results can be used as baseline for further monitoring programs and for planning better strategies to protect Italian MPAs. However, a better understanding of the effectiveness of MPAs and the impacts of chemicals on marine biodiversity is needed.

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Assessment of soft-bottom Polychaeta assemblage affected by a spatial confluence of impacts: Sewage and brine discharges

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Mediterranean coastal areas are subject to significant pressures caused by human activity. Sewage effluent is considered one of the most common anthropogenic disturbances of marine benthic communities and it has long been recognized as one of the principal causes of faunal change in near-shore benthic environments (Pearson and Rosenberg, 1978; Lercari and Defeo, 2003), and it can also lead to severe habitat degradation in marine environments (Hunter and Evans, 1995). There is a considerable body of knowledge of temporal and spatial benthic population and community dynamics in relation to certain types of disturbance, especially organic enrichment (Pearson and Rosenberg, 1978; Rhoads and Germano, 1986; Cardell et al., 1999).

In recent years a new activity has appeared that may affect coastal areas: seawater desalination plants. In the Mediterranean, the total production of fresh water from seawater is about 4.2 million m^3/day (17% of the worldwide production). Spain, with 7%, is the largest producer in the region and about 70% of the Spanish plants are located on the Mediterranean coast (Lattemann and Höpner, 2008), and most of these employ reverse osmosis technology. The introduction of a desalination plant will inevitably be associated with several potential adverse environmental impacts particularly on marine ecosystems (Alameddine and El-Fabel, 2007). The main impact on marine communities of reverse osmosis desalination plants is caused by the discharge of an effluent of very high salinity (70–90 psu). The magnitude of the impact will depend both on the size of the plant and on the sensitivity of the ecosystem that receives the spill (Höpner and Windelberg, 1996). However, it may mainly affect marine benthic communities due to the high density of the brine discharge which remains on the bottom.

In addition, there is the increasing possibility that benthic communities will feel the impact of both sewage outfall and brine discharge when and wherever these processes merge. Growing pressure on the coastal zone has increased the number of large-scale, diffuse and chronic impacts operating on coastal and estuarine ecosystems. Multiple sources and types of impacts interacting over a range of spatial scales complicate the assessment and management of such ecosystems (Hewitt et al., 2005).

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Benthic invertebrates are often used as bioindicators to detect and monitor environmental changes, because of their rapid responses to natural and or anthropogenic caused stress (Pearson and Rosenberg, 1978; Simboura and Zenetos, 2002; Perus et al., 2004). Polychaetes are one of the most useful marine organisms to detect pollution. Numerous authors consider polychaetes as being the taxonomic group with the highest level of sensitivity to alterations of the soft substrata (Grassle and Grassle, 1977; Bellan, 1984; Ros et al., 1990; Del-Pilar-Ruso et al., 2008) due to their extraordinary ability to adapt to a whole range of habitats and environmental variation (Fauchald and Jumars, 1979). Therefore, polychaetes are well suited as indicators of environmental changes, since this group contains both sensitive and tolerant species and they are found throughout the whole gradient from pristine to heavily disturbed areas. The presence or absence of specific polychaetes in marine sediments, therefore, provides an excellent indication of the condition or health of the benthic environment (Ryggs, 1985; Tsutsumi, 1990; Pocklington and Wells, 1992). The role of polychaete as indicators for assessing organic pollution is well known (Reish, 1957; Bellan, 1964; Pearson and Rosenberg, 1978; Tsutsumi, 1990; Bitar, 1982; Pocklington and Wells, 1992; Elias et al., 2003). However, there are few reports dealing with the effects of brine on polychaete assemblage and with its role as indicators of environmental changes associated to this impact (Del-Pilar-Ruso et al., 2008). The aim of this survey was to assess how the combination of sewage and brine discharges affects the soft-bottom benthic polychaete assemblage.

The present study has focused on a part of the San Pedro coast (southeast Spain) where a sewage outfall and a brine discharge merge (Fig. 1). The sewage outfall has been in place for decades and produces a flow of 5000 m^3/day in winter and 20,000 m^3/day in summer, with wastewater secondary treatment (lagoons). The brine discharge of the SWRO desalination plant presents a new impact, where the reverse osmosis technique is employed. It began operations in January 2006 with a discharge of 80,000 m^3/day, but by October 2006 its production had doubled. The discharge emerges from the mouth of a pipeline in a deep zone (around 30 m), which shows low levels of hydrodynamic activity and it is

