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PRIMARY RESEARCH PAPER

Effects of sewage pollution on the structure of rocky shore macroinvertebrate assemblages

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Abstract The urgency to find efficient indices and indicators to prevent further deterioration of coastal areas is one of the hot topics in today's scientific publication. However, a detailed knowledge of community responses to anthropogenic impacts is essential to sustain those indices. The studies on the response of benthic community to sewage pollution on intertidal rocky shores are generally based on visual census and do not take into account the tidal levels. In order to fulfil this gap in this study: (i) the sampling was performed by destructive sampling, with all individuals identified to the species level; (ii) the sampling was done at all levels of the intertidal (sublittoral fringe, eulittoral, and littoral fringe). Sewage pollution changed the environmental variables and the abundance of macroinvertebrates, being *Mytilus galloprovincialis*, *Melarhaphe neritoides*, and *Chthamalus montagui* the species most responsible for the dissimilarities observed. Effects were different on the three intertidal zones: community structure changed in the sublittoral fringe; suspension-feeders abundances and species richness increased in the eulittoral; no differences were detected in the littoral fringe. Moreover, the results confirm that the presence of sewage discharges tended to benefit suspension feeders, and that the sensitive species were replaced by opportunistic ones.

Keywords Rocky shore · Intertidal · Macrofauna · Sewage

Introduction

Half the world's population lives along the coastline and consequently the habitats located in those areas are under great human pressure. This includes a great variety of toxic contaminants from agricultural, industrial, and urban activities (Little et al., 2010). Sewage discharges are among the most common anthropogenic impacts on rocky shores, resulting in organic and nutrient enrichment (Arévalo et al., 2007).

In Europe, sewage can receive secondary (organic matter removed) or tertiary (nutrients and bacteria removed) treatment, prior to being discharged directly into the shore, or at some distance from the shore, through pipeline systems. In nearly half of the

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countries, the majority of sewage treatment plants only include primary and secondary treatment (http://epp.eurostat.ec.europa.eu). As a result, nutrient enrichment and bacteria concentrations become a major concern in the preservation of marine ecosystems. With this in mind, the European Water Framework Directive (WFD) seeks to prevent further deterioration of the European coastal waters by evaluating the ecological status of all the water bodies. For coastal areas, this evaluation should be based on both physicochemical elements and biological indicators such as phytoplankton, macroalgae, benthic macrofauna, and seagrasses. Above all, benthic invertebrates are considered powerful indicators of marine pollution due to their sedentarism, long lives, easy sampling, and to the existence of extensive literature on their distribution in specific environments and on their response to different environmental stresses (Reish et al., 1999; Fano et al., 2003; Blanchet et al., 2008).

The responses of benthic invertebrate assemblages from the intertidal areas of rocky shores to sewage pollution are poorly understood (Johnston & Roberts, 2009; Dauvin et al., 2010; Bustamante et al., 2012). Nevertheless there are several studies focusing on intertidal populations of polychaetes (Dauer & Conner, 1980; Elías et al., 2006; Jaubet et al., 2011), molluscs (Bishop et al., 2002; Terlizzi et al., 2005a; Vallarino and Elías, 2006; Atalah & Crowe, 2012) or crustaceans (Calcagno et al., 1998; De-la-Ossa-Carretero et al., 2010). But studies dealing with the effect on the entire intertidal benthic community are very rare (Littler & Murray, 1975; López-Gappa et al., 1990; Archambault et al., 2001, Klein & Zhai, 2002; 1993). Moreover, several of those studies do not have the most appropriate sampling design to detect human disturbances (e.g., using only one reference site). Furthermore, in rocky shores the species are distributed in bands, creating a vertical zonation, where physical and biotic factors diverge, and communities varied in terms of species richness and composition (Hawkins & Jones, 1992; Little et al., 2010). However, earlier studies have only focused on one of the tidal level (López-Gappa et al., 1990, 1993; Klein & Zhai, 2002). Finally, previous research was mainly based on visual census, which has the disadvantage of underestimating species with smaller dimensions (Littler & Murray, 1975; Archambault et al., 2001). Nevertheless, previous studies have already pointed out important results, such as the changes in the structure and functioning of the community, and the replacement of sensitive species by opportunistic ones due to the presence of sewage discharges.

With all this in mind, the aim of this paper was to study the effects of sewage pollution on hard bottom macrofauna assemblages compared with control locations not exposed to this human threat. This study was carried out across all intertidal zones (littoral fringe, eulittoral, and sublittoral fringe) to assess the consistency of patterns.

