

Confounding response of macrofauna from a confluence of impacts: brine and sewage pollution

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Abstract

Throughout the shores of the world, a confluence of different sources of pollution is common, e.g., through outlets releasing brine, industrial, and domestic sewage. In this study, we assessed whether a combined disposal of domestic sewage and brine altered the patterns of abundance and assemblage structure of subtidal macrofauna inhabiting sandy seabeds on the south coast of Gran Canaria (Canary Islands, NE Atlantic Ocean). Samples were collected in two surveys (May 2008 and January 2009) at three distances (0, 15, and 30 m) away from the discharge point. Macrofaunal abundances showed different patterns with varying proximity from the disposal point in the two surveys. In May 2008, lower abundances were observed at 0 m (53.89±46.82 ind.) than at 15 m (120±91.7 ind.) and 30 m (283.89±189.33 ind.) away from the disposal point. In January 2009, however, higher abundances were observed at 0 m (131.33±58.69 ind.) than at 30 m (100±24.44 ind.) and 15 m (84.78±58.39 ind.) away from the disposal point. Significant differences in macrofaunal assemblage structure were found with varying proximity from the discharge point in both surveys. The effect of proximity from the disposal point was confounded by changes in sedimentary composition between surveys, which contributed to explain differences in macrofaunal abundances and assemblage structure with varying proximity from the discharge point. As a result, confounding drivers of macrofaunal assemblage structure, here, changes in sediment composition, accounted for changes in macrofaunal abundances and assemblage structure with varying proximity from a combined disposal point of brine and sewage.

Key words: macrofauna, amphipoda, polychaeta, brine, sewage, Canary Islands

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1 Introduction

Human activities may produce chemical and physical perturbations that affect coastal benthic assemblages. One of the most important forms of marine pollution is eutrophication, resulting from sewage discharges (Gray, 1981; McIntyre, 1995). This may induce changes in species richness, abundance, biomass, and assemblage structure (Méndez, 1997; Cardell et al., 1999; Elías et al., 2004), but effects can vary depending on the response variables considered and the type of data analysis used (Terlizzi et al., 2005; Fraschetti et al., 2006). The first phase of eutrophication may produce an increase in the abundance and biomass of benthic individuals. Further organic loading may cause hypoxia, or even anoxia, with exclusively the most resistant species surviving (Hily et al., 1986). Thus, organic pollution can be studied using indicator or opportunistic organisms (e.g., the polychaetes *Capitella capitata*, *Scolecopsis fuliginosus*, and *Polydora ligni*, and the mollusc *Abra alba*) (Tsutsumi, 1987; Méndez et al., 1998) that are frequently used as bioindicators for marine monitoring studies because of their rapid response to sewage disposals (del Pilar Ruso et al.,

2007; de-la-Ossa-Carretero et al., 2010).

In recent decades, freshwater scarcity has become a common issue across areas with low precipitation regimes. Seawater desalination has been proposed as an alternative, and so the number of constructed, as well as projected, desalination plants has increased considerably (Latteman and Höpner, 2008). Currently, there are more than 12 500 desalination plants worldwide across 120 countries, and the overall world capacity is ca. 42×10^6 m³/d of potable water (GWI, 2006).

Multiple sources and types of impacts interacting over a range of spatial and temporal scales complicate the monitoring assessment and management of coastal ecosystems (Hewitt et al., 2005). To our knowledge, there are no previous studies on the combined effects of brine and sewage on macrofauna. Del Pilar Ruso et al. (2009) studied the confluence of the former two impacts; however, the brine and sewage were from two different pipelines, separated ca. 900 m. Gacia et al. (2007) showed high concentrations of nutrients, especially nitrates, in the surroundings of the brine disposal. However, its origin was unclear, and it could even be an indirect consequence of desalination

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processes. Unfortunately, the former study only focused on the seagrass *Posidonia* meadow, and no infaunal data were obtained.

In the Canarian archipelago, a common solution for the release of sewage effluents is the utilization of pipelines from desalination plants. Thus, a confluence of impacts is a common situation on subtidal sandy substrates, particularly around tourist areas located on the south coast of Tenerife and Gran Canaria. In this context, the aim of this study was to assess the effect of proximity from a disposal discharge point (brine and sewage) on the abundance and assemblage structure of macrobenthic faunal assemblages living on surrounding sandy seabeds.

2 Materials and methods

2.1 Study area and sampling design

This study was conducted around Tarajalillo treatment plant, located on the south coast of Gran Canaria (27°45'46"N, 15°33'18"W) (Fig. 1). The plant has a brine and sewage outfall running approximately 500 m offshore. The diameter of the outfall is 25 cm, and discharges through an open "mouth" at a depth of 11 m on a sandy seabed. The volume of daily disposal discharge is ca. 14 000 m³/d. The disposal point discharges brine from a desalination plant (ca. 80%) and domestic sewage (ca. 20%). Samples were taken at 0, 15, and 30 m away from the brine discharge point through three radial transects. All samples were taken by means of SCUBA divers. Samples at 0 m were as adjacent to the brine discharge point as possible. Cores (20 cm inner diameter) were pushed into the sediment, to a depth of 30 cm. Six replicates were collected haphazardly for faunistic determinations at each distance, while three additional cores were collected for analysis of abiotic variables. Sampling was repeated twice, in May 2008 and January 2009.

