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Assessing reliable indicators to sewage pollution in coastal soft-bottom communities

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Abstract Physicochemical characteristics of sediment and benthic communities were studied in the proximity of seven sewage outfalls with differences in flow and wastewater treatment in the Western Mediterranean Sea. Redox potential was the only abiotic parameter which showed a pattern related with distance to outfalls, whereas granulometry, percentage of organic matter, metal concentrations or pH did not show changes related with outfall presence. Benthic community analysis proved to be the most suitable monitoring tool. The results showed that the highest impacted stations corresponded with those closest to outfall with the highest flow and only pre-treatment, whilst a decrease of this tendency was detected in the locations where secondary treatment takes place. Meta-analysis showed a decrease of amphipods and tanaids abundance as well as redox potential, as the indicators with the clearest response to sewage presence.

Keywords Monitoring · Benthos · Sewage · Mediterranean Sea · Wastewater treatment · Meta-analysis

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Introduction

The need for prevention of the environment from being adversely affected by the disposal of insufficiently treated urban wastewater has led to the development of Urban Waste Water Treatment Directive 91/271/EEC. It is expected that measures aimed at the protection of the coastal environment in conjunction with the additional benefits from the reuse of water will lead to an improvement in the level of wastewater treatment in the near future. Identification and characterisation of the locations affected by sewage discharge is necessary in order to detect the effectiveness of wastewater management.

Although in most European countries the residual waters are treated in wastewater treatment plants, some of them only use primary treatment (solids settlement) to reduce oils, grease, fats, sand, grit and coarse solids. Disposal may still contain heavy metals, bacteria and increased amounts of suspended particulate organic matter (Bothner et al. 2002; Smith and Shackley 2006).This effluent could produce physical and chemical changes in sediments that has been addressed by several authors (Bald et al. 2005; Best et al. 2007; Simboura and Reizopoulou 2008).

On the other hand, the benthic communities often reflect the effects of pollution, and they are widely used in the monitoring effects of marine pollution (Gray et al. 1990). The relationships

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between benthic assemblages and the effect of contaminants have been described extensively in the literature (Borja et al. 2006; Estacio et al. 1997; Pearson and Rosenberg 1978; Warwick et al. 1990; Del-Pilar-Ruso et al. 2007). However, the fauna composition is also affected by natural variables and requires specialized taxonomic and statistical expertise to analyse and interpret correctly its process in such a way that benthic analysis could be reduced or omitted in certain cases, due to sampling and identification costs (Bilyard 1987; Dauvin et al. 2003; Warwick 1993). This has led that classical approaches such as physicochemical water analyses were considered more favourable to achieve results within short timescales (Sanchez-Moyano et al. 2006). A possible alternative to decreasing this cost problem and "natural noise" is to reduce taxonomic efforts in order to consider the taxonomic sufficiency, where the identification of taxa only needs to be carried to the taxonomic level necessary for the purpose of the study (Ellis 1985). If the abundance and composition of taxa differ in polluted and unpolluted areas, little or no relevant information may be lost by identifying animals to higher taxa (Gray et al. 1990; Herman and Heip 1988; Sanchez-Moyano et al. 2006; Warwick 1988, 1993; Warwick et al. 1990).

Along the Spanish Mediterranean Coast, there are numerous sewage discharges. These disposal sites have been active for several decades and represent a source of continuous pollution showing a marked increase in disposal during the summer period caused by seasonal population rise due to tourism. Several municipal treatment plants discharge treated wastewater with different degrees of sewage treatment and different flows. Most of these outfalls discharge at similar depth and into a similar benthic community along Spanish Western Mediterranean coast. It is characterized as medium-to-fine sand communities of *Spisula subtruncata*, one of the communities more frequently distributed in shallow soft-bottom non-vegetated areas from Western Mediterranean (Cardell et al. 1999). Consequently, it is an ideal site for investigating the effect of pollutants (de-la-Ossa-Carretero et al. 2009). Physicochemical characteristics of sediment and benthic communities affected by seven outfalls were analysed. The aims of this analysis are to assess sewage discharge effects through these two different approaches in order to establish general trends for a widely distributed community and to determine the reliability of physical parameters and benthic fauna as indicators for the monitoring of the sewage pollution.

Material and methods

The study area was located off the Comunidad Valenciana Coast (NE Spain, Western Mediterranean), where seven locations affected by sewage outfalls were analyzed. These outfalls correspond to the villages of Vinaroz (location I), Benicarló (location II), Peñíscola (location III), Alcossebre (location IV), Torreblanca (location V), Gandia (location VI) and Oliva (location VII; Fig. 1). Wastewater was discharged through submarine pipelines at a depth of approximately 15 m. Wastewater treatment plants from locations I, II, III and IV utilise only a pre-treatment process, which includes an automated mechanically raked screen, a sand catcher and grease trap. Whereas, secondary treatment, consisting of biological treatment of activated sludge, was implemented in locations V, VI and VII. Monthly data of flow and water quality of sewage disposal (suspended solids, biological oxygen demand (BOD), chemical oxygen demand (COD), phosphates, nitrates and turbidity) were provided us by CONSOMAR S.A and Entitat de Sanejament d'Aigues (Table 1).

For each location, three distances from the discharge (0, 200 and 1,000 m) were sampled, with two sites, following the coastline in order to keep a constant depth at each location. All samples were collected in July during five consecutive years (2004 to 2008). Four Van Veen grab samples (400 cm^2) were obtained at each station. Three samples were sieved through a 0.5-mm screen and preserved in 10% formalin for the study of the benthic community. Other sample was used to characterize the sediment. Grain size analysis was assessed by standard sieve fractionation (Holme and McIntyre 1984). Redox potential and pH were analysed using a CRISON 507 pH meter. Organic content of dry sediment was estimated as the loss of weight after ashing. Granulometric analysis was

