



The effects of sewage discharge on shallow hard substrate sessile assemblages

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Abstract

On rocky shores, sewage discharges can modify natural distribution patterns of sessile organisms. The impact of sewage on shallow hard substrate assemblages has been assessed along SW Apulian coast (Ionian Sea, Italy), providing a framework to evaluate the benefits of future sewage displacement to deeper waters. Four locations (three controls and one putatively impacted) were selected and three sites were chosen at each location. Each site was sampled by 10 replicate photographic records. Univariate analyses revealed that the outfall did not affect the spatial distribution of number of taxa, total cover and abundance of some dominant taxa (mostly algae, sponges and bryozoans). The outfall negatively influenced the natural distribution pattern of filamentous green algae, whilst some algae (i.e. Gelidiales and *Colpomenia sinuosa*) were exclusively present at the impacted location. Multivariate analyses revealed that the outfall heavily modified the natural pattern of variability in the structure of the assemblage. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Sewage discharge; Subtidal assemblages; Rocky substrates; Spatial variability; Multiple controls; Mediterranean Sea

1. Introduction

Differences in physical and biological conditions are the main cause of variation in marine communities, both in time and space (Paine and Levin, 1981; Dayton, 1984; Sousa, 1984). Physical processes can include disturbances such as wave action, temperature, irradiance or salinity whilst biological processes may include settlement, recruitment, predation, and competition. Anthropogenic disturbance has the potential to alter the patterns of natural variability at various scales of organisation within the community. Thus, it is crucial to understand the patterns of natural variability to distinguish them from the variability induced by human disturbance on marine habitats (Underwood, 1993, 1994).

Sewage effluent is often discharged via outfall into shallow subtidal habitats (McIntyre, 1995; Koop and Hutchins, 1996). This can result in significant effects on marine biota (Pearson and Rosenberg, 1978; Underwood and Peterson, 1988). Several biological assem-

blages, including plankton and fish, have been monitored to assess the effects of sewage pollution (Smith et al., 1999; Guidetti et al., 2002 and references therein). However, the bulk of available information derives from studies on soft-bottom communities (Dauer and Conner, 1980; Austen et al., 1989). The advantages in using soft-bottom communities for biomonitoring purposes are reviewed by Warwick (1993); it is generally acknowledged that they are easier to sample quantitatively, and show detectable responses to anthropogenic impact. Contamination of soft-bottom by sewage discharges may result in changes in the abundance, biomass and diversity of the organisms (Pearson and Rosenberg, 1978), bioaccumulation of organic and inorganic compounds (Phillips, 1978) and alteration of trophic interactions among species (Otway et al., 1996 and references therein). Few papers described the effects of sewage on macro-benthic assemblages living on hard substrata and most of them focused on the intertidal, e.g. Fairweather (1988). Moreover, some authors (e.g. Lopez-Gappa et al., 1990) analysed differences between an impacted location and a single control, with evident problems of pseudoreplication and consequent confounding in the logical interpretation of results (Underwood, 1997).

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In the subtidal, most of the information derives from Australia, where the effects of sewage pollution on macrobenthos living on hard substrata were studied both in shallow and deep waters (Chapman et al., 1995; Underwood and Chapman, 1996; Roberts, 1996a,b; Roberts et al., 1998).

Along the coasts of the Mediterranean Sea, despite a large amount of domestic and industrial sewage is discharged to the sea (UNEP, 1989), no published account exists concerning the effects of sewage effluents on macrobenthos living on rocky subtidal substrates. The aim of the present study is to investigate the effects of a sewage discharge on the spatial variability of benthic subtidal rocky assemblages.

2. Methods

Sampling operations were performed at four locations (one putatively impacted by the outfall and three randomly selected controls) (Fig. 1).

The outfall serves more than 30,000 inhabitants. Waste waters (with volumes discharged varying from 200 to 600 m³ h⁻¹) are supposed to be biologically and chemically treated before discharging. However, especially during summer, technical problems often prevent the complete treatment.

