomic groups or into morphological groups. The accuracy in the quantification of percentage coverage of single taxa from the slides was facilitated by the lack of an algal canopy (eg *Cystoseira* spp., Dictyotales) at any of the sample sites. Full taxonomic details of the variables recorded are reported in Appendix A in the supplementary information.

Statistical analyses

For the analysis of the effects of the substratum, the experimental design consisted of five factors, *viz* Time (Ti, 2 levels, fixed), Location (Lo, 3 levels, random and orthogonal), Site (Si, 2 levels, random, nested in Lo), Substratum (Su, 4 levels, fixed and orthogonal) and Roughness (Ro, 2 levels fixed and orthogonal), with $n = 5$ replicates for each combination of factors (480 samples and 20 variables). A distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson 2001a; McArdle and Anderson 2001) was performed to test the hypothesis of no effect of nature

and roughness of substrate on the multivariate assemblages colonizing the tiles. The analysis was based on Bray–Curtis dissimilarities (Bray and Curtis 1957) on square root transformed data to smooth out the contribution of conspicuous colonizing species to the multivariate patterns (Warwick 1993). Each term in the analysis was tested by 4999 random permutations of appropriate units (Anderson 2001b; Anderson and Ter Braak 2003). To reanalyze the effects of the nature of the substratum and its roughness irrespective of spatial processes associated with the geographical position of sites, the analysis was also conducted using the distances between each site as covariable (Benedetti-Cecchi and Osio 2007). Real distances were obtained by a GIS software (Arcview 8.1) using the rank order of the position of shores around the Salento peninsula (starting from P. Cesareo). Multivariate patterns of differences between substrata were visualized for each sampling times by non-metric multidimensional scaling (nMDS) (Kruskal and Wish 1978) of $Su \times Si(Lo)$ centroids. The percentage contribution of each taxon to the dissimilarity between site and between types of substrata for each sampling date was calculated (SIMPER analysis, Clarke 1993) and variables/taxa contributing for at least the 5% to recorded values of dissimilarity were selected as important differentiators. All multivariate analyses were performed using the computer program PRIMER v6 (Clarke and Gorley 2006), including the add-on package PERMANOVA+ (Anderson et al. 2008).

Analysis of variance (ANOVA) was used to test for single response variables (ie total abundance, number of taxa and abundance of important differentiators), and the same hypotheses described above for the whole assemblage. Prior to analyses, the assumption of homogeneity of variances was checked using Cochran's *C*-test and, if required, data were appropriately transformed to remove heteroscedasticity (Winer et al. 1991). When homogeneity of variances could not be achieved by transformation, data were analyzed nonetheless, as ANOVA is robust for departure from this assumption when there are many independent replicates and sizes of samples are equal, as in this case (Underwood 1997). Results were, however, interpreted with caution by judging significance more conservatively ($\alpha = 0.01$). Only terms relevant to the hypotheses (ie those involving the factors substratum and roughness) were tested and terms involved in significant higher-order interactions were not analyzed. All ANOVAs were performed using GMAV version 5 software (University of Sydney, Australia).

PERMANOVA (120 samples and 33 variables) and nMDS of individual observation units based on Bray–Curtis dissimilarities, on transformed data (square root), were also used in the analysis of natural spatial

patterns of assemblages for the two sampling dates. In this case, the design consisted of three factors: Time (Ti, 2 levels, random), Location (Lo, 3 levels, random and orthogonal) and Site (Si, 2 levels, random, nested in Lo).

Estimate of sediment load

On the basis of previous knowledge of ecological features at the three locations, and given the crucial role of sedimentation in affecting settlement processes and colonization on rocky surfaces (for a review see Airoldi 2003), three sediment traps (PVC pipe) were located at each location in order to estimate the mean regime of sedimentation in the field for the whole period of the experiment. Each pipe had a H/D ratio > 10 (height 60 cm; diameter 4 cm; $H/D = 15$), to retain a wide size fraction of sediment (Schiel et al. 2006) and to limit the danger of resuspension effects. Traps were emptied at the end of the experiment by pouring the contents into a bucket, which was then sealed and taken back to the laboratory. The contents were poured through sieves of different sizes to separate the size fractions and then transferred onto filter paper. Samples were dried for 72 h at 50°C and then weighed. Three meshes were utilized, *viz* 1, 0.5 and 0.063 mm, the last fraction employed a GF/F filter (0.7 μm), which was dried at 450°C for 4 h.