Materials and methods

Study site and sampling procedures

The study was conducted in Peniche peninsula, located on the central western coast of Portugal (Fig. 1). In this peninsula, a sewage treatment plant was built in 1998. The outfall releases secondary-treated effluents. It serves a human population of 40,000 and discharges the effluent directly into the intertidal area of the rocky shore. The lack of pre-impact data led to the choice of an ACI (after control/impact) experimental design. Consequently, three sampling areas, about 1-km distance from each other, were selected: an impacted area, near the sewage discharges (Imp) and two reference areas (R1 and R2) (Fig. 1). Ideally, the sampling design should have one reference area located on each site of the impacted area. However, due to the location of the impacted area in the tip of the Peninsula it was not possible. Nevertheless, data analyses showed that there were no significant differences between the two reference areas, supporting the selection of those areas. All sampling areas had comparable environmental conditions, with regard to slope, orientation, wave exposure and type of substrate (Fig. 1). Within each sampling area three intertidal zones were sampled: the littoral fringe, the eulittoral, and the sublittoral fringe. This pattern of zonation has been mentioned for the coastal intertidal areas of the Portuguese (Boaventura et al., 2002a, b). The littoral fringe is characterized by the presence of *Melarhaphe neritoides* and encrusting lichens; the eulittoral zone is dominated by barnacles and mussels; and the sublittoral fringe is dominated by red algae in central and southern regions of Portugal (Boaventura et al., 2002a, b). For each intertidal zone, five quadrats (12 × 12 cm) were randomly selected and organisms were collected by scraping the selected area using a spatula and a chisel.

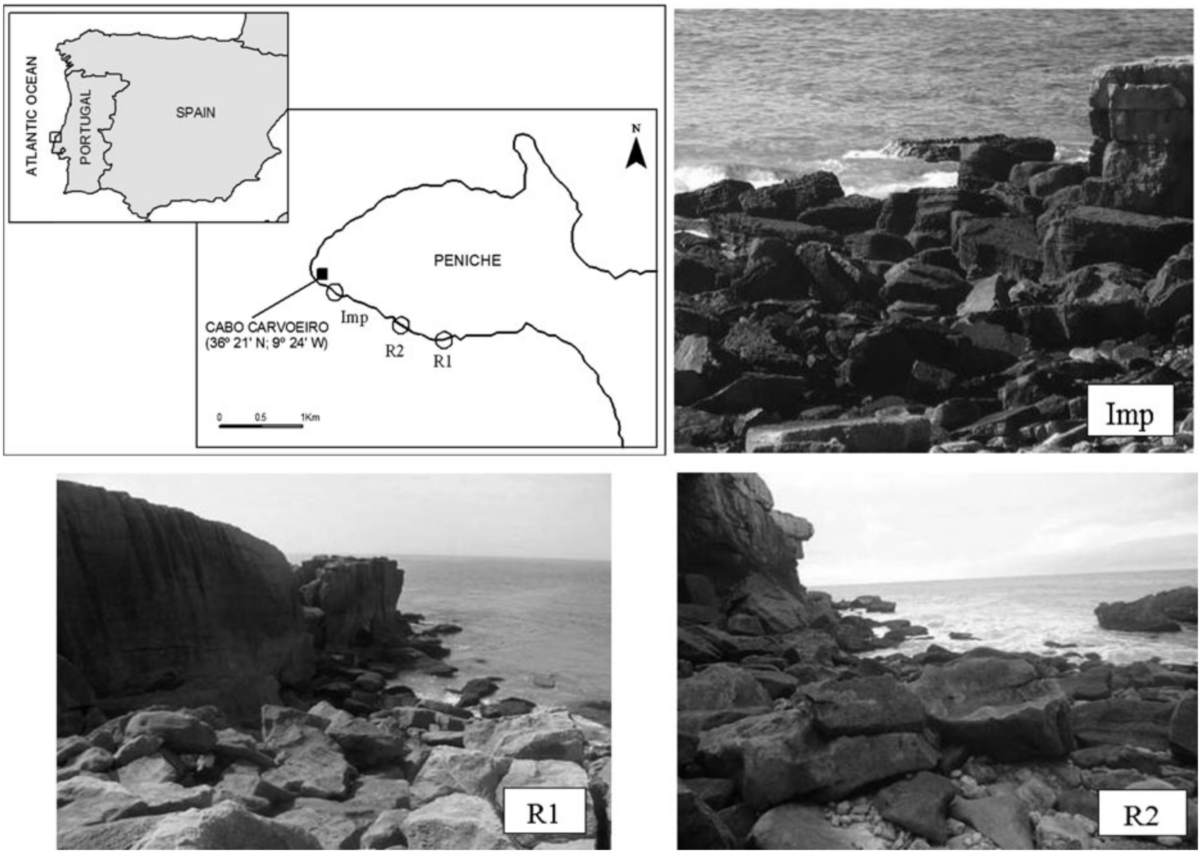

Fig. 1 Map of Peniche peninsula, western coast of Portugal, showing the location and photos of the sampling areas: *Imp* impacted area, *R1* and *R2* reference areas

As the studied area presents a temperate climate, four sampling dates representative of each season were chosen in order to account for the temporal variation (February, April, July, and November 2010). It was not our goal to study the differences between seasons but to capture the natural yearly variation of the community's response. During the sampling programme in all areas, environmental variables (dissolved oxygen, temperature, salinity, and pH) were measured in the seawater. Also, water samples were collected in order to determine in the laboratory the concentrations of nutrients, total-suspended solids (TSS), and bacteria (total coliforms).