All locations were approximately 3–5 km from each other and situated on sub-vertical rocky reef at a depth of 5 m. Three sites were randomly selected for each

location. Sites at each location were approximately 100–300 m apart. At the putatively impacted location, one site was about 10 m from the outfall, the remaining two were on its right and left, respectively. The assemblages were sampled by a Nikonos V underwater camera and flash. Ten random 16 × 23 cm quadrats were taken for each site. The choice of the size of the sample and the level of replication, as well as the number of sites within locations, derived from previous cost-benefit studies aimed at optimise the sample efforts (Underwood, 1997).

The slides were analysed under a binocular microscope by superimposing a transparent sub-dividing grid of 25 equal sized squares. The percentage cover by conspicuous organisms were quantified by giving each individual taxon a score ranking from 0 to 4 in each smaller square, and then summing scores for all the smaller squares where the taxon was present. Final values were expressed as percentage. Organisms filling less than 1/4 square were given an arbitrary value of 0.5 (Dethier et al., 1993). To help determine the taxa recorded in quantitative photo-quadrats, a comprehensive field collection was done and species identity confirmed by specialist. The voucher collection was lodged at the Museum of Marine Biology of Porto Cesareo (Lecce, Italy).

To test for differences between the putatively impacted location and the controls, total number of taxa, total cover, and the abundance of the most representative taxa (i.e., those contributing, on average, more than 3% to total cover) were calculated for each replicate quadrat, and analysed by a Beyond BACI design (Underwood, 1991, 1992, 1993). Beyond BACI models were developed to distinguish between natural variability that characterises assemblages and variability-induced by a particular form of disturbance. In our case, since no pre-impact data was available, an after-control impact (ACI) design was utilised (Chapman et al., 1995; Roberts, 1996a; Glasby, 1997). The experimental design here used, therefore, allowed to quantify the effects of the sewage discharge at a variety of scales, ranging from kilometres (among locations), hundreds of meters (among sites within locations), meters (among replicates within sites).

Homogeneity of variances was tested by Cochran *C*-test (Underwood, 1997). In some cases, variances proved to be heterogeneous and could not be stabilised. Nevertheless, analysis of variance (ANOVA) is considered sufficiently robust to the departures from the assumption, particularly with balanced designs and many independent estimates of sample variance (as in our case). Thus, the untransformed data were used and results were interpreted with the more conservative probability level of 0.01 (Underwood, 1997).

Multivariate statistical analyses (PRIMER 5 software package from Plymouth Marine Laboratory, UK) were used to describe differences in assemblage at the different spatial scales considered. Data were transformed by double square root to arrange all taxa in the same range

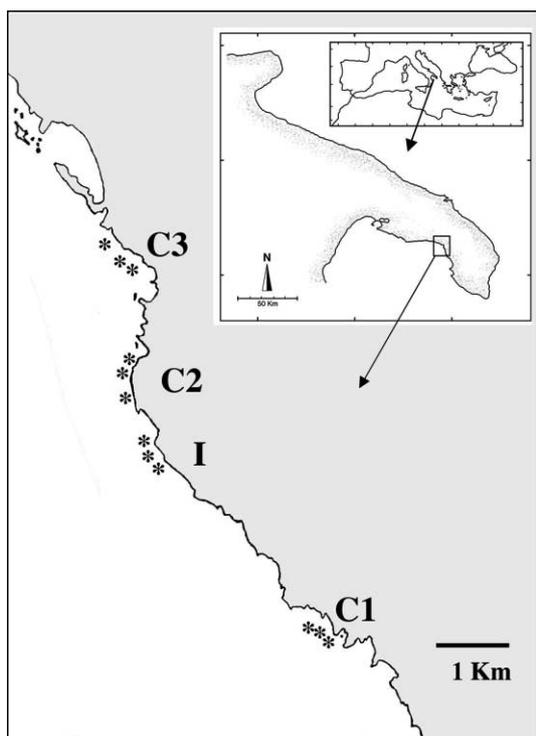


Fig. 1. Study area and position of impacted (I) and control (C1, C2 and C3) locations. The * indicates the position of sites in each location.

of abundance. Bray–Curtis similarity matrix was calculated and used to generate a 2-dimensional plot with the non-metric multidimensional scaling (nMDS) technique (Clarke, 1993; Clarke and Warwick, 1994). Two-way analysis of similarity (ANOSIM) was used to test the null hypothesis of no differences among locations and among sites within each location.