Results

Effect of the substratum

PERMANOVA highlighted the significance of the interaction terms $Ti \times Si(Lo) \times Su$ and $Ti \times Lo \times Su$, indicating that differences across substratum type varied across times at the scale of both sites and locations (Table 1). Pairwise comparisons of the four substrata within each $Ti \times Si(Lo)$ combination (Table 2) suggested that the overall pattern of differences among substrata were mainly driven by a higher similarity between concrete and sandstone which, in turn differed globally from limestone and granite. These latter two substrata were significantly different in the majority of tests. This general pattern of difference among substrata emerged after 9 months exposure (Time 2) and, although varying in magnitude between sites, was more evident at Locations 2 and 3.

PERMANOVA results were clearly depicted by the nMDS ordination of the centroids of the substrata (Figure 2). During the earlier colonization phase, differences among substrata emerged (Figure 2, T1) and after 9 months, assemblages present on limestone and granite were clearly different from those on the other two substrata (Figure 2, T2). However, the level of separation varied among locations. Specifically,

Table 1. Multivariate analysis (PERMANOVA) based on Bray–Curtis dissimilarities (square root transformed), conducted on a whole set of variables (20 taxa × 480 samples) of experimental units.

Source of variation	d.f.	MS	F	P (perm)	MS _{DEN}
Time = Ti	1	133,510.21			
Location = Lo	2	85,223.97			
Site(Lo) = Si(Lo)	3	10,420.77			
Substratum = Su	3	5,224.06			
Roughness = Ro	1	2,381.72			
Ti × Lo	2	27,295.15			
Ti × Si(Lo)	3	6,270.02			
Ti × Su	3	1,127.79			
Ti × Ro	1	1,201.41	1.70	0.2814	Ti × Lo × Ro
Lo × Su	6	1,305.92	1.65	0.1378	Si(Lo) × Su
Lo × Ro	2	1,093.62	0.84	0.5442	Si(Lo) × Ro
Si(Lo) × Su	9	790.65	1.42	0.055	Res
Si(Lo) × Ro	3	1,296.64	2.32	0.0076	Res
Su × Ro	3	891.77	1.64	0.2096	Lo × Su × Ro
Ti × Lo × Su	6	2,683.73	3.34	0.002	Ti × Si(Lo) × Su
Ti × Lo × Ro	2	707.94	1.63	0.2352	Ti × Si(Lo) × Ro
Ti × Si(Lo) × Su	9	802.98	1.44	0.0454	Res
Ti × Si(Lo) × Ro	3	434.95	0.78	0.6814	Res
Ti × Su × Ro	3	204.82	0.37	0.9398	Ti × Lo × Su × Ro
Lo × Su × Ro	6	543.47	0.97	0.5076	Si(Lo) × Su × Ro
Si(Lo) × Su × Ro	9	561.57	1.01	0.435	Res
Ti × Lo × Su × Ro	6	558.78	1.19	0.3176	Ti × Si(Lo) × Su × Ro
Ti × Si(Lo) × Su × Ro	9	469.02	0.84	0.738	Res
Residual	384	558.50			
Total	479				

Each test was performed using 4999 permutations of appropriate units. The term used for the denominator mean square in each case is given in column MS_{DEN}. Terms already involved in significant higher-order interactions were not analysed.

Table 2. Results of multivariate pairwise comparisons between the four substrata at each site within location and time.