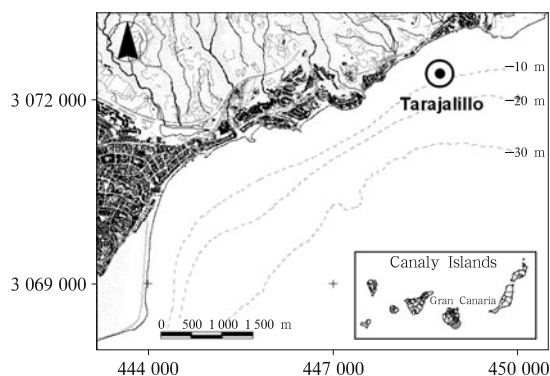


Fig. 1. Map of the study area showing the sampling location.

2.2 Analysis of environmental variables

Since sediment features (e.g., grain size and organic matter content) can notoriously influence soft-bottom macrofaunal assemblages (Gray, 1981; Pearson and Rosenberg, 1978), we quantified these two attributes as a way to estimate their potential effects on the patterns of abundance and assemblage structure of macrofauna with varying proximity from the brine discharge point. To assess the granulometric composition of the sediment, ca. 100 g of sediment from each sample was oven dried at 105°C, passed through a graded series (2, 1, 0.5, 0.25, 0.125 and 0.063 mm) of sieves, and then dry weighed (Buchanan, 1984). The method of Walkley and Black (1934) was

used to determine the organic matter content of the sediment. Total nitrogen was determined following the Kjeldahl method (Bradstreet, 1965), and phosphorus concentration was calculated using a spectrum-photometric method (Murphy and Riley, 1962).

2.3 Identification of macrofauna

Samples were preserved in a 10% seawater formaldehyde solution, and subsequently decanted through a 0.5 mm mesh sieve. The fraction remaining was separated into different taxonomical groups under a binocular microscope, and preserved in 70% ethanol. Macrofaunal specimens were determined to species level, whenever possible, by means of a binocular microscope, or even in a LEICA DMLB microscope equipped with Nomarski interference contrast (Riera, 2004).

2.4 Statistical analysis

Differences in macrofaunal assemblage structure with varying proximity from the brine discharge point (i.e., distance of 0, 15, and 30 m) through the two surveys (May 2008 and January 2009) were tested by means of a permutational MANOVA that included the following factors: "Distance" (fixed factor) and "Year" (random factor and orthogonal to "Distance"). The same model, but in a univariate context, tested for differences in overall macrofaunal abundance and the abundance of the most abundant taxa (the mollusc *Loripes lacteus*, the amphipod *Ampelisca brevicornis*, and the tanaid *Apseudes talpa*). Although variances remained heterogeneous in all cases, we decreased the increase in Type I error by reducing the α value to 0.01 (Underwood, 1991); ANOVA is robust for such departures for balanced studies. Post-hoc tests were used, when appropriate, to resolve differences among levels of significant factors.

To visualize affinities in assemblage structure, nMDS (non-metric multidimensional scaling) was carried out on square root transformed abundance data via the Bray-Curtis similarity index. Only centroids (i.e., means) for each distance (0, 15, and 30 m) were plotted to facilitate visualization of multivariate patterns in the ordination space. A distance-based redundancy analysis (db-RDA; Legendre and Anderson, 1999) tested whether variation in any of the measured abiotic variables significantly contributed to explain variation in the macrofaunal assemblage structure with varying proximity to the brine discharge point. Multivariate multiple regression using the DISTLM routine (Anderson, 2001) then tested the significance of these relationships by fitting a linear model based on Bray-Curtis dissimilarities on square root transformed abundance data. To retain variables with good explanatory power, as a result of colinearity among variables, the AIC routine was used as a selection criterion (the smaller the value the better the model; Legendre and Anderson, 1999). Analyses were based on a "forward" selection procedure. All multivariate procedures were carried out via the PRIMER 6.0 & PERMANOVA+ statistical package.

3 Results

3.1 Environmental variables

No seasonal variations in the discharge volume were observed during the two sampling surveys (May 2008 and January 2009). Salinity at the discharge point typically ranged from 49–53 throughout the entire study. At 30 m away from the brine disposal point, salinity ranged from 36.6–36.7 (Table 1). No eutrophication (organic matter, nitrogen, and phosphorus) effect was observed in sediments affected by the disposal, though an

Table 1. Mean (\pm SE) values of environmental variables with varying proximity (0, 15, and 30 m) from the brine discharge point