The putative impact of the outfall on community patchiness (i.e., the small-scale variability among replicates) was also tested. The average similarity within each site (from the Bray–Curtis similarity matrix) was considered as a measure of variability among replicate quadrats (Underwood and Chapman, 1998). Asymmetrical ANOVA was then performed to test the hypothesis that variability within sites in control location differed from that observed at the impacted sites.

3. Results

A total of 57 taxa were identified, encompassing algae, hydrozoans, anthozoans, serpulids, gastropods, bivalves, barnacles, bryozoans, and ascidians. Of them, 41 were identified to species level. The remainder were kept at level of genera (6), families (2) and complex of many species with similar morphology (e.g. filamentous green algae, filamentous brown algae, encrusting calcified red algae). A detailed account of the spatial pattern of macrobenthic assemblages living in this habitat can be found in Frascchetti et al. (2001).

The spatial distribution patterns of total cover and total number of taxa are reported in Fig. 2. The results of Beyond BACI analyses testing for spatial differences in these two variables are summarised in Table 1. Total

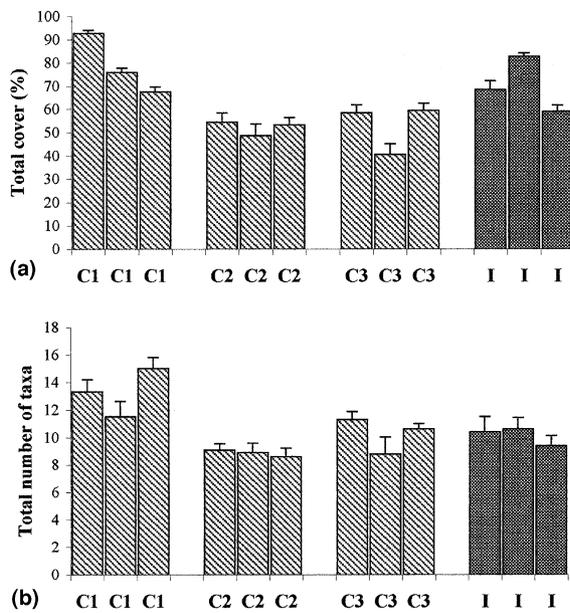


Fig. 2. Mean total percent cover (a) and total number of taxa (b) at each of the three sites in each location (\pm S.E., $n = 10$).

Table 1
Summaries of the two-ways nested ANOVAs comparing spatial variation in total cover, number of taxa and some of selected taxa

Source	df	Total cover			No. of taxa			Filamentous brown algae			Encrusting calcified red algae			Filamentous green algae			
		MS	F	F vs	MS	F	F vs	MS	F	F vs	MS	F	F vs	MS	F	F vs	
Locations = L	3																
Am Cs	2	6876.14	**	Residuals	152.14	**	Residuals	519.68	**	Residuals	728.61	**	Residuals	72.04	NS	Residuals	
I vs Cs	1	1786.68	NS	Among Cs	9.68	NS	Among Cs	1436.01	NS	Among Cs	936.67	NS	Among Cs	201.00	**	Residuals	
Sites (L)	8																
Sites (Cs)	6	952.03	**	Residuals	15.96	NS	Residuals	208.4	**	Residuals	62.36	NS	Residuals	248.68	**	Residuals	
Sites (I vs Cs)	2	1435.30	NS	Sites (Cs)	4.13	NS	Sites (Cs)	406.03	NS	Sites (Cs)	14.04	NS	Residuals	1.63	NS	Sites (Cs)	
Residual	108	105.88		Residuals	6.92		Residuals	35.57		Residuals	30.94		Residuals	33.23			

Cs: controls; I: impacted location; AmCs: among controls; NS: not significant; ** $p < 0.01$.

cover values showed significant differences among controls both at site and location level ($p < 0.01$). For total number of taxa, differences among controls were detected only at location level. However, in both cases there were no significant differences between the impact sites and locations vs the average of the controls. The same analyses were performed for the most dominant taxa. Some results are reported in Fig. 3 and Table 1 as a summary of the observed trends. Filamentous brown algae (grouping species of the genera *Ectocarpus* and *Sphacelaria*) significantly differed among and within controls but no difference was detected between the impact sites and locations vs the average of the controls. The sponge *Crambe crambe*, the bryozoan *Schizoporella longirostris* and the encrusting calcified red algae (grouping *Lithophyllum frondosum*, *Lithophyllum incrustans*, *Mesophyllum alternans* and *Peyssonnelia polymorpha*) only differed among control locations. Only in the case of filamentous green algae (grouping species of the genera *Bryopsis*, *Cladophora* and *Chaetomorpha*) significant differences were found between the impact location and the average of the controls.