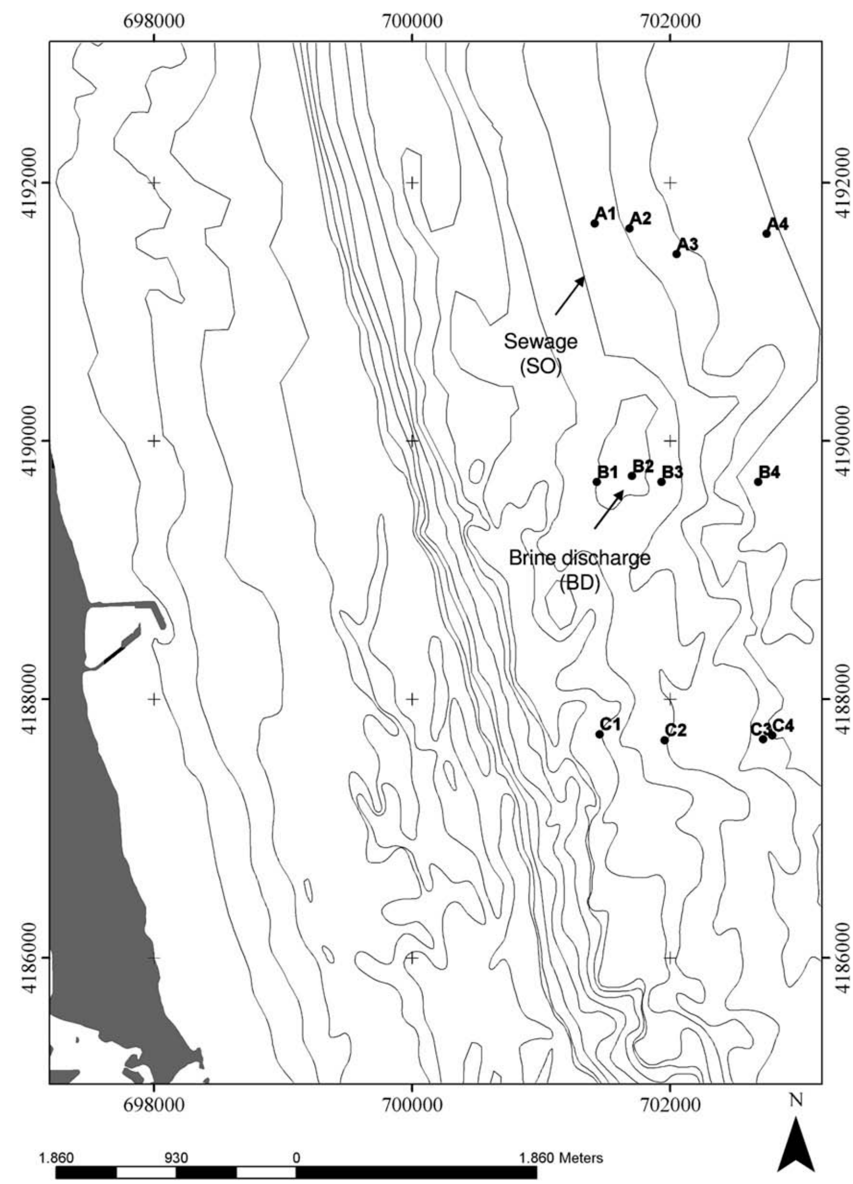

Fig. 1. Map of the study area showing the sampling stations. Transects A, B and C are separated 2 Km each other. Sites 1, 2 and 3 are separated 250 m each other and site 4 is separated 750 m from site 3, except between C3 and C4, that the distance had to be shorter. (SO = sewage outfall and BD = brine discharge).

also characterized by its high salinity (70 psu) and low nutrient content.

We compared a grid of 12 sites around the two discharge areas, in a depth range of 29–38 m, during autumn over a three year period (T1 = 2005, T2 = 2006 and T3 = 2007). With respect to the desalination activity, one sampling period took place before the brine discharge was in place (T1) and the others afterwards (T2 and T3). Three perpendicular transects to the coast were established and called A, B and C, each at a distance of 2 Km from each other. Four stations were located at each transect (1, 2, 3 and 4). The distances between 1 and 2 and between 2 and 3 were 250 m. The distance between 4 and 2 was 1 km. Finally, the distance between C3 and C4 was shorter due to sampling problems (the initial C4 sampling site was rocky bottom, therefore it was changed). The brine discharge outfall takes place next to B2 while the sewage outlet is situated between stations A1 and B1 (Fig. 1). Main currents in the area run parallel to the shore line. Near bottom currents move mainly in a southerly direction but superficial currents move mainly towards the North, with an average speed of 10.03 cm/s. Directly in front of the brine discharge the effluent moves towards the North-east because of its higher density. According to this current pattern we would expect that transect A would have be influenced more by the sewage discharge while stations B2 and B3 would have be influenced more by the brine discharge.

