

Structural and functional response of meiofauna rocky assemblages to sewage pollution

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Abstract

Despite meiofauna being one of the most popular tool for detecting the ecological effects of different sources of impact, the application of structured experimental designs to this benthic component is typically neglected, especially in subtidal rocky shores. In this study, an asymmetrical design was used to detect hard-bottom meiofaunal abundance and biodiversity response to sewage discharge. Sampling was carried out at a depth of 3–4 m by SCUBA diving, by means of a modified manual corer. Six replicate cores were collected at three sites (80–100 m apart), at each of three locations (one purportedly impacted [*I*] and two controls [*Cs*]). A total of 84,994 specimens were collected. The outfall affected meiofaunal assemblages in terms of taxon richness, by determining a significant reduction of the number of taxa in the disturbed location, and community structure, by causing functional changes in terms of a decrease of the abundance of nematodes and an increase of the hydrozoan component. Nematodes and syllid polychaetes showed significant lower average abundance at *I*. Multivariate analyses showed that both meiofaunal assemblage and syllids were significantly different at *I* compared with *Cs*. The sewage outfall also affected patterns of spatial distribution at the scale of site (100 m apart) and of replicate units (centimetres apart), both in syllids and in nematodes. Our results provide evidence that the selection of multiple controls is crucial to prevent the widespread risk of Type II error, highlighting the need of more accurate experimental designs when dealing with meiofauna.

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1. Introduction

Patterns of distribution of populations and assemblages in most marine systems are extremely variable at several spatial and temporal scales (Levin, 1992; Wiens et al., 1993; Frascetti et al., 2005). Natural patterns of variability, in terms of mean values of response variables and in measures of spatial variance, can be significantly modified by human activities (Underwood, 1992, 1994; Chapman et al., 1995; Benedetti-Cecchi, 2001; Balestri et al., 2004; Terlizzi et al., 2005a,b). This is becoming increasingly evi-

dent from the attention to sampling design in recent studies evaluating environmental impacts. An adequate sampling design coupled with new statistical tools can provide a powerful basis for the analysis of species' responses to anthropogenic disturbance (Anderson and Thompson, 2004). However, it is still unclear whether the findings deriving from a limited number of case studies can be of general value in the assessment of environmental impacts. This lack of generality stems from the limited array of habitat types investigated, whereas several habitats and ecological systems are left unstudied. Plankton assemblages and, in general, benthic mobile fauna are less tractable from an experimental point of view than benthic sessile assemblages, so that pseudo-replication in space and time is still common. Consequently, monitoring programs aimed at detecting impacts on populations and assemblages may

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confound the effects of human interventions with other sources of variation not specifically linked to the source of disturbance under investigation, unless rigorous experimental designs are used.

Meiofaunal organisms are well suited for environmental impact assessment studies because the characteristics of their life cycles (small size, high turnover, and lack of larval dispersion) (Higgins and Thiel, 1988) make them sensitive to environmental disturbance (from organic enrichment to mining) (Vincx and Heip, 1991; Danovaro et al., 1995; Kennedy and Jacoby, 1999; Mirto et al., 2000, 2002; Mirto and Danovaro, 2004; Ahnert and Schriever, 2001; La Rosa et al., 2001). Meiofauna are generally described as featured by heterogeneous spatial patterns of distribution (Coull, 1988; Steyaert et al., 2003), but the quantification of this patchiness is rarely conducted by using appropriate experimental designs (Li et al., 1997). Moreover, the effects of anthropogenic disturbance are generally tested by comparing one impacted location with a single control location, thereby failing to separate natural variability from variability induced by the disturbance itself (see Lardicci et al., 1999 as an exception).

In coastal marine environments, sewage discharge is a common source of human disturbance and its effects have been studied on several ecological systems (macrofauna from soft substrates, fish assemblages, seagrass, sessile and mobile fauna from hard substrates, and epiphytes on the endemic Mediterranean seagrass *Posidonia oceanica*). Results showed that sewage can change structural and functional attributes of biodiversity, but effects can vary depending on the response variables considered and the types of data analysis used (Pearson and Rosenberg, 1978; Chapman et al., 1995; Smith et al., 1999; Otway et al., 1996; Guidetti et al., 2003; Balestri et al., 2004; Piazzini et al., 2004; Terlizzi et al., 2005a,b). In this study, we examined the possible impact of a sewage outfall on a rocky subtidal meiofaunal assemblage in Southern Italy.