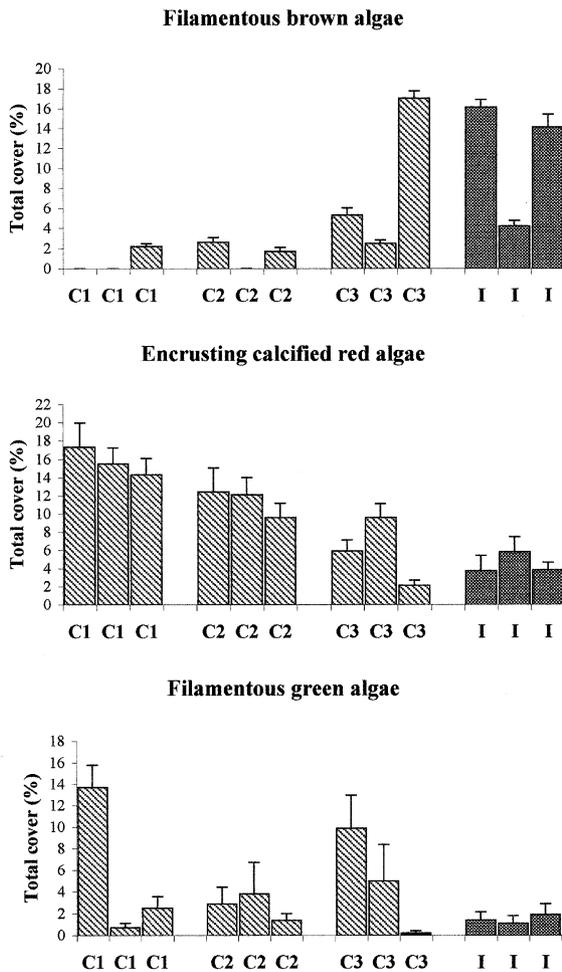


Fig. 3. Mean % cover for some of most dominant taxa at each of the three sites in each location (\pm S.E., $n = 10$).

Table 2
Two-ways nested ANOSIM

Global R among locations = 0.704** Global R among sites = 0.269**		
Pairwise test	R values	Significance level %
C1 vs C2	0.826	10
C1 vs C3	0.63	10
C2 vs C3	0.333	10
I vs C1	1	10
I vs C2	1	10
I vs C3	0.963	10

Global R is the value of the ANOSIM test calculated among all locations and all sites within each location. Pairwise test indicates differences between specific pair of locations. Pairwise R value is calculated on a number of random permutations equal to 10, allowing a level of significance of 0.1. C1, C2, C3: control locations; I: impacted location.

The group of Gelidiales (grouping red algae of the genera *Pterocliadiella* and *Gelidium*) and the brown algae *Colpomenia sinuosa* were exclusively found at the outfall. In these cases, no statistical test was needed to reveal how the outfall heavily influenced their distribution. Gelidiales, in particular, were characterised by very high percentage of covering, reaching the mean value of 32 (± 6.3 S.E., $n = 10$) in the site closest to the outfall.

Two-way nested ANOSIM showed significant differences both within each location and among the four locations (Table 2). Post hoc pairwise comparisons exhibited the highest differences between the impact location and controls. Differences between controls also existed, even if with lower values of R (Table 2). Nevertheless, since pairwise comparisons were obtained on a number of possible permutations equal to 10 (significance level of 10%), these results have to be interpreted with caution.

