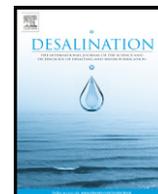




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Variability of macrofaunal assemblages on the surroundings of a brine disposal

Rodrigo Riera ^{a,*}, Fernando Tuya ^b, Eva Ramos ^a, Myriam Rodríguez ^a, Óscar Monterroso ^a

^a Centro de Investigaciones Medioambientales del Atlántico (CIMA SL), Arzobispo Elías Yanes, 44, 38206 La Laguna, Tenerife, Canary Islands, Spain

^b BIOGES, Universidad de Las Palmas de G.C., 35017 Las Palmas de Gran Canaria, Canary Islands, Spain

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ABSTRACT

Desalination plants generate large volumes of hypersaline brine, likely affecting recipient communities. We aimed to assess whether proximity to a brine discharge point altered the abundance, assemblage structure and diversity of macrobenthic fauna. Collection of samples took place twice (May 2008 and January 2009) at 0, 15 and 30 m away from a brine discharge point. Total macrofaunal abundance increased with increasing distance from the brine discharge point, though the magnitude of differences was inconsistent between successive years, probably as a result of a change in particle size distribution. Proximity to the brine discharge point also altered patterns in macrofaunal assemblage structure. The macrofaunal species density was higher at 15 and 30 m than at 0 m. In conclusion, proximity to a brine discharge point significantly altered the ecological pattern of macrobenthic fauna, though disentangling the effect of the increase in salinity from particle size distribution remains undetermined.

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1. Introduction

The number of desalination plants in regions with freshwater limitations has been increasing over the last decades, particularly in the southern countries of the northern hemisphere, to cover the necessities of their populations and industrial and agricultural activities as well [1,2]. Irrespective of whether the process used is 'reverse osmosis' or 'distillation', desalination plants generate notable volumes of hypersaline brine, which are then discharged back into the sea and may, therefore, affect recipient biological communities [15]. The 'reverse osmosis' method is the preferred desalination process, mainly due to the low energy and space consumption and the reduction in the cost of obtained potable water [17,30,34].

When the brine is discharged into the sea, the density difference between the brine and the seawater induce the formation of a stratified system. As a result, the brine creates a bottom layer that can subsequently affect benthic communities living in environments under natural salinity conditions [13,19]. The release of brine (~44–90 PSU salinity) is generally accompanied by different chemical products (e.g. antiscalant, antifouling, hydrochloric acid, ferric chloride, sodium hexametaphosphate, etc.) used during desalination processes [26,33]. The magnitude of the impact over those biological communities living in the surroundings of any brine discharge point depends on both the volume of the brine disposal, which typically depends on the size of the desalination plant, as well as on the sensitivity of those

communities that are recipient of the effluent [7,26]. Available information on the effects of these hypersaline effluents over faunal assemblages are, however, extremely limited [9,10,35]. These potential impacts may be minimised by selecting an appropriate discharge location, and/or through a previous dilution of the effluent. It is also important, moreover, to establish a carefully designed monitoring plan to assess the dispersion of any brine plume over time, in order to adopt appropriate measurements whenever necessary [17,18,25]. The aim of this study was, therefore, to assess the effect of proximity to a brine discharge point over the abundance, assemblage structure and diversity of macrobenthic faunal assemblages living on soft bottoms.

2. Materials and methods

2.1. Study area and sampling design

This study was conducted around Las Burras desalination plant, located south off Gran Canaria (27°45'55 N, 15°33'01 W), which began to operate in 1999 (Fig. 1). The plant has a brine outfall approximately 300 m long running offshore. The diameter of the outfall is ~60 cm, and discharges through an open 'mouth' at 7 m depth on a soft bottom. The volume of seawater collected for desalination is approximately 42,000 m³ day⁻¹, with an estimated production of potable water of ca. 25,000 m³ day⁻¹. As a result, the volume of brine discharged everyday is ca. 17,000 m³ day⁻¹. Salinity at the brine discharge point ranged between 47 and 50 PSU throughout the entire study (Table 1). At 30 m away from the brine disposal

* Corresponding author.

