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Sensitivity of amphipods to sewage pollution

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Amphipods are considered a sensitive group to pollution but here different levels of sensitivity were detected among species, by analysing the impact of five sewage outfalls, with different flow and treatment levels, on amphipod assemblages from the Castellon coast (NE Spain). Sewage pollution produced a decrease in the abundance and richness of amphipods close to the outfalls. Most of the species showed high sensitivity, particularly species such as *Bathyporeia borgi*, *Perioculodes longimanus* and *Autonoe spiniventris*, whereas other species appeared to be more tolerant to the sewage input, such as *Ampelisca brevicornis*. These different responses could be related to burrowing behaviour, with fossorial species being more sensitive and domicolous species being less affected. Benthic amphipods, which live in direct contact with sediment, are widely used for bioassay and numerous species are usually employed in ecotoxicology tests for diverse contaminants. In order to consider amphipods for monitoring and biodiversity programmes, it is important to establish the degree of sensitivity of each species to different sources of pollution.

1. Introduction

The Order Amphipoda is an abundant and ecologically important component of soft-bottom marine benthic communities (Thomas, 1993). This high abundance and wide distribution suggest that amphipods could often play major roles in the ecology of these habitats (Conlan, 1994). Benthic amphipods meet several criteria that render them highly recommendable for inclusion in marine monitoring programmes and in sediment ecotoxicology tests. They are ecologically and trophically important, numerically dominant, exhibit a high degree of niche specificity, are tolerant to varying physico-chemical characteristics in sediment and water, have relatively low dispersion and mobility capabilities, live in direct contact with the sediment, have a documented sensitivity to pollutants and toxicants compared to other benthic organisms and indeed they have been considered capable of accumulating toxic substances (Reish, 1993; Thomas, 1993; Gómez Gesteira and Dauvin, 2000; Dauvin and Ruellet, 2009).

Despite it being established that most amphipods are sensitive to different kinds of pollutions (Dauvin, 1987, 1998; Gómez Gesteira and Dauvin, 2000; Dauvin and Ruellet, 2007), sensitivity to pollution for the same taxonomic group may differ from one species to another (Afli et al., 2008). In this way, Reish and Barnard (1979) observed that some amphipod species are more tolerant than others to organic pollution, and Bellan-Santini (1980) observed changes in compositions of amphipods inhabiting rocky environments related to the degree of pollution. There are >6300 species of Gammaridean amphipods (Gruner, 1993) and little is known about the ecology of most species. Conlan (1994) reviewed the role of amphipods in environment disturbance and compiled only biological information for less than 3% of all the described species. The wide distribution in both marine and fresh waters, together with the high abundance in both benthic and pelagic environments, means that there is a need for knowledge regarding the specific sensitivity of different species or at least of more abundant species. The application and use of amphipods as a biological indicator is limited by a comprehensive taxonomic and natural history knowledge (Thomas, 1993) that explains the degree of sensitivity of each species.

Moreover, ecological factors must also be considered when evaluating the potential information value of various amphipod groups. The sensitivity of a benthic species is dependent on the organism's living habits, such as burrowing behaviour and feeding strategy (Simpson and King, 2005; King et al., 2006). Tube builder amphipods may exhibit different habitat requirements and dispersion capabilities than burrowers (Thomas, 1993) and different

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trophic groups may be influenced by different routes of exposure to contaminants. These attributes must be taken into account when considering amphipods for monitoring and biodiversity programmes.

Shallow soft-bottom non-vegetated areas of the western Mediterranean Sea are commonly inhabited by the medium-to-fine sand community of *Spisula subtruncata* (Cardell et al., 1999). This community colonises exposed or semi-exposed sublittoral habitats, from the beach environment to depth of 30 m (Sardá et al., 1996). Although this community generally contains low numbers of individuals and low biomass values, a high abundance and diversity of amphipods has been reported (Bakalem et al., 2009). This widely distributed community is common off the Castellon coast (NE Spain), where several municipal treatment plants discharge wastewater. In this study, we examined the impact of five sewage outfall sites, with different flows and wastewater treatment processes, on amphipod populations.

The main objective of this paper is to test the effect of these outfalls on amphipod populations in order to characterise the sensitivity of different species and the relation with burrowing and feeding behaviour.

2. Materials and methods

In the study area off the Castellon coast (NE Spain), five locations affected by sewage outfalls along 40 km of coast were analysed (Fig. 1). These outfalls correspond to the villages of Vinaroz (location I), Benicarló (location II), Peñíscola (location III), Alcossebre (location IV) and Torreblanca (location V). Wastewater was discharged through submarine pipelines at a depth of approximately 15 m. The mean sewage flow was 222,597 m^3/month; the highest flow was registered at location II (502,612 m^3/month) whereas the lowest was registered at location V (43,256 m^3/month). Wastewater treatment plants from locations I, II, III and IV utilise a pre-treatment process, which includes an automated mechanically raked screen, a sand-catcher and a grease trap. Secondary treatment was only implemented at location V, consisting of the biological treatment of activated sludge with biological aerated filters, producing better values for water quality parameters in this location. Sediment characteristics (granulometry, percentage of organic matter and redox potential) and data related to the flow and water quality of sewage disposals have previously been presented by de-la-Ossa-Carretero et al. (2009, 2011). The study area has a constant water depth, homogeneous bottom sediment and uniform benthic communities. This homogeneous area with an established pollution gradient represents an ideal site for investigating links between macrofaunal assemblages and the effect of contaminants (de-la-Ossa-Carretero et al., 2008, 2009, 2010a,b; Del-Pilar-Ruso et al., 2010).

For each location, three distances from the discharge (0, 200 and 1000 m) were sampled, establishing two stations for each distance, keeping a constant depth of approximately 15 m (Fig. 1). Samples were collected during July, coinciding with the highest rate of sewage disposal, for a period of five years from 2004 to 2008. Three Van Veen grab samples (400 cm^2) were obtained at each station. Samples were sieved through a 0.5 mm screen, and preserved in 10% formalin. Amphipods were sorted and preserved in 70% ethanol for subsequent identification. These were identified using the Mediterranean amphipod fauna inventory established by Bellan-Santini et al. (1982, 1989, 1993, 1998), except for the genus *Bathyporeia*, which was identified according to d'Udekem d'Acoz and Vader (2005).

An analysis of variance (ANOVA), with location and distance as fixed factors and year as a random factor, was used in order to test differences in abundance, the Shannon–Wiener diversity index and the abundance of key species. Prior to ANOVA, the homogeneity of variance was tested using Cochran's test. Data were ln $(X+1)$ transformed when variances were significantly different. The SNK test (Student–Newman–Keuls) was used to determine which samples were responsible for the differences.

Non-parametric multivariate techniques were used to compare the composition of species and to determine key species that are mainly affected by sewage presence. 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 mean annual abundance values, in order to cluster stations according to

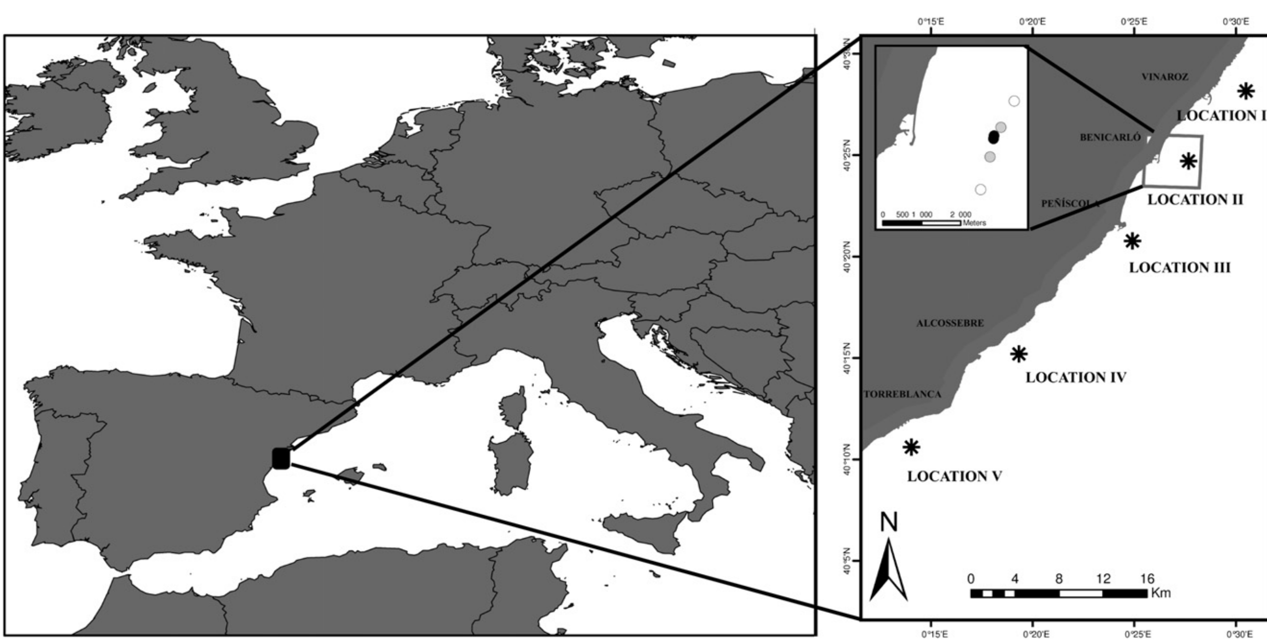

Fig. 1. Study area. Location of the five pipelines. Sampling stations of location II (colour of circle is related to distance to outfall (black: 0 m, grey: 200 m and white: 1000 m to outfall)).