Location	Time	Site	Substratum comparisons					
			Li vs Co	Li vs Gr	Li vs Sa	Co vs Gr	Co vs Sa	Gr vs Sa
P.to Cesareo (L1)	T1	S1		**		*		
		S2					*	
Otranto (L2)	T2	S1		*				
		S2				*		
	T1	S1		*	*	***		***
		S2		*	*			
T.re Guaceto (L3)	T2	S1	*	***	*	*	*	*
		S2	*	***	**	***	**	***
	T1	S1	**	*		***		*
		S2					*	
	T2	S1	**	**	***	***		***
		S2	***	***	***	***	**	***

Li = limestone, Co = concrete, Gr = granite, Sa = sandstone; *P < 0.05; **P < 0.01; ***P < 0.001.

Location 1 revealed very different patterns, showing smaller differences among the different substrata with respect to what was observed at the other two locations. Multivariate analysis also revealed the significance of the interaction term Si(Lo) × Ro. Pairwise comparisons on this interaction, however, showed significant differences for the factor ‘roughness’ only at one site at Location 2. Repeating the analyses including distances between sites as the

covariate, to remove the effects of spatial processes associated with the geographical position of sites, did not change the overall pattern described above.

The identity and abundance of a suite of algae drove changes in the structure of the assemblages observed through multivariate analyses. SIMPER (see Appendix B in the supplementary information for more details [Supplementary material is available via a multimedia link on the online article webpage.]

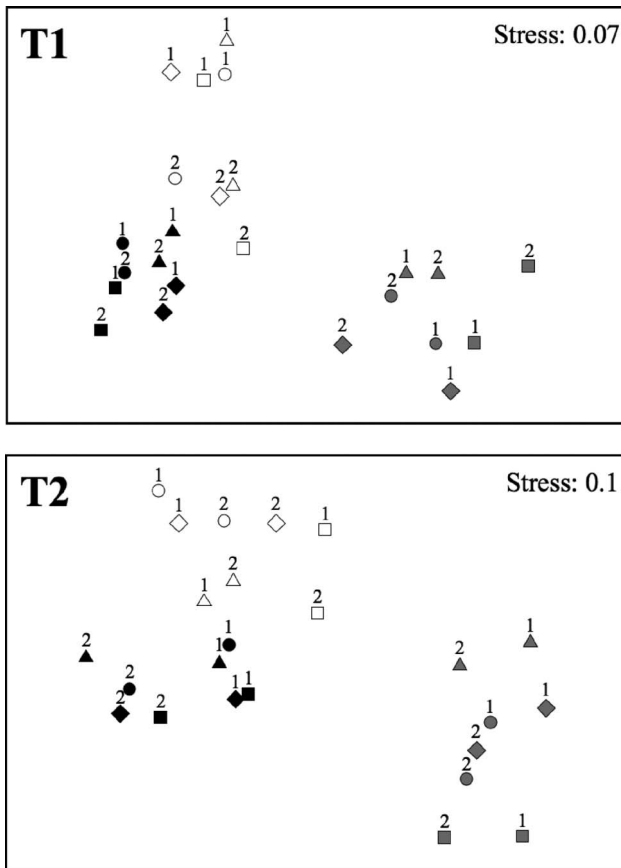


Figure 2. Non-metric multidimensional scaling (nMDS) on the basis of the Bray–Curtis dissimilarity measure of substratum centroids in both sites (1 and 2) for each location in the two sampling dates (T1 and T2). Black = Location 1; Gray = Location 2; White = Location 3; Δ = limestone; \circ = concrete; \square = granite; \diamond = sandstone.

showed that differences mainly depended on the type of algae, namely Green Filamentous Algae (GFA), Encrusting Coralline Rhodophytes (ECR) and Dark Filamentous Algae (DFA). GFA contributed to differences among substrata especially in the initial colonization phases. After 9 months, such differences depended mainly on ECR and DFA. The contribution of other algal taxa, namely Articulate Corallines (AC), Dictyotales (DIC), *Mesogloia vermicularis* (MES), *Caulerpa racemosa* (CAU) and Ceramiales (CER) was less relevant or exclusive to a single location. The contribution of invertebrates (mainly hydroids) to dissimilarities among substrata was generally low, except for Location 2 where their contribution was >10%.