The differences in the structure of the benthic assemblages between the impact and controls are portrayed in the nMDS ordination (Fig. 4). This was

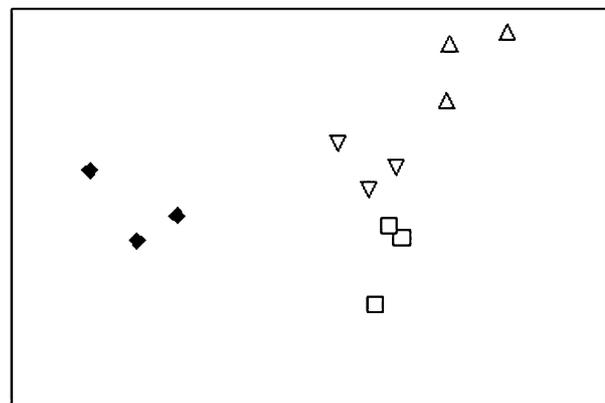


Fig. 4. Non-metric multidimensional scaling of site's centroids (stress = 0.08). (◆): impacted sites (I location); (Δ): sites of the first control (C1); (▽): sites of second control (C2) (□): sites of third control (C3).

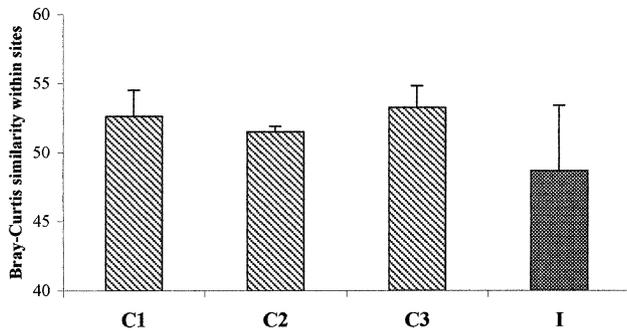


Fig. 5. Mean Bray-Curtis similarity among quadrats at impacted and control locations (\pm S.E., $n = 3$).

Table 3

Values of Bray-Curtis similarity among individual quadrats calculated for each of the three sites at each location

	C1	C2	C3	I
1	59.8	50.5	53	39.6
2	51.9	51.1	48.4	66
3	47	52.9	58.3	40.2

C1, C2, C3: control locations; I: impacted location.

obtained plotting the centroids for each site (i.e. the mean values for all quadrats) to better represent differences among and within locations. Plotting centroids, though not considering variability within each site, improved the stress values, providing a better representation of the differences among samples. The ordination model indicates rather high differences among and within controls and a quite clear separation between impacted location and controls.

The asymmetrical ANOVA performed on average similarity among replicate quadrats within each site (see Section 2) did not detect significant differences between the average of controls and the impact. However, the impact location accounted for the highest values of S.E. (Fig. 5). Table 3 shows that both the lowest and the highest values of similarity (i.e. the highest and the lowest levels of variability among replicates) were found at the impacted location. The MDS configuration on fourth-root transformed data, clearly separated, at the impacted location, the site closest to the outfall from the adjacent ones (Fig. 6). In these latter sites a much higher scattering of individual quadrats was also observed.

4. Discussion

This study showed that the sewage discharge had no significant effects on total cover and total number of species. These results are in accordance with Roberts et al. (1998), who studied, in Australian temperate habitats, sewage-exposed rocky assemblages at different depths, before and after the outfall commissioning.

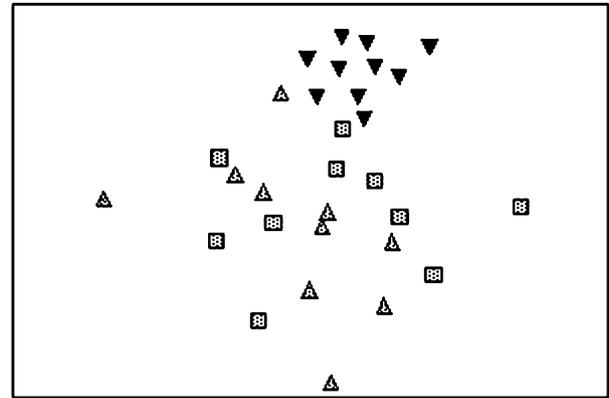


Fig. 6. Non-metric multidimensional scaling ordinations of individual quadrats at the three sites of the impacted location (stress = 0.18): (▼): site 2 (about 10 m apart from the outfall); (▲) and (■) = site 1 and 3 (about 100 m apart from the outfall).