Meiofaunal assemblages are known to live on hard substrates in association with periphytic and epiphytic algae and attached epibiota, but the abundance, diversity, and colonizing abilities of hard substrate meiofauna have been poorly documented (Gibbons, 1988a,b; Danovaro and Frascchetti, 2002; Atilla et al., 2003; Mirto and Danovaro, 2004). Here, we used an asymmetrical sampling design to compare patterns of meiofauna abundance and assemblage structure at one location exposed to sewage discharge to two reference locations. Sampling was conducted at a hierarchy of spatial scales, ranging from sampling units (tens of centimetres apart) to location (kilometres apart). More specifically, the study was conducted on nematodes and syllids (polychaetes), which together account for 50% of total abundance of the benthic assemblages investigated. They share approximately the same size and are featured by similar life-cycle traits (from dispersal to reproduction). Test of hypotheses were examined in univariate and multivariate context in terms of changes of mean values and spatial variance.

2. Materials and methods

2.1. Study site and sampling design

The area of interest is located along the Apulian coast of Italy (Ionian Sea, 17°55' N, 40°11' E). Specific information about the study site, the type and volumes of discharged wastewaters is reported elsewhere (Terlizzi et al., 2002). The effects of this particular sewage outfall have been already quantified on spatial and temporal patterns of sessile benthos, fish and mobile fauna (Guidetti et al., 2003; Terlizzi et al., 2005a,b). Three main outcomes derive from those studies: (1) sewage discharge can modify habitat complexity, causing significant differences in the algal assemblage at the impacted location with respect to reference conditions; (2) the outfall alters spatio-temporal patterns of distribution in sessile organisms, fish, and mobile fauna; (3) the discharge influences both structural features and functional traits of assemblages, such as trophic groups of fish fauna. Results from those studies also substantiate that analysis of small-scale variability (i.e. the residual variance among units of observation) has important implications in environmental impact assessment.

An asymmetrical sampling design was used to test the effect of the sewage outfall on meiofauna. Sampling was undertaken in November 2002, at a depth of 3–4 m, in three locations separated by at least 3 km. Of these, one was directly adjacent to the discharge (hereafter indicated as *I*) and two were randomly selected as reference locations (Controls, hereafter indicated as *Cs*). At each of the three locations, three sites, separated by 80–100 m were also randomly chosen.

SCUBA divers sampled each site with a modified manual corer. This consisted of a transparent Plexiglas cylinder (int. diam., 8.4 cm, 14-cm length) open on the bottom (with a soft and 1-cm thick rubber O-ring to better adapt the opening to rough surface) and closed at the top. The corer had two windows. The first, 2 cm above the bottom, enabled the diver to scrape the hard bottom surface using an internal spatula. The second, at the bottom, enabled an efficient closure of the cylinder, immediately after the scraping operations. This sampler allows for the efficient removal of both the macroalgae and the rock surface beneath the algae, thus avoiding underestimation of meiofaunal densities, which is known to occur when only epiphytic algae are sampled (Danovaro and Frascchetti, 2002). Six replicate samples were collected at each site for a total of 54 sampling units.

2.2. Analysis of meiofaunal biodiversity

Each sampling unit was fixed with buffered formaldehyde (4% final concentration in sodium tetraborate) and then sieved through 1000 µm mesh (to retain macrobenthos and macroalgae) and 37 µm mesh (to retain smaller meiofauna). The sample fraction retained by a 37 µm mesh

net was added to Ludox HS 40 (density 1.31 g cm^{-3}) for density centrifugation extraction ($10'$, 800g , for 3 times) from sediment (Heip et al., 1985). All specimens were counted and classified by taxon (e.g. nematodes, copepods, polychaetes, hydroids, and other organisms) under a stereomicroscope using Delfuss cuvettes.

Nematodes and polychaetes were further examined for a more accurate taxonomic analysis. Among the polychaetes, syllids, a group characterized by a consolidate taxonomic knowledge and by high sensitiveness to pollution (Giangrande et al., in press), were identified to species level in the full set of samples. Nomenclature followed Licher (1999) and San Martín (2003). Nematodes (always $n > 100$ per each unit) were identified to the genus level in three randomly selected replicate samples out of the six collected at each site. Nomenclature followed Platt and Warwick (1983, 1988), Warwick et al. (1998) and recent literature dealing with new nematode genera and species. Nematodes were also classified by trophic group according to Wieser's (1953) original groupings as follows: (1A) buccal cavity absent or fine and tubular-selective bacterial feeders; (1B) large but unarmed buccal cavity-non-selective deposit feeders; (2A) buccal cavity with scraping tooth or teeth-epistrate (diatom) feeders; (2B) buccal cavity with large jaws-predators/omnivores.