Laboratory procedures

All material collected from scrapings was sieved through a 500-µm mesh and all individuals were identified to the lowest possible taxonomic level and counted to determine density. Water samples were filtered (Whatman GF/F glass-fibre filter) and stored frozen at −18°C until analysis. Analyses followed the standard methods described in Limnologisk Metodik (1985) (for ammonia and phosphate), in Strickland & Parsons (1972) (for nitrate and nitrite), and ESS Method 340.2 for total suspended solids (ESS 1993). Total coliforms in the water samples were determined using the membrane filtration technique. A 20-fold dilution was used for the water samples from the impacted area. The samples were cultivated onto CHROMOCULT® coliform agar (Merck), with an incubation period of 24 h at 37°C. At the end of this period CFU were counted.

Statistical analysis

Environmental data were transformed (square-root) and normalized and a principal component analyses (PCA) composed by the Euclidean distance was used for the ordination of the sampling units based on the physico-chemical data.

A distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was carried out separately for each intertidal zone (littoral fringe, eulittoral, and sublittoral fringe) to test for differences in the structure of the invertebrate assemblages between impacted and reference conditions. The model consisted of two factors: Time (4 levels, random, orthogonal) and Location (1 impacted and 2 reference areas, fixed, orthogonal). In both cases, the design was asymmetrical (Underwood, 1991) due to the presence of a single impacted location. Therefore, the location term, and all terms involving location, was partitioned into two portions: the 1-degree-of-freedom contrast of Imp-v-Rs and the variability between reference locations (Rs). The same partitioning was performed for the residual variability for observations within Imp (Res Imp) within Rs (Res Rs). Appropriate denominators for F ratios were identified from expected mean squares and tests were constructed following the logic of asymmetrical design (see Terlizzi et al., 2005b). All analyses were based on Bray–Curtis similarity of square-root transformed data, and each term in the analysis was tested by 4,999 random permutations of appropriate units. To visualize multivariate patterns, differences in the structure of the community among treatment levels were visualized by principal coordinate (PCO) analyses on the basis of Bray–Curtis similarities. Species classes found in each intertidal zone were displayed as vectors in the PCO plots.

The total number of species (S) was calculated for each observation unit, using the DIVERSE routine contained in the PRIMER statistical package. Univariate permutational analyses of variance (Anderson, 2001) were carried out on several variables using the same experimental design as described above for the multivariate analyses. The variables were: number of species, total faunal density, density of species classes (Gastropoda, Bivalvia, Crustacea, Polychaeta, and Polyplacophora) and abundance of *Corallina* spp. Univariate analyses were performed using PERMANOVA, with Euclidean distances as the measure of similarity.

For all statistical tests, the significance level was set at $P \leq 0.05$. All calculations were performed using the PRIMER v 6 software package (Clarke & Gorley, 2006).

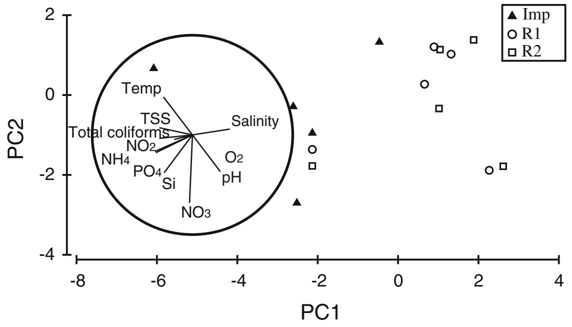

Fig. 2 PCA analyses displaying sampling areas (*R1*, *R2* reference areas, *Imp* impacted area) and environmental variables in the two first principal components (salinity; *O_2* dissolved oxygen, pH; *NO_3* nitrate, *Si* silica, *NO_2* nitrite, *NH_4* ammonia, *PO_4* phosphate, bacteriological analysis; *TSS* total-suspended solids and *Temp* seawater temperature)

Results

Environmental variables

The environmental variables were markedly different when comparing impacted and reference areas (Fig. 2). The PCA plot obtained using the environmental variables showed a gradient produced by the presence of the outfall. The first axis of the PCA explained 56.5% of the total variance and the second 19.1%. The temperature of the seawater was higher in the impacted area, while dissolved oxygen, salinity, and pH were lower in the sewage-affected areas. The concentrations of nutrients, especially ammonia and phosphate, and total-suspended solids (TSS) were also higher near the sewage discharges (supplementary material).

Intertidal macroinvertebrate assemblages

Littoral fringe

Only the gastropod *Melarhaphe neritoides* and (occasionally) the isopod *Ligia oceanica* were found in this level. PERMANOVA analysis did not detect any significant differences between impacted and reference areas, but only a significant temporal variability (Table 1). However, *M. neritoides* was slightly more abundant in the impacted area (8,590 ind/m^2 ± 2518) than in the reference areas (R1 = 6,951 ind/m^2 ± 2108; R2 = 6,475 ind/m^2 ± 1,082),

Table 1 PERMANOVA results on littoral fringe assemblages

Source of variability	df	Littoral fringe		
		MS	*F*	*P*
Time = Ti	3	7960.5	8.044	**0.0002**
Location = Lo	2	420.3	0.325	0.7392
Imp-v-Rs	1	834.9	0.729	0.4458
Rs	1	5.77	0.004	0.8574
Ti × Lo	6	1293.2	1.307	0.2728
Ti × Imp-v-Rs	3	1145.8	1.150	0.3416
Ti × Rs	3	1440.5	1.860	0.1444
Res	48	989.6		
Res Imp	16	1419.9		
Res Rs	32	744.5		

Significant results are given in bold (see text for further details)

although the differences were not statistically significant.