However, Roberts (1996b) and Underwood and Chapman (1996) provided some evidences of an influence of sewage effluents on the total number of taxa.

The sewage discharge significantly modified the pattern of distribution of some taxa. Filamentous green algae were constantly present along the surveyed coast but their abundance significantly decreased at the outfall. Conversely, the impacted location was characterised by the exclusive presence of the algae of the genera *Gelidium*, *Pterocladia* and *Colpomenia*, indicating a strong effect of sewage discharge on their distribution and confirming the species as typical of organically polluted waters (Chrysosvergis and Panayotidis, 1995).

For the remaining taxa, the univariate analyses revealed that the outfall did not significantly modify their natural patterns of spatial distribution. However, for some taxa, the lack of statistically significant results occurred, despite some differences between the outfall and controls were evident in the graphs. In these cases the obtained F ratios and associated p values approximated to the tabulated one. This finding suggested that a more powerful test (obtainable, with more resource, by increasing the number of replicates) would have probably enabled to detect an impact on more taxa than that found (Osenberg et al., 1996).

Sewage discharge altered the relative contribution of these taxa to the structure of the assemblage. This is suggested by the multivariate approach that clearly displayed differences between impacted location and controls.

The effects of sewage pollution on marine biological assemblages have been often studied using univariate (Otway et al., 1996; Roberts, 1996b; Underwood and Chapman, 1996) or multivariate (Clarke, 1993; Smith and Simpson, 1993) methods. Fewer studies benefited from a combination of them (Chapman et al., 1995; Roberts et al., 1998). Underwood and Peterson (1988),

and Warwick (1993) emphasised the need to use both techniques of analysis as an integrate approach. As in the work present here, the use of different analyses makes detecting of effects on different components of the assemblage more likely.

A common feature of both types of analyses was the significant differences among controls. Thus, the study stressed out the importance of selecting multiple controls in detecting ecological impact of human activities (Underwood, 1994; Glasby and Underwood, 1998). Our attempts at pairwise comparing the impact location vs each available control (although sampled in many replicates) led to conflicting results and to wrong (or confusing) interpretations about the effects of the sewage on the structure of the assemblage.

Results also provided evidence that sites located about 100–300 m apart from the outfall were similar to each other and they differed from the site located at the close proximity of the sewage. This finding is probably attributable to the distance from the source of pollution and could provide some indication about how the assemblages respond to the dilution of pollutants.

It is also likely that sewage discharge contributed to modify the pattern of variability of assemblage structure at small-scale. Caswell and Cohen (1991) hypothesised that disturbance induces higher variability in communities. Warwick and Clarke (1993) recorded increased variability among replicate samples from several different marine communities exposed to different source of stress, thus providing support to the hypothesis and suggesting it may represent a general rule (but see also Chapman et al., 1995 for different findings). In the present case, the relationship between small-scale variability of assemblage structure and disturbance caused by sewage discharge seems to be not linear. The highest value of similarity was recorded in the site closest to the outfall, the lowest in the two sites located about 100–300 m apart from the outfall. Intermediate values were found at control sites. However, these observations are lacking of replication during time. It should be noted that, in some cases, sewage pollution could influence the temporal dynamics of the community, besides the spatial distribution and the structure of the assemblage (Underwood and Chapman, 1996; Roberts et al., 1998). Therefore, further research is in progress to clarify the consistency during time of the observed differences between the impacted and control locations. This also will provide a framework to evaluate the benefits of future sewage displacement to deeper waters.

Acknowledgements

Research funded by the Regione Puglia (INTERREG Greece–Italy project), Amministrazione Provinciale di Lecce (Biodiversità and Porto Cesareo projects) and

MURST (COFIN project). P. D'ambrosio, and C. Vaglio provided invaluable assistance during field work. S. Bevilacqua assisted with analysis of the photographic slides. M. Cormaci, E. Cecere, L. Matinato, C. Longo and F. Ferdeghini helped in identifying dubious specimens of algae, sponges and bryozoans. L. Benedetti-Cecchi and F. Micheli commented on experimental design and statistical analysis. R. Coleman revised an earlier version of the manuscript.

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