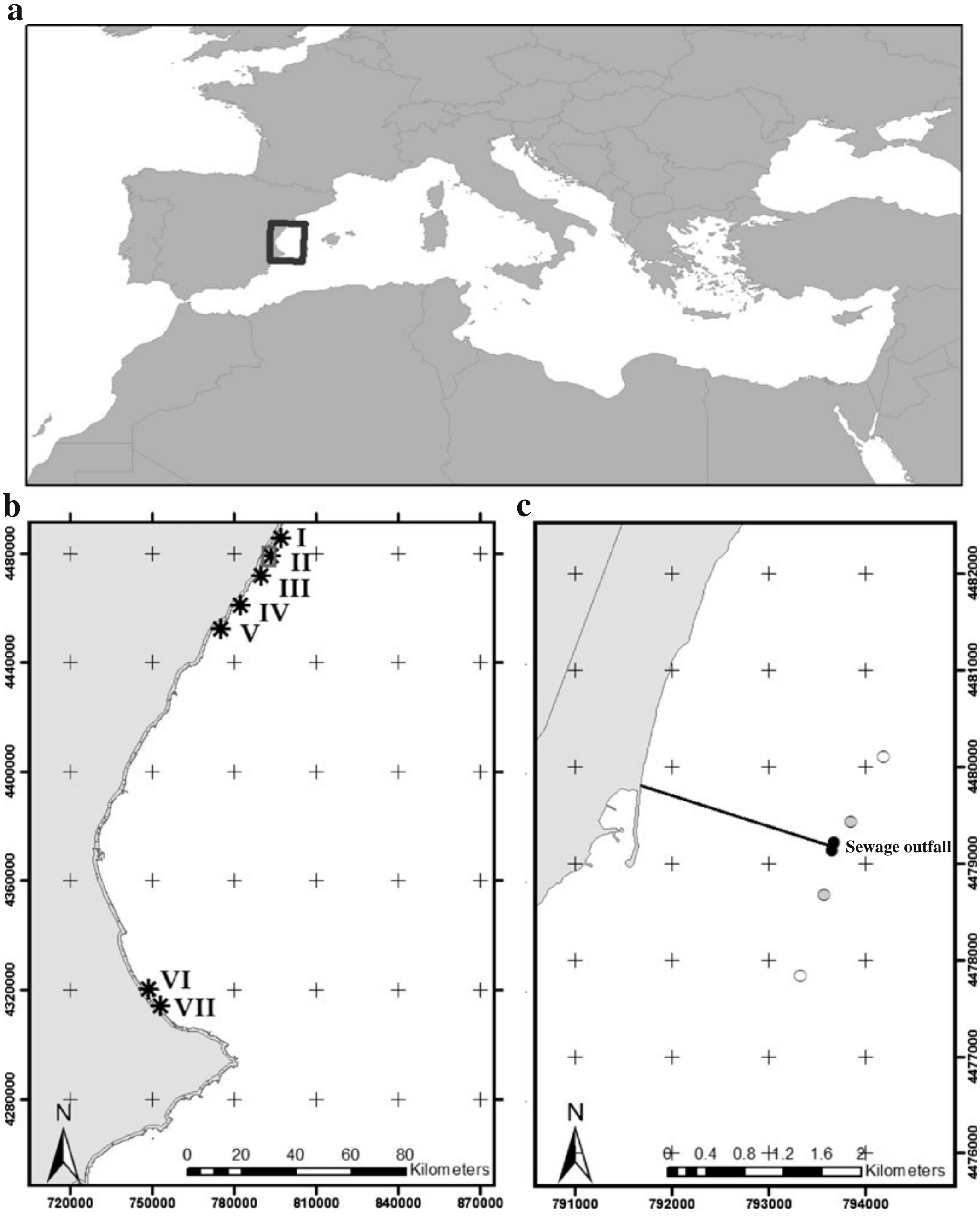

Fig. 1 **a** Study area, **b** location of the seven, **c** sampling stations of location II (*colour of circle* is related to distance to outfall (*black* 0 m, *grey* 200 m and *white* 1,000 m to outfall). UTM Zone 30N

performed all years—whereas redox potential, pH and organic content were analysed from 2005 to 2008. Redox potential, pH and organic content values were examined using 3-factor analyses of variance (ANOVA) with distance, location and year as factors. Prior to ANOVA, the homogeneity of variance was tested using Cochran's test. Data were $\sqrt{X+1}$ transformed when variances were significantly different, and if variance remained heterogeneous, untransformed data were analysed by reducing significance level. SNK test (Student–Newman–Keuls) was used to determine which samples were implicated in the differences.

During the last campaign (2008), heavy metal concentrations (Cd, Cu, Pb, As, Zn, Cr and Ni) were analysed. Sediment samples were digested with acid HNO_3 solution, and three replicates were analysed by inductively coupled plasma mass spectroscopy THERMO ELEMENTAL VG PQ. Heavy metal concentrations were examined using 2-factor ANOVA with distance and location as factors.

Changes in benthic community were analysed using high taxonomic level, in order to consider the taxonomic sufficiency (Ellis 1985), although species composition was considered when it comes to interpret results. Non-parametric multivariate techniques were used to compare abundance of different taxonomic groups present at each station from the study area. All multivariate analyses were performed using the PRIMER version 6 statistical package (Clarke and Warwick 1994). Triangular similarity matrices were calculated through the Bray–Curtis similarity coefficient using abundance values. The values were previously dispersion weighted in order to reduce "noise" produced by taxa whose distribution is erratic and whose abundance shows high variance between replicates (Clarke et al. 2006). Graphical representation of multivariate patterns of infaunal assemblages was obtained by non-metric multidimensional scaling (nMDS). ANOSIM was used to test the differences between distances at each location. Similarities percentage analyses (SIMPER) of abundances was used to determine the infaunal groups with higher percentage contribution in dissimilarity between distances.

ANOVA with distance, location and year as factors were used in order to test differences in abundance of benthic groups indicated by the SIMPER analysis.

BEST procedure was used to determine the parameter combination most correlated with benthic changes between sampled stations, in order to link benthic community analyses to sediment and wastewater variables. Spearman correlation between similarity matrices of samples of outfall points based on the abundances of benthic community, data of sediment (granulometric analy-

Table 1 Characteristics of the sewage outfalls analysed: outfall depth, sewage treatment, and monthly average of flow and measurements of water quality disposal (suspended solids, BOD (biologic oxygen demand), COD (chemical oxygen demand), phosphates, nitrates, conductivity and turbidity). Annual average of monthly samples

Location	Outfall depth (m)	Sewage treatment	Wastewater parameters								
			pH	Flow (m^3/month)	S.S. (mg/l)	BOD (mg/l)	COD (mg/l)	Pt (mg/l)	Nt (mg/l)	Cond.	Turb. (NTU)
I	15.81	Pre-treatment	7.54	226799	262.47	358.61	624.08	55.58	11.11	2609	217.53
II	14.58	Pre-treatment	7.58	502612	266.72	209.72	479.17	30.87	12.98	10539	153.36
III	15.5	Pre-treatment	7.39	286127	171.44	112.72	236.64	16.70	4.04	6787	115.39
IV	14	Pre-treatment	7.45	54190	228.92	252.22	448.53	41.56	6.59	2382	154.92
V	14	Biological treatment	7.56	43256	9.92	10.31	34.83	10.20	1.02	3050	5.44
VI	16.84	Biological treatment	7.63	1358387	10.36	12.97	37.39	14.73	2.57	4688	4.67
VII	15.5	Biological treatment	7.64	118423	13.53	19.14	41.25	16.44	3.33	1503	9.83

Data from CONSOMAR S. A. and Entitat de Sanejament d' Aigües sis, redox potential, pH and organic content) and wastewater data (flow, suspended solids, BOD, COD, phosphates, nitrates and turbidity) were determined.

Meta-analysis was applied in order to assess which parameter, among physical characteristics and abundance of taxa, responds better to sewage presence. Meta-analysis is a set of methods designed to synthesis the results of disparate studies (Hedges and Olkin 1985), in this case, different locations and sampling years. For meta-analysis of studies with continuous measures, such as physical parameters or benthos abundance, a standardized difference between treatments means is typically used (Cooper and Hedges 1994). We used Hedges' g statistic (Hedges and Olkin 1985) as measure of effect size (standardized differences in mean of sediment parameters or taxa abundance between outfalls sites and sites at 1,000 m to the outfall).