	May 2008		
	0 m	15 m	30 m
Pore water salinity	48.7 \pm 1.4	38.9 \pm 0.9	36.7 \pm 0.2
Water column salinity	50.8 \pm 3.5	39 \pm 0.8	36.6 \pm 0.1
Water column temperature/ $^{\circ}$ C	20.93 \pm 0.5	20.8 \pm 0.4	20.6 \pm 0.3
Water column pH	8.19 \pm 0.1	8.17 \pm 0.02	8.16 \pm 0.1
Chlorophyll/ μ g \cdot L $^{-1}$	1.4 \pm 0.4	1.1 \pm 0.2	0.9 \pm 0.3
Sediment: total nitrogen/mg \cdot kg $^{-1}$	<1	<1	<1
Sediment: total phosphorus/mg \cdot kg $^{-1}$	2.66 \pm 1.04	2.87 \pm 0.47	1.30 \pm 0.52
Sediment: organic matter/%	0.64 \pm 0.18	0.25 \pm 0.07	0.32 \pm 0.06
Total coliforms/CFU \cdot 100 ml $^{-1}$	51.33 \pm 66.16	0.67 \pm 0.58	4.33 \pm 6.66
Escherichia coli/CFU \cdot 100 ml $^{-1}$	4.33 \pm 3.78	0	0
Sediment: gravels/%	18.91 \pm 18.07	0.44 \pm 0.39	0.23 \pm 0.40
Sediment: very coarse sands/%	19.67 \pm 7.47	0.35 \pm 0.31	0.30 \pm 0.36
Sediment: coarse sands/%	14.69 \pm 8.74	0.48 \pm 0.45	0.23 \pm 0.40
Sediment: medium sands/%	2.99 \pm 1.42	0.88 \pm 0.95	0.53 \pm 0.32
Sediment: fine sands/%	11.66 \pm 5.38	25.49 \pm 12.97	25.50 \pm 13.06
Sediment: very fine sands/%	23 \pm 9.42	57.32 \pm 8.10	57.28 \pm 4.67
Sediment: silt/clay/%	9.06 \pm 4.90	15.04 \pm 4.73	15.92 \pm 8.19
	January 2009		
	0 m	15 m	30 m
Pore water salinity	47.9 \pm 2.1	38.5 \pm 1.1	36.8 \pm 0.8
Water column salinity	50.4 \pm 3.4	38.8 \pm 1.0	36.9 \pm 0.4
Water column temperature/ $^{\circ}$ C	19.7 \pm 0.4	19.25 \pm 0.3	19.11 \pm 0.4
Water column pH	8.17 \pm 0.2	8.16 \pm 0.2	8.16 \pm 0.1
Chlorophyll/ μ g \cdot L $^{-1}$	1.1 \pm 0.5	0.9 \pm 0.4	0.8 \pm 0.4
Sediment: total nitrogen/mg \cdot kg $^{-1}$	1.30 \pm 0.10	1.23 \pm 0.06	1.25 \pm 0.07
Sediment: total phosphorus/mg \cdot kg $^{-1}$	3.60 \pm 0.35	4.03 \pm 1.88	3.13 \pm 0.85
Sediment: organic matter/%	0.63 \pm 0.38	0.86 \pm 0.56	1.26 \pm 0.53
Total coliforms/CFU \cdot 100 ml $^{-1}$	130 \pm 35.37	116 \pm 26.46	181.67 \pm 27.30
Escherichia coli/CFU \cdot 100 ml $^{-1}$	0.67 \pm 1.15	23.33 \pm 40.41	1 \pm 1.73
Sediment: gravels/%	6.39 \pm 2.95	9.93 \pm 5.17	19.26 \pm 7.16
Sediment: very coarse sands/%	12.71 \pm 4.22	11.66 \pm 5.53	26.11 \pm 1.63
Sediment: coarse sands/%	9.84 \pm 1.80	8.20 \pm 3.73	14.82 \pm 3.53
Sediment: medium sands/%	4.26 \pm 0.32	6.02 \pm 1.41	5.08 \pm 0.42
Sediment: fine sands/%	27.83 \pm 1.70	31.85 \pm 7.64	16.43 \pm 3.85
Sediment: very fine sands/%	38.61 \pm 6.67	31.80 \pm 2.49	17.84 \pm 2.98
Sediment: silt/clay/%	0.37 \pm 0.20	0.55 \pm 0.16	0.46 \pm 0.05

increase in microorganism assemblages was observed in the first survey (May 2008) (Table 1).

3.2 Macrofaunal assemblages

A total of 6 965 individuals were collected throughout the study, belonging to 14 taxonomic groups (Amphipoda, Cnidaria, Cumacea, Decapoda, Echinodermata, Isopoda, Leptostraca, Misidacea, Mollusca, Nemertea, Oligochaeta, Ostracoda, Polychaeta, and Tanaidacea) (Table 2). The most abundant groups were molluscs (2 215 ind., 31.80% of the overall abundance), followed by amphipods (1 564 ind., 22.45%) and polychaetes (1 118 ind., 16.05%). In terms of species, the most abundant taxa were the mollusc *Loripes lacteus* (1 803 ind., 25.88% of the overall abundance), followed by the amphipod *Ampelisca brevicornis* (1 278 ind., 18.35%) and the tanaid *Apseudes talpa* (1 197 ind., 17.19%). Samples at 0 m away from the discharge point were dominated by the amphipod *Ampelisca brevicornis*, the mollusc *Loripes lacteus*, the oligochaete *Grania* sp., the polychaetes *Platynereis dumerilii* and *Rhynchospio glutaea*, and the tanaid *Apseudes talpa* (Table 2).

Macrofaunal abundance did not significantly vary with in-

creasing distance from the disposal point (Fig. 2a; one-way ANOVA: "Distance", $F=10.90$, $p=0.373$ 8; Table 3). In turn, these differences were temporally inconsistent with distance from the disposal point (one-way ANOVA: "Distance \times Year", $F=15.93$, $p=0.236$ 8; Table 3). Significant differences in total macrofaunal abundance were found between surveys (May 2008 and January 2009) (Fig. 2a; one-way ANOVA: "Year", $F=350.1$, $p=0.000$ 2; Table 3). In May 2008, lower abundances were observed at 0 m (53.89 \pm 46.82 ind.) than at 15 m (120 \pm 91.7 ind.) and 30 m (283.89 \pm 189.33 ind.) away from the disposal point. In January 2009, however, higher abundances were observed at 0 m (131.33 \pm 58.69 ind.), than at 30 m (100 \pm 24.44 ind.) and 15 m (84.78 \pm 58.39 ind.) away from the disposal point. The most abundant species, the mollusc *Loripes lacteus*, showed marginally significant differences with proximity from the disposal point (Fig. 2b; one-way ANOVA: "Distance", $F=36.97$, $p=0.037$ 6; Table 3), with lower abundances at 0 m than at 15 and 30 m away. Other abundant species, i.e., the amphipod *Ampelisca brevicornis* and the tanaid *Apseudes talpa*, showed significant differences with varying proximity from the disposal point, with the lowest abundances at the outfall (Fig. 2b and c;