sewage effect, regardless of temporal variability. The values were previously dispersion weighted in order to reduce “noise” produced by species with an erratic distribution, and whose abundance indicates a great variance between replicates (Clarke et al., 2006). A graphical representation of multivariate patterns of amphipod assemblages was obtained by non-metric multidimensional scaling (nMDS). Similarity percentage analysis (SIMPER) of abundances was used to determine the species with a higher percentage of dissimilarity between stations. Correspondence analysis was used to identify the relationships among the distribution pattern of amphipod assemblages and sediment characteristics (granulometry, organic matter and redox potential). The output was displayed as a biplot, in which the plotted points for stations can be related to sediment characteristics that are represented as arrows. The strength of the correlation of a variable is reflected in the length of the arrow, and its association is reflected in the acuteness of the angle with the axis. This displays the relationships among stations and environmental variables on one plot.

Meta-analysis was applied in order to assess the sensitivity levels of species indicated by the SIMPER analysis. Meta-analysis is a set of methods designed to synthesise the results of disparate studies (Hedges and Olkin, 1985), in this case different locations and sampling years. To carry out a meta-analysis of studies with continuous measures, such as amphipod abundance, a standardised difference between treatments is typically used (Cooper and Hedges, 1994). We used Hedges' g statistic (Hedges and Olkin, 1985) as a measure of effect size (standardised differences in mean of sediment parameters or species' abundance between outfall sites and sites at 1000 m from the outfall).

Species were classified according to trophic groups and behaviours. We used the trophic group classification suggested by Mearns and Word (1982), who simplify it into four main trophic groups (TG): TG-1 (suspension feeders), TG-2 (carrion feeders), TG-3 (surface deposit feeders and those species that are both suspension feeders and surface deposit feeders) and TG-4 (subsurface deposit feeders that feed on sedimentary detritus and bacteria).

Table 1
Amphipoda species collected. *N*: number of specimens collected. Feeding: classification in feeding behaviour. D: deposit feeders, F: filter feeders, O: omnivorous, P: predators, S: scavengers, G: grazer; TG: trophic group assigned according to Mearns and Word (1982): TG-1 (suspension feeders), TG-2 (carrion feeders), TG-3 (species that are both suspension feeders and surface deposit feeders) and TG-4 (subsurface deposit feeders). Behaviour: burrowing behaviour, dom.: domicolous, fos.: fossorial and int.: interstitial. Bibliography where classification was found was indicated. References: 1: MarBEF Data System ERMS, Bellan-Santini and Costello (2001), 2: Crawford (1937), 3: Enequist (1949), 4: Krapp-Schickel and Krapp (1975), 5: Lincoln (1979), 6: Word (1980), 7: Wildish and Peer (1981), 8: Grosse et al. (1986), 9: Bellan-Santini and Dauvin (1988), 10: Eleftheriou and Basford (1989), 11: Marti (1989), 12: Jimeno (1993), 13: Beare and Moore (1994), 14: Bellan-Santini et al. (1998), 16: Grandi et al. (2007) and Guerra-García and Tierno de Figueroa (2009). EG: Ecological Group of AMBI (Borja et al., 2000, http://www.ambi.azti.es) and BENTIX (Simboura and Zenetos, 2002; http://www.hcmr.gr).

Families	Species	N	Feeding	TG	Behaviour	References	EG AMBI/ BENTIX
Ampeliscidae	*Ampelisca brevicornis*	269	D, F	3	Dom.	1, 9, 5, 3, 14	I/II
	Ampelisca diadem	301	D, F	3	Dom.	1, 9, 5, 15, 14	II/II
	Ampelisca sarsi	56	D, F	3	Dom.	1, 9, 5, 3, 14 (as *Ampelisca* sp.)	I/II
	Ampelisca spinier	25	D, F	3	Dom.	1, 9, 5, 3, 14 (as *Ampelisca* sp.)	I/–
	Ampelisca tenuicornis	208	D, F	3	Dom.	1, 9, 14	I/I
	Ampelisca typica	1215	D, F	3	Dom.	1, 9, 5, 3, 14	I/I
Amphilochidae	*Amphilochus brunneus*	23	D	4	Int.	1, 12 (as *Amphilochus* sp.)	II/–
Amphithoidae	*Amphithoe ramondi*	1	D, G	4	Dom.	12, 4, 14	III/II
Aoridae	*Aora spinicornis*	503	D	4	Dom.	12, 14	I/I
	Autonoe spiniventris	5343	D, F	3	Dom.	1, 14 (as Aoridae)	I/I
	Microdeutopus versiculatus	1	D, F	4	Dom.	12, 14 (as Aoridae)	I/–
Argissidae	*Argissa hamatipes*	10	D	4	Int.	1, 7	–/I
Atylidae	*Atylus massiliensis*	20	P, S	2	Int.	1, 12 (as *Atylus* sp.)	I/II
Bathyporeiidae	*Bathyporeia borgi*	321	D, F	3	Fos.	14, 9 (as *O. nana*)	I/I
	Bathyporeia guilliamsoniana	250	D	4	Fos.	14, 9	I/I
Caprellidae	*Pariambus typicus*	991	D	4	Int.	16, 2	III/–
	Phtisica marina	295	D	4	Int.	16, 2	I/II
Cheirocratidae	*Cheirocratus sundevalli*	28	D	4	Int.	1, 3, 2	I/–
Corophiidae	*Medicorophium longisetosum*	316	D, F	3	Dom.	1, 14, 11, 2, 8 (as *Corophium* sp.)	–/I
	Medicorophium runcicorne	825	D, F	3	Dom.	1, 14, 11, 2, 8 (as *Corophium* sp.)	III/I
	Siphonoecetes sabatieri	1904	D, F	3	Dom.	1, 11	I/I
Dexaminidae	*Dexamine spinosa*	1	D	4	Int.	1, 12	III/I
Eusiridae	*Apherusa alacris*	11	D, F	3	Int.	9	I/I
Isaeidae	*Microprotopus maculatus*	57	O, P, S	2	Dom.	1, 2	I/I
Ischyroceridae	*Ericthonius punctatus*	30	F	1	Dom.	1, 11	I/I
Leucothoidae	*Leucothoe inicisa*	143	D	4	Fos.	2, 12 (as *Leucothoe* sp.)	I/I
	Leucothoe oboa	150	D	4	Fos.	2, 12 (as *Leucothoe* sp.)	I/II
Lysianassidae	*Hippomedon massiliensis*	146	D	4	Int.	1, 3, 12 (as Lysianasidae)	I/II
	Lepidepecreum longicorne	46	O, P, S	2	Int.	1, 3, 12 (as Lysianasidae)	–/–
	Orchomenella nana	99	O, P, S	2	Int.	1, 12 (as *Orchemene* sp.)	II/–
	Tryphosites longipes	6	D	4	Int.	1, 3, 5, 12 (as Lysianassidae)	I/I
Megaluropidae	*Megaluropus massiliensis*	111	F	1	Fos.	1, 2 (as *M. agilis*)	I/–
Melitidae	*Elasmopus pocillamus*	3	D	4	Int.	12, 11	–/–
Oedicerotidae	*Monoculodes gibbosus*	12	P	2	Fos.	1, 13, 8	I/I
	Perioculodes longimanus	1981	D	4	Fos.	8, 1, 3, 11	II/I
	Synchelidium haplocheles	84	D,F, G	3	Fos.	1, 3, 8	I/II
Photidae	*Gammaropsis maculata*	16	D,F	3	Dom.	1, 8 (as *Photis* sp.)	I/I
	Megamphopus cornutus	217	D,F	3	Dom.	1, 8 (as *Photis* sp.)	I/–
	Photis longicaudata	2	D,F	3	Dom.	1, 8, 2, 6	I/I
	Photis longipes	894	D,F	3	Dom.	8, 1, 6	I/II
Phoxocephalidae	*Harpinia crenulata*	6	D	4	Fos.	1, 3, 8	I/I
	Harpinia pectinata	20	D	4	Fos.	1, 3, 8	I/I
	Metaphoxus fultoni	67	F	1	Fos.	1, 2, 8	I/–
Urothoidae	*Urothoe pulchella*	636	D	4	Fos.	14, 1, 11, 10	I/II

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Regarding behaviour, species were classified into three types: domicolous (species that build tubes), fossorial (species that burrow using their periopods) and interstitial (species that live in the interstices between grains of sand). The classification for each species was obtained from the current bibliography (Table 1). Analysis of variance (ANOVA) was used in order to test differences in abundance percentages of groups from both classifications. The appropriate transformation for the analysis of this kind of data is the arc-sin of the square-root of the proportion.