Eulittoral

PERMANOVA analysis detected significant differences between impacted and reference areas, but such patterns were not consistent in time as a significant Ti × Imp-v-Rs interaction was observed (Table 2). Similarly, total faunal density also varied significantly (Table 2), being superior in the impacted area (Fig. 3). The differences in total faunal density were not consistent in time either (Table 2). The PCO plot (Fig. 4) showed that the community structure was similar in all sampling areas, but that the densities of Bivalvia and, especially, Gastropoda and Crustacea were higher near the sewage affected areas. In Table 3, it can be observed that those differences were statistically significant although not consistent in time (significant Ti × Imp-v-Rs interaction). In addition, as can be seen in Table 4, *Chthamalus montagui*, *M. neritoides*, and *Mytilus galloprovincialis* were the most important species in differentiating assemblages.

Regarding species richness (Table 2) it can be observed a significant interaction Imp-v-Rs, indicating that the number of species in the eulittoral was higher near the sewage affected areas (Fig. 3). As can be seen in Table 4, several species were observed only in the impacted area: *Littorina saxatilis*, *Lasae adansoni*, *Dynamene* spp., and *Hyale pontica*). Finally, *Patella* spp. were the only species more abundant in the reference areas (Table 4).

Sublittoral fringe

The sublittoral fringe presented the highest densities and species richness (Fig. 3). This level was dominated by the red algae *Corallina* spp., that was more abundant near the sewage discharges (Tables 6, 7). Concerning the macrofauna, the assemblages differed significantly between impacted and reference areas, but such patterns were not consistent in time as a significant Ti × Imp-v-Rs interaction was observed (Table 5). Identically, the differences in total faunal density between impacted and reference areas were not consistent in time (Table 5). Concerning species richness it was observed no differences between sampling areas (Table 5). However, the PCO plot (Fig. 5) showed that the structure of the community changed along the sampling areas: more Bivalvia, Crustacea, and Polychaeta were found in the impacted area, while Gastropoda and Polyplacophora were more common in the reference areas. Those differences were statistically significant although not consistent in time (Table 6). In addition, as can be seen in Table 7, *M. galloprovincialis* was the most abundant species in the impacted areas, while *Rissoa parva* was the dominant species in the reference areas. Several species of gastropods were present in the sublittoral fringe (Table 7). Some species were equally abundant along all sampling areas (as *Nucella lapillus* or *Gibbula umbilicalis*). However, the majority were mainly present in the reference areas, namely *Rissoa parva*, *Skeneopsis planorbis*, or *Tricolia pullus*. On the contrary, bivalves (as *Mytilus galloprovincialis*, *Musculus costulatus*, or *Lasae adansoni*) were more abundant near the sewage discharges, with the exception of *Modiolus modiolus* (Table 7). Regarding crustaceans, *Hyale perieri*, *Idotea pelagica*, *Dynamene* spp., and *Tanais dulongii* showed higher abundances near the sewage discharges (Table 7). Nevertheless, other species seemed to prefer the reference areas (*Pirimela denticulata*, *Campecopea lusitanica*). The same pattern was also observed for the polychaetes (Table 7): *Eulalia viridis* or *Sabellaria alveolata* were more abundant in the impacted area and *Perinereis* spp. or *Syllis gerlachi* were more abundant in the reference areas. Finally, nematoda and nemertinea seemed to prefer the impacted area, while Polyplacophora were more abundant in the reference areas (Table 7). Other classes, although with lower numbers, like echinoidea and ophiuridea were only observed in the reference areas.

Table 2 PERMANOVA results on community structure, total faunal density and number of species for the eulittoral

Source of variability	df	Community structure			Total faunal density			Number of species		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Time = Ti	3	2647.9	3.073	**0.0014**	489.5	5.187	**0.0046**	0.483	4.636	**0.007**
Location = Lo	2	18126	10.118	**0.0052**	7343.1	10.233	**0.015**	5.155	29.514	**0.003**
Imp-v-Rs	1	32673.7	12.002	**0.025**	14627	10.383	0.0542	9.859	35.405	**0.0274**
Rs	1	3579.3	4.158	0.055	59.25	2.248	0.2352	0.451	6.363	0.1002
Ti × Lo	6	1791.5	2.079	**0.0064**	717.6	7.603	**0.002**	0.175	1.677	0.1434
Ti × Imp-v-Rs	3	2722.2	2.979	**0.002**	1408.8	15.692	**0.0002**	0.278	2.556	0.0622
Ti × Rs	3	860.8	0.802	0.5834	26.35	0.2436	0.865	0.071	0.64	0.5882
Res	48	861.6			94.38			0.104		
Res Imp	16	438.5			66.87			0.091		
Res Rs	32	1073.1			108.14			0.111		