Table 2. Mean abundance (\pm SE) of macrofaunal taxa (0.1 m²) with varying proximity (0, 15, and 30 m) from the brine discharge point

Group	Species	May 2008			January 2009		
		0 m	15 m	30 m	0 m	15 m	30 m
Amphipoda	<i>Ampelisca brevicornis</i>	0.11 \pm 0.33	22 \pm 38.44	72.22 \pm 109.73	34 \pm 30.98	9.88 \pm 9.74	3.77 \pm 7.53
Amphipoda	<i>Amphilochus neapolitanus</i>	0	0	0	0	0	0.22 \pm 0.44
Amphipoda	<i>Ampithoe rubricata</i>	0	0	0.22 \pm 0.44	0.77 \pm 1.56	0.22 \pm 0.67	0
Amphipoda	<i>Aora typica</i>	0	0.33 \pm 0.71	0.22 \pm 0.44	0.33 \pm 1.0	0	0
Amphipoda	<i>Bathyporeia elegans</i>	0	3 \pm 5.48	1.11 \pm 1.76	0	0	0
Amphipoda	<i>Corophium</i> sp.	0	0	0	0	0	0.22 \pm 0.44
Amphipoda	<i>Corophium</i> sp. 1	0	0	0	0.11 \pm 0.33	0	0
Amphipoda	<i>Dexamine spinosa</i>	0	1.11 \pm 1.69	2.22 \pm 5.95	0	0	0
Amphipoda	<i>Ericthonius brasiliensis</i>	0	0.11 \pm 0.33	0	0	0	0
Amphipoda	<i>Harpinia antennaria</i>	0	0.66 \pm 1.12	0.55 \pm 1.01	0	0	0
Amphipoda	<i>Leptocheirus pectinatus</i>	0	0	1.11 \pm 2.98	0	0	0.66 \pm 0.87
Amphipoda	<i>Leucothoe spinicarpa</i>	0	0	0.11 \pm 0.33	0	0	0
Amphipoda	<i>Megamphopus cornutus</i>	0	0	0	0	0	0.44 \pm 0.88
Amphipoda	<i>Pariambus typicus</i>	0	0.22 \pm 0.44	0.44 \pm 0.73	0	0	0
Amphipoda	<i>Pereionotus testudo</i>	0	0	0.33 \pm 1.0	0	0	0
Amphipoda	<i>Photis longicaudata</i>	0.11 \pm 0.33	0.88 \pm 1.36	1.33 \pm 1.80	0.22	0.33 \pm 0.50	1.22 \pm 1.30
Amphipoda	<i>Phtisica marina</i>	0	0.55 \pm 1.01	0.22 \pm 0.67	0	0	0.11 \pm 0.33
Amphipoda	<i>Urothoe marina</i>	0	5.66 \pm 10.20	1.55 \pm 2.30	0	0	0.11 \pm 0.33
Amphipoda	<i>Urothoe pulchella</i>	0	3.22 \pm 4.44	0.66 \pm 1.0	0.66 \pm 0.87	0.11 \pm 0.33	0
Cnidaria	<i>Anemona</i> sp.	0	0.11 \pm 0.33	0	0	0	0
Cumacea	<i>Iphinoe canariensis</i>	0	2.88 \pm 4.83	5.11 \pm 5.06	1.22 \pm 1.79	0.11 \pm 0.33	0.22 \pm 0.44
Decapoda	<i>Albunea carabus</i>	0	0	0.11 \pm 0.33	0	0	0
Decapoda	<i>Anapagurus laevis</i>	0	0	0.11 \pm 0.33	0	0	0
Decapoda	<i>Athanas nitescens</i>	0	0	0.11 \pm 0.33	0	0	0
Decapoda	<i>Callianassa tyrrenha</i>	0	0	0	0.11 \pm 0.33	0.33 \pm 0.50	0
Decapoda	<i>Cycoes cristata</i>	0	0.11 \pm 0.33	0	0	0	0
Decapoda	<i>Hippolyte inermis</i>	0	0	0.11 \pm 0.33	0	0	0
Decapoda	<i>Hippolyte longirostris</i>	0	0	0.22 \pm 0.67	0	0	0
Decapoda	<i>Ilia</i> cf. <i>spinosa</i>	0	0	0.11 \pm 0.33	0	0	0
Decapoda	<i>Liocarcinus corrugatus</i>	0	0	0.11 \pm 0.33	0	0	0
Decapoda	<i>Liocarcinus zariquieyi</i>	0	0.33 \pm 0.50	0.11 \pm 0.33	0	0	0
Decapoda	<i>Macropodia</i> sp.	0	0	0.11 \pm 0.33	0	0	0
Decapoda	<i>Pagurus anachoretus</i>	0	0.11 \pm 0.33	0.44 \pm 0.73	0	0.22 \pm 0.67	0.11 \pm 0.33
Decapoda	<i>Philoscheras bispinosus</i>	0	0.11 \pm 0.33	0.11 \pm 0.33	0	0	0
Decapoda	<i>Pisa carinimana</i>	0	0	0.11 \pm 0.33	0	0	0
Decapoda	<i>Processa canaliculata</i>	0	0.22 \pm 0.44	0.11 \pm 0.33	0	0	0
Decapoda	<i>Upogebia pusilla</i>	3.33 \pm 3.97	0.33 \pm 0.71	0.88 \pm 1.54	0	0	0.33 \pm 0.50
Decapoda	<i>Xantho poressa</i>	0	0	0	0.11 \pm 0.33	0.33 \pm 0.67	0
Echinodermata	<i>Amphipholis squamata</i>	0	0	0.22 \pm 0.67	0	0	0
Isopoda	<i>Anthura gracilis</i>	0	0.66 \pm 1.12	0.44 \pm 0.88	0	0	0
Isopoda	<i>Cymodoce truncata</i>	0	0	0.11 \pm 0.33	0	0	0
Isopoda	<i>Eurydice pulchra</i>	0	0.33 \pm 1.0	0	0	0	0
Leptostraca	<i>Nebalia</i> cf. <i>clausi</i>	0.88 \pm 1.36	0	0	0.11 \pm 0.33	0	0.11 \pm 0.33
Misidacea	<i>Gastrosaccus sanctus</i>	0	0.33 \pm 0.71	0.66 \pm 0.87	0.11 \pm 0.33	0	0
Mollusca	<i>Abra alba</i>	0.11 \pm 0.33	0.33 \pm 1.0	1.22 \pm 2.11	0	0	0
Mollusca	<i>Acanthocardia tuberculata</i>	0	1.11 \pm 1.54	0.77 \pm 0.83	0	0	0
Mollusca	<i>Amaea formosissima</i>	0.22 \pm 0.67	0	0	0	0	0
Mollusca	<i>Bela ornata</i>	0	0.11 \pm 0.33	0.11 \pm 0.33	1.00	1.22 \pm 1.92	0
Mollusca	<i>Corbula gibba</i>	0	0	0.11 \pm 0.33	0	0	0
Mollusca	<i>Cylichna propecyllindracea</i>	0	0.66 \pm 1.41	1.77 \pm 2.91	0	0	0
Mollusca	<i>Gibberula miliaria</i>	0	0.11 \pm 0.33	0.66 \pm 1.66	0	0	0
Mollusca	<i>Gibberula secreta</i>	0	0.22 \pm 0.67	0.11 \pm 0.33	0	0	0.22 \pm 0.67
Mollusca	<i>Gibberula</i> sp.	0	0	0.11 \pm 0.33	0	0	0
Mollusca	<i>Granulina guancha</i>	0	0	0.11 \pm 0.33	0	0	0
Mollusca	<i>Jujubinus exasperatus</i>	0	0.11 \pm 0.33	0	0	0	0
Mollusca	<i>Linga adansonii</i>	1.33 \pm 1.41	1.55 \pm 2.60	0.33 \pm 0.50	1.11 \pm 1.27	4.11 \pm 3.89	12.33 \pm 3.50
Mollusca	<i>Loripes lacteus</i>	20.66 \pm 27.08	28.55 \pm 48.90	109 \pm 130.73	3.88 \pm 4.23	21.44 \pm 16.12	16.77 \pm 7.14