The maturity index (MI) was calculated according to the weighted mean of the individual genus scores: $MI = \sum v(i)f(i)$, where $v(i)$ is the c-p (colonisers-persisters) value of genus i as given in the appendix of Bongers et al. (1991) and $f(i)$ is the frequency of that genus.

2.3. Statistical analyses

Asymmetrical univariate analyses of variance (Underwood, 1992; Glasby, 1997) were used to test for differences in number of individuals, number of species or taxa, and meiofaunal trophic groups at I versus C_s .

The experimental design consisted of two factors: Location (L, 1 disturbed and 2 controls) and Site (S, 3 levels, random, nested in Location), with $n = 6$ or 3, depending on the analysed data set (see above). For the analyses, the Location term was partitioned into two portions: the one degree-of-freedom contrast I -v- C_s and the variability between C_s . The overall mean square of the term Site (Location) (S(L)) was similarly partitioned into S(I) and S(C_s). Also, the residual variation was divided into the residual variability for observations within I (Res I) and the residual variability for observations within C_s (Res C_s). The logic followed for the identification of the appropriate denominators for F -tests is given elsewhere (see Table 1 in Terlizzi et al., 2005a). Prior to analyses the homogeneity of variance was tested by Cochran's C test and data were appropriately transformed, if necessary. When transformation did not remove heteroscedasticity, the analyses were based on untransformed data but results were interpreted with a more conservative significance level of $\alpha = 0.01$ (Underwood, 1997). The analyses were done

using the GMAV 5 software (University of Sydney, Australia).

Distance-based permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) based on Bray-Curtis dissimilarities (Bray and Curtis, 1957) on untransformed data (54 samples and 16 variables, for the multivariate assemblage; 54 samples and 38 variables for syllids; 27 samples and 68 variables for nematodes) was used to estimate variation in the multivariate assemblages between I -v- C_s . The analyses tested the same hypotheses described above for univariate analyses but in a multivariate context (Terlizzi et al., 2005a). PERMANOVAs were done using the computer programs DISTLM.exe and PERMANOVA.exe (Anderson, 2004, 2005).

Graphical representations of multivariate patterns of meiofauna assemblage, nematodes and syllids were obtained by non-metric multi-dimensional scaling (nMDS) ordinations of centroids of each site. nMDS plots were based on Bray-Curtis dissimilarities. Centroids were calculated using principal coordinates (Anderson, 2001).

The similarity percentages procedure (done with the PRIMER SIMPER routine) was used to identify the percentage contribution that each species made to the observed value of the Bray-Curtis dissimilarity between I -v- C_s (Clarke, 1993). A cut-off criterion was applied to allow identification of a subset of species whose cumulative percentage contribution reached 90% of the dissimilarity value.

3. Results

3.1. Meiofaunal abundance and assemblage structure

A total of 84,994 specimens were collected. Total meiofaunal abundance ranged from 926.6 ± 66.1 to 2174.3 ± 292.6 individuals 55 cm^{-2} . The number of taxa ranged between 10 ± 0.33 and 13 ± 0.31 per sample (Fig. 2a and b). Nematodes were the most abundant group (37% of the total), followed by copepods (30%), polychaetes (12%) and minor groups (i.e. hydroids, amphipods, isopods and oligochaetes).

Of the total meiofauna, 31,846 specimens were nematodes. Of these, 1200 specimens were identified as belonging to 67 genera of 23 families. The trophic structure was characterised by the dominance of specimens belong to the 2A group (epistrate feeders, 21 and 28 genera at I and C_s , respectively), which accounted for 67% of total nematode abundance, followed by 1A (8 and 12 genera at I and C_s , respectively), 2B (7 and 13 at I and C_s , respectively) and 1B (7 and 5 genera at I and C_s , respectively).

Among polychaetes, syllids accounted for the 12% of the total abundance and was the most abundant family. Overall, 1263 syllid polychaetes belonging to 31 species were identified. The most abundant taxa were adult specimens belonging to the Exogoninae subfamily (usually smaller sized syllids, e.g. *Brania*, *Exogone*, *Salvatoria*, *Sphaerosyllis*), and juvenile specimens belonging to other subfamilies

(mainly Syllinae e.g. *Haplosyllis*, *Syllis*, *Trypanosyllis*). The species that showed the highest abundance were *Salvatoria clavata* (Claparède, 1863), *Exogone (Exogone) naidina* (Örsted, 1845), *Syllis prolifera* (Krohn, 1852), *S. limbata* (Claparède, 1868), *Sphaerosyllis hystrix* (Claparède, 1863), *S. rosea* (Langerhans, 1879) and *S. gerlachi* (Hartmann-Schröder, 1960). Detailed taxonomic lists of nematode genera and syllid species are available on request to the corresponding author.