3. Results

A total of 17,643 specimens were collected and identified as 44 species, belonging to 38 genera and 22 families. Among these, *Autonoe spiniventris* was the most abundant species which contributed to 30.3% of total abundance, followed by *Perioculodes longimanus* (11.2%) and *Siphonoecetes sabatieri* (10.8%). Other species contributed less than 0.1%, such as *Photis longicaudata*, *Elasmopus pocillamanus* or *Harpinia crenulata*; or indeed only one specimen was collected, *Amphithoe ramondi*, *Microdeutopus versiculatus* or *Dexamine spinosa*. New records from the Mediterranean Spanish coast and a new species of *Medicorophium longisetosum* sp. nov. (Myers et al., 2010) were also described from this collection (de-la-Ossa-Carretero et al., 2010a,b).

The highest population density detected was 2833 individuals/m^2, in a station situated at 1000 m from the outfall of location I, whereas the lowest abundance was obtained in a station situated at the outfall of location III (47 individuals/m^2). The abundance of amphipods showed a decrease at 0 m from outfall stations of locations I, II, III and IV (Fig. 2). Significant differences were detected in the interaction between the three factors due to the fact that this decrease was not detected in 2007 at any location or in 2005 at location I (Table 2). The Shannon–Wiener diversity index reached values of 1.11–2.66. As well as abundance, the diversity

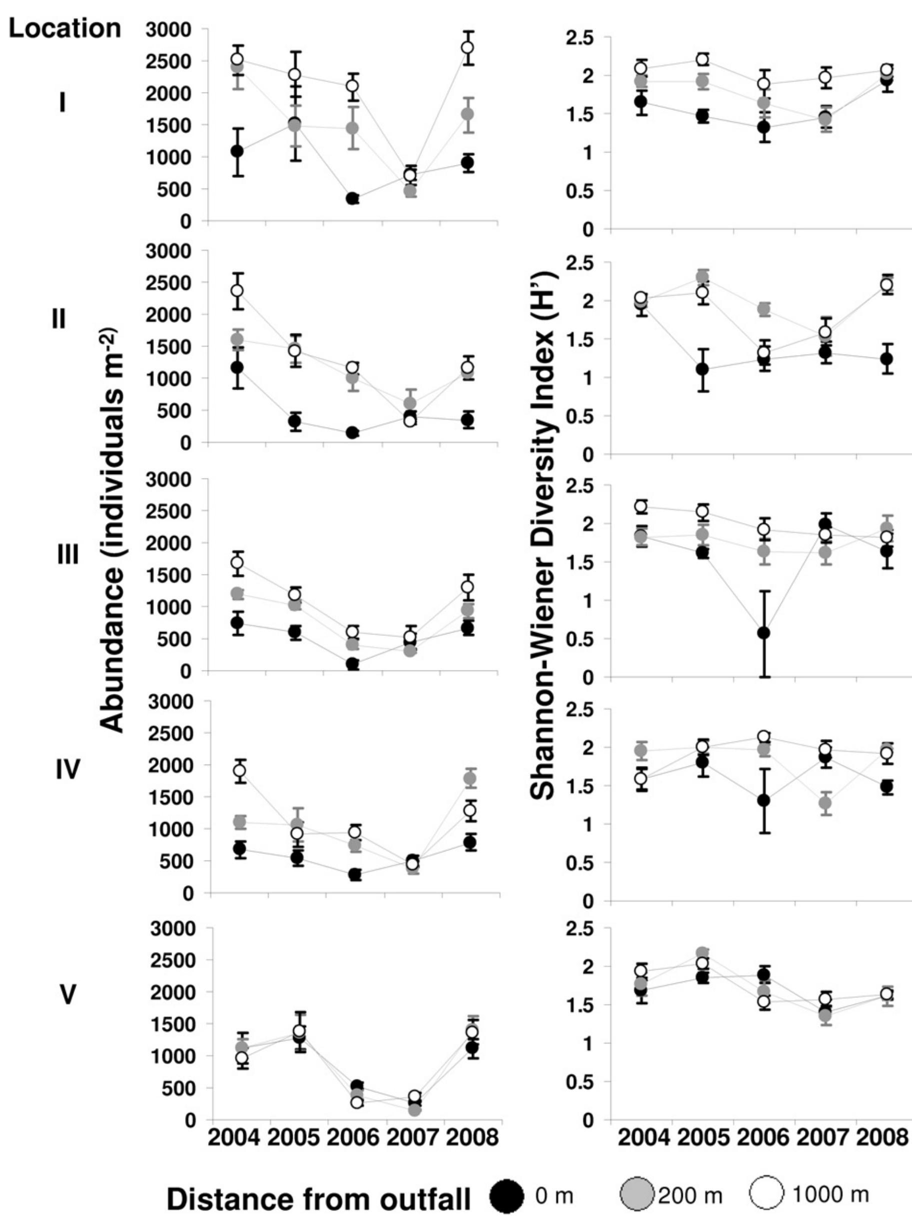

Fig. 2. Mean and standard error of amphipod abundances and Shannon–Wiener diversity index at each location (I–V), year (2004–2008) and distance to the outfall (0, 200 and 1000 m).

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Table 2
Results of ANOVA for abundance (individuals/m^2) and Shannon–Wiener diversity index for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007, 2008), Res = residual, df: degrees of freedom, *F* of each factor = mean square factor/mean square residual because all the factors are orthogonal.

	DF	Abundance		Shannon–Wiener diversity	
		F	*P*	*F*	*P*
Distance	2	13.20	0.00	7.11	0.02
Location	4	8.77	0.00	0.25	0.91
Year	4	74.55	0.00	16.88	0.00
Dis. × Loc.	8	3.86	0.00	1.11	0.38
Dis. × Year	8	5.26	0.00	5.42	0.00
Loc. × Year	16	3.87	0.00	2.77	0.00
Dis. × Loc. × Year	32	1.81	0.01	3.37	0.00
Res	375				
Tot	449				

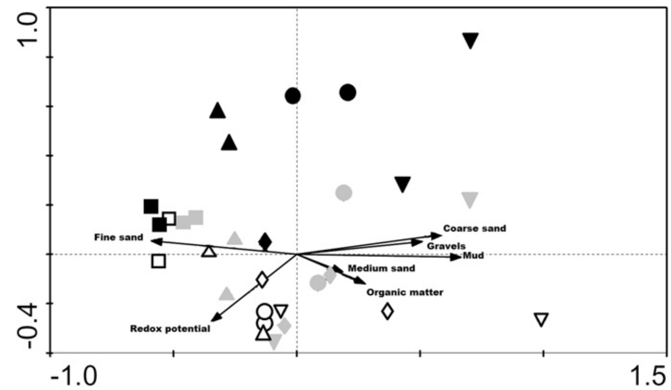

Fig. 4. Results of correspondence analysis biplot. Points correspond to stations differentiating location (I: ▼, II: •, III: ◆, IV: ▲ and V: ■) and distance to the outfall (black: 0, grey: 200 and white: 1000 m). Arrows indicate sediment characteristics. Axis I and axis II had eigenvalues of 0.193 and 0.088, respectively.

index showed a decrease in outfalls (Fig. 2), with significant differences in the interaction of the three factors. Differences in distances were detected at locations I, II, III and IV for certain years. In this way, despite annual variability, an effect of sewage presence was detected at locations I, II, III and IV.

MDS plots of the mean annual abundances of amphipod species showed a segregation of the stations, from the sites closest to the outfalls on the right hand side to those furthest away on the left hand side (Fig. 3), except for those closest to the location V outfall which is nearest to stations situated at 200 m and 1000 m (Group C). Meanwhile, a gradient corresponding to a latitudinal pattern, from location I to location V, could be observed rising from the bottom to the top of the MDS. Based on similarities in different stations, seven groups were established (Fig. 3). Among these groups, E, F and G corresponded with stations sited in disposals, whereas group B included the majority of stations sited at 200 and 1000 m from the outfalls.

Correspondence analysis results (Fig. 4) reflected that segregation of stations closest to outfalls was related to potential redox, whereas gradient corresponding to a latitudinal pattern was related to granulometry variability and changes in the percentage of organic matter.

The SIMPER routine indicated that contribution to the average Bray–Curtis dissimilarity between groups of outfall stations and group B were mainly due to the following species: *Autonoe spiniventris*, *Perioculodes longimanus*, *Photis longipes*, *Bathyporeia borgi*, *Siphonoecetes sabatieri*, *Medicorophium runcicorne*, *Urothoe pulchella*, *Phtisica marina*, *Ampelisca typica*, *Aora spinicornis*, *Pariambus typicus* and *Ampelisca brevicornis*.

A forest plot showed the differences in the abundance of these species between stations sited at 0 m and at 1000 m from the outfall (Fig. 5). The species that showed the clearest sensitivity to sewage presence were *Bathyporeia borgi*, *Perioculodes longimanus* and *Autonoe spiniventris*; whereas *Siphonoecetes sabatieri* did not show a negative response and *Ampelisca brevicornis* showed a certain positive response to sewage presence.