E-mail address: rodrigo@cimacanarias.com (R. Riera).

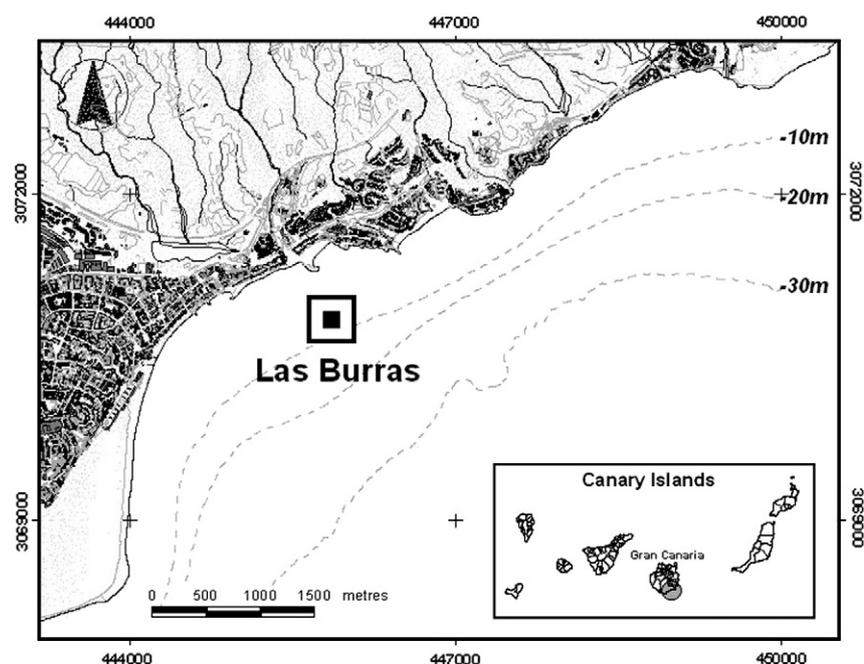


Fig. 1. Map of the study area, showing the location of the brine discharge point.

point, salinity ranged at natural values, i.e. between 36.6 and 36.8 PSU (Table 1). Indeed, a dilution from 75 to 38 PSU within 20 m from a brine outlet has been registered in a zone adjacent to

Table 1

Mean (\pm SE) values of abiotic variables located at different distances (0, 15 and 30 m) from the brine discharge point.