ANOVA carried out on the mean percentage cover, the mean number of taxa and on the mean abundance of taxa identified by SIMPER analysis, are summarized in Table 3. For the analyses, invertebrates were

collapsed into a single group (INV). The number of taxa showed significant differences in the interaction term $Su \times Si(Lo)$, indicating an effect of substratum variation between sites. Roughness did not show a significant effect either for the mean percentage cover or for the number of taxa.

ANOVA showed a significant effect of substratum on GFA, inconsistent in time and between sites, and a significant interactive effect of roughness and substratum on Dictyotales. In contrast, DFA highlighted a significant effect of both substratum and roughness, which varied between sites and time. There was a main effect of the substratum on the coverage by the alga *Laurencia* spp. Ceramiales and invertebrates were influenced significantly by both roughness and substratum, with effects changing in time and between sites. Interestingly, *post hoc* comparisons of roughness within levels of the $Ti \times Si(Lo)$ interaction showed the highest values of mean percentage cover for invertebrates in T2 on tiles with a rough surface (Table 3, Figure 3b).

Quantification of natural spatial patterns

PERMANOVA (Table 4) showed the significance of the term $Ti \times Si(Lo)$, indicating a complex pattern of variability between natural assemblages, with differences between sites within locations that varied in time. At the larger scale, the analyses also revealed the significance of the $Ti \times Lo$ interaction term, an outcome that was clearly portrayed by nMDS ordination of individual observation units (Figure 4) where difference among location, though varying in magnitude with time, clearly emerged.

Estimate of sediment load

The mean sediment load at the three locations is reported in Figure 5. The graphs show the proportions for the three granulometric fractions to be consistent among locations, but the results highlighted that sediment loads were many orders of magnitude greater at T.re Guaceto than at the other two locations.

Discussion

This study demonstrates that the role played by the characteristics, ie roughness and nature, of the introduced substratum in affecting initial settlement and subsequent stages of colonization differs across spatial scales. Differences in assemblage structure among sites and locations overwhelm any difference consequent on substratum type or roughness. The effects of the substratum can vary significantly at spatial scales of hundreds of meters to kilometres,

Table 3. Summary of ANOVA tests for the effects of substratum on selected variables.

Sources of variation	AC	DFA	DIC	ECR	GFA	LAU	CER	INV	Coverage %	N° Taxa
Su						*				
Ro										
Ti × Su										
Ti × Ro										
Lo × Su								**		
Lo × Ro								*		
Si(Lo) × Su										*
Si(Lo) × Ro			**							
Su × Ro			*							
Ti × Lo × Su							*			
Ti × Lo × Ro										
Ti × Si(Lo) × Su			*		*			*		
Ti × Si(Lo) × Ro							*	***		
Ti × Su × Ro										
Lo × Su × Ro										
Si(Lo) × Su × Ro										
Ti × Lo × Su × Ro										
Ti × Si(Lo) × Su × Ro		*								
Cochran' test (C)	0.275	0.062	0.058	0.072	0.069	0.072	0.050	0.061	0.051	0.053
	P < 0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS
Transformation	None	ln(X+1)	ln(X+1)	Sqrt(X+1)	None	ln(X+1)	Sqrt(X+1)	None	None	None

Only tests relevant to hypotheses are reported. Terms already involved in significant higher-order interactions were not analysed. Time = Ti, location = Lo, site = Si, substratum = Su, roughness = Ro; *P < 0.05; **P < 0.01; ***P < 0.001; NS = not significant (see Appendix 1 for abbreviations of taxa).

indicating that relevant ecological processes operate differently at those scales. Under these conditions, making predictions about the outcome of employing artificial substrata in the marine subtidal is still difficult (Connel 2000; Bulleri 2005), but understanding the effects of deployment of man-made structures on marine biodiversity is important because their introduction is rapidly increasing in coastal areas (Vaselli et al. 2008). The possible effects due to the introduction of new substrata along the coast need to be quantified (Airoidi et al. 2005) to avoid the use of materials that differ substantially from local natural substrata (Perkol-Finkel et al. 2006).