Abundance tends to decrease for most of these species at sites closest to the outfall (Fig. 6). However, ANOVA results showed differences among species for the analysed factors (Table 3). Species such as *Autonoe spiniventris* or *Perioculodes longimanus* showed significant differences in the interaction between distance and location. This difference was due to a decrease of abundance at 0 m from the outfall in all locations except location V. In the same way, *Urothoe pulchella* showed a significant difference for interactions between distance and location, obtaining a decrease at stations closest to the outfall of locations I, II and IV. *Bathyporeia borgi*, *Phtisica marina* and *Aora spinicornis* decrease at 0 m in all locations, showing significant differences for the factor distance. This decrease was less clear at location V. A similar pattern was observed in abundance for *Photis longipes*, where significant differences were

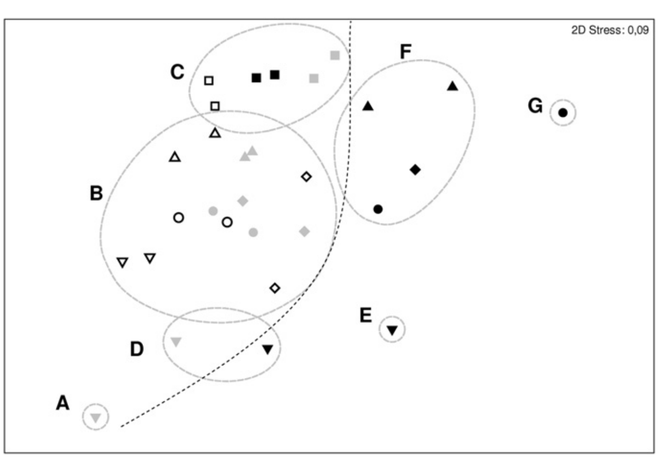

Fig. 3. MDS ordination of annual mean amphipod abundance (individuals/m^2) and associated stress value. Differentiating location (I: ▼, II: •, III: ◆, IV: ▲ and V: ■) and distance to the outfall (black: 0, grey: 200 and white: 1000 m). Letters (A, B, C, D, E, F and G) indicate groups based on similarity.

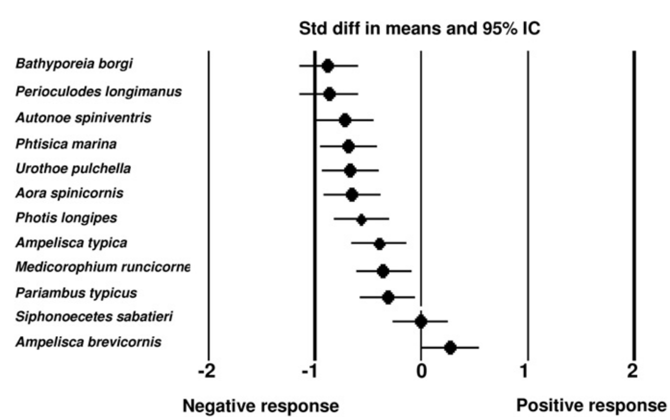

Fig. 5. Forest plot of effect sizes for Amphipoda species abundances (standardised differences in abundances between sited at 0 m and 1000 m) based on different locations and years. The vertical line represents no difference and the error bars are equivalent to 95% confidence intervals.

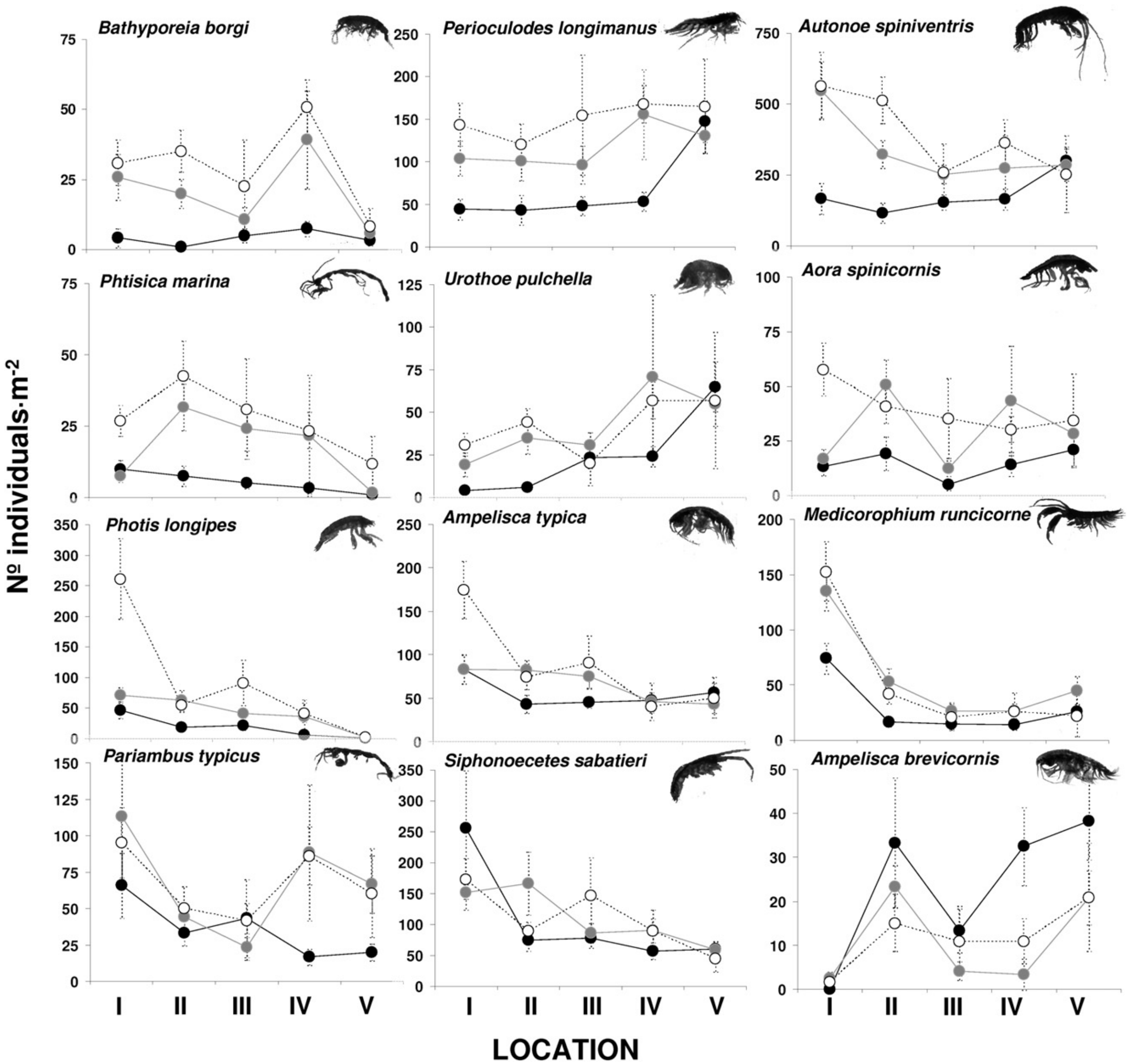

Fig. 6. Annual mean abundances (individuals/m^2) and standard errors of more contributing Amphipoda species in each location and distance to the outfall (black: 0, grey: 200 and white: 1000 m).

not detected every year for the factor distance. Interaction for the three factors was detected in species *Ampelisca typica* and *Pariambus typicus*, whose abundances decrease in sites near outfalls but only in certain locations and years. The abundance of other species, such as *Siphonoecetes sabatieri* and *Medicorophium runcicorne*, decrease at 0 m but only in certain years, in fact an increase of *S. sabatieri* was detected at an outfall station at location I. Finally, *Ampelisca brevicornis* showed significant differences for the interaction distance × location, due to an increase of abundance at location IV at sites 0 m from the outfall.

Regarding trophic group percentages (Fig. 7, Table 4), significant differences for factor distance were observed in TG-1 and TG-4 due to a decrease in stations near outfalls, and a difference close to significance was detected in TG-3 due to an increase in outfall stations. Meanwhile, differences for interaction between distance and year were detected in TG-2, showing a decrease near outfalls in 2004 and an increase in 2005 at outfall stations. Significant differences between locations were also detected.

Burrowing behaviour groups (Fig. 8, Table 4) showed differences in factor distance for domicolous and fossorial groups. While domicolous species showed higher percentages in stations near outfalls, fossorial species percentages decreased. Differences in interaction between location and year were detected in the three classes, due to an increase in fossorial species at locations IV and V with respect to locations I, II and III, where domicolous species showed higher percentages.

4. Discussion

Amphipods showed a general sensitivity to sewage outfalls, decreasing their abundance and diversity near these discharges, from locations I to IV. This decrease was not observed near location

Table 3
Results of ANOVA for abundance (individuals/m^2) of each species for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007, 2008), Res = Residual, df: degrees of freedom, *F* of each factor = mean square factor/ mean square residual because all the factors are orthogonal.