to be continued

Continued from Table 2

Group	Species	May 2008			January 2009		
		0 m	15 m	30 m	0 m	15 m	30 m
Mollusca	<i>Lucinella divaricata</i>	0	0.66±1.32	1.22±1.48	0	0.33±0.71	0
Mollusca	<i>Mangelia costulata</i>	0	0	0.33±1.0	0	0	0
Mollusca	<i>Megaximus appendiculata</i>	0.11±0.33	0	0	0	0	0.22±0.67
Mollusca	<i>Nassarius cuvierii</i>	0.11±0.33	0.44±1.01	2.11±3.41	0.11±0.33	1.11±1.96	1.22±1.09
Mollusca	<i>Nassarius incrassatus</i>	0	0.11±0.33	0	0	0	0.11±0.33
Mollusca	<i>Nassarius reticulatus</i>	0.11±0.33	0	0	0.22±0.44	0.44±1.01	0.11±0.33
Mollusca	<i>Natica dillwynii</i>	0	0	0.11±0.33	0	0	0
Mollusca	<i>Parvicardium exiguum</i>	0	0	0.55±0.73	0	0	0
Mollusca	<i>Parvicardium scriptum</i>	0	0	0	0.11±0.33	0	0
Mollusca	<i>Plagiocardium papillosum</i>	0	0.44±1.33	0	0	0	0
Mollusca	<i>Polinices lacteus</i>	0	0	0.11±0.33	0	0	0
Mollusca	<i>Solemya togata</i>	0	0	1.88±4.01	0.55±0.73	0.11±0.33	0.11±0.33
Mollusca	<i>Spisula subtruncata</i>	0	0	0	0.11±0.33	0.33±0.71	0
Mollusca	<i>Tellina donacina</i>	0	0	0.22±0.44	0	0	0
Mollusca	<i>Thracia papyracea</i>	0	0	0.55±0.73	0	0	0
Mollusca	<i>Thyasira plexuosa</i>	0	0.11±0.33	0	0	0	0
Mollusca	<i>Tricolia pullus canarica</i>	0	0	0.11±0.33	0	0	0
Nemertea	<i>Nemertea</i> sp. 1	1.11±0.60	0.44±1.01	0	0	0.33±0.50	0
Oligochaeta	<i>Grania</i> sp.	3.66±4.85	1.11±2.26	0	6.88±15.22	0.44±1.01	2.11±2.47
Oligochaeta	<i>Tubificidae</i> sp. 1	0.55±0.73	0	0	0	0	0
Ostracoda	<i>Cypridina mediterranea</i>	0	9.44±14.20	32.55±40.32	0.88±1.54	0	0.11±0.33
Ostracoda	<i>Cypridina norvergica</i>	0	3.55±4.45	6.88±4.40	0.22±0.44	0	0.11±0.33
Ostracoda	<i>Ostracoda</i> sp.	0.11±0.33	0.33±0.50	0.22±0.44	0	0	0
Polychaeta	<i>Aonides oxycephala</i>	0.33±0.71	0.22±0.67	0.88±2.32	0.11±0.33	0	0.11±0.33
Polychaeta	<i>Aponuphis bilineata</i>	0	7±10.81	12.11±8.42	2±2.12	0.66±0.87	3.11±2.62
Polychaeta	<i>Armandia cirrosa</i>	0	0.11±0.33	0.22±0.44	0	0	0
Polychaeta	<i>Bispira viola</i>	0	0.11±0.33	0	0	0	0
Polychaeta	<i>Branchiomma vesiculosum</i>	0	0.11±0.33	0	0	0	0
Polychaeta	<i>Capitellido</i> sp. 1	0.11±0.33	0	0	0	0	0
Polychaeta	<i>Demonax brachychona</i>	0	0	0.11±0.33	0	0	0
Polychaeta	<i>Glycera dayi</i>	0.56	0.11±0.33	0.55±1.33	0.22±0.67	0.22±0.44	0
Polychaeta	<i>Glycera</i> sp.	0	0	0	0.11±0.33	0.11±0.33	0
Polychaeta	<i>Goniada emerita</i>	0.11±0.33	0	0	0	0	0
Polychaeta	<i>Harmothoe</i> sp.	0	0.11±0.33	0	0	0	0
Polychaeta	<i>Harmothoe</i> sp. 1	0	0	0.33±0.50	0	0	0
Polychaeta	<i>Malacoceros filiginosus</i>	0.22±0.67	0.44±1.33	0.11±0.33	0	0	0
Polychaeta	<i>Nematoneis unicornis</i>	0	0	0	0.11±0.33	0	0
Polychaeta	<i>Nephtys caeca</i>	0	0.77±1.09	1.11±1.27	0	0	0
Polychaeta	<i>Nephtys cirrosa</i>	0	0	0	0	0.11±0.33	0.11±0.33