Significant results are given in bold (see text for further details)

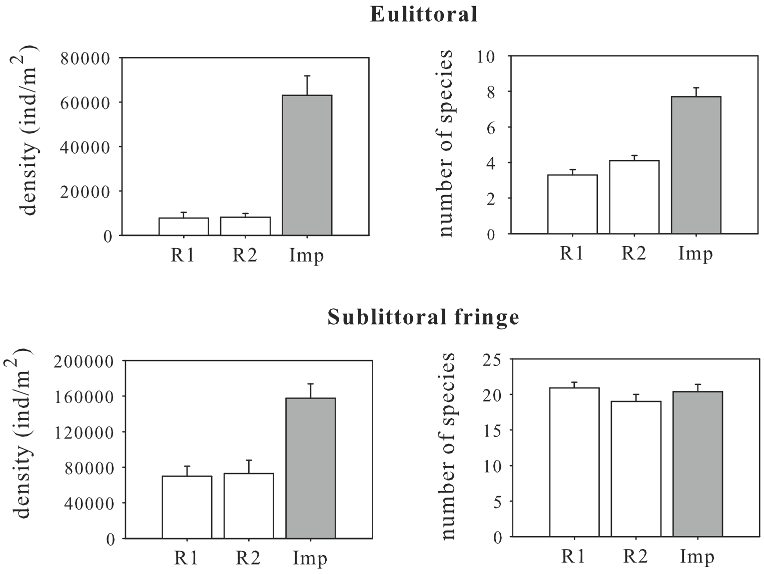

Fig. 3 Changes in density (ind/m^2 ± SE) and number of species in the eulittoral and sublittoral fringe. *Open bar* R1 and R2—reference areas, *closed bar* Imp—impacted area

Discussion

Environmental data

The effects of the sewage discharges in the environmental variables have already been noticed in previous studies. Roberts et al. (1998) and Elías et al. (2009) detected an increase in the concentration of nutrients and suspended solids. López-Gappa et al. (1990, 1993) observed an increase in the temperature of the seawater and total coliforms and a decrease in pH, salinity, and dissolved oxygen values in Argentina shores. As a rule, during the treatment of the sewage, gross solids are eliminated from the effluents (primary treatment), followed by the removal of the organic matter (secondary treatment). Finally, bacteria and nutrients are taken out of the effluents (tertiary treatment). However, several sewage treatment plants are only prepared to secondary treatment, and consequently the increase in the temperature of seawater and the decrease in dissolved oxygen, salinity and pH are expectable. Likewise, the lack of tertiary treatment also explains the higher concentrations of total-suspended solids, total coliforms, and nutrients near the sewage affected areas. Concerning nutrients, the highest concentrations were found for ammonia and

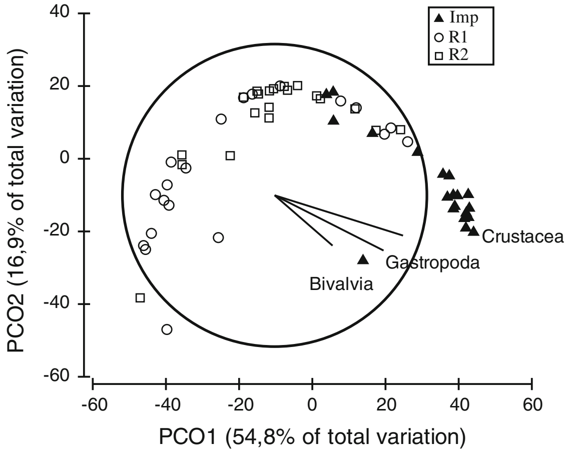

Fig. 4 Principal coordinates ordination (PCO) plots of eulittoral assemblages at both impacted (*filled symbols*) and reference areas (*empty symbols*) on the basis of Bray–Curtis similarities of the square-root transformed data

phosphate, which is also expectable, since domestic and industrial effluents are the main contributors for the eutrophication of marine waters.

Intertidal macroinvertebrate assemblages

Littoral fringe

The littoral fringe is the level located further away from the sewage discharge, which can explain the lower effects observed in abundance and species richness. Two species were found in this level: the gastropod *Melarhaphe neritoides* and the isopod *Ligia oceanica*. *L. oceanica* was only found in the impacted area. This isopod is a mobile omnivore that feeds on particulate organic matter and detritus (Littler & Murray, 1975; Fish & Fish, 2001) that might explains its higher abundance near the sewage discharges. On the contrary, no differences were found for *M. neritoides* between sampling areas. However, to assess the effect of sewage discharges on the population of *M. neritoides* it is necessary to study the population along all tidal height, and not only in the littoral fringe. Planktonic larvae settle on the lower levels of rocky shores and then start moving upshore, resulting in a shell size gradient (Cabral-Oliveira et al., 2009). The results obtained in this work confirmed previous findings (Cabral-Oliveira et al., 2009) where the density of *M. neritoides* was higher in the impacted area as a result of massive settlement. Also in this work was found a higher number of juveniles in the eulittoral near the outfall. The higher concentration of nutrients near sewage discharges will lead to a larger quantity of microalgae on the rocky surfaces, on which *M. neritoides* feed. This could be attractive to the settling of *M. neritoides* larvae. However, this discrepancy in not found in the density values of the adults (in the littoral fringe). The greater density of juveniles in the eulittoral near the sewage discharges could lead to greater competition for food and space, and, consequently, to this significant mortality.