	DF	*B. borgi*		*P. longimanus*		*A. spiniventris*	
		F	*P*	*F*	*P*	*F*	*P*
Distance	2	17.73	0.00	39.21	0.00	5.40	0.03
Location	4	8.07	0.00	4.98	0.01	0.13	0.97
Year	4	8.52	0.00	48.02	0.00	62.84	0.00
Dis. × Loc.	8	1.67	0.14	2.30	0.05	5.42	0.00
Dis. × Year	8	2.31	0.02	0.88	0.53	7.08	0.00
Loc. × Year	16	1.38	0.15	2.79	0.00	8.55	0.00
Dis. × Loc. × Year	32	1.26	0.16	1.32	0.12	1.24	0.17
Res	375						
Tot	449						
		P. marina		*U. pulchella*		*A. spinicornis*	
Distance	2	13.51	0.00	5.20	0.04	16.29	0.00
Location	4	2.12	0.13	3.84	0.02	1.28	0.32
Year	4	8.16	0.00	15.26	0.00	5.39	0.00
Dis. × Loc.	8	1.29	0.28	4.27	0.00	1.71	0.14
Dis. × Year	8	1.86	0.06	1.72	0.09	1.36	0.21
Loc. × Year	16	3.91	0.00	2.31	0.00	2.81	0.00
Dis. × Loc. × Year	32	0.98	0.50	0.99	0.49	1.31	0.12
Res	375						
Tot	449						
		P. longipes		*A. typica*		*M. runcicorne*	
Distance	2	10.46	0.01	1.53	0.27	1.38	0.30
Location	4	8.49	0.00	2.21	0.11	6.77	0.00
Year	4	14.95	0.00	28.91	0.00	12.67	0.00
Dis. × Loc.	8	2.00	0.08	0.85	0.56	1.04	0.43
Dis. × Year	8	2.69	0.01	1.16	0.32	4.19	0.00
Loc. × Year	16	6.00	0.00	3.23	0.00	5.14	0.00
Dis. × Loc. × Year	32	1.24	0.18	2.27	0.00	1.24	0.18
Res	375						
Tot	449						
		P. typicus		*S. sabatieri*		*A. brevicornis*	
Distance	2	7.78	0.01	3.14	0.10	1.96	0.20
Location	4	0.45	0.77	1.46	0.26	5.18	0.01
Year	4	22.66	0.00	9.72	0.00	1.45	0.22
Dis. × Loc.	8	1.74	0.13	1.63	0.15	2.32	0.04
Dis. × Year	8	1.45	0.17	3.19	0.00	1.16	0.32
Loc. × Year	16	4.76	0.00	4.31	0.00	2.83	0.00
Dis. × Loc. × Year	32	1.50	0.04	1.28	0.15	0.97	0.52
Res	375						
Tot	449						

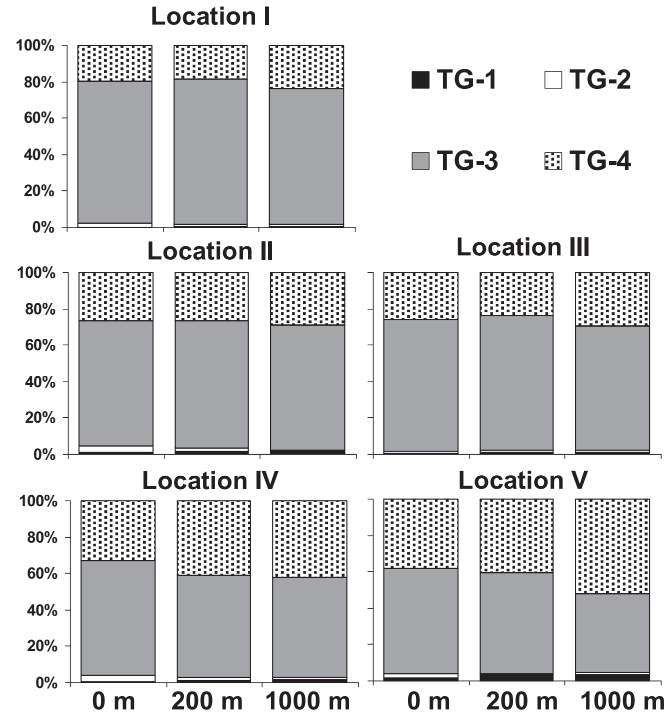

Fig. 7. Percentage of number of individuals for each trophic group at each location (I–V) and distance (0, 200 and 1000 m). Classification according to Mearns and Word (1982). TG-1 (suspension feeders), TG-2 (carrion feeders), TG-3 (surface deposit feeders and those species that are both suspension feeders and surface deposit feeders) and TG-4 (subsurface deposit feeders that feed on sedimentary detritus and bacteria).

V, since this was the location with the lowest flow and the only one where biological treatment of activated sludge takes place (de-la-Ossa-Carretero et al., 2008, 2009, 2010a,b; Del-Pilar-Ruso et al., 2010). Changes in amphipods assemblages were related to a decrease of redox potential in stations close to outfalls, indicating a possible hypoxia situation (de-la-Ossa-Carretero et al., 2011). Hypoxia causes mortality in many invertebrates, and crustaceans are especially sensitive to this lack of oxygen (Gray et al., 2002). This sensitivity of Order Amphipoda contrasts with the response of class Polychaeta to some of these sewage outfalls (Del-Pilar-Ruso et al., 2010). Polychaete diversity decreases in stations affected by pre-treated sewage, but only at locations with medium and high flow rates, not in stations with low flow such as location IV (Alcossebre) where amphipods showed a sensitive response. Moreover, an abundance of certain opportunistic polychaetes can increase close to outfalls, particularly location II (Benicarlo), where high flow and low water quality values have been reported. This inverse response of amphipods and opportunistic polychaetes has previously been employed to develop the Benthic Opportunistic Polychaetes Amphipods index (BOPA/BO2A), in order to infer environmental status from the assessment of the state of the benthic community in the implementation of the European Water Framework Directive (WFD, 2000/06/EC) (Dauvin and Ruellet, 2007, 2009; de-la-Ossa-Carretero and Dauvin, 2010). The effectiveness of the BO2A index for monitoring sewage outfalls was previously analysed in de-la-Ossa-Carretero et al. (2009).

Published literature has established that amphipods are more sensitive to polluted sediments than to other benthic organisms (Gómez Gesteira and Dauvin, 2000; Dauvin and Ruellet, 2007, 2009), with a general decrease of amphipod abundance and diversity when pollution increases (Bellan-Santini, 1980; Conlan, 1994). Thus, its sensitivity to certain types of pollution, such as oil pollution, was clearly established (Gómez Gesteira and Dauvin, 2000). Moreover, crustacean amphipods are widely used for bioassay and numerous species are usually employed in ecotoxicology tests for contaminants such as polycyclic aromatic hydrocarbons (PHAs), polychlorinated biphenyls (PCBs), organochlorine pesticides (DDT), heavy metals, ammonium or nitrite (Riba et al., 2003; Anderson et al., 2008; Ramos-Gómez et al., 2009). However, despite this general sensitive trend of amphipods, not all species showed the same level of sensitivity. While some species such as *Bathyporeia borgi*, *Autonoe spiniventris* or *Perioculodes longimanus* showed a homogeneous negative response to disposal presence, reducing its abundance in stations sited near the outfalls, other species may show an unclear pattern or, indeed, certain tolerance to sewage presence.

AMBI and BENTIX, another two biotic indices developed for WFD (Borja et al., 2000; Simboura and Zenetos, 2002), cluster benthic species since this response to pollution. Though most amphipods were classified in the sensitive group, some amphipod species were recorded as tolerant (Table 1); and we can find some discrepancies between both classifications; e.g. *Autonoe spiniventris* is classified as tolerant in BENTIX, whereas AMBI, as observed in our results, established this species as sensitive. The response of a given

Table 4
Results of ANOVA for abundance (individuals/m^2) of each percentage of trophic and burrowing behaviour group, for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007, 2008), Res = residual, df: degrees of freedom, *F* of each factor = mean square factor/mean square residual because all the factors are orthogonal.