Four replicates were taken using a Van Veen grab (0.04 m^2). Three samples were sieved through a 0.5 mm mesh screen, and fixed in 10% buffered formalin, preserved in 4% formalin for later sorting and identification of the polychaete assemblage to the family level. An additional sample was used to characterize the sediment (organic matter, pH, and granulometric analysis). The pH was measured, immediately after its collection in the field, in undisturbed sediment using a pH-meter Crisom with a sensor 52-00. Once in the laboratory, the sediment was dried in an oven for granulometric analysis. One sub-sample was used for granulometric analysis following Buchanan methodology (1984) and another sub-sample was used to obtain organic matter percentage. Organic matter content of dry sediment was estimated by loss of mass on ignition after being ashes at 400 °C for 4 h. Bottom salinity values were obtained at each station during the sampling campaigns by means of a multiparametric probe Hydrolab H20.

Non-parametric multivariate techniques were used to detect possible changes in the structure of polychaete assemblage in relation to both different impacts. All multivariate analyses were performed using the PRIMER statistical package (Clarke and Warwick, 1994). Triangular similarity matrices were calculated using the Bray–Curtis similarity coefficient using abundance values; all three replicates at each sampling station were pooled (Clarke and Warwick, 1994). Graphical representation of multivariate patterns of polychaete assemblage was obtained by non-metric multidimensional scaling (nMDS) and the possible relationship between polychaete assemblage and abiotic factors was determined using the BIO-ENV procedure. Sperman correlation between data and abiotic factors (sediment grain size, organic matter, pH, depth and salinity) were determined using the RELATE procedure (Clarke, 1993) and similarity percentages (SIMPER) procedure was used to determine the percentage contribution of each polychaete family. Information from this last procedure may be useful to detect possible indicator families for these two different discharges. Studies of marine benthic communities have shown that pollution impacts can be detected at high taxonomic levels, thus saving considerable time and cost (Dethier and Schoch, 2006; Dauvin et al., 2003; Del-Pilar-Ruso et al., 2008). Non-parametric multivariate techniques were also applied to the abundance data of trophic categories. The classification of taxa into trophic categories was based on Fauchald and Jumars (1979). To analyse changes in polychaete assemblage, abundance values, Shannon–Wiener diversity index and Margalef richness index of Polychaeta assemblage were obtained at each sampling station and for each sampling period.

A total of 6698 individuals grouped in 41 families were analyzed. The family Paraonidae was the most abundant (20.86%), followed by Lumbrineridae (13.09%), Syllidae (10.05%), Cirratulidae (8.36%), Magelonidae (5.84%), Sabellidae (5.30%) and Nephtyidae (5.27%). The polychaete assemblage was dominated by the non-selective deposit feeders (26.36%) and the omnivore (25.03%) trophic strategy.

As multivariate analysis appears to be an especially sensitive tool for detecting changes in the structure of the faunal community (Warwick and Clarke, 1991; Clarke and Warwick, 1994; Olsgard et al., 1997; Clarke, 1999) we applied the MDS plot (Fig. 2), and we observed changes in the structure of polychaete assemblage in the study area. On the one hand, we identified two major groups. One group (group I (GI)), established by sites A1, A2, A3, B1 and B4, was characterized as muddy bottom. Stations A1, A2 and A3 presented around 90% of silt and clay while in B1 and B4 this fraction ranged between 65% and 80% (Fig. 3). The other group (group II (GII)) included stations A4, B2, B3, C1, C2, C3 and C4 and it was characterized by the heterogeneity of the sediments (Fig. 3). Group I was categorized by the dominance of the families Paraonidae, Lumbrineridae, Cirratulidae and Magelonidae (61.38% of similarity). Non-selective deposit feeder was the most representative trophic strategy in this group with a contribution percentage of the similarity (C) of 45.33% (Fig. 4). However, the families Syllidae, Lumbrineridae, Paraonidae, Onuphidae, Eunicidae, Nephtyidae and Cirratulidae were responsible for 71.00% of the similarity in group II (49.17%) Omnivore strategy (C: 43.38%) characterized this group (Fig. 4). The families with a higher contribution to the dissimilarity between GI and GII (64.82%) were Paraonidae, Lumbrineridae, Cirratulidae and Magelonidae which were the most representative taxa in GI while the family Syllidae was the most representative taxa in GII (Table 1).

A segregation of site A3 in T2 was also observed with respect to group I (Fig. 2). The dissimilarity (68.67%) was due to the decrease of the abundance of Paraonidae, Cirratulidae and Lumbrineridae while the families Magelonidae and Cossuridae disappear in A3. There was also high dissimilarity between A3 and B2 (56.70%). The families Magelonidae and Paraonidae dominated in B2 while A3 was dominated by Lumbrineridae, Capitellidae and Cirratulidae.