In the present study, assemblages colonizing limestone, which is the substratum occurring naturally at the three locations, differed significantly at the end of the experiment from assemblages colonizing the other three substrata, although the patterns varied in space. The highest dissimilarities were observed between limestone and granite tiles and those made of concrete or sandstone. Thus, species may select particular substrata during colonization. In addition, the patterns observed could depend on specific attributes of the substrata that may determine larval choice such as color (Swain et al. 2006; Finlay et al. 2008), texture (Swain et al. 2000; Prendergast et al. 2008) and a high porosity, shared between concrete and sandstone

compared to the other two substrata (James and Underwood 1994; Berntsson et al. 2000). These factors could differentially influence post-settlement survival, creating differences among substrata, independent of active discrimination.

Elucidating the mechanisms leading to the establishment of different assemblages on natural and artificial substrata represents a crucial step for improving the design of artificial structures replacing natural habitats, as discussed by Bulleri (2005). The outcomes of the present study have implications for the management of coastal areas. At the local scale, they reinforce the view that the introduction of artificial substrata could cause significant changes in the identity and relative abundance of species. On a larger scale, they highlight that those changes vary in an inconsistent manner from place to place, suggesting unpredictable results when baseline knowledge of the systems is scant (Doak et al. 2008).

Explaining ecological patterns requires an understanding of the interactions among processes occurring at different scales. The present study showed that the effects of the introduction of artificial substrata can be largely context-dependent. Rock type and topographic complexity can be considered as small-scale processes embedded within processes acting at larger scales. This result was rather unexpected since the roughness of the

substratum was expected to be a crucial factor driving species richness (eg Johnson et al. 1998; Blanchard and Bourget 1999; Kostylev et al. 2005). In the present

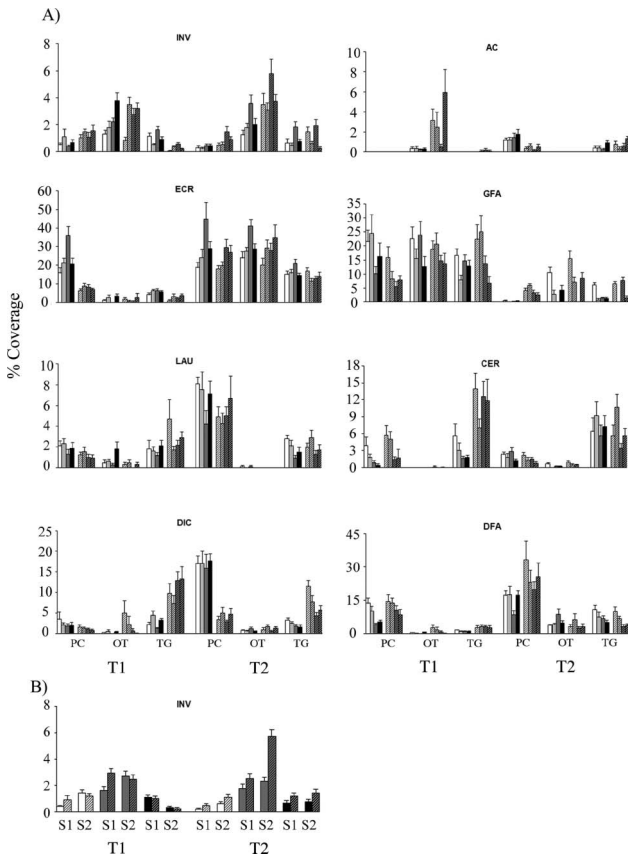


Figure 3. A: Mean % coverage of the most representative taxa (\pm SE, $n = 10$) for each substratum across sites at the two sampling dates (T1 and T2). Data are plotted for each location (PC = P.to Cesareo; OT = Otranto; TG = T.re Guaceto). White = limestone; light gray = concrete; dark gray = granite; black = sandstone, \square Site 1, \blacksquare Site 2. B: Mean % coverage of invertebrates (\pm SE, $n = 20$) for both levels of complexity (\square , Smooth, \blacksquare , Rough) in each site (S1, S2) at the two sampling dates. Data are plotted for each location (White = PC; Gray = OT; Black = TG).

study, only invertebrates showed a significantly higher percentage cover on tiles with higher levels of topographic complexity. This result was especially evident in the later colonization stage after 9 months and confirms previous results from the literature that invertebrates can be affected even by small variations in topographic complexity (eg Bourget et al. 1994; Beck 1998, 2000; Herbert and Hawkins 2006).