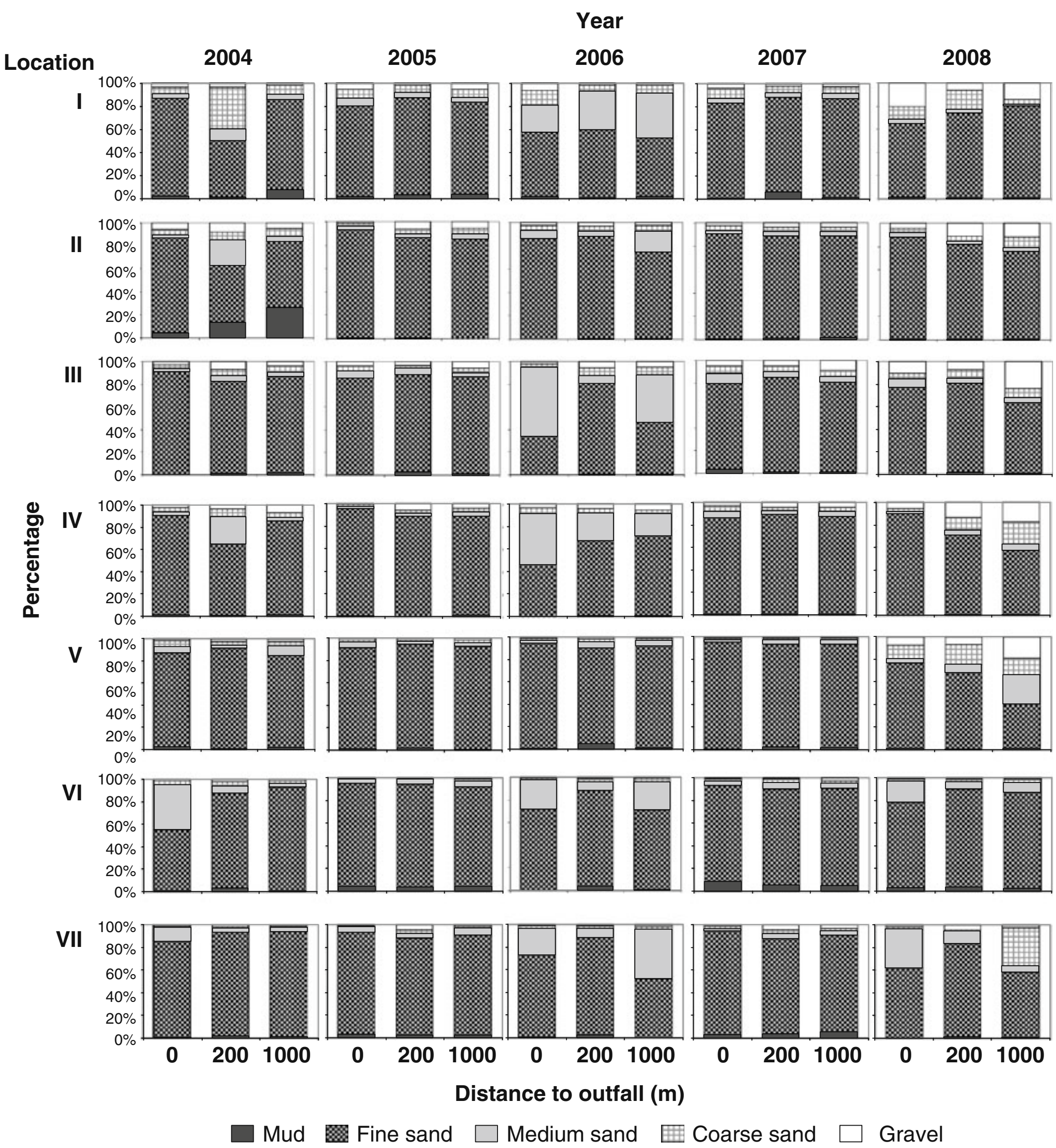

Fig. 2 Grain size distribution for the three distances (0, 200 and 1,000 m) at each location (I, II, III, IV, V, VI and VII) for 5 years (2004, 2005, 2006, 2007 and 2008)

Results

The study area was characterized by fine-sand sediments (0.125–0.25 mm). There was a low variability of the granulometry which did not seem to be related to outfall presence (Fig. 2). Only punctual changes were detected, such as increases of coarse sand at 200 m to the outfall in location I