Table 3 PERMANOVA results on species classes of eulittoral assemblages

Source of variability	df	Gastropoda			Bivalvia			Crustacea		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Time = Ti	3	2922.3	20.919	**0.0002**	521.34	3.6754	**0.0132**	331.94	1.6743	0.1906
Location = Lo	2	13361	5.7909	**0.0376**	5198.7	20.933	**0.0054**	7362.3	7.7163	**0.0272**
Imp-v-Rs	1	26647	5.806	0.1156	10393	21.484	**0.0298**	14541	8.09	0.0766
Rs	1	74.779	2.9653	0.1684	4.8348	0.37273	0.56	183.25	1.6535	0.287
Ti × Lo	6	2307.2	16.516	**0.0002**	248.35	1.7509	0.1282	954.13	4.8125	**0.0006**
Ti × Imp-v-Rs	3	4589.2	34.808	**0.0002**	483.74	3.671	**0.018**	1797.4	9.317	**0.0006**
Ti × Rs	3	25.218	0.17759	0.9182	12.972	0.61168	0.6158	110.82	0.4425	0.7314
Res	48	139.69			141.85			198.26		
Res Imp	16	135.07			383.12			93.879		
Res Rs	32	142.01			21.207			250.45		

Significant results are given in bold (see text for further details)

Table 4 Density (ind/m^2 ± SE) of the most common species found in the eulittoral

Species	R1	R2	Imp
Littorina saxatilis	0	0	73 ± 28
Melarhaphe neritoides	1906 ± 806	2809 ± 1136	31778 ± 5908
Patella spp.	694 ± 101	649 ± 61	264 ± 36
Lasae adansoni	0	0	1101 ± 354
Mytilus galloprovincialis	122 ± 72	38 ± 18	2203 ± 839
Campecopea hirsuta	14 ± 8	0	236 ± 90
Chthamalus montagui	5052 ± 1945	4451 ± 983	25875 ± 3666
Dynamene sp.	0	0	90 ± 49
Hyale perieri	21 ± 7	118 ± 23	410 ± 98
Hyale pontica	0	0	45 ± 45

Table 5 PERMANOVA results on community structure, total faunal density and number of species for the sublittoral fringe

Source of variability	df	Community structure			Total faunal density			Number of species		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Time = Ti	3	8887.8	11.935	**0.005**	381.3	5.486	**0.0034**	1.449	7.617	**0.004**
Location = Lo	2	12125	4.16	**0.005**	1907.4	2.583	0.1462	0.311	0.898	0.4524
Imp-v-Rs	1	21449	6.186	**0.0278**	3814.7	2.638	0.196	0.047	0.172	0.6926
Rs	1	2801.4	1.186	0.3816	0.0086	0.00028	0.9168	0.575	1.369	0.4118
Ti × Lo	6	2914.7	3.914	**0.0002**	738.31	10.623	**0.0002**	0.346	1.82	0.112
Ti × Imp-v-Rs	3	3467.3	3.951	**0.0002**	14461.1	21.939	**0.0002**	0.273	1.295	0.2832
Ti × Rs	3	2362.1	2.499	**0.0004**	30.52	0.4087	0.744	0.419	1.835	0.1504
Res	48	744.7			69.5			0.19		
Res Imp	16	343.3			59.16			0.113		
Res Rs	32	945.4			74.67			0.229		

Significant results are given in bold (see text for further details)

Eulittoral

Previous studies (Archambault et al., 2001) suggested that sewage discharges have little impact in organisms that live in the eulittoral. However, different conclusions can be drawn with the present work.

In the eulittoral the higher abundances found near the sewage affected areas were explained by the larger number of gastropods (*Melarhaphe neritoides*), bivalves (*Mytilus galloprovincialis*), and crustaceans (*Chthamalus montagui*). *M. galloprovincialis* and *C. montagui* had higher densities near the sewage discharges, in all levels. Both species are filter-feeders and space occupiers (Hawkins & Jones, 1992). Attending to the higher amount of suspended solids near the sewage discharges it was predictable to find higher densities of filter-feeders where the availability of food is higher.

Concerning the so-called not dominant species, it was observed higher abundances of the bivalve *Lasae adansoni*, the isopods *Campecopea hirsuta* or *Dynamene* spp., and the small periwinkles *Littorina saxatilis* near the sewage-affected areas. This can be due to the higher amount of empty barnacle's cases, which are the habitat of those species (Fish & Fish, 2001). As a result, species richness increased in the impacted area. Previous authors (Magurran & McGill, 2010) have already pointed out that periodic disturbance might increase biodiversity by adding more resources to the habitat and by promoting the coexistence of species adapted to different conditions.