Polychaeta	<i>Onuphis eremita</i>	0	0.33±0.71	0.11±0.33	0.11±0.33	0.11±0.33	0.22±0.44
Polychaeta	<i>Periquesta canariensis</i>	0	0.66±0.71	0	0.22±0.44	0.22±0.44	0.11±0.33
Polychaeta	<i>Pista cristata</i>	0	0	0	0	0.22±0.44	0
Polychaeta	<i>Platynereis dumerilii</i>	14.11±13.23	0.77±0.83	0.33±1.0	6.22±7.89	20.55±29.53	22.77±11.97
Polychaeta	<i>Poecilochaetus serpens</i>	0	0.11±0.33	0.22±0.67	0	0	0
Polychaeta	<i>Prionospio</i> sp.	0	0	0	0	0.11±0.33	0.11±0.33
Polychaeta	<i>Prionospio steenstrupii</i>	0	0.22±0.44	0	1±1.32	0	0.22±0.67
Polychaeta	<i>Psammolyce arenosa</i>	0	0.11±0.33	0	0	0	0
Polychaeta	<i>Pseudomistides limbata</i>	0	0	0	0	0.11±0.33	0
Polychaeta	<i>Rynchospio glutaea</i>	2±2.24	0.44±0.88	0	11.33±15.83	1.33±1.58	1.44±1.74
Polychaeta	<i>Schistomeringos albomaculata</i>	0	0	0	0	0	0.11±0.33
Polychaeta	<i>Scolecopsis squamata</i>	0	0	0.11±0.33	0	0	0
Polychaeta	<i>Scoloplos (Leodamas)</i> sp.	0.22±0.44	0.55±1.01	0.44±1.33	0	0	0
Polychaeta	<i>Scoloplos armiger</i>	0	0.11±0.33	0.11±0.33	0.55±0.73	0	0
Polychaeta	<i>Sigalion squamatum</i>	0	0.55±1.01	1.33±1.94	0.11±0.33	0	0
Polychaeta	<i>Spio filicornis</i>	0.22±0.67	0	0.22±0.44	0.11±0.33	0.11±0.33	0
Polychaeta	<i>Streptosyllis bidentata</i>	0.22±0.67	0	0.22±0.44	0.11±0.33	0	0
Polychaeta	<i>Syllis prolifera</i>	0.11±0.33	0	0	0	0	0
Tanaidacea	<i>Apseudes talpa</i>	2.55±3.81	13.77±20.37	11.22±17.92	55.88±40.50	19.22±15.31	30.33±26.15
Tanaidacea	<i>Leptochelia dubia</i>	0.55±1.01	0.44±0.73	1.22±2.44	0.22±0.44	0.11±0.33	0.33±0.50
Tanaidacea	<i>Tanais dulongii</i>	0	0.11±0.33	0	0	0.11±0.33	0

Table 3. Results of multi- and univariate ANOVA testing for differences in macrofaunal assemblage structure, overall macrofaunal abundance, and *Ampelisca brevicornis*, *Apseudes talpa*, and *Loripes lacteus* abundances with varying proximity to the brine discharge point (“Distance”, fixed factor) through years (“Year” fixed factor and orthogonal to “Distance”)

Source of variation	DF	Assemblage structure			Overall macrofaunal abundance			<i>A. brevicornis</i> abundance			<i>A. talpa</i> abundance			<i>L. lacteus</i> abundance		
		MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>
Distance (<i>D</i>)	2	57 703	35.93	0.003 8 ¹⁾	21 577	10.90	0.373 8	13 320	21.53	0.007 4 ¹⁾	6 071.7	186.2	0.003 6 ¹⁾	25 430	36.97	0.037 6 ¹⁾
Year (<i>Y</i>)	1	110 028	68.51	0.000 2 ¹⁾	69 316	350.1	0.000 2 ¹⁾	17 800	28.78	0.001 2 ¹⁾	11 360	348.4	0.000 2	70 013	101.8	0.003 6 ¹⁾
<i>D</i> × <i>Y</i>	2	48 153	29.98	0.007 8 ¹⁾	31 533	15.93	0.236 8	10 550	17.06	0.046 2	6 303.9	193.3	0.000 2	24 657	35.85	0.040
Residual	12	16 061.1			19 800			6 185.5								

Note: ¹⁾ Significant differences ($p < 0.05$).