With regard to the physical characteristics (Table 2 and Fig. 3) of the study area correlations of selected environmental variables with polychaete assemblage were obtained through the BIO-ENV procedure; the best combination included gravel, medium sand, mud, and salinity (corr. = 0.597). The RELATE procedure also indi-

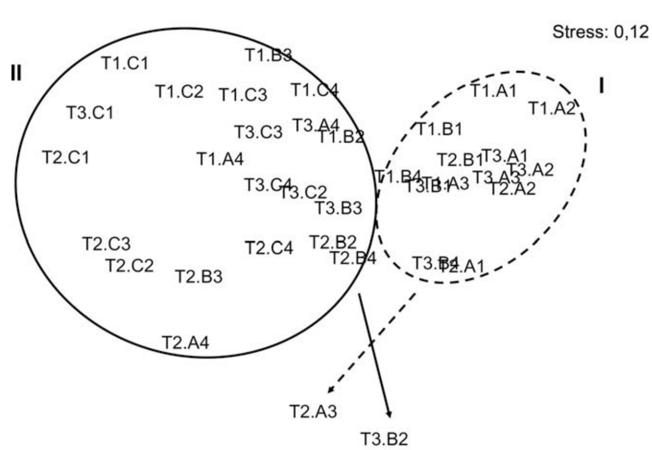

Fig. 2. MDS plot using Bray–Curtis similarities, using non-transformed polychaete abundance data. Each group is represented (I = group I and II = group II).