Finally, the limpets *Patella* spp. were more abundant in the reference areas in the eulittoral. However, the opposite was observed for the density of the juveniles found in the sublittoral fringe. Intraspecific

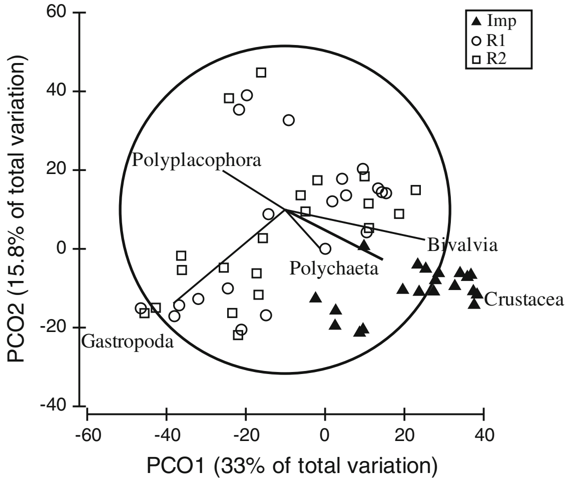

Fig. 5 Principal coordinates ordination (PCO) plots of sublittoral fringe assemblages at both impacted (*filled symbols*) and reference areas (*empty symbols*) on the basis of Bray–Curtis similarities of the square-root transformed data

competition normally occurs in the rocky intertidal environment when space or food resources are not enough or when recruitment occurs in high densities leading to crowding (Boaventura et al., 2002b). Due to the organic enrichment near the sewage discharges, the competition should be mainly for space. The higher abundance of juveniles near the sewage discharges increases the competition for space, and consequently only a small number of individuals survive and become adults.

Sublittoral fringe

The sublittoral fringe presented the highest densities and number of species. Although the number of species was similar in all sampling areas, there were qualitative differences in the species present in the impacted and reference areas.

Table 6 PERMANOVA results and pair-wise comparisons on species classes of sublittoral fringe assemblages and *Corallina* spp. total cover

Source of variability	df	Gastropoda			Bivalvia			Crustacea		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Time = Ti	3	10011	43.77	**0.0002**	5863.2	10.729	**0.0002**	124.89	10.577	**0.0002**
Location = Lo	2	2155.8	1.237	0.3608	28418	5.9869	**0.0362**	1108.7	8.2816	**0.0192**
Imp-v-Rs	1	4310.8	1.324	0.3428	56815	7.185	0.094	2167.3	9.616	0.071
Rs	1	0.83306	0.00362	0.8024	21.724	0.0137	0.8982	50.111	1.1829	0.4064
Ti × Lo	6	1742.9	7.62	**0.0002**	4746.7	8.6861	**0.0002**	133.88	11.338	**0.0002**
Ti × Imp-v-Rs	3	3256	14.51	**0.0002**	7907.8	13.26	**0.0002**	225.4	15.75	**0.0002**
Ti × Rs	3	229.82	0.70886	0.5602	1585.7	2.6659	0.064	42.363	4.9273	**0.0034**
Res	48	228.73			546.47			11.808		
Res Imp	16	37.756			449.79			18.228		
Res Rs	32	324.22			594.81			8.5977		

Source of variability	df	Polychaeta			Polyplacophora			*Corallina* spp.		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Time = Ti	3	104.23	9.4527	**0.0002**	129.7	3.1626	**0.035**	293.75	5.1134	**0.003**
Location = Lo	2	78.316	1.3759	0.3238	1228.7	6.8843	**0.0274**	678.97	6.1068	**0.0378**
Imp-v-Rs	1	122.89	1.31	0.3286	2286.4	12.03	0.0576	1196.2	189.9	**0.0308**
Rs	1	33.746	1.6834	0.3374	171	1.0249	0.362	161.79	0.7488	0.494
Ti × Lo	6	56.92	5.1619	**0.0002**	178.48	4.3522	**0.0012**	111.18	1.9354	0.0866
Ti × Imp-v-Rs	3	93.792	7.826	**0.0008**	190.12	3.745	**0.0162**	6.2979	0.092	0.9622
Ti × Rs	3	20.047	2.1584	0.1128	166.84	3.0763	**0.0372**	216.07	3.4567	**0.0294**
Res	48	11.027			41.01			57.446		
Res Imp	16	14.505			14.562			47.325		
Res Rs	32	9.2877			54.233			62.507		

Significant results are given in bold (see text for further details)

Table 7 Biomass (g/m^2 ± SE) of *Corallina* spp. and density (ind/m^2 ± SE) of the most common species found at the sublittoral fringe