	DF	Trophic group				Burrowing behaviour		
		TG-1	TG-2	TG-3	TG-4	Dom.	Int.	Foss.
		F^p	F^p	F^p	F^p	F^p	F^p	F^p
Distance	2	$4.83^{0.04}$	$0.50^{0.62}$	$3.95^{0.06}$	$7.03^{0.02}$	$4.70^{0.04}$	$0.83^{0.47}$	$16.29^{0.00}$
Location	4	$5.33^{0.01}$	$0.46^{0.77}$	$9.15^{0.00}$	$10.51^{0.00}$	$8.01^{0.00}$	$0.49^{0.74}$	$15.40^{0.00}$
Year	4	$1.39^{0.21}$	$3.45^{0.01}$	$18.44^{0.00}$	$17.69^{0.00}$	$15.84^{0.00}$	$10.49^{0.00}$	$6.78^{0.00}$
Dis. × Loc.	8	$0.17^{0.99}$	$0.34^{0.95}$	$0.73^{0.66}$	$0.77^{0.63}$	$0.77^{0.63}$	$1.64^{0.15}$	$1.03^{0.44}$
Dis. × Year	8	$0.99^{0.44}$	$3.07^{0.00}$	$1.59^{0.13}$	$1.24^{0.27}$	$1.65^{0.11}$	$1.32^{0.23}$	$0.94^{0.48}$
Loc. × Year	16	$0.82^{0.67}$	$1.65^{0.05}$	$3.94^{0.00}$	$3.21^{0.00}$	$4.23^{0.00}$	$2.72^{0.00}$	$2.85^{0.00}$
Dis. × Loc. × Year	32	$1.18^{0.23}$	$1.22^{0.19}$	$1.32^{0.12}$	$1.21^{0.20}$	$1.35^{0.10}$	$1.23^{0.18}$	$1.64^{0.02}$
Res	375							
Tot	449							

species is dependent on the kind of perturbation (Bustos-Baez and Frid, 2003) and classification of species along a sensitivity-tolerance continuum is thus a very difficult task and still a matter of debate (Labrune et al., 2006; Grémare et al., 2009). Abundance of *Grandidierella japonica* increased in Richmond harbour (Swartz et al., 1994), although it has been reported as reliable in bioassays and its sensitivity in sediment toxicity test methods from areas adjacent to wastewater outfalls and indeed harbours has been proved (Nipper et al., 1989). *Phtisica marina* is capable of resisting stress conditions in harbours and calm zones (Conradi et al., 1997; Sánchez-Moyano and García-Gómez, 1998; Guerra-García and García-Gómez, 2001), but we detected a certain sensitivity of this caprellid, whose abundance decreased in outfall stations. According to the AMBI list, this species is sensitive, whereas the BENTIX classification reported this species as tolerant. Another caprellid species, *Pariambius typicus* showed a certain sensitivity to sewage presence, but it was classified with some tolerance in the AMBI and BENTIX lists. In this way different considered response of each

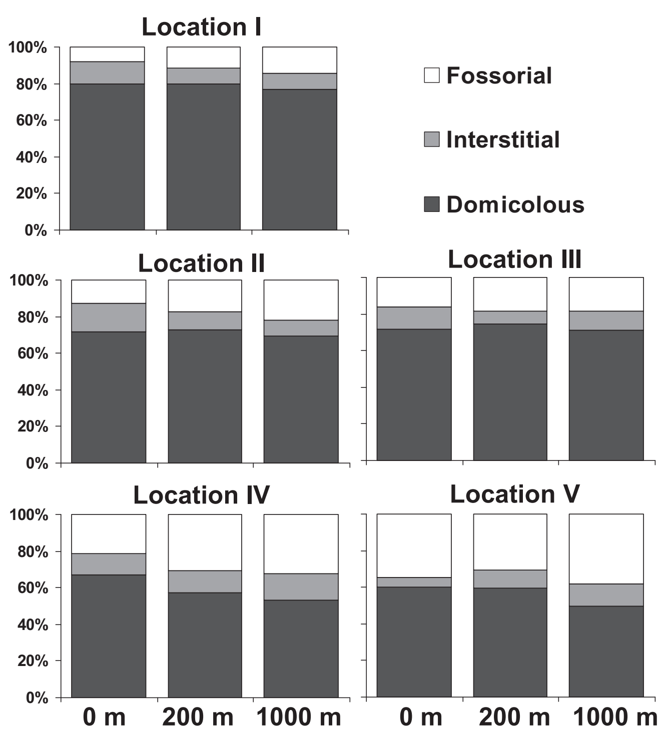

Fig. 8. Percentage of number of individual for each burrowing behaviour group at each location (I–V) and distance (0, 200 and 1000 m).

amphipod species could produce disagreement among biotic indices, such us *Erichtonius brasiliensis* and *Pseudolirius kroyerii*, classified as tolerant species by MEDOCC, another biotic index, but as indifferent or sensitive by other indices (Pinedo et al., 2011).

Although the genus *Ampelisca* has been reported as well adapted to environmental stress (Lowe and Thompson, 1997; Ingole et al., 2009), several species in the genus *Ampelisca* are used for sediment toxicity assessment. In fact, *Ampelisca abdita* is recommended and used in the US bioassay tests (EPA, 1990; Thomas, 1993) but it was reported as numerical dominant in polluted areas (Santos and Simon, 1980) and its highly variable seasonal abundances mean that it is not considered suitable as an assessment indicator. In our case, despite the fact that *Ampelisca brevicornis* has been reported as a good test organism in sediment toxicity analysis (Riba et al., 2003; Ramos-Gómez et al., 2009) we have detected a certain tolerance of this species to sewage discharge. After the high pollution of the muddy fine sand community of the Bay of Morlaix (western English Channel), and the destruction of the dominant *Ampelisca* species, *A. brevicornis* was one of those which colonised the benthic polluted community the quickest: one year after at the more polluted site (Rivière de Morlaix) and two years later in a less polluted site (Pierre Noire) (Dauvin, 1998, 2000). *Ampelisca brevicornis* shows a large ecological distribution from muddy sand to gravel and from the intertidal to subtidal sediments which gives an advantage over other species of *Ampelisca*. The AMBI and BENTIX lists showed discrepancies in the classification of this species; while AMBI classified it as sensitive BENTIX classified it as tolerant.

Within the *Corophium* genus, *Corophium volutator* was chosen by OSPAR for use in the standard sediment test for offshore chemical products (OSPAR, 1995), and can be considered as sensitive to metal contamination (Warwick, 2001). Conversely, Norkko et al. (2006) described the opportunistic behaviour of this species following experimental defaunation on an intertidal location on the Swedish west coast. In fact, certain species of the *Corophiidae* family exhibit greater production near sewer outfalls (Lowe and Thompson, 1997). *Corophium ellisi* productivity depends on oxygen conditions, and Grizzle (1984) found higher densities in a sewage polluted station on the east coast of Florida. *Corophium salmonis* is also somewhat pollution tolerant (Arvai et al., 2002). Among the Corophiidae family found in our study, *Medicorophium runcicorne* and *Siphonoecetes sabatieri* showed a decrease in abundance at outfall stations but not for all years. An increase of *S. sabatieri* is observed at outfall stations of location I. AMBI and BENTIX lists classified the *Corophium* genera with certain tolerance, whereas *Siphonoecetes* was sensitive in both indices. *Siphonoecetes* sp. are known to feature unstable population dynamics resulting in sharp peaks in abundance correlated with the nature of the sediment or interaction with other species (Cunha et al., 2000; Bigot et al., 2006). Therefore, sensitivity of specimens from this family could be ambiguous as they may be affected by locally environmental conditions.

Regarding feeding and burrowing behaviour, our results showed higher sensitivity to sewage presence in fossorial species than domicolous species, whereas trophic group species showed only slight differences in sensitivity with lower sensitivity in species that are both suspension and deposit feeders. Previously, King et al. (2006) have reported less sensitivity in tube-dwelling amphipods than in epibenthic amphipods, as well as more tolerance in filter feeding species. They recommended using epibenthic amphipods as indicators given that they are more sensitive than infaunal tube dwellers. Several reasons could explain that amphipods with distinct burrowing behaviour respond differently (Anderson et al., 2008). Tube builder amphipods are more isolated from sediment contaminants than free-burrowing species and the tube construction may reduce interstitial water contact with this species. On the other hand, sewage presence may alter the biochemical composition of sediments (Cotano and Villate, 2006), producing organic enrichment, and its posterior degradation could lead to lower oxygen concentrations (Gray et al., 2002). Domicolous amphipods are capable of pumping oxygenated water down into their burrows and tubes, whereas fossorial species depend on dissolved oxygen penetrating into the sediment by molecular diffusion. Moreover, Okladen et al. (1984) reported the possibility that burrowing amphipods actively avoided polluted sediment and chose more desirable sediment.

In summary, the Order Amphipoda is generally sensitive to sewage pollution, showing a decrease in abundance and diversity in stations close to outfalls in the studied locations, except at the location with the lowest sewage flow and better wastewater treatment processes. However, affected species showed some differences in level of sensitivity. This varied response could be due to burrowing and feeding behaviour, in such a way that suspension and surface deposit feeders and tube builders showed less sensitivity to disposal presence than others, and are thus even able to increase in abundance, as occurs with *Ampelisca brevicornis* which showed a weak positive response.

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References

Afli, A., Ayari, A., Zaabi, S., 2008. Ecological quality of some Tunisian coast and lagoon locations, by using benthic community parameters and biotic indices. Estuarine, Coastal and Shelf Science 80, 269–280.

Anderson, B.S., Lowe, S., Phillips, B.M., Hunt, J.W., Vorhees, J., Clark, S., Tjeerdema, R.S., 2008. Relative sensitivities of toxicity test protocols with the amphipods *Eohaustorius estuarius* and *Ampelisca abdita*. Ecotoxicology and Environmental Safety 69, 24–31.