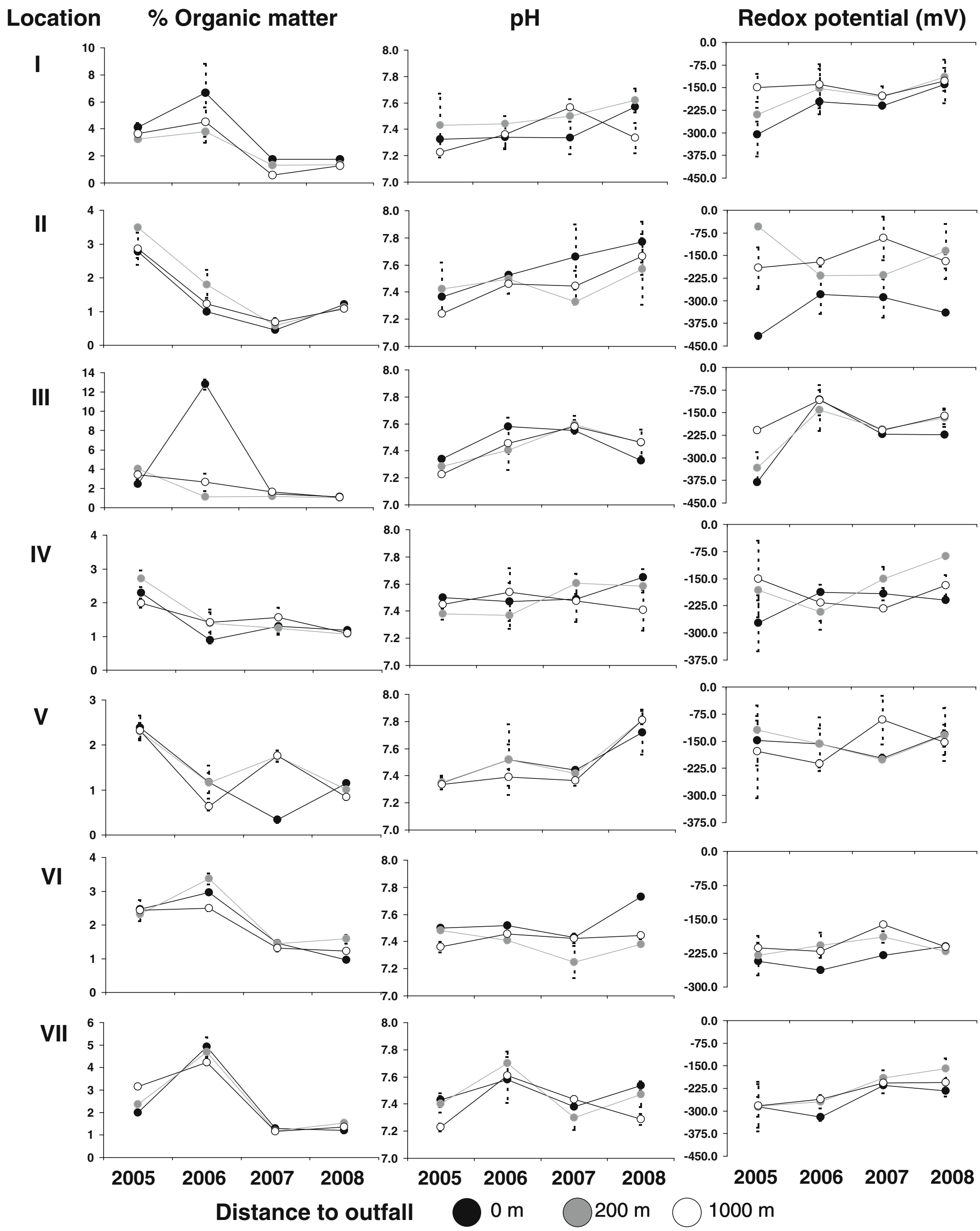

Fig. 3 Sediment parameters (Redox potential, pH and % organic matter) for three distances to the outfall and four years in the seven locations: I, II, III, IV, V, VI and VII

in 2004 and at 1,000 m to the outfall in location VII in 2008; increases of mud at 1,000 m to the outfall in location II in 2004 and increases of medium sand in some stations in year 2006 (Fig. 2).

With regard to the other analysed parameters (Fig. 3), only redox potential showed a pattern related to distance to outfall. In this way, significant differences (Table 2) were detected for interaction between distance and location due to the reduced conditions of the sediment in stations closer to the location II outfall. Significant differences were also detected for interaction between location and year. These differences were due to the lowest values obtained in locations III and VII in 2005 and in locations II and IV in 2006.

With regard to pH, significant differences (Table 2) were only detected for the interaction between location and year due to the higher values obtained in 2008 in locations II and V. In the same way, percentage of organic matter did not clearly correlate with distance to the outfall, but significant differences for interaction distance, location and year were detected (Table 2). Differences among distances were due to an organic matter increase, at 0 m to the outfall in location I in 2006 and in location III in 2005 and 2006 and to a decrease in the station close to the outfall in location V in 2007. The higher percentages obtained in locations I, III, VI and VII in 2006 produced differences among locations.

Increase in heavy metal concentrations were not clearly detected in stations closest to the outfalls (Fig. 4, Table 3); only copper and cadmium increased significantly at outfalls from locations IV and II, respectively. In most of the cases, the opposite pattern was observed, with a decrease of metal concentrations that was detected at stations sited 0 m to the outfalls producing a significant interaction between distance and location.

Infaunal taxonomic composition showed a gradation of stations from sites further away to the outfalls to those closest in certain locations

Table 2 Results of ANOVA for sediment parameters (redox potential, pH and % organic matter) for the factors location, distance and year

	Source	*df*	MS	*F*	*P* value
Redox potential (mV)	Distance	2	52320.46	9.51	*
	Location	6	19071.37	2.43	ns
	Year	3	22707.22	6.27	***
	Dis. × Loc	12	9503.45	2.53	*
	Dis. × Year	6	5502.93	1.52	ns
	Loc. × Year	18	7848.29	2.17	*
	Dis. × Loc. × Year	36	3755.83	1.04	ns
	RES	84	3624.35		
	Transformation	–			
pH	Distance	2	0.0602	3.39	ns
	Location	6	0.0237	0.44	ns
	Year	3	0.2599	15.71	***
	Dis. × Loc	12	0.0184	1.18	ns
	Dis. × Year	6	0.0178	1.07	ns
	Loc. × Year	18	0.0541	3.27	***
	Dis. × Loc. × Year	36	0.0155	0.94	ns
	RES	84	0.0165		
	Transformation	–			
% Organic matter	Distance	2	8.8624	0.63	ns
	Location	6	28.1208	1.77	ns
	Year	3	128.7655	178.45	***
	Dis. × Loc	12	8.5683	0.89	ns
	Dis. × Year	6	13.9597	19.35	***
	Loc. × Year	18	15.8758	22	***
	Dis. × Loc. × Year	36	9.6692	13.4	***
	RES	420	0.7216		
	Transformation	+			

df degrees of freedom, *MS* medium squares

F of each factor = MS factor/MS residual.

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Transformation: dash (–) indicates that there is no transformation; plus (+) indicates that there is no homogeneity of variance and levels of significance were $*p < 0.01$; $**p < 0.001$, $***p < 0.0005$

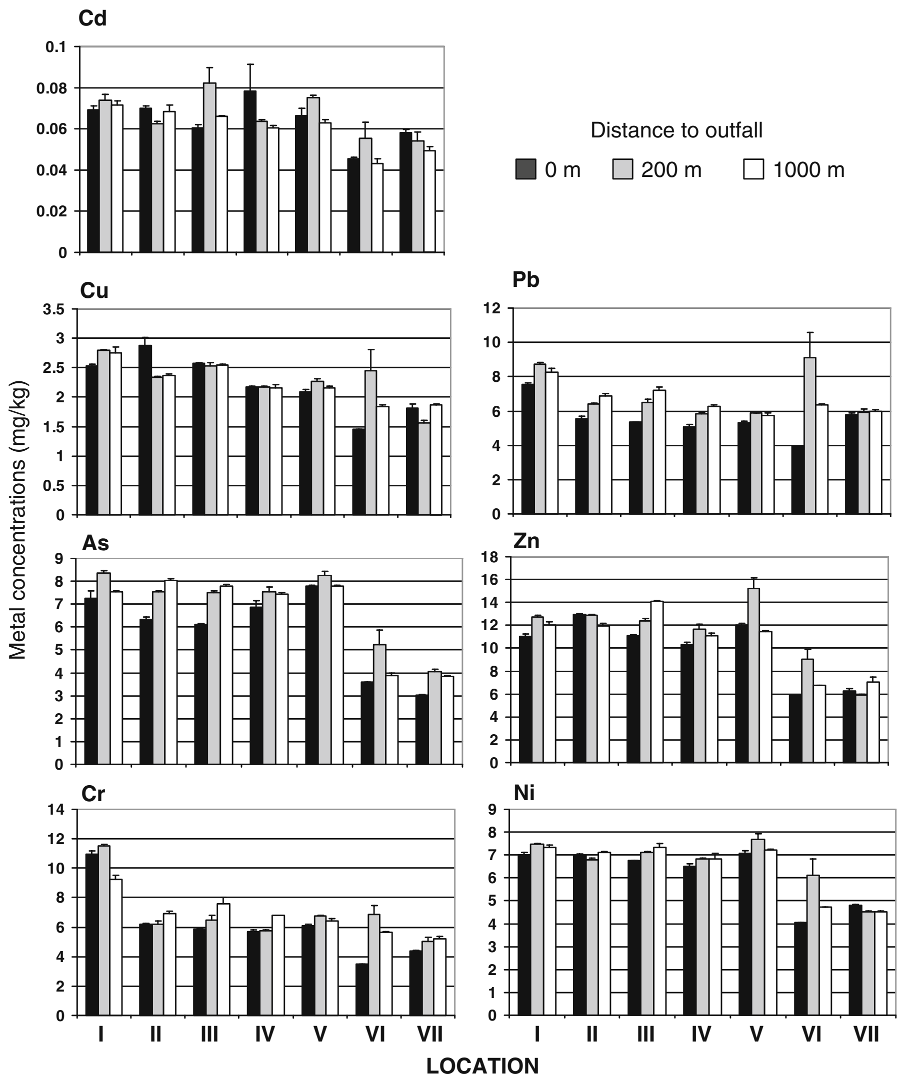

Fig. 4 Heavy metal concentrations for three distances to the outfall in the seven locations: I, II, III, IV, V, VI and VII