Species	R1	R2	Imp
Corallina spp.	918 ± 105	721 ± 126	1398 ± 125
Barleeia unifasciata	1903 ± 481	1420 ± 336	5889 ± 1514
Gibbula umbilicalis	45 ± 15	21 ± 10	45 ± 14
Nucella lapillus	59 ± 23	42 ± 14	56 ± 31
Odostomia eulimoides	101 ± 77	69 ± 33	0
Patella spp.	66 ± 26	45 ± 22	514 ± 113
Rissoa parva	29472 ± 10775	32990 ± 12265	2653 ± 910
Skeneopsis planorbis	5674 ± 1514	2854 ± 778	1208 ± 432
Tricolia pullus	472 ± 239	410 ± 159	122 ± 48
Lasae adansoni	278 ± 183	0	2906 ± 601
Modiolus modiolus	10427 ± 2322	10205 ± 2755	10281 ± 1038
Musculus costulatus	819 ± 203	375 ± 105	1538 ± 313
Mytilus galloprovincialis	15823 ± 4349	19944 ± 5468	109250 ± 17727
Campecop lusitanica	101 ± 60	90 ± 64	7 ± 5
Dynamene sp.	170 ± 37	233 ± 73	4243 ± 1010
Hyale perieri	819 ± 219	288 ± 127	2028 ± 595
Hyale pontica	28 ± 15	10 ± 10	170 ± 130
Idotea balthica	14 ± 11	97 ± 56	0
Idotea pelagica	118 ± 36	111 ± 41	722 ± 208
Ischyromene lacazei	63 ± 34	38 ± 35	934 ± 390
Pirimela denticulata	42 ± 12	149 ± 38	6.9 ± 48
Tanais dulongii	306 ± 114	56 ± 19	1618 ± 474
Eulalia viridis	69 ± 20	35 ± 12	160 ± 35
Perineris cultrifera	97 ± 44	97 ± 37	17 ± 10
Platynereis dumerilli	35 ± 15	42 ± 14	87 ± 38
Sabellaria alveolata	94 ± 32	52 ± 19	233 ± 124
Syllis amica	448 ± 135	115 ± 59	42 ± 25
Syllis gerlachi	132 ± 94	156 ± 75	0
Nematoda	108 ± 78	76 ± 56	483 ± 141
Nemertinea	6.9 ± 5	0	2170 ± 1226
Acanthochitona crinita	347 ± 108	677 ± 212	42 ± 20
Acantho fascicularis	590 ± 105	979 ± 198	111 ± 40
Echinoidea	38 ± 17	76 ± 33	0
Ophiuridea	17 ± 14	63 ± 28	0

In the impacted area, Bivalvia become the dominant class, which can be explained by the feeding mode. Being filter-feeders it was predictable to find higher densities near the sewage-affected areas, rich in suspended solids.

Regarding the other taxonomic groups (Gastropoda, Crustacea, Polychaeta, and Polyplacophora) there were species that seem to prefer the reference areas, and others that were more abundant in the impacted area. Nevertheless, the majority of gastropods and chitons seem to prefer the reference areas, while Crustacea and Polychaeta seem to prefer the impacted area. Polyplacophora, like most gastropod species, seem to prefer non-polluted habitats. Earlier studies (Airoldi, 2003; Terlizzi et al., 2005a) have advanced the possible explanation that the increase of suspended solids near the sewage discharges could change the sedimentation rates, which may have negative effects on gastropods. Atalah & Crowe (2012) have also found consistent differences in the assemblages of molluscs related with nutrient enrichment, which suggests that those assemblages can be potential indicators of pollution in coastal areas.

Concerning marine worms, the result was predictable, since other studies on polychaetes assemblages have also observed an increase in density, biomass, and average number of species near the sewage-affected areas (Anger, 1975; Pearson & Rosenberg, 1978; Dauer & Conner, 1980). Identically, Nematoda and nemertinea are more abundant near sewage discharges due to their ability to exploit the available food resources (Fraschetti et al., 2006, and references therein). As a result, these groups were classified as tolerant by Borja et al. (2000).

Crustaceans are generally very sensitive to pollution (De-la-Ossa-Carretero et al., 2010, and references therein). The content of organic matter and the availability of oxygen are some of the factors used to explain the sensitivity of crustacean species. In this study, although the increase of organic matter and decrease in the availability of oxygen near the sewage affected areas, the majority of crustacean species seem to prefer the impacted area. The most common crustaceans found in the sublittoral fringe were *Hyale perieri*, *Dynamene* spp., and *Tanais dulongui*. Earlier studies have already found higher densities of these species of amphipods and tanaids near sewage discharges (Adami et al., 2004; Kalkan et al., 2007). These higher abundances in the sewage-affected areas can be explained by the higher amount of *Corallina* sp. (Fish & Fish, 2001), that acts as refuge and increases the heterogeneity of the substrate. Nevertheless, some species of crustaceans were more abundant in the reference areas (e.g., *Pirimela denticulata*, *Campecopea lusitanica*).

Some other classes only appeared in the reference areas, like echinoidea and ophiuridea. This pattern was somehow expected since these groups are known to be very sensitive (Borja et al., 2000).

Based on all stated above the presence of sewage discharges seem to: (i) change the environmental variables; (ii) increase the densities of macroinvertebrates, being *Mytilus galloprovincialis*, *Melarhaphe neritoides*, and *Chthamalus montagui* the species most responsible for the dissimilarities; (iii) have a different impact in the three intertidal zones. In the sublittoral fringe the community structure was changed. In the eulittoral suspension-feeders abundances and species richness increased. In the littoral fringe no effect was observed; (iv) change the feeding guilds moving forward the dominance of suspension feeders; (v) promote a qualitative change by the replacement of sensitive species by tolerant ones.

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