3.2. Univariate analyses

The results of the asymmetrical analyses of variance are summarised in Table 1. There was no significant difference between *I-v-Cs* in the mean number of meiofauna specimens. Differences were detected among sites within the disturbed location (*S(I)*) but not among sites within control locations (*S(Cs)*) (Table 1, Fig. 1). This was interpreted as a possible effect of the outfall in enhancing spatial heterogeneity at the scale of sites. Significant differences between the controls were also observed.

The number of individuals of nematodes differed between *I-v-Cs* (Table 1, Fig. 2). Differences among sites were detected within the disturbed location but not among sites within control locations (Table 1). Comparison of residual variance indicated no significant differences in the variability among replicates between *I-v-Cs*. Differences between *I-v-Cs* were not detected for the number of nematode genera, even though an effect of the outfall in modifying spatial patterns at the scale of sites was suggested by the significance of the term *S(Cs)* and by the non-significance of the term *S(I)* (Table 1).

Differences between *I-v-Cs* were found significant for the mean number of individual syllids. The comparison of residual variance revealed significantly more residual variation at *I* compared to *Cs* at the scale of replicate units (Table 1, Fig. 1). There was no evidence of impact on this group in terms of number of species. However, an increased amount of variation was observed at the scale of sites close to the outfall. The same greater variability was observed among residuals at *I*.

3.3. Multivariate analyses

Results of the multivariate analyses are reported in Table 2 and illustrated in Fig. 2. Asymmetrical PERMANOVA revealed a significant effect of the sewage on meiofaunal assemblage as a whole. In addition, assemblages differed significantly among sites at *I* and not at *Cs*. Visual inspection of nMDS plot clearly depicted the result obtained by PERMANOVA, with impacted sites well separated from control sites and more scattered than those at *Cs* (Fig. 2). SIMPER analyses showed that the concurrent effect of decreasing nematode abundance and increasing hydrozoans abundance (mainly polyps belonging to the family of Campanularidae) in the impacted location caused major differences between *I-v-Cs*.

Table 1
Asymmetrical analyses of variance comparing the disturbed location (*I*) with control locations (*Cs*) for number of individuals, number of genera, number of species of total meiofauna, nematodes and syllids

Source	Meiofauna						Nematodes						Syllids						Nematodes																
	Number of individuals						Number of individuals						Number of individuals						Number of species						Number of genera										
	df	MS	F	P	F _{denom}		df	MS	F	P	F _{denom}		df	MS	F	P	F _{denom}		df	MS	F	P	F _{denom}		df	MS	F	P	F _{denom}						
Location = L	2																																		
<i>Cs</i>	1	0.66	19.40	0.00	Res	Res	0.07	1.35	0.25	0.22	<i>S(Cs)</i>	28.44	1.69	0.26	<i>S(Cs)</i>	1	6.72	1.85	0.24	<i>S(Cs)</i>	1	8.16	1.21	0.31	<i>S(L)</i>	19.59	1.52	0.26	<i>S(L)</i>	1	8.16	1.21	0.31	<i>S(L)</i>	
<i>I-v-Cs</i>	1	2.55	3.85	0.30	<i>Cs</i>	<i>S(L)</i>	30.35	50.44	0.00	0.03	<i>S(L)</i>	2469.16	7.37	0.03	<i>S(L)</i>	19.59	1.52	0.26	<i>S(L)</i>	19.59	1.52	0.26	<i>S(L)</i>	19.59	1.52	0.26	<i>S(L)</i>	19.59	1.52	0.26	<i>S(L)</i>	19.59	1.52	0.26	<i>S(L)</i>
Site(L) = <i>S(L)</i>	6	0.08	2.38	0.04	Res	Res	0.60	11.57	0.00	0.00	Res	335.05	5.43	0.00	Res	12.89	2.80	0.02	Res	12.89	2.80	0.02	Res	12.89	2.80	0.02	Res	12.89	2.80	0.02	Res	12.89	2.80	0.02	Res
<i>S(Cs)</i>	4	0.01	0.29	0.88	Res	Res	0.01	0.10	0.98	0.00	Res <i>Cs</i>	413.47	5.26	0.00	Res <i>Cs</i>	16.81	6.60	0.01	Res	16.81	6.60	0.01	Res	16.81	6.60	0.01	Res	16.81	6.60	0.01	Res	16.81	6.60	0.01	Res
<i>S(I)</i>	2	0.22	6.56	0.00	Res	Res	1.80	34.52	0.00	0.01	Res <i>I</i>	178.21	6.39	0.01	Res <i>I</i>	5.06	0.58	0.57	Res <i>I</i>	5.06	0.58	0.57	Res <i>I</i>	5.06	0.58	0.57	Res <i>I</i>	5.06	0.58	0.57	Res <i>I</i>	5.06	0.58	0.57	Res <i>I</i>
Res	45	0.03					0.05				61.67				4.60				18	3.63															
Res <i>Cs</i>	30	0.03					0.04				78.56				2.54				12	4.22															
Res <i>I</i>	15	0.03					0.07				27.90				8.72				6	1.63															
Cochran's							0.21 (n.s.)				0.3 (n.s.)				0.54				0.58																
<i>C</i> -test							ln(x + 1)				None				None				(<i>P</i> < 0.01)																
Transformation							1.75 (n.s.)				2.81				3.43				2.59 (n.s.)																
Two tailed <i>F</i> -test							1.75 (n.s.)				2.81				3.43				2.59 (n.s.)																