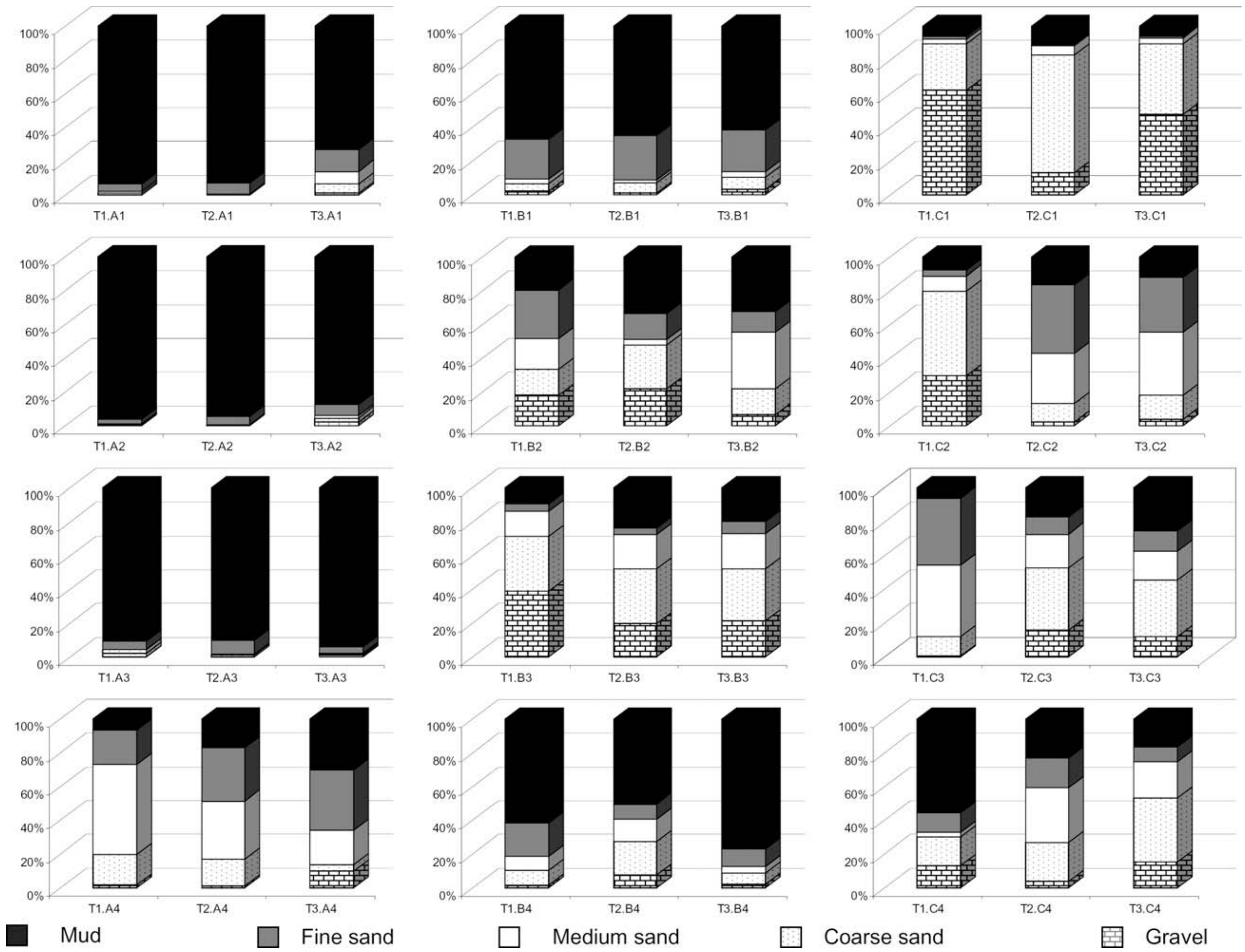

Fig. 3. Granulometric percentage at each station during the sampling period (T1 = 2005, T2 = 2006 and T3 = 2007; grain sizes: gravel: >2 mm, coarse sand: 2 mm–500 μm, medium sand: 500 μm–250 μm, fine sand: 250–63 μm and mud: <63 μm).

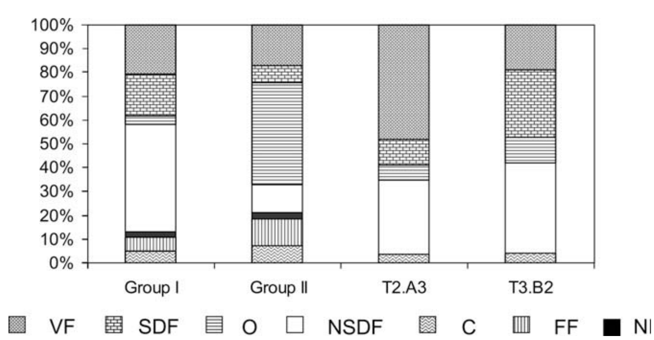

Fig. 4. Percentage of different trophic categories at each group established. VF = varied trophic strategy feeders; SDF = selective deposit feeders; O = omnivore; NSDF = non-selective deposit feeders; C = carnivore; FF = filter feeders; NI = no identified).

cated the relationship between abiotic factors and polychaete assemblage structure (Rho = 0.612; $P = 0.001$). Analysing each factor, a positive correlation is observed between the abundances of polychaete families and the factors: depth (Rho = 0.178; $P = 0.005$), organic matter (Rho = 0.17; $P < 0.02$), gravels (Rho = 0.26; $P < 0.002$), coarse sands (Rho = 0.39; $P = 0.001$), medium sands (Rho = 0.258; $P = 0.001$), fine sands (Rho = 0.242; $P = 0.003$) and mud (Rho = 0.592, $P < 0.01$). Higher mud percentage is shown in GI than in GII; while the gravel, coarse, medium and fine sand percentage were higher in GII. Although organic matter did not show strong correlation, it was higher in GI (mean values of 6.26 ± 1.4%) than in GII (3.25 ± 0.63%). The depths showed a gradient from site 1 to site 4. With respect to salinity, the highest values were obtained in the proximity of the brine outfall 39.2 psu in T3.B3, 39.6 psu in T2.B2; and 40 psu in T3.B2.

We have observed that the spatial distribution of polychaete assemblage inhabiting soft bottom environments is widely related with abiotic factors, and mainly with sediment type. In this sense, our study area could be classified into two types of habitat. Firstly, a habitat characterized by a high presence of mud fraction; more than 90% in the majority of the sites located close to the sewage discharge, with a higher percentage of organic matter (group I). The fact that these sites present high percentages of finer sediments could be related to the existence of a sewage discharge outlet which has been in operation for decades. The sewage input can contribute to an increase in the organic carbon content characteristics of an area, and the proportion of silts and clays may reach bottom communities (Gray, 1992; Heip, 1995). Suspended solid may deposit on the bottom and it can contribute to an increase in the proportion of silts, changing the sediment type in the sites close to the sewage outfall in the study area. However, it is not possible to only correlate the organic matter proportion observed with the presence of the sewage due to the high organic matter differences between sampling periods. These high percentages may also be the result of a specific accumulation of seagrass detritus. Sublittoral soft bottom habitats are especially vulnerable to sewage

Table 1
Summary of the results of SIMPER. Dissimilarities among the main groups obtained by means of the MDS procedure (Av. Abund. = average abundance (Ind/m^2), Cum.% = percentage of cumulative contribution, A.D. = average dissimilarity).