Assemblages that developed on tiles included algae mainly belonging to three morphological groups, viz encrusting, turf and canopy species. Turf-forming species belonging to the order Ceramiales (a typical opportunistic species, Terlizzi et al. 2000) were well represented especially in T.re Guaceto, where the presence of a small river was likely to have influenced the high sedimentation rate, which could have had a detrimental effect on the entire algal assemblage. Assemblages dominated by algal turf, ie filamentous, corticated erect and calcareous articulated algae (Steneck and Dethier 1994) are often associated with trapped sediments (Airoldi 2001; Irving and Connell 2002). Several authors have suggested that the increase in human activities along the shores worldwide (Glasby and Connell 1999) coupled with an increase in sediment load (Airoldi and Cinelli 1997) can result in a reduced vertical structure of algal assemblages. This has been attributed to the loss of canopy-forming species that are replaced by turf-forming species. In areas with high sediment deposition, the effects of microtopography can be reduced by the accumulation of fine silt in the minute crevices and depressions, possibly decreasing settlement of spores (Norton and Fetter 1981; Irving and Connell 2002) and consequently causing a reduction in the algal standing crop. High sediment deposition was observed especially at T.re Guaceto, where the sediment load was many orders of magnitude greater than that found at the other two locations. However, the presence of sediments was also recorded in P.to Cesareo and Otranto, which may explain the general lack of significant effects

Table 4. PERMANOVA to test for differences in natural assemblages among and within locations through time.

Source of variation	d.f.	MS	F	P (perm)	MS _{DEN}
Time = Ti	1	6,319.76			
Location = Lo	2	31,090.02			
Site(Lo) = Si(Lo)	3	4,147.85			
Ti \times Lo	2	4,271.56	3.03	*	Ti \times Si(Lo)
Ti \times Si(Lo)	3	1,411.38	1.92	**	Res
Residual	108	733.51			
Total	119				

The analysis is based on Bray-Curtis dissimilarities (square root transformed), conducted on whole set of variables (33 taxa \times 120 samples) found in the three locations investigated. Each test was performed using 4999 permutations of appropriate units. The term used for the denominator mean square in each case is given in column MS_{DEN}. Terms already involved in significant higher-order interactions were not analysed; * $P < 0.05$; ** $P < 0.01$.