Two tailed *F*-tests compared spatial variances at the scale of replicate units between *I* and *Cs*.

Nematode assemblages (Table 2) did not differ between *I-v-Cs*. Although there was no significant effect detected by PERMANOVA, the nMDS shows quite a clear separation between sites near the sewage and those of *Cs* (Fig. 2). Different patterns of variability among sites were however detected between *I-v-Cs*.

Global differences between *I-v-Cs* were observed in syllid assemblages (Table 2, Fig. 2). *Exogone naidina*, *Syllis prolifera*, *Salvatoria clavata* and *S. limbata* were identified by SIMPER as important in determining differences between *I-v-Cs*. Differently from nematodes, no difference was detected in the structure of the assemblage at the scale of site between *I-v-Cs*.

4. Discussion

Several studies have highlighted the sensitivity of meiofauna to various kinds of human activities (Sandulli and De Nicola-Giudici, 1990, 1991; Austen et al., 1994; Danovaro et al., 1995; Mazzola et al., 1999; Mirto et al., 2000, 2002). The lack of larval dispersion and the close dependence on local habitat features make meiofauna potentially highly sensitive to environmental change, including modification of habitat complexity at very small spatial scales (Blome et al., 1999; Sandulli and Pinckney, 1999). The general outcome from the studies mentioned above is that anthropogenic disturbance alters meiofaunal abundance, diversity and biomass, and species composition, but that the direction of changes is not always univocal (sometimes increasing, sometimes decreasing the mean value) in the impacted assemblages with respect to the controls. Information about meiofaunal responses to sewage discharge is rather limited (Sandulli and De Nicola-Giudici, 1990,

1991), in spite of the increasing potential influence of this specific type of urban pollution in coastal marine environments. Studies that have been conducted on soft substrates indicated that the effect of sewage discharge could determine substantial changes in the structure of meiofaunal assemblages, increasing nematodes and decreasing harpacticoid copepods (Sandulli and De Nicola-Giudici, 1990, 1991). These changes were explained with the increased organic content (i.e., potential food sources) in the sediments, associated with the presence of the sewage discharge.

Univariate and multivariate analyses utilised in the present study revealed a number of significant idiosyncratic responses of meiofauna to sewage disturbance. Results, in fact, indicate that the sewage discharge affected meiofaunal assemblages in terms of taxon richness, determining a significant reduction of the number of taxa in the impacted samples, and community structure, causing a decrease in the relative dominance of nematodes and an increase of hydrozoans. The increase of the relative dominance of hydroids near the sewage outfall indicates a functional change in the assemblage related with the increase of the trophic component of sessile micropredators. At the same time, the sewage did not cause any significant changes in terms of total number of individuals.

As far as syllids are concerned, a significant effect of sewage discharge was observed both on the structure of the assemblage and on the number of individuals, but not in terms of number of species. Spatial heterogeneity was also significantly modified by the presence of the sewage outfall.

A reduced variability among replicate units was observed near the outfall for the number of individuals,