Families	Av. abund.	Av. abund.	Cum.%
	Group I	T2.A3	AD:68.67
Paraonidae	653.57	58.33	35.15
Magelonidae	167.26	0.00	45.41
Cirratulidae	201.19	25	55.28
Lumbrinereidae	319.05	166.67	64.39
Cossuridae	88.69	0.00	69.82
	Group II	T3.B2	AD:73.79
Syllidae	269.17	0.00	20.06
Magelonidae	37.50	166.67	30.89
Onuphidae	110.83	0.00	38.70
Paraonidae	116.25	108.33	45.76
Sabellidae	100.83	0.00	52.34
Cirratulidae	91.25	0.00	58.73
Lumbrinereidae	130.83	58.33	64.92
Nephtyidae	89.58	16.67	70.59
Eunicidae	80.00	8.33	75.99
	Group I	Group II	AD:64.82
Paraonidae	653.57	116.25	24.29
Syllidae	16.07	269.17	36.08
Lumbrinereidae	319.05	130.83	45.80
Cirratulidae	201.19	91.25	53.36
Magelonidae	167.26	37.50	58.73
	T2.A3	T3.B2	AD:56.70
Magelonidae	0.00	166.67	36.36
Lumbrinereidae	166.67	58.33	60.00
Paraonidae	58.33	108.33	70.91
Capitellidae	58.33	25.00	78.18
Cirratulidae	25.00	0.00	83.64

Table 2
Summary of physical characteristics (depth (m), pH, organic matter (%), and salinity (psu)) at each station during each sampling period (2005, 2006 and 2007).

Station	Year	Depth	pH	organic matter	Bottom salinity
A1	2005	34.5	6.98	9.10	36.90
	2006	34.7	7.69	3.19	37.13
	2007	34.3	7.84	3.29	36.80
A2	2005	35.1	6.8	12.61	36.90
	2006	35.3	7.71	3.52	37.13
	2007	35	7.48	3.48	36.80
A3	2005	35.6	7.04	6.47	36.90
	2006	35.5	7.82	3.62	37.25
	2007	35.8	7.59	3.52	37.20
A4	2005	36.8	7.34	2.55	36.90
	2006	36.4	7.85	1.72	37.63
	2007	36.5	7.38	2.13	37.20
B1	2005	33.4	7.29	18.43	36.90
	2006	33.1	7.88	2.98	38.50
	2007	32.8	7.38	2.34	38.80
B2	2005	33.7	7.47	9.15	36.90
	2006	33.5	7.35	1.85	39.63
	2007	33.6	7.45	1.99	40.00
B3	2005	32.2	7.12	3.91	36.90
	2006	32.7	7.65	1.65	38.50
	2007	32.3	7.8	1.55	39.20
B4	2005	37.8	7.06	6.61	36.90
	2006	37.6	7.74	2.16	37.63
	2007	37.9	7.76	2.27	37.20
C1	2005	29.7	7.49	4.94	37.40
	2006	30.3	7.89	1.28	37.50
	2007	29.2	7.66	1.29	37.60
C2	2005	31.6	7.31	4.81	37.40
	2006	33	7.72	1.77	37.50
	2007	33.4	7.77	1.66	37.60
C3	2005	32.6	7.37	1.89	37.40
	2006	36.1	7.78	1.89	37.38
	2007	34.9	7.6	1.79	37.60
C4	2005	35.1	7.27	14.14	37.40
	2006	36	7.77	1.88	37.38
	2007	35.6	7.76	1.93	37.60

input, since they are very different from eutrophic environments, principally in relation to three main sedimentological factors: both organic matter and silt contents, as well as the degree of oxygenation (Gray, 1992; Heip, 1995). These factors may act as either an energy source or a stress factor for an ecosystem, altering its productivity and community development favouring the dominance of the community by a few more resistant organisms (Odum, 1985, 1988) such as those we have detected in this area. This habitat tends to containing high abundances of deposit feeder polychaetes such as Paraonidae, Cirratulidae and Magelonidae. Finer sediments are known to be colonized by deposit feeders (Pearson and Rosenberg, 1978). In spite of the presence of this impact, polychaete assemblage seem to remain more stable with time due to the fact that sewage outfall has been in operation for decades. The influence of the sewage may also reach station B1 but the high presence of mud in station B4 could not be related with the presence of the sewage outfall due to the fact that this station is beyond the area of influence of the sewage, and it is probably more related to depth. It may be observed that this station is situated along the boundary between both groups (Fig. 2).

Secondly, we have detected another habitat characterized by the heterogeneity of the sediments, with high percentages of gravel, coarse, medium and fine sand and lower percentage of mud at each site (group II). In this type of habitat we have observed that a higher number of polychaete families, such as Syllidae, Lumbrineridae, Paraonidae, Onuphidae, Eunicidae, Nephtyidae, and Cirratulidae coexist due to the complexity of the habitat that favours higher values of richness and biodiversity than in those habitats that have lost complexity as a result of environmental stresses (Dean and Connell, 1987). Furthermore, coarse sediments are known to provide a wide range of interstitial spaces which constitute a suitable habitat for these families (Dauvin, 1988; Westheide, 1990; Moreira et al., 2006; Guzmán-Alvis and García, 2006) due to the fact that the space between grains makes the search and capture of potential prey easier for carnivores, such as Lumbrineridae, Onuphidae, Nephtyidae and omnivore families such as Syllidae and Eunicidae (Parson et al., 1995).