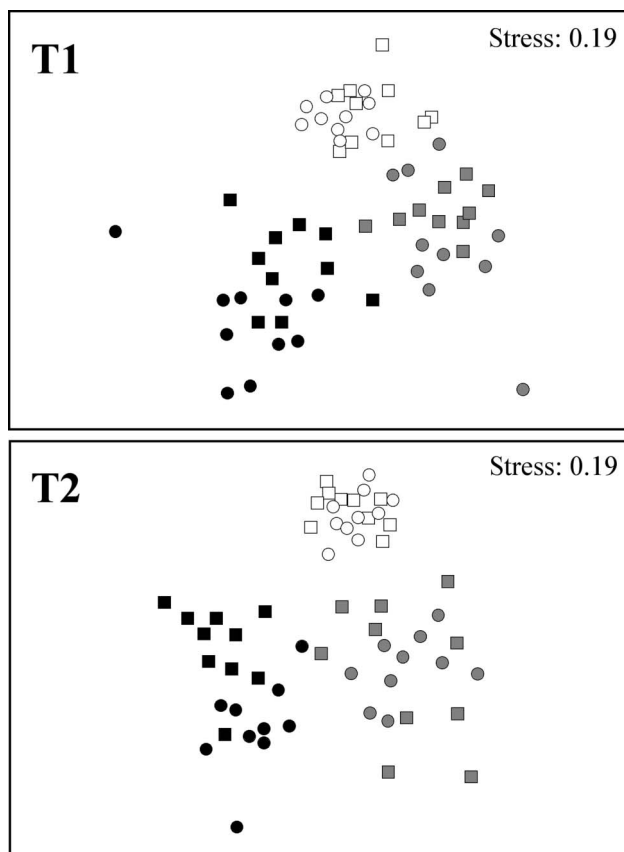


Figure 4. Non-metric multidimensional scaling (nMDS) on the basis of the Bray–Curtis dissimilarity measure of individual observation units in both sites (circles and squares) for each location in the two sampling dates (T1 and T2). Black = Location 1; Gray = Location 2; White = Location 3.

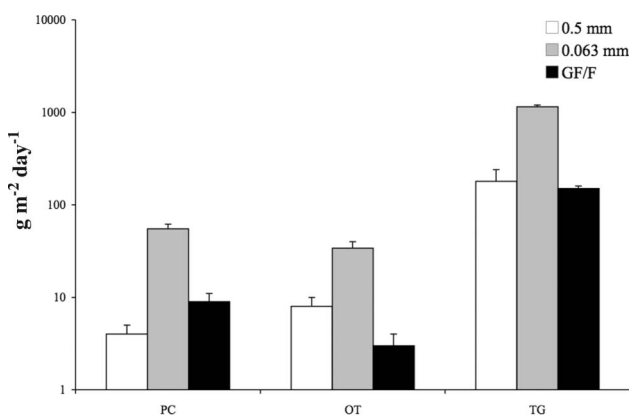


Figure 5. Mean sediment load in the three locations (logarithmic scale). Colors represent the different mesh utilized during filtration of samples to separate the different granulometric fractions. PC = P.to Cesareo; OT = Otranto; TG = T.re Guaceto.

of roughness on patterns of colonization, with the exception of invertebrates. Sedimentation can therefore be viewed as a large-scale process, with the potential to affect species and assemblages also at a small scale, which drives changes in the diversity and abundance of subtidal macroalgae (Kendrick 1991; Airoldi and Virgilio 1998; Umar et al. 1998).

Another factor that might have indirectly contributed to different patterns of colonization at the various locations could be related to the potential effect of herbivores, such as sea urchins. Sea urchins can exert significant control on macroalgal stands (Palacín et al. 1998; Shears and Babcock 2002) and their unselective grazing on new settlers has been shown to play a crucial role in colonization processes (McClanahan et al. 1996). However, although sea urchins were occasionally encountered at the experimental sites, their scarce abundance facilitated efficient removal from tiles throughout the duration of the experiment.

The authors are aware that repeating the experiment at different dates and for a longer time could provide more robust results. However, since resources were limited, it was felt more important to widen the spatial scales traditionally used in these kinds of experiments to ascertain the possibility of scaling up the results from studies carried out on a small scale. The next step will therefore be the design of future long-term experimental monitoring in order to understand whether patterns in time and across scales are consistent.

The results indicated that predicting the consequences of the introduction of man-made structures in coastal areas could be a difficult task. Large-scale processes interact with processes acting at smaller scales, causing idiosyncratic patterns of settlement and colonization of artificial substrata. Further investments in large-scale and long-term studies are needed to adequately manage the intrusion of artificial substrata in marine systems and to effectively mitigate their impact on coastal areas.

Acknowledgements

This research was conducted as part of GG's PhD dissertation. Financial support was provided by MURST (COFIN and FIRB projects). The authors acknowledge the support of the European Union, viz. the SESAME integrated project, the Centro Euro-Mediterraneo per i Cambiamenti Climatici (CMCC) project and the MARBEF Network of Excellence 'Marine Biodiversity and Ecosystem Functioning', which is funded in the Community's Sixth Framework Programme (contract no. GOCE-CT-2003-505446). The authors also acknowledge the staff of the Marine Protected Areas of Torre Guaceto and Porto Cesareo. Comments and criticism from two anonymous reviewers greatly improved the final version of the manuscript. The experiments comply with the current laws of the country in which they were performed.

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