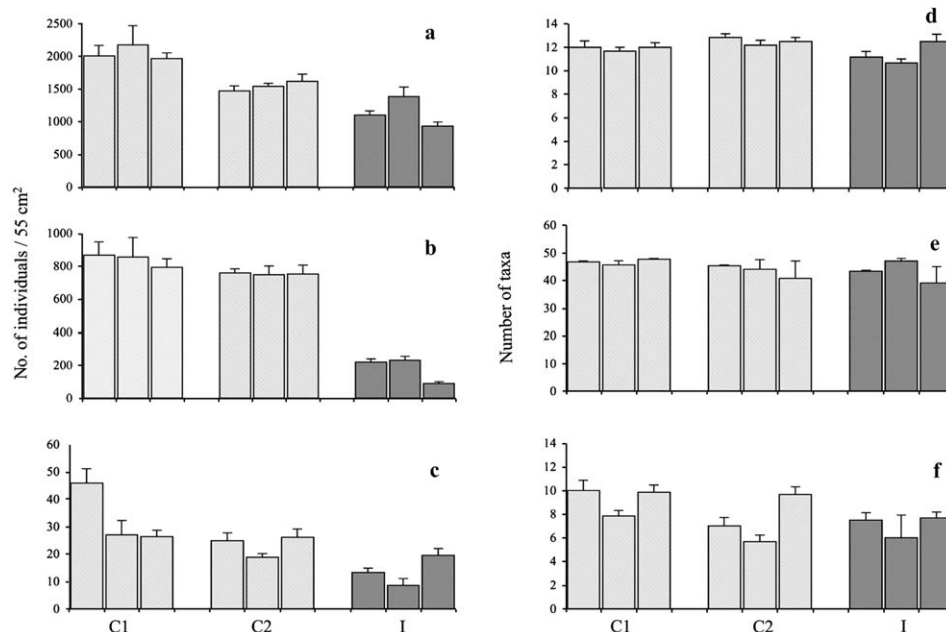


Fig. 1. Mean (+SE, $n = 18$) number of individuals for (a) total meiofaunal assemblage, (b) nematodes, (c) syllids. Number of taxa of (d) meiofauna, (e) nematodes, (f) syllids at the impacted locations (I) and at the two controls (C1 and C2).

while no differences in spatial variance were observed at the scale of sites (100 m). Spatial patterns in the number of species revealed significant differences both at the scale of replicate units and at the scale of sites.

Analyses at higher taxonomic resolution revealed that nematodes were influenced by the sewage in terms of reduced number of individuals, but not in terms of number of genera or taxonomic composition. Nematodes experienced a significant decrease in abundance, while syllids were characterised both by a decrease in the number of individuals and a change in the structure of the assemblage at *I* compared to *Cs*.

The decrease of nematode abundance was unexpected because available literature suggests that often nematodes

increase in abundance due to their ability to exploit the food released from sewage discharge (Vidakovic, 1983; Arthington et al., 1986; Bongers et al., 1991; Bongers and Ferris, 1999). In addition, an increase in the sedimentation rate can cause a higher abundance of nematodes and an increase of the number of deposit-feeding genera, but this was not the case in the present study. Changes observed in this study are possibly related with the modified structural complexity of the substrate, caused by changes in macroalgae assemblage composition near the sewage outfall. In fact, the sewage discharge caused the dominance by red algae of the family of Gelidiaceae, the preferred habitat of crustaceans (Gibbon, 1988b), and by filamentous brown algae of the genera *Ectocarpus* and *Sphacelaria* (Terlizzi et al., 2002). Macroalgal species composition and fractal dimension are known to affect meiofaunal abundance and distribution on subtidal hard substrates (Gee and Warwick, 1994; Danovaro and Fraschetti, 2002). Such a change in algal complexity is likely to be responsible also for changes in the structure of molluscan assemblages in an environmental impact study conducted in the same area (Terlizzi et al., 2005b). However, the descriptive nature of our experiment and the lack of temporal replication do not allow drawing any conclusion on the cause-effect relationships of the observed patterns.

The maturity index did not change significantly between *I-v-Cs* (3.5 and 3.2–3.3 for impact and both controls, respectively). The maturity index was rather high compared with data reported in other urban pollution studies (e.g., Bongers and Ferris, 1999) suggesting that nematode assemblages were mainly composed by “persisters” (Bongers et al., 1991) both at *I* and *Cs*. This pattern suggested that the sewage discharge did not affect nematode assemblages favouring “colonisers” (Bongers et al., 1991) that are characterised by a short life-cycle, higher colonization ability and tolerance to disturbances. The lack of significant differences in the maturity index between *I* and *Cs* suggested that both locations were characterised by nematodes with similar life histories. This pattern was also confirmed by the fact that in both locations, nematode assemblages were dominated by the same trophic guild (>60%, *epistrate feeders*—2A) that generally is associated with high maturity index values (Bongers et al., 1991). To our knowledge, there are no published accounts on the response of syllids to anthropogenic disturbance, and thus no comparisons can be made with previous information.