Table 3 Results of ANOVA for heavy metal concentrations for the factors distance and location

Source	*df*	Cd	Cu	As	Cr	Pb	Zn	Ni
		F^p	F^p	F^p	F^p	F^p	F^p	F^p
Distance	2	4.1^{ns}	1.65^{ns}	65***	30.35***	33.47***	33.5***	12.95***
Location	6	13.78***	43.87***	381***	210.06***	19.09***	191.1***	117.41***
Dis.x Loc	12	2.5*	7.66***	5.19***	15.01***	7.02***	10.16***	5.35***
RES	105							
Transformation		+	+	+	+	+	+	+

F of each factor = MS factor/MS residual

df degrees of freedom

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Transformation: plus (+) indicates that there is no homogeneity of variance and levels of significance were $*p < 0.01$; $**p < 0.001$, $***p < 0.0005$

(Fig. 5a). This segregation was observed in the locations where wastewater was only pre-treated (black circles in nMDS plots), especially in locations II and IV where this pattern was clear noticed (Fig. 5c and e). On the other hand, stations corresponding to outfalls where wastewater was previously biological treated (black triangles in nMDS plots) were closer to stations at 200 and 1,000 m to the outfalls (grey and white squares in nMDS plots). This layout was clearly marked in location V (Fig. 5f), where variability in community structure was related to a temporal pattern.

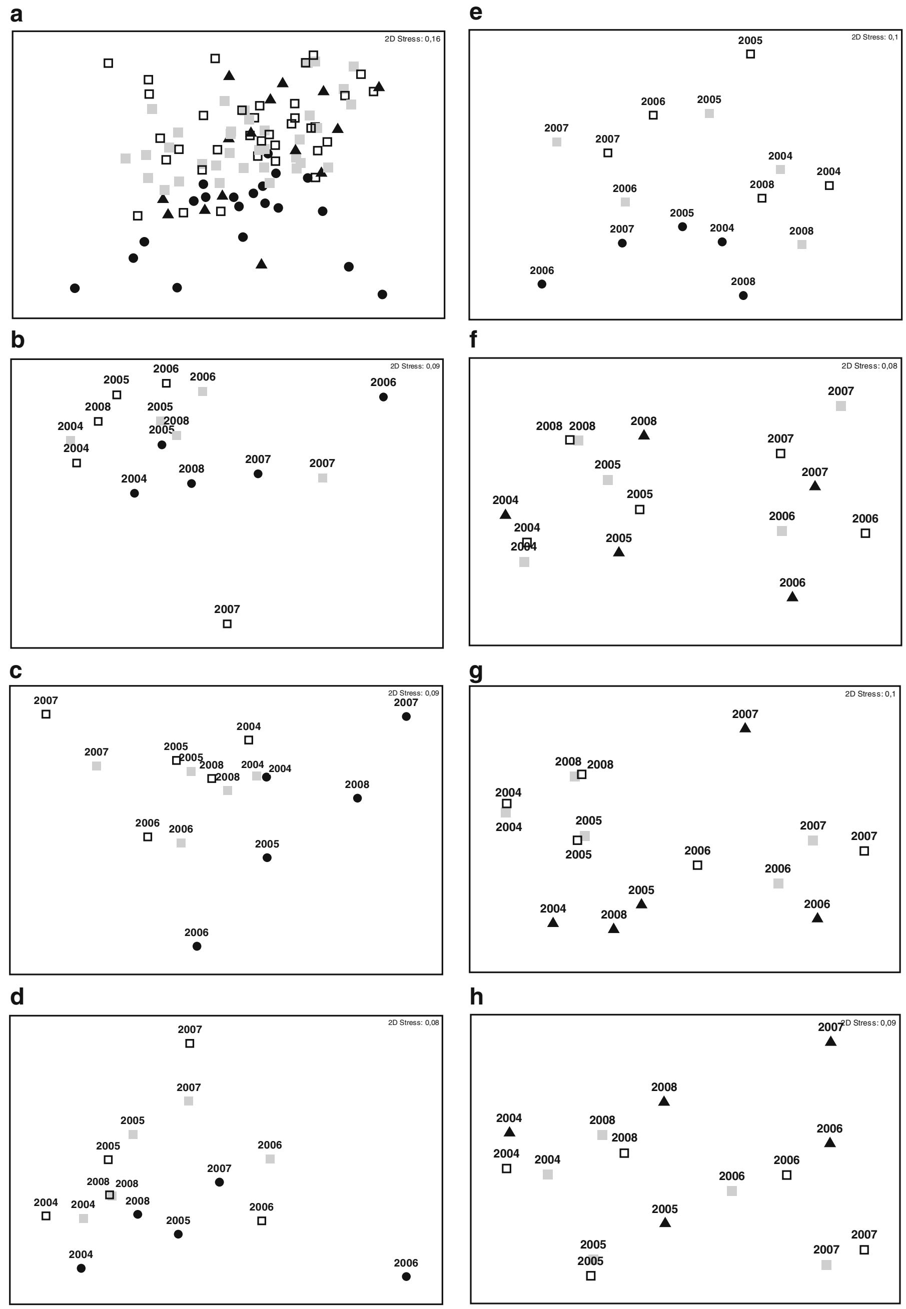

Fig. 5 MDS ordination of infauna abundance (indiv./m^2) and associated stress values for **a** all locations and each location **b** I, **c** II, **d** III, **e** IV, **f** V, **g** VI and **h** VII. Three distances to the outfall (0, 200 and 1,000 m) and 5 years (2004, 2005, 2006, 2007 and 2008). *Colour* and *shape* are related to distance to outfall and different treatment levels: *filled circles* 0 m to outfall with pre-treatment, *filled triangles* 0 m to outfall with biological treatment, *grey squares* 200 m and *white squares* 1,000 m to outfall

Table 4 R statistics values and significance level of statistic (R^p) from Pairwise test of two-way crossed ANOSIM of infauna abundance (indiv./m^2) for the factor distance to the outfall (0, 200 and 1,000 m) across all years group at each location (I, II, III, IV, V, VI and VII)

Location	Pairs of distances			
	0 vs 200 m	0 vs 1,000 m	200 vs 1,000 m	Global *R*
I	0.40*	0.65***	0.25*	0.41***
II	0.60*	0.80***	0.25^{ns}	0.48***
III	1.00***	0.25^{ns}	−0.11^{ns}	0.24^{ns}
IV	0.70**	0.55*	0.20^{ns}	0.44***
V	0.15^{ns}	−0.30^{ns}	−0.40^{ns}	−0.13^{ns}
VI	1.00***	0.00^{ns}	−0.05^{ns}	0.20^{ns}
VII	0.55*	0.35^{ns}	−0.25^{ns}	0.20^{ns}

ns no significant difference
$*p < 0.05$, $**p < 0.01$, $***p < 0.001$

In order to establish differences between distances at each location, a two-way crossed ANOSIM with distance and year as factors was run. It showed significance difference between distance groups (Global R) in locations I, II and IV (Table 4). Pairwise test (Table 4) showed that differences attributed to distance were mainly due to differences detected between stations closest the outfall and sites at 200 and 1,000 m to the outfall. On the other hand, no significant differences were detected between 200 and 1,000 m, except in location I whose *R* value was lower.