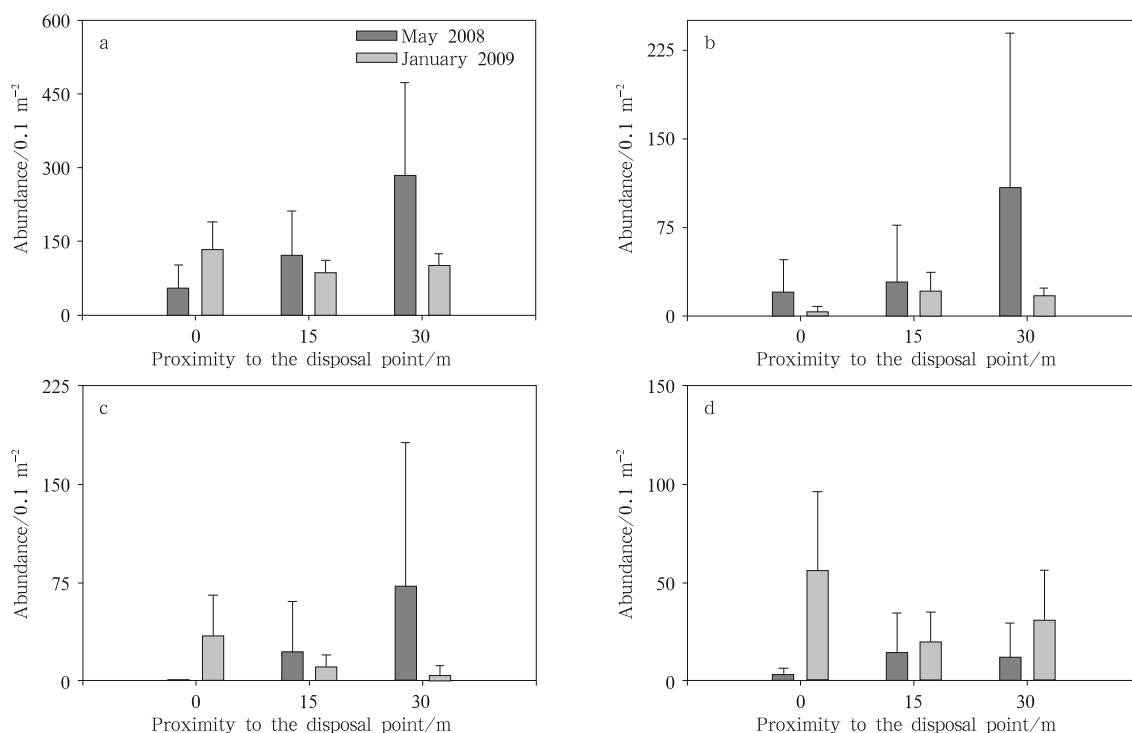
**Fig. 2.** Mean abundance (\pm SE) of overall meiofauna (a); mean abundance (\pm SE) of *Loripes lacteus* (b); mean abundance (\pm SE) of *Ampelisca brevicornis* (c); and mean abundance (\pm SE) of *Apseudes talpa*, with varying proximity (0, 15, and 30 m) from the disposal point (d). Black bars represents May 2008 and grey bars January 2009.

Table 3).

“Distance” from the disposal point significantly affected macrofaunal assemblage structure (PERMANOVA, pseudo- $F=35.93$, $p=0.003 8$). These differences were, however, inconsistent between surveys (PERMANOVA, “Distance×Year”, pseudo- $F=29.98$, $p=0.007 8$) (Fig. 3; Table 3). The first two axes from the db-RDA explained 32.16% of the total variation in macrofaunal assemblage structure (Fig. 4). The percentage of very fine sands was positively correlated with the first axis, which accumulated 21.2% of the total variability of macrofaunal assemblage structure. The second axis of the db-RDA was negatively correlated with silt and clay content and positively correlated with coarse sands. The former two variables together with the percentage of very fine sands were selected as those mostly contributing to explain variation in macrofaunal assemblage structure (Table 4), as a result of strong colinearity among several abiotic variables and principally among the percentages of the sedimentary classes. Samples at 0 m had different gran-

ulometric composition: silt and clay dominated in May 2008, while coarse sands dominated in January 2009. In May 2008, samples at 15 and 30 m showed differences in grain size composition with 0 m locations, whilst in January 2009 no differences among 0, 15, and 30 m samples were observed (Fig. 4). These results confirmed that variation in sediment grain size determined the structure of macrofaunal assemblages around the discharge point to a great extent.