In this study, a significant amount of variability between controls was also detected in several analyses. This is a very common result in impact studies where multiple controls are included (Lardicci et al., 1999; Terlizzi et al., 2002; Piazzini et al., 2004 as examples from different habitats in the Mediterranean Sea) and is dependent on the large spatial variability usually characterizing natural systems. This result has relevant statistical consequences: when variation among controls was significant, the main contrast *I-v-Cs* was examined over the term *Cs*. As a result of the low number of degrees of freedom involved (only 1 df both at the

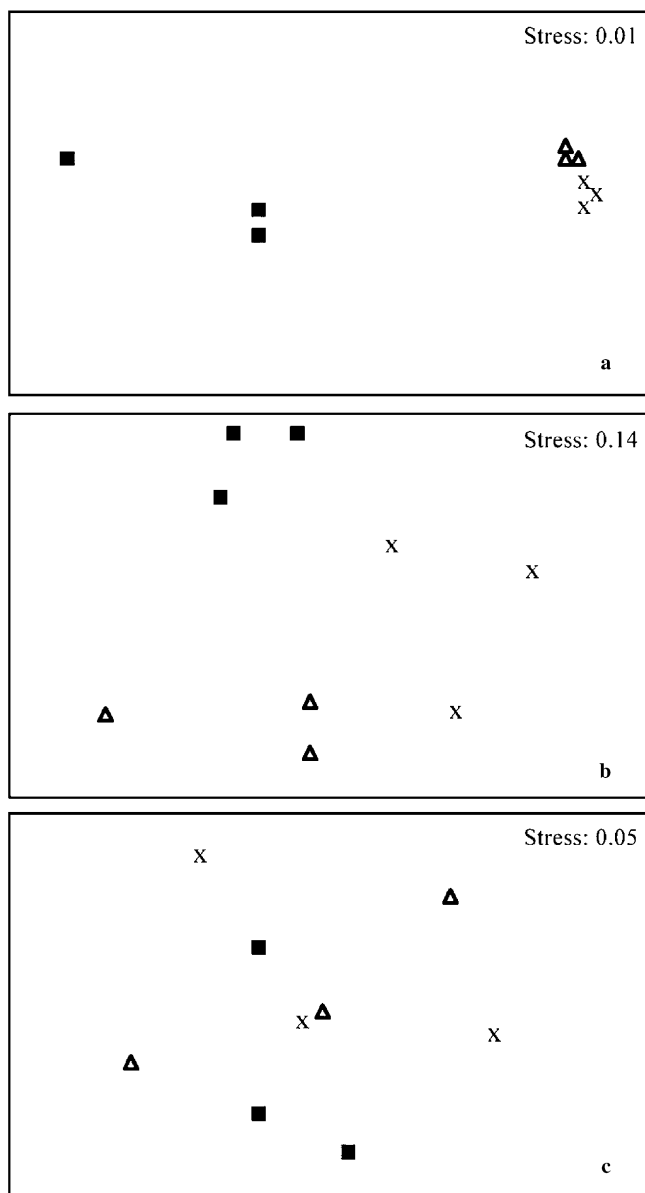


Fig. 2. Results of the non-metric multidimensional scaling ordination (nMDS plot) on Bray–Curtis dissimilarity measure of centroids for each site. (a) Total meiofauna; (b) nematodes and (c) syllids.

Table 2
PERMANOVA based on the Bray–Curtis dissimilarities of the multivariate data set (total meiofauna: 16 taxa; nematodes: 68 genera; Syllids: 31 species) (data untransformed)