SIMPER showed that abundance of Amphipoda, Bivalvia, Polychaeta, Cumacea, Tanaidacea, Cephalochordata and Ophiuroidea were the taxa with higher average contribution to the overall dissimilarity between the stations closer to the outfalls and stations at 1,000 m to the outfall in locations I, II and IV(Table 5). Most of these taxa obtained average dissimilarity contributions higher than standard deviation, except Bivalvia or Ophiuroidea that showed a high standard deviation, being less consistent discriminating taxa.

Plotting mean abundance of these relevant taxa, we observed the general trends of each main taxa with proximity to outfalls from locations where significance differences between distances were detected. The abundance of Polychaeta, Amphipoda, Tanaidacea, Cumacea, Bivalvia, Ophiuroidea and Cephalochordata for each year and distance from outfall of locations I, II and IV is shown in Fig. 6.

Table 5 Percentage of contributions (Contrib.%) and ratio average contributions/standard deviation (Diss/SD) from each taxa to the average Bray–Curtis dissimilarity and between pairs of distance groups (0 vs 1000 m) for locations I, II and IV

Taxon	LOCATION I		LOCATION II		LOCATION IV	
	Contrib.%	Diss/SD	Contrib.%	Diss/SD	Contrib.%	Diss/SD
Amphipoda	32.7	1.6	18.3	1.5	13.8	1.4
Bivalvia	15.7	0.7	16.5	0.9	22.4	1.0
Polychaeta	11.0	1.3	27.5	1.0	9.7	1.4
Cumacea	7.4	1.0	4.8	0.9	9.9	1.2
Tanaidacea	4.5	1.1	5.6	1.3	7.5	1.2
Cephalochardata	3.6	1.1	4.0	1.1	9.3	1.1
Ophiuroidea	5.1	0.9	4.8	0.8	3.4	1.1
Decapoda	2.9	1.1	2.6	1.3	3.8	1.1
Gasteropoda	2.7	0.9	2.6	1.0	3.8	1.5
Nematoda	1.9	0.7	3.1	0.7	3.6	0.8
Nemertea	2.7	1.2	1.9	1.2	3.1	1.1

Taxa were ordered in decreasing mean contribution of the three locations

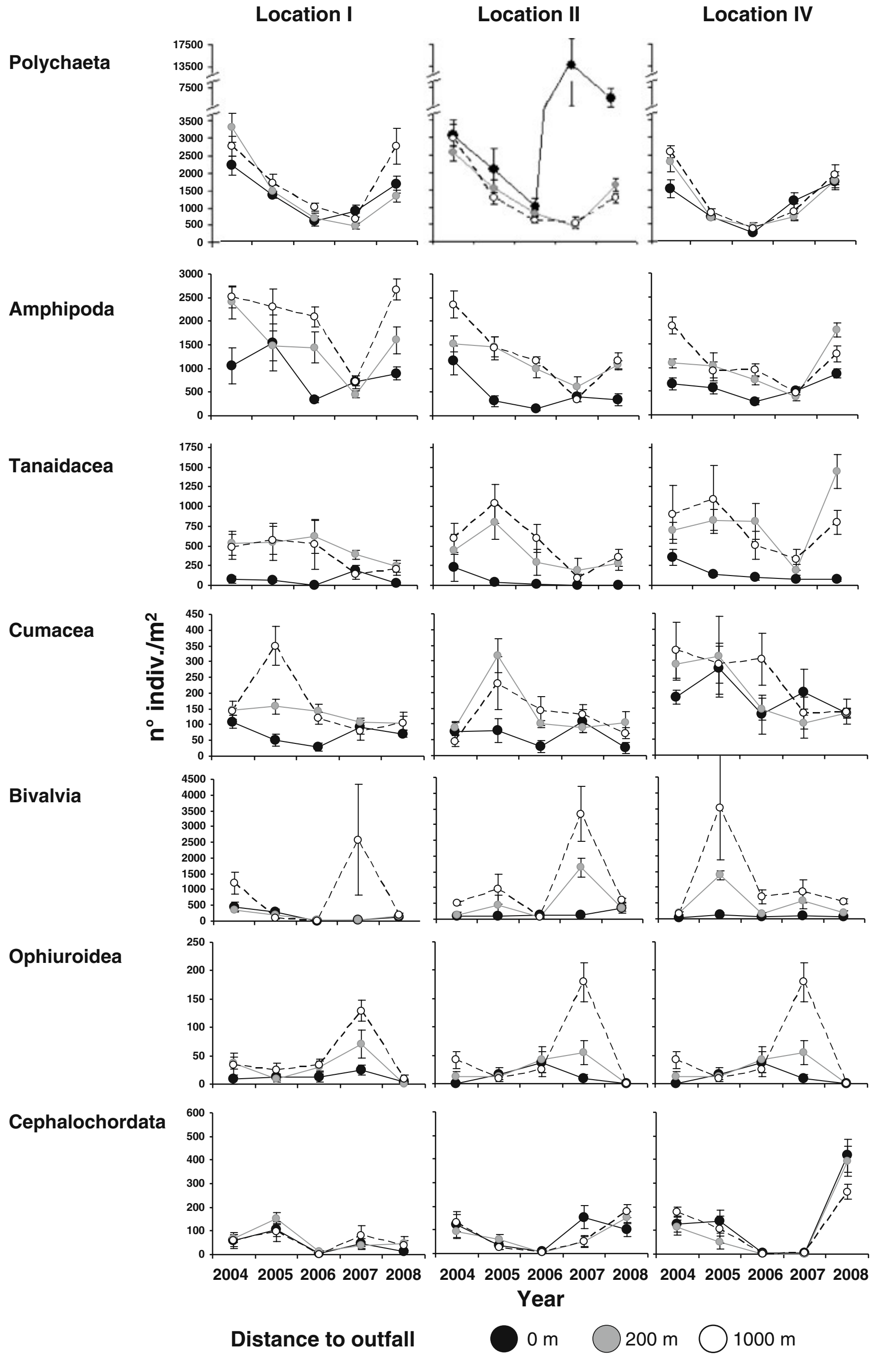

Fig. 6 Mean abundances (indiv./m^2) and standard errors of Polychaeta, Amphipoda, Tanaidacea, Cumacea, Bivalvia, Cephalochordata and Ophiuroidea in locations I, II and IV, year (2004 to 2008) and distance to the outfall (0, 200 and 1,000 m)