As has been mentioned above, the study area is also affected by a brine discharge. This discharge takes place close to one of the sites (B2) included in this heterogeneous group (GII). We have observed that desalination activity causes a decrease in abundance, and richness and biodiversity in polychaete assemblage in the vicinity of the discharge, where the influence of the discharge is higher (Fig 5). The decrease of these parameters was observed in all the study area between T1 and T2, although it was more evident in B2. However, a recovery of these values in T3 was observed in all the sites, except in those adjacent to the brine discharge outlet where lowest values of the parameters were obtained. Therefore, it is reasonable to assume that although these changes are not related to the presence of brine since they affect all the stations, the absence of recovery in the station B2 may be related with the brine discharge. This decrease is shown to be more evident over time (Fig. 5). These results are similar to those obtained in other surveys where the effect of brine discharge on soft bottom communities was also assessed (Castriota et al., 2001; Del-Pilar-Ruso et al., 2007, 2008).

With respect to the structure of polychaete assemblage in this particular site affected by higher salinity, we have observed a temporal substitution (Fig. 6) of a polychaete assemblage characterized by the presence of the families Sabellidae, Paraonidae, Lumbrineridae, Cirratulidae, Syllidae, Nephtyidae, Capitellidae, Onuphidae,

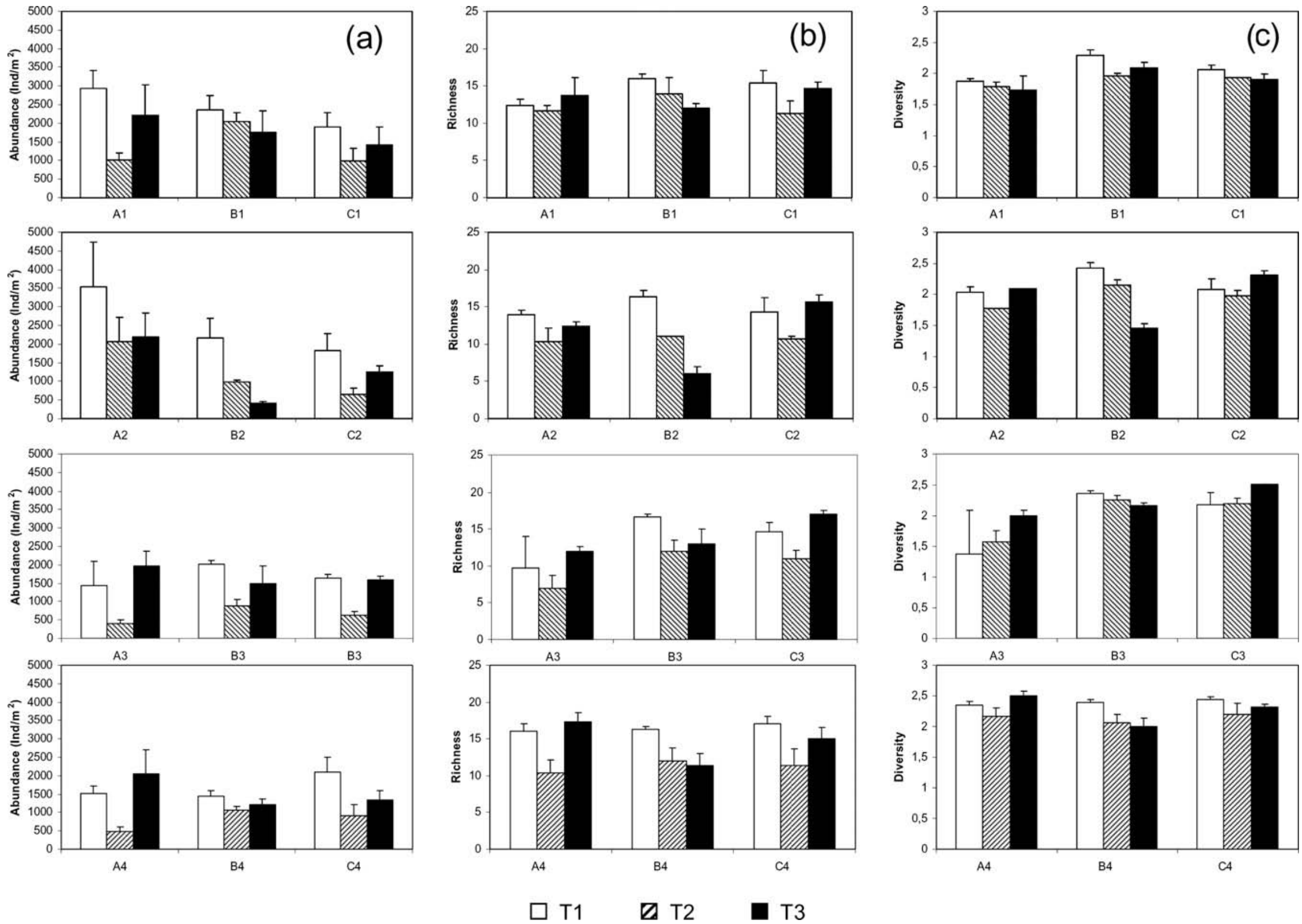

Fig. 5. (a) Mean (±SE) abundance; (b) mean (±SE) richness and (c) mean (±SE) diversity of Polychaete assemblage at all stations, for each year (2005, 2006, and 2007).

Table 3
Summary of the results of SIMPER. Dissimilarities of site B2 along the three sampling periods with respect to the sites included in the heterogeneous group (Av. Abund = average abundance (Ind/m^2), contrib.% = percentage of contribution of each family, A.D = average dissimilarity; Heterogeneous group = all sites included in group II, except B2).

Heterogeneous group		T1.B2	AD:47.62	T2.B2	AD:50.79	T3.B2	AD:70.74
Families	Av. abund	Av. abund	Contrib.%	Av. abund	Contrib.%	Av. abund	Contrib.%