4 Discussion

The results of this study showed that shifts in grain size composition were mainly responsible for shifts in macrofaunal assemblage structure. Hence, this effect may mask ultimate interpretations about the importance of combined disposal (brine and sewage) into recipient macrofaunal assemblages. The effects of combined brine and sewage disposal on macrofaunal assemblages differed depending on each taxonomic group, even among families within the same taxonomic group

Table 4. Results of multivariate multiple regression testing the relationship between the measured set of environmental variables (Table 1) and macrofaunal assemblage structure

Variable	AIC	SS (trace)	Pseudo-F	<i>p</i>	Proportion of explained variation
Very fine sands	138.26	7 290.5	3.733 7	0.001 6 ¹⁾	0.189 2
Silt/clay	137.77	4 046.6	2.231 9	0.014 4 ¹⁾	0.105 0
Coarse sands	137.41	3 339.5	1.959 8	0.050 6	0.086 7
Total nitrogen	137.2	2 755.6	1.697 7	0.103	0.071 5

Notes: To retain variables with explanatory power, the AIC procedure was chosen as the model selection criterion (sequential tests; Legendre and Anderson, 1999). ¹⁾ *p*-values denote significant values ($p < 0.01$). All data were fourth-root transformed.

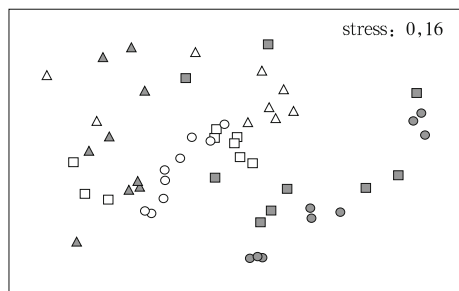


Fig. 3. Ordination plot (nMDS, stress=0.16) showing similarities in macrofaunal assemblage structure with varying proximity (0, 15, and 30 m) from the discharge point. Centroids for each distance are plotted. Triangles represent 0 m, squares 15 m, and circles 30 m. Grey represents May 2008 and white January 2009.

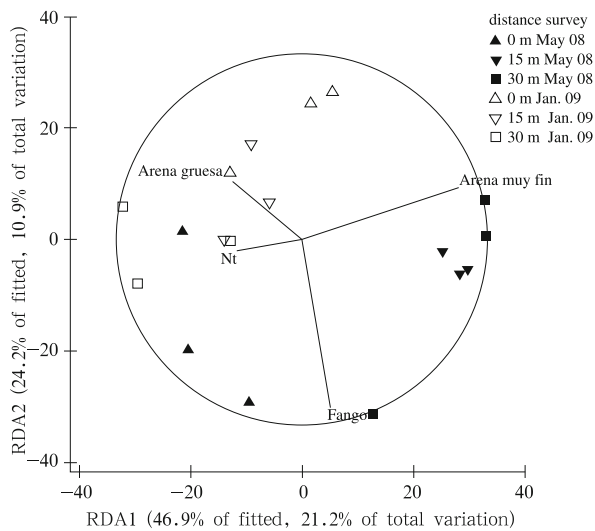


Fig. 4. Distance-based redundancy analysis (db-RDA) biplot of the first and second axes relating those environmental variables that better explain macrofaunal assemblage structure with varying proximity from the outfall (see Table 1). Centroids for each distance and survey are plotted. Triangles represent 0 m, squares 15 m and circles 30 m. Black represents May 2008 and white January 2009.

(e.g., polychaetes) (del Pilar Ruso et al., 2009). Macrofaunal nematodes (>0.5 mm) replaced a typical macrofaunal assemblage composed by polychaetes, crustaceans, and molluscs on sandy seabeds affected by brine (salinity>39) (del Pilar Ruso et al., 2007). Several opportunistic species were abundant at the outfall, such as the mollusc *Loripes lacteus*, the

oligochaete *Grania* sp., and the polychaete *Platynereis dumerilii*. Although the bivalve *L. lacteus* has been recorded in areas affected by a high concentration of organic matter (Guerra-García and García-Gómez, 2004), this species showed the lowest abundances at 0 m away from the disposal point in our study, because of the dominance of large-sized sedimentary types (e.g., very coarse sands and gravels). However, other species, such as the polychaetes *Platynereis dumerilii* and *Rhynchospio glutaea* and the oligochaete *Grania* sp., were favoured by the effects of combined disposal, showing higher abundances at 0 m. Other species showed no clear trend and their abundances were more related to sedimentary changes than to combined disposal, such as the amphipod *Ampelisca brevicornis* and the tanaid *Apseudes talpa* that were very abundant in fine and very fine sands (0 m locations in May 2009).

In the present study, the combined discharge (brine and sewage) did not significantly affect macrofaunal abundances among 0, 15, and 30 m sites. However, significant differences in overall macrofaunal abundance were found between sampling periods (May 2008 and January 2009) due to differences in sedimentary composition, with higher content of gravels and very coarse sands in the first survey. The pattern of macrofaunal abundances was the opposite in May 2008 (0<15<30 m) compared to January 2009 (0>15>30 m). Sediment-mediated interactions are common in infaunal communities, rendering the sediment less suitable for other organisms (Herbert Wilson, 1991). For example, suspension-feeders (e.g., *Apseudes talpa*) are more common in sandy substrates, whilst deposit-feeders (e.g., oligochaetes) show preferences for mud substrates; it has been demonstrated as a limitation of growth of suspension-feeders in sediments dominated by deposit-feeders (Rhoads and Young, 1970).

Macrofaunal assemblage structure was significantly affected by the combined outfall, however, these differences were inconsistent throughout the two sampling periods. Sedimentary changes were likely caused by differences in the magnitude of hydrodynamic parameters, such as differences in swell height or period of rough seas that typically occur in winter (December–March) in the Canary archipelago. Thus, the second survey was affected by swells (AEMET, 2009).

In summary, our results revealed confounding drivers of macrofaunal assemblage structure, here, changes in sediment composition, to explain changes in macrofaunal abundances and assemblage structure with varying proximity from a combined disposal point of brine and sewage.

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