ANOVA detected significant differences in the interaction among the three factors in Polychaeta, Amphipoda, Bivalvia and Ophiuroidea (Table 6). An increase of polychaetes abundance was detected in the stations closer to the outfall in location II (years 2007 and 2008), whereas amphipods, bivalves and ophiurids decrease certain years in this station. Regarding Amphipoda, a decrease was detected in locations I and II, all years except 2007, and in location IV, years 2004

Table 6 Results of ANOVA for abundance (individues/m^2) of Polychaeta, Amphipoda, Tanaidacea and Bivalvia for the factors location (I, II and III), distance from outfall (0, 200 and 1000 m) and year (2004, 2005, 2006, 2007 and 2008)

Source	*df*	Polychaeta	Amphipoda	Tanaidacea	Cumacea	Bivalvia	Ophiuroidea	Cephalochordata
		F[p]	F[p]	F[p]	F[p]	F[p]	F[p]	F[p]
Distance	2	1.50^{ns}	12.26**	27.81***	4.10*	5.37*	5.79*	0.00^{ns}
Location	2	3.50^{ns}	10.90*	6.14*	4.83*	0.27^{ns}	2.69^{ns}	0.34^{ns}
Year	4	3.23*	36.33***	10.39***	2.71*	7.70***	36.78***	49.05***
Dis.x Loc	4	2.94^{ns}	1.68^{ns}	1.26^{ns}	3.14*	0.27^{ns}	0.38^{ns}	0.84^{ns}
Dis.xYear	8	2.53^{ns}	4.89	3.12**	2.75**	3.46**	3.76***	0.96^{ns}
Loc.xYear	8	1.56^{ns}	3.15**	3.09**	2.04*	4.67***	1.08^{ns}	19.92***
Dis.xLoc.xYear	16	1.82*	2.00*	1.41^{ns}	0.99^{ns}	2.19**	3.66***	1.25^{ns}
RES	225							
Transformation		+	+	$\sqrt{(x+1)}$	$\sqrt{(x+1)}$	+	$\sqrt{(x+1)}$	$\sqrt{(x+1)}$

RES residual, *df* degrees of freedom
F of each factor = MS factor/MS residual
$*p < 0.05$, $**p < 0.01$, $***p < 0.001$
Transformation: $\sqrt{(x+1)}$, data were $\sqrt{(x+1)}$ transformed when variances were significantly different; plus (+) indicates that there is no homogeneity of variance and levels of significance were $*p < 0.01$; $**p < 0.001$, $***p < 0.0005$

and 2008. Bivalvia decreases near the outfall in locations I and II, 2007, and in location IV, 2005, while a decrease of ophiurids was detected in locations I and II, year 2004 and 2007 and in location IV, year 2007 (Fig. 6). In the case of Tanaidacea, significant differences were detected for interactions between the pairs of factors distance–year and location–year (Table 6). Differences between distances were due to lower abundance in the stations closer to the outfall all years except 2007 (Fig. 6). Whereas, differences between locations were due to higher abundances obtained in location IV with respect to locations I and II. With regard to Cumacea abundances, significant differences were detected in interactions distance–location, distance–year and location–year (Table 6). Differences in interaction distance–location were due to a decrease in stations near outfalls in locations I and II (Fig. 6). This decrease was detected in years 2005, 2006 and 2008. Finally, abundance of Cephalochordata showed differences in the interaction (location and year). Whereas, the highest abundances were detected in years 2004 and 2005 in location IV, in year 2007 the abundances of Cephalochordata increased in location I and II (Fig. 6; Table 6).

BEST procedure showed that percentage of mud; redox potential and percentage of organic matter were the combination of parameters of sediment most related to changes detected in

Table 7 BEST results

Spearman correlation of benthic data with sediment and wastewater parameters

	No.Vars	Correlation	Selections
Sediment parameters	3	0.043	% Mud; Redox potencial; % organic matter
	3	0.038	% medium sand; Redox potencial; % organic matter
	4	0.037	% Mud; % medium sand; Redox potencial; % organic matter
	2	0.037	Redox potencial; % organic matter
	2	0.035	% Mud
Wastewater parameters	5	0.333	Pt, suspended solids, conductivity, Nt pH
	5	0.330	Pt suspended solids DQO conductivity, pH
	4	0.330	Pt suspended solids conductivity, pH
	4	0.329	Suspended solids conductivity, Nt pH
	4	0.327	Suspended solids DQO conductivity, pH

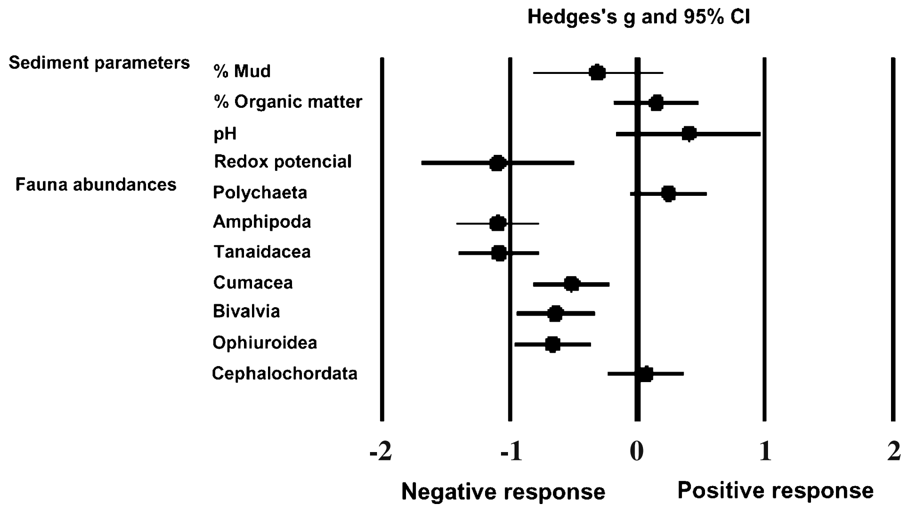

Fig. 7 Forest plot of effect sizes for sediment parameters and taxa abundances (standardized differences in parameters and abundances between sited at 0 and 1000 m) based on different locations and years. The *vertical line* represents no difference and the *error bars* are equivalent to 95% confidence intervals

fauna composition (Table 7). However, all parameters obtained a weak correlation level and did not show significant correlation (Rho = 0.043, $p = 0.91$).

Regarding wastewater parameters, phosphates, suspended solids, conductivity, nitrates and pH were the parameter combination best explaining community variability of stations closer to sewage outfalls. These combination obtained significant correlation. The parameters with the highest Spearman correlation level were phosphates (Rho = 0.258), suspended solids (Rho = 0.250) and DQO (Rho = 0.219); whereas ph (Rho = 0.086) and flow (Rho = 0.073) obtained the lowest correlation level.

Figure 7 displays forest plots of the differences in physical parameters and taxa abundance between the stations sited at 0 and 1,000 m to the outfall. The clearest response to sewage presence was the decrease in amphipods and tanaids abundance. Among abiotic parameters, redox potential showed also a negative response though it had wide confidence intervals. A less clear negative response was observed in ophiuroids, bivalves and cumaceans abundance; whereas polychaetes and cephalochordates showed a slight positive response.