Arvai, J.L., Levings, C.D., Harrison, P.J., Neill, W.E., 2002. Improvement of the sediment ecosystem following diversion of an intertidal sewage outfall at the Fraser river estuary, Canada, with emphasis on *Corophium salmonis* (amphipoda). Marine Pollution Bulletin 44, 511–519.

Bakalem, A., Ruellet, T., Dauvin, J.C., 2009. Benthic indices and ecological quality of shallow Algeria fine sand community. Ecological Indicators 9, 395–408.

Beare, D.J., Moore, P.G., 1994. Observations on the biology of a rare British marine amphipod: *Monoculodes gibbosus* (Crustacea: Amphipoda: Oedicerotidae). Journal of Marine Biological Association of the United Kindong 74, 193–201.

Bellan-Santini, D., 1980. Relationship between populations of amphipods and pollution. Marine Pollution Bulletin 11, 224–227.

Bellan-Santini, D., Costello, M.J., 2001. Amphipoda. In: Costello, M.J., Emblow, C.S., White, R. (Eds.), European Register of Marine Species. A check-list of the marine species in Europe and a bibliography of guides to their identification. Publications Scientifiques du Muséum National d'Histoire Naturelle, Paris. Patrimoines Naturels, vol. 50, pp. 295–308. www.marbef.org/data/erms.php (.

Bellan-Santini, D., Dauvin, J.C., 1988. léments de synthèse sur les *Ampelisca* du nord-est Atlantique. Crustaceana 13, 20–60.

Bellan-Santini, D., Karaman, G., Krapp-Schickel, G., Ledoyer, M., Myers, A., Ruffo, S., Schiecke, U., 1982. The Amphipoda of the Mediterranean. Part 1. Gammaridea (Acanthonozomatidae to Gammaridae). In: Ruffo, S. (Ed.), Mémoires de l'Institut Océanographique, Monaco, 13, pp. 1–364.

Bellan-Santini, D., Diviacco, G., Krapp-Schickel, G., Myers, A., Ruffo, S., 1989. The Amphipoda of the Mediterranean. Part 2. Gammaridea (Haustoriidae to Lysianassidae). In: Ruffo, S. (Ed.), Mémoires de l'Institut Océanographique, Monaco, 13, pp. 365–576.

Bellan-Santini, D., Karaman, G., Krapp-Schickel, G., Ledoyer, M., Ruffo, S., 1993. The Amphipoda of the Mediterranean. Part 3. Gammaridea (Melphidipiidae to Talitridae)-Ingolfiellidea-Caprellidea. In: Ruffo, S. (Ed.), Mémoires de l'Institut Océanographique, Monaco, 13, pp. 577–813.

Bellan-Santini, D., Karaman, G., Ledoyer, M., Myers, A., Ruffo, S., Vader, W., 1998. The Amphipoda of the Mediterranean part 4. In: Ruffo, S. (Ed.), Mémoires de l'Institut Océanographique, Monaco, 13, pp. 815–959.

Bigot, L., Conand, C., Amouroux, J.M., Frouin, P., Bruggemann, H., Grémare, A., 2006. Effects of industrial outfalls on tropical macrobenthic sediment communities in Reunion Island (Southwest Indian Ocean). Marine Pollution Bulletin 52, 865–880.

Borja, A., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within european estuarine and coastal environments. Marine Pollution Bulletin 40, 1100–1114.

Bustos-Baez, S., Frid, C., 2003. Using indicator species to assess the state of macrobenthic communities. Hydrobiologia 496, 299–309.

Cardell, M.J., Sardà, R., Romero, J., 1999. Spatial changes in sublittoral soft-bottom polychaete assemblages due to river inputs and sewage discharges. Acta Oecologica 20, 343–351.

Clarke, K.R., Warwick, R.M., 1994. Changes in the Marine Communities: an Approach to Statistical Analysis and Interpretation. Natural Environment Research Council, UK.

Clarke, K.R., Chapman, M.G., Somerfield, P.J., Needham, H.R., 2006. Dispersion-based weighting of species counts in assemblage analysis. Marine Ecology Progress Series 320, 11–27.

Conlan, K.E., 1994. Amphipod crustaceans and environmental disturbance: a review. Journal of Natural History 28, 519–554.

Conradi, M., López-González, P., García-Gómez, C., 1997. The amphipod community as a bioindicator in Algeciras Bay (Southern iberian Peninsula) based on a spatio-temporal distribution. Marine Ecology Progress Series 18, 97–111.

Cooper, H., Hedges, L., 1994. The Handbook If Research Synthesis. Russel Sage Foundation, New York.

Cotano, U., Villate, F., 2006. Anthropogenic influence on the organic fraction of sediments in two contrasting estuaries: a biochemical approach. Marine Pollution Bulletin 52, 404–414.

Crawford, G.I., 1937. Notes on the distribution of burrowing isopoda and amphipoda in various soils on the sea bottom near plymouth. Journal of the Marine Biological Association of the United Kingdom 21, 631–643.

Cunha, M.R., Moreira, M.H., Sorbe, J.C., 2000. The amphipod *Corophium multisetosum* (Corophiidae) in Ria de Aveiro (NW Portugal). 2. Abundance, biomass and production. Marine Biology 137, 651–660.

Dauvin, J.C., 1987. Evolution a' long terme (1978–1986) des populations d'Amphipodes des sables fins de la Pierre Noire (Baie de Morlaix, Manche Occidentale) apre's la catastrophe de l'Amoco Cadiz. Marine Environmental Research 21, 247–273.

Dauvin, J.C., 1998. The fine sand *Abra alba* community of the Bay of Morlaix twenty years after the Amoco Cadiz oil spill. Marine Pollution Bulletin 36, 669–676.

Dauvin, J.C., 2000. The muddy fine sand *Abra alba-Melinna palmata* community of the Bay of Morlaix twenty years after the Amoco Cadiz oil spill. Marine Pollution Bulletin 40, 528–536.

Dauvin, J.C., Ruellet, T., 2007. Polychaete/amphipod ratio revisited. Marine Pollution Bulletin 55, 215–224.

Dauvin, J.C., Ruellet, T., 2009. The estuarine quality paradox: is it possible to define an ecological quality status for specific modified and naturally stressed estuarine ecosystems? Marine Pollution Bulletin 59, 38–47.

de-la-Ossa-Carretero, J.A., Dauvin, J.C., 2010. A comparison of two biotic indices, AMBI and BOPA/BO2A, for assessing the ecological quality status (EcoQS) of benthic macro-invertebrates. Transitional Water Bulletin 4, 12–24.

de-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Giménez-Casalduero, F., Sánchez-Lizaso, J.L., 2008. Effect of sewage discharge in *Spisula subtruncata* (da Costa, 1778) populations. Archives of Environmental Contamination and Toxicology 54, 226–235.

de-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Giménez-Casalduero, F., Sánchez-Lizaso, J.L., 2009. Testing BOPA index in sewage affected soft-bottom communities in the north-western Mediterranean. Marine Pollution Bulletin 58, 332–340.

de-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Giménez-Casalduero, F., Sánchez-Lizaso, J.L., 2010a. Sensitivity of tanaid *Apseudes latreillei* (Milne-Edwards) populations to sewage pollution. Marine Environmental Research 69, 309–317.

de-la-Ossa-Carretero, J.A., Dauvin, J.C., Del-Pilar-Ruso, Y., Giménez-Casalduero, F., Sánchez-Lizaso, J.L., 2010b. Inventory of benthic amphipods from fine sand community of the Iberian Peninsula east coast (Spain), western Mediterranean, with new records. Marine Biodiversity Records 3, e119.

de-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Giménez-Casalduero, F., Sánchez-Lizaso, J.L., 2011. Assessing reliable indicators to sewage pollution in coastal soft-bottom communities. Environmental Monitoring and Assessment. doi:10.1007/s10661-011-2105-8.

Del-Pilar-Ruso, Y., de-la-Ossa-Carretero, J.A., Giménez-Casalduero, F., Sánchez-Lizaso, J.L., 2010. Sewage treatment level and flow rates affect polychaete assemblages. Marine Pollution Bulletin 60, 1930–1938.

d'Udekem d'Acoz, C., Vader, W., 2005. The Mediterranean *Bathyporeia* revisited (Crustacea, Amphipoda, Pontoporeiidae), with the description of a new species. Bollettino del Museo Civico de Storia Naturale di Verona 29, 3–38.

Enequist, P., 1949. Studies on the soft-bottom amphipods of the Skagerrak. Zool Bidr fran Uppsala 28, 299–492.

EPA (Environmental Protection Agency), April 1990. Biological criteria: National program guidance for surface waters. EPA-440/5-90-004. EPA, Washington D.C., 57 pp.

Eleftheriou, A., Basford, D.J., 1989. The macrobenthic infauna of the offshore northern North Sea. Journal of Marine Biological Association of United Kingdom 69, 123–143.

Gómez Gesteira, J.L., Dauvin, J.C., 2000. Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. Marine Pollution Bulletin 40, 1017–1027.