Sabellidae	96.76	275.00	12.65	0.00	7.28	0.00	6.40
Paraonidae	102.31	283.33	11.56	200.00	11.07	108.33	6.78
Lumbrineridae	119.91	275.00	10.24	183.33	9.16	58.33	5.49
Cirratulidae	84.26	233.33	10.01	75.00	5.04	0.00	5.94
Syllidae	283.33	241.67	9.31	41.67	19.50	0.00	21.10
Nephtyidae	82.87	183.33	6.53	116.67	6.13	16.67	5.21
Capitellidae	37.04	141.67	6.36	41.47	0	25.00	0
Onuphidae	118.52	25.00	5.18	58.33	5.67	0.00	8.18
Magelonidae	33.80	66.67	2.86	75.00	4,60	116.67	11.11
Eunicidae	78.70	91.67	2.52	91.67	3.70	8.33	5.24

Magelonidae and Eunicidae, before the beginning of the desalination activity (Autumn 2005 (T1)), for another dominated mainly by Magelonidae and Paraonidae, after the second year of the activity (Autumn, 2007 (T3)) (Table 3), where the salinity values reach 40. These changes occur even though the sediment type remained consistent throughout the study period. For this reason it is assumed that these changes in the composition of the assemblage are due to the presence of the brine.

Moreover, changes of polychaete assemblage show the existence of a gradient of sensitivity to high salinity in Polychaeta families (Del-Pilar-Ruso et al., 2008). Sabellidae disappears at first perturbation state (time 2), so this would seem to be the most sensitive family, followed by Cirratulidae, Syllidae and Onuphidae which need more exposure to disappear completely (time 3). Nephtyidae, Eunicidae and Capitellidae do not disappear but decrease in time 3. However, Paraonidae and Magelonidae appear to be the most resistant to salinity increase. Our survey shows similar behavioural patterns to those that were observed in a previous study (Del-Pilar-Ruso et al., 2008), in particular with the families Syllidae, Paraonidae and Capitellidae in spite of the different discharge characteristics (depth, hydrodinamics etc.). Further study will be necessary to detect the critical level of sensitivity of each Polychaeta family and to improve our knowledge of this new impact.

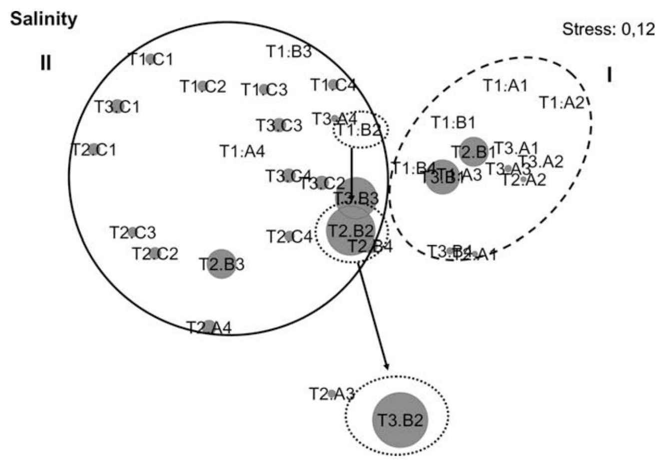

Fig. 6. MDS plot where temporal changes in polychaete assemblage in B2 are highlighted. Salinity values are directly proportional to the bubble size.

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