Discussion

Sewage discharges may alter the biochemical composition of sediments (Cotano and Villate 2006), producing organic enrichment and physical changes towards finer-grained sediment (Pearson and Rosenberg 1978). Moreover, posterior degradation of the organic matter could lead to lower oxygen concentrations and hypoxia situations (Gray et al. 2002). However, in this study, granulometry, organic matter percentage and pH did not show any change related with sewage discharge. The medium-to-fine sand communities, such as the study area, are always influenced by high hydrodynamism and a low input of organic matter, which can partially or totally clean the bottom, by removing the surface layer and concealing the disposal effect (Cardell et al. 1999). Nevertheless, redox potential decreased in the outfall with higher flow and low treatment, showing a possible hypoxia situation near this disposal site. This effect in redox potential was not detected around outfalls marked by higher values of water quality. In the same way, heavy metal concentrations did not show a clear increase near disposals studied. According to Gonçalves and Souza (1997), urban wastewaters have a typical composition, with high contents of solids and nutrients and low concentrations of metals, hydrocarbons and pesticides. However, high heavy metal concentrations were detected in other outfalls, as in sewage disposals from Sydney (Matthai and Birch 2000). These increases had been detected with presence of industrial activities, as photography (Bothner et al. 2002), oil and chemical industries (Verlecar et al. 2006) or mining activity (Riba et al. 2004). Sewage from studied outfalls should not be mixed with industrial wastewaters, being mainly of domestic origin and avoiding high concentrations of metals. Moreover, sandy habitats influenced by high hydrodynamics, where the study was carried out, are not expected to accumulate toxic levels of contaminants.

Despite the fact that the physical analysis of sediment did not show a clear disturbance effect, benthic community analysis detected changes between stations close to outfalls from locations I, II and IV, and stations further afield. Assessment of patterns in structure of marine benthic assemblages has several advantages over other experimental or field methods for detection of anthropogenic disturbance (Elias et al. 2005). The benthos can integrate conditions over a period of time rather than reflecting conditions just at the time of sampling, so benthic organisms should be more useful in assessing local effects in monitoring programs than classical approaches such as physicochemical analyses of sediment. In fact, changes detected in this work are correlated with the flow of sewage disposal, treatment level and water quality. Significant differences between distance groups were not detected in locations III, V, VI and VII. Wastewater was previously treated by biological treatment in locations V, VI and VII, and although wastewater was only pre-treated in location III, water quality parameters showed better values in this location than in locations I, II and IV. Water quality of sewage disposal (suspended solids, phosphates, nitrates and turbidity) showed correlated differences with the variations in the benthic community structure. The highest impacted stations correspond with those closest to the outfall characterised by the highest flow of sewage effluents in which only pre-treatment is received, location II. Meanwhile, the community near the location V sewage outfall showed lowest changes related with distance to the outfall, since this is the location with the lowest flow and where secondary treatment takes place. At this location, water quality parameters of sewage disposal showed the best values where requirements for discharges from urban wastewater treatment plants which dump in sensitive areas, set by Directive 91/271, were complied to (phosphates $<$ 2 mg/l, nitrates $<$ 15 mg/l, BOD $<$ 25 mg/l O_2, COD $<$ 125 mg/l O_2, suspended solids $<$ 35 mg/l) (de-la-Ossa-Carretero et al. 2009).

Multivariate analysis appears to be an especially sensitive tool for detecting these changes (Clarke 1993; Clarke and Ainsworth 1993; DelValls et al. 1998; Del-Pilar-Ruso et al. 2007; Warwick and Clarke 1991). Benthic community and multivariate analysis allowed us to monitor the influence of sewage disposals, differentiating sites affected by sewage disposal and differentiating a decrease of this effect with an increase in wastewater treatment and a decrease of the flow discharged.

Among taxa which contributed in these differences, the crustacean groups—amphipods and tanaids—demonstrated higher sensitivity to sewage outfall presence. The increased abundance of amphipods as one moves away from the discharge site, suggests that the environment was less stressful, since amphipods are more sensitive to pollution than other marine species (Arvai et al. 2002; Cesar et al. 2004; Dauvin and Ruellet 2007; de-la-Ossa-Carretero et al. 2009; Riba et al. 2004). Tanaidacea assemblages of this study were dominated by *Apseudes latreillei*. Response of this species was previously established as high sensitive to presence of outfalls (de-la-Ossa-Carretero et al. 2010), although it sometimes has been reported as being a tolerant species (Sanz-Lázaro and Marín 2006; Bouchet and Sauriau 2009). As regards to cumaceans, they showed response to sewage disposals, though its sensitivity was lower than in amphipods or tanaids, since its abundance only decreased near outfalls at locations I and II, where higher flows were registered.

On the other hand, Polychaeta group contains both sensitive and tolerant species, and they are found along the whole gradient from pristine to heavily disturbed areas (Olsgard et al. 2003). Some Polychaeta species showed significantly greater numbers of individuals at the sewage-affected sites while other species densities showed no difference or in some cases even a decrease (Dauer and Conner 1980). In our study, it was found that an increase of abundance of Polychaeta in stations near the outfall from location II was due to an increase in the abundance of the family Dorvilleidae. Similarly, some Bivalvia species have been considered to be very tolerant to organic matter enrichment and other species avoided the polluted stations (Guerra-García and García-Gómez 2004). *S. subtruncata* whose high sensitivity to sewage discharge was previously tested (de-la-Ossa-Carretero et al. 2008) dominates bivalve populations in medium to fine sand communities from the Western Mediterranean. The decrease of Bivalvia abundances near outfalls in certain cases may be related with the dominance of *S. subtruncata*. In the same way, Ophiuroidea showed sensitivity to sewage presence because a decrease of its abundance was detected near outfalls the year when higher densities where obtained in sites at 200 and 1,000 m to the outfall. However, temporal and spatial variability of both taxa could cause that their discriminating capability was not as consistent as other taxa. Finally, the Cephalochordata *Branchiostoma lanceolatum*, despite being considered a sensitive species, did not show a clear response to sewage presence; in fact, only interannual variations and variability among locations were obtained some years. These interannual changes were also detected in abundances of other taxa; however, sewage effect was detected throughout the whole study period. Moreover, sewage discharges did not vary from year to year; therefore, these changes seem to be due to interannual natural variability. For this reason, assessing the impacts of sewage disposals requires that the outfalls and multiple control sites be sampled contemporaneously.

Meta-analysis allowed obtaining general patterns from large datasets from different locations and years. Amphipods and tanaids abundance and, with respect to abiotic parameters, redox potential showed the clearest response. Benthos has the advantage that it gives an integrated view of the long-term conditions at a site, whereas chemical and physical analytical methods provide only a ‘snapshot’ of conditions at the time of sampling (Saiz-Salinas 1997). Chemical analysis could fail assessing outfall disposals, the large amount of possibilities: organic enrichment, heavy metals, chlorinated pesticides, PCB, PAHs, ammonia, steroids…could complicate finding the correct variable or combination of variables affected by the disposal. In fact the only abiotic variable which showed certain response to outfall presence, redox potential, depends on biotic process as organic compounds decomposition.

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