Grandi, V., Montanari, G., Lera, S., Simonini, R., 2007. Distribution, life Cycle and behaviour of the amphipod Ampelisca diadema, a potentail new species for ecotoxicological test on marine sediments. Biologia Marina Mediterranea 14, 136–138.

Gray, J.S., Wu, R.S., Or, Y.Y., 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. Marine Ecology Progress Series 238, 249–279.

Grémare, A., Labrune, C., VandenBerghe, E., Amouroux, J.M., Bachelet, G., Zettler, M.L., Vanaverbeke, J., Fleischer, D., Bigot, L., Maire, O., Deflandre, B., Craeymeersch, J., Degraer, S., Dounas, C., Duineveld, G., Heip, C., Herrmann, M., Hummel, H., Karakassis, I., Kedra, M., Kendall, M., Kingston, P., Laudien, J., Occhipinti-Ambrogi, A., Rachor, E., Sardá, R., Speybroeck, J., Van Hoey, G., Vincx, M., Whomersley, P., Willems, W., Wlodarska-Kowalczuk, M., Zenetos, A., 2009. Comparison of the performances of two biotic indices based on the MacroBen database. Marine Ecology Progress Series 382, 297–311.

Grizzle, R.E., 1984. Pollution indicator species of macrobenthos in a coastal lagoon. Marine Ecology Progress Series 18, 191–200.

Grosse, D.J., Pauley, G.B., Moran, D., 1986. Species Profiles: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (Pacific Northwest): Amphipods. U.S. Fish Wildlife Service Biological Report 82(11.69). U.S. Army Corps of Engineers, TR EL-82-4. U.S. Fish Wildlife Service.

Gruner, H.E., 1993. Klasse Crustacea. In: Gruner, H.E., Kaestner (founder), A. (Eds.), Wirbellose Tiere, 4. Teil. Arthropoda. Lehrbuch der Speziellen Zoologie, Band I. Verlag Gustav Fischer, Jena, 448–1030 pp.

Guerra-García, J.M., García-Gómez, J.C., 2001. The spatial distribution of Caprellidea (Crustacea: Amphipoda): a stress bioindicator in Ceuta (North Africa, Gibraltar Area). Marine Ecology PSZN 22, 357–367.

Guerra-García, J.M., Tierno de Figueroa, J.M., 2009. What do caprellids (Crustacea: Amphipoda) feed on? Marine Biology 156, 1881–1890.

Hedges, L.V., Olkin, L., 1985. Statistical Methods for Meta-Analysis. Academic Press, Orlando.

Ingole, B., Sivadas, S., Nanajkar, M., Sautya, S., Nag, A., 2009. A comparative study of macrobenthic community from harbours along the central west coast of India. Environmental Monitoring and Assessment 154, 135–146.

Jimeno, A., 1993. Contribución al estudio de los Anfípodos de las costas mediterráneas catalanas: estudio faunístico, ecológico, biológico y biogeográfico. PhD thesis, University of Barcelona. Spain.

King, C.K., Gale, S.A., Hyne, R.V., Stauber, J.L., Simpson, S.L., Hicke, C.W., 2006. Sensitivities of Australian and New Zealand amphipods to copper and zinc in waters and metal-spiked sediments. Chemosphere 63, 1466–1476.

Krapp-Schickel, G., Krapp, F., 1975. Quelques traits de l'écologie d'amphipodes et de pycnogonides provenant d'un ilot nord-adriatique. Vie et Milieu (B) 25, 1–31.

Labrune, C., Amouroux, J.M., Sarda, R., Dutrieux, E., Thorin, S., Rosenberg, R., Grémare, A., 2006. Characterization of the ecological quality of the coastal Gulf of Lions (NW Mediterranean). A comparative approach based on three biotic indices. Marine Pollution Bulletin 52, 34–47.

Lincoln, R.J., 1979. British Marine Amphipoda: Gammaridea. British Museum (Natural History), London, UK, ISBN 0-565-00818-8, vi, 658 pp.

Lowe, S., Thompson, B., 1997. Identifying Benthic Indicators for San Francisco Bay, Regional Monitoring Program, Annual Report. San Francisco Estuary Institute, Oakland, CA.

Marti, A., 1989. Anfípodos del litoral de Alboraya-Albuixech (Golfo de Valencia, Mediterráneo Occidental). Estudio faunístico y ecológico. Degree thesis. University of Valencia. Spain.

Mearns, A.J., Word, J.Q., 1982. Forecasting effects of sewage solids on marine benthic communities. In: Mayer, G.F. (Ed.), Ecological Stress and the New York Bight: Science and Management. Estuarine Research Federation, Columbia, pp. 495–512.

Norkko, A., Rosenberg, R., Thrush, S.F., Whitlatch, R.B., 2006. Scale- and intensity-dependent disturbance determines the magnitude of opportunistic response. Journal of Experimental Marine Biology and Ecology 330, 195–207.

Nipper, M.G., Greenstein, D.J., Bay, S.M., 1989. Short- and long-term sediment toxicity test methods with the amphipod *Grandidierella japonica*. Environmental and Toxicology Chemistry 8, 1191–1200.

Okladen, J.M., Oliver, J.S., Flegal, A.R., 1984. Behavioral responses of a phoxocephalid amphipod to organic enrichment and trace metals in sediment. Marine Ecology 14, 253–257.

OSPAR, 1995. PARCOM Protocols on Methods for the Testing of Chemicals Used in the Offshore Oil Industry. OSPAR, ISBN 0946956448, p. 35.

Pinedo, S., Jordana, E., Salas, F., Subida, M.D., García Adiego, E., Torres, J., 2011. Testing MEDOCC and BOPA indices in shallow softbottom communities in the Spanish Mediterranean coastal waters. Ecological Indicators. doi:10.1016/j.ecolind.2011.07.024.

Ramos-Gómez, J., Martín-Díaz, M.L., DelValls, T.A., 2009. Acute toxicity measured in the amphipod *Ampelisca brevicornis* after exposure to contaminated sediments from Spanish littoral. Ecotoxicology 18, 1068–1076.

Reish, D.R., 1993. Effects of metals and organic compounds on survival and bioaccumulation in two species of marine gammaridean amphipod, together with a summary of toxicological research on this group. Journal of Natural History 27, 781–794.

Reish, D.R., Barnard, J.L., 1979. Amphipods (Arthropoda:Crustacea:Amphipoda). In: Hart, C.W., Fuller, S.L.H. (Eds.), Pollution Ecology of Estuarine Invertebrates. Academic Press, New York, pp. 1–406.

Riba, I., DelValls, T.A., Forja, J.M., Gómez-Parra, A., 2003. Comparative Toxicity of Contaminated Sediment from a Mining Spill Using two Amphipods Species: *Corophium volutator* (Pallas, 1776) and *Ampelisca brevicornis* (A. Costa, 1853). Bulletin of Environmental Contamination and Toxicology 71, 1061–1068.

Sánchez-Moyano, J.E., García-Gómez, J.C., 1998. The arthropod community, especially Crustacea, as a bioindicator in Algeciras Bay (Southern Spain) based on a spatial distribution. Journal of Coastal Research 14, 1119–1133.

Santos, S.L., Simon, J.L., 1980. Response of soft-bottom benthos to annual catastrophic disturbance in a south Florida estuary. Marine Ecology Progress Series 3, 347–355.

Sardá, R., Pinedo, S., Martin, D., 1996. Seasonal dynamics of macroinfaunal key speciess inhabiting shallow soft-bottoms in the Bay of Blanes NW Mediterranean. Acta Oecologica 20, 315–326.

Simboura, N., Zenetos, A., 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. Mediterranean Marine Science 3/2, 77–111.

Simpson, S.L., King, C.K., 2005. Exposure-pathway models explain causality in whole-sediment toxicity tests. Environmental Science Technology 39, 837–843.

Swartz, R.C., Cole, F.A., Lamberson, J.O., Ferraro, S.P., Schults, D.W., DeBen, W.A., Lee Jr II, H., Ozretich, R.J., 1994. Sediment toxicity, contamination and amphipod abundance at a DDT- and dieldrin-contaminated site in San Francisco Bay. Enviromnental Toxicology and Chemistry 13, 949–962.

Thomas, J.D., 1993. Biological monitoring and tropical biodiversity in marine environments: a critique with recommendations, and comments on the use of amphipods as bioindicators. Journal of Natural History 27, 795–806.

Warwick, R.M., 2001. Evidence for the effects of metal contamination on the intertidal macrobenthic assemblages of the Fal Estuary. Marine Pollution Bulletin 42, 145–148.

Wildish, D.J., Peer, D., 1981. Tidal current speed and production of benthic macrofauna in the lower Bay of Fundy. Canadian Journal of Fisheries and Aquatic Sciences 40, 309–321.

Word, J.Q., 1980. Classification of benthic invertebrates into infaunal trophic index feeding groups. In: Biennal Report 1979–1980 Southern California Coastal Water Research Project, Long Beach, CA, US, pp. 103–121.

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