Source	df	SS	MS	<i>F</i>	<i>P</i>	MS _{denom}	Permutable units
<i>Total meiofauna</i>							
Location = L	2	17,024.87	8512.44				
<i>Cs</i>	1	1749.01	1749.01	10.06	<i>0.090</i>	S(<i>Cs</i>)	6 S(<i>Cs</i>) cells
<i>I-v-Cs</i>	1	15,275.86	15,275.86	26.30	<i>0.000</i>	S(L)	9 S(L) cells
Site(L) = S(L)	6	3485.59	580.93	3.40	0.007	Residual	54 raw data units
S(<i>Cs</i>)	4	695.26	173.82	1.13	0.340	Res <i>Cs</i>	36 raw data units
S(<i>I</i>)	2	2790.32	1395.16	6.83	0.000	Res <i>I</i>	18 raw data units
Residual	45	7698.17	171.07				
Res <i>Cs</i>	30	4632.93	154.43				
Res <i>I</i>	15	3065.24	204.35				
<i>Nematodes</i>							
Location = L	2	11,956.00	5978.00				
<i>Cs</i>	1	6841.25	6841.25	2.99	<i>0.025</i>	S(<i>Cs</i>)	6 S(<i>Cs</i>) cells
<i>I-v-Cs</i>	1	5114.75	5114.75	0.75	<i>0.670</i>	<i>Cs</i>	2 <i>Cs</i> cells
Site(L) = S(L)	6	14,594.34	2432.39	2.12	0.000	Residual	54 raw data units
S(<i>Cs</i>)	4	9150.71	2287.68	2.22	0.000	Res <i>Cs</i>	36 raw data units
S(<i>I</i>)	2	5443.64	2721.82	1.97	0.060	Res <i>I</i>	18 raw data units
Residual	18	20,643.07	1146.84				
Res <i>Cs</i>	12	12,371.99	1031.00				
Res <i>I</i>	6	8271.08	1378.51				
<i>Syllids</i>							
Location = L	2	16,618.77	8309.38				
<i>Cs</i>	1	5566.68	5566.68	2.07	<i>0.080</i>	S(<i>Cs</i>)	6 S(<i>Cs</i>) cells
<i>I-v-Cs</i>	1	11,052.09	11,052.09	3.65	<i>0.001</i>	S(L)	9 S(L) cells
Site(L) = S(L)	6	18,190.98	3031.83	2.39	0.000	Residual	54 raw data units
S(<i>Cs</i>)	4	10,768.11	2692.03	2.98	0.000	Res <i>Cs</i>	36 raw data units
S(<i>I</i>)	2	7422.86	3711.43	1.84	0.010	Res <i>I</i>	18 raw data units
Residual	45	57,054.25	1267.87				
Res <i>Cs</i>	30	26,841.59	894.72				
Res <i>I</i>	15	30,212.66	2014.18				

Each test was done using 4999 random permutations. *P*-values in italics were obtained using 4999 Monte Carlo samples from the asymptotic permutation distribution (Anderson and Robinson, 2003).

numerator and denominator of the *F* ratio), the power of these tests was very low and only large differences between the disturbed and reference locations could have been detected as significant. As such, it is still possible that the impact is acting also on the number of nematode genera and/or on the structure of nematode assemblages (as suggested by the nMDS plot), though the low power of the test prevented its detection. This further reinforces the idea that traditional selection of a single control in environmental impact studies can lead to confound different sources of variability, stressing the need of carefully planned sampling designs for unambiguous assessments of environmental impacts (Benedetti-Cecchi, 2001).

The need for hierarchical sampling designs to quantify the extent to which natural patterns of spatial variance can be modified by human interventions is not new in the assessment of environmental impacts (Bishop et al., 2002). Nonetheless, anthropogenic impacts on meiofauna are typically studied by sampling at only 1 spatial scale

and, generally, at regularly spaced intervals from the point of discharge. This severely limits the quantification of the spatial extent of an impact, and prevents the possibility to test whether the impact has the potential to modify the spatial variability in the impacted location with respect to controls. Several studies quantified the effects of different sources of impacts on measures of spatial variance of benthic organisms (Warwick and Clarke, 1993; Chapman et al., 1995; Balestri et al., 2004; Terlizzi et al., 2005a,b). With few exceptions, all these studies focused on benthos of hard substrata. The effects of anthropogenic disturbance do not always consist in an enhancement of spatial variance. This was also the case in our study. In a study of the impact of thermal pollution on macro- and meiofauna, Lardicci et al. (1999), found higher values of multivariate dispersion among replicate units in impacted sites than in sites within controls. The mechanisms underlying the observed changes in spatial patterns are difficult to ascertain, as they do not always occur in the same direction.

Only the acquisition of larger data set (with the incorporation of detailed experimental manipulations) will be able to clarify the meaning of this kind of response under stressful conditions.

5. Conclusions

Several studies have attempted to identify the effect of anthropogenic disturbance or environmental gradients without appropriate spatial replication. This is the case with studies carried out in estuarine subtidal soft bottoms (Albertelli et al., 1999), coastal meiofauna assemblages influenced by a river plume (Danovaro et al., 2000), and meiofauna of shallow sediments subjected to the impact of fish-farming (Mazzola et al., 1999, 2000; Mirto et al., 2000, 2002) and to an oil-induced disturbance (Danovaro et al., 1995, 1999). Additional studies, based on clearly stated hypotheses and appropriate experimental designs are urgently needed for those ecological compartments (such as meiofauna) which have been largely neglected in this kind of ecological studies, despite their potential for elucidating the issue of environmental impact assessment. This first comparison indicates that response from subtidal hard substrates can be largely different from those obtained for a similar source of impact on other habitats or environmental contexts. Such evident differences stress the need of integrating in this context the investigation of ecological processes influenced by disturbance through the use of manipulative experiments capable of overcoming the intrinsic limitation of the correlative approach.

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