	May 2008		
	0 m	15 m	30 m
Pore water salinity	45.6 \pm 1.2	38.7 \pm 0.7	36.6 \pm 0.2
Water column salinity	48.9 \pm 2.7	40.1 \pm 1.2	36.7 \pm 0.2
Water column temperature ($^{\circ}$ C)	20.64 \pm 0.3	20.61 \pm 0.04	20.61 \pm 0.04
Water column pH	8.17 \pm 0.02	8.17 \pm 0.02	8.17 \pm 0.02
Chlorophyll-a (μ g/l)	0.3 \pm 0.2	0.2 \pm 0.2	0.2 \pm 0.2
Sediment: total nitrogen (mg/kg)	<1	<1	<1
Sediment: total phosphorus (mg/kg)	7.80 \pm 2.33	3.93 \pm 0.55	3.13 \pm 0.77
Sediment: organic matter (%)	0.39 \pm 0.06	0.25 \pm 0.01	0.21 \pm 0.06
Sediment: gravels (%)	1.28 \pm 0.61	0.34 \pm 0.17	0
Sediment: very coarse sands (%)	8.28 \pm 2.05	0.99 \pm 0.85	0
Sediment: coarse sands (%)	46.67 \pm 5.15	4.99 \pm 3.80	0
Sediment: medium sands (%)	16.84 \pm 4.13	4.56 \pm 2.78	0.26 \pm 0.13
Sediment: fine sands (%)	6.67 \pm 0.96	28.10 \pm 2.19	21.07 \pm 5.03
Sediment: very fine sands (%)	14.26 \pm 3.82	48.33 \pm 6.63	61.08 \pm 4.12
Sediment: silt/clay (%)	6.01 \pm 1.47	12.68 \pm 1.29	17.61 \pm 1.16
	January 2009		
	0 m	15 m	30 m
Pore water salinity	45.1 \pm 1.4	38.5 \pm 0.6	36.4 \pm 0.3
Water column salinity	48.4 \pm 2.5	40.1 \pm 1.3	36.8 \pm 0.3
Water column temperature ($^{\circ}$ C)	19.54 \pm 0.4	19.12 \pm 0.2	19.11 \pm 0.3
Water column pH	8.15 \pm 0.03	8.16 \pm 0.02	8.17 \pm 0.03
Chlorophyll-a (μ g/l)	0.2 \pm 0.1	0.2 \pm 0.2	0.2 \pm 0.1
Sediment: total nitrogen (mg/kg)	1 \pm 0.1	1.2 \pm 0.2	1.2 \pm 0.3
Sediment: total phosphorus (mg/kg)	6.17 \pm 1.83	4.33 \pm 0.55	9.30 \pm 4.26
Sediment: organic matter (%)	0.48 \pm 0.02	0.63 \pm 0.10	0.46 \pm 0.07
Sediment: gravels (%)	2.33 \pm 1.18	36.98 \pm 17.72	5.11 \pm 3.51
Sediment: very coarse sands (%)	9.98 \pm 1.78	9.78 \pm 2.34	17.71 \pm 2.72
Sediment: coarse sands (%)	50.97 \pm 3.14	31.43 \pm 14.46	55.49 \pm 3.59
Sediment: medium sands (%)	14.94 \pm 3.32	14.22 \pm 3.93	17.50 \pm 1.23
Sediment: fine sands (%)	8.79 \pm 2.92	3.98 \pm 2.33	2.30 \pm 1.05
Sediment: very fine sands (%)	12.47 \pm 5.68	2.97 \pm 2.06	1.33 \pm 1.01
Sediment: silt/clay (%)	0.51 \pm 0.11	0.63 \pm 0.2	0.56 \pm 0.18

the study area [38], and so samples at 30 m away from the outfall can be considered as controls.

Collection of samples took place at a distance of 0, 15 and 30 m away from the brine discharge point. Collections at 0 m were as close to the brine discharge point as possible; a slight underestimation of the real distance was then assumed, though in terms of sampling design we will refer this level as “0 m” throughout the entire manuscript. Sediment cores (20 cm inner diameter) were pushed into the sediment, with the help of a hammer, to a depth of 30 cm. Nine replicates ($n=9$) were collected randomly for faunistic determinations at each distance, while three cores at each distance were additionally collected for analysis of abiotic variables. Since sediment features, such as size and the content of organic matter, influence soft-bottom macrofaunal assemblages [23,32], we proceeded to quantify these two attributes as a way to estimate their effects over the patterns of abundance and assemblage structure of macrofauna located at different distances from the brine discharge point. The sediment characteristics might co-vary with salinity, since they could be indirectly affected by either the brine plume or directly by the physical presence of the outfall. The level of replication was based on a previous study [36]. Sampling was repeated twice: at May 2008 and January 2009, to determine whether patterns were consistent when measured at different times.

2.2. Analysis of abiotic factors

To assess the particle size distribution of the sediment, ca. 100 g of sediment from each sample was oven dried at 105 $^{\circ}$ C, passed through a graded series (mesh size of 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm and 0.063 mm) of sieves, and then dry weighed [8]. The method of [41] was used to determine the organic matter content of the sediment through dichromate oxidation. Total nitrogen was determined following the Kjeldahl method [6], by digestion with sulphuric acid, and total phosphorus concentration was calculated using a spectrum-photometric method [31]. Water column parameters (salinity, temperature, pH and Chlorophyll concentration: Chl-a) were determined by means of a SBE9plus CTD along a vertical profile from the seabed to the sea surface; we took measurements on the bottom and at the surface as the most representatives (Table 1). Water samples

Table 2

Mean abundance (\pm SE) of macrofaunal taxa (0.1 m²) located at different distances (0, 15 and 30 m) from the brine discharge point.

Group	Taxa	May 2008			January 2009		
		0 m	15 m	30 m	0 m	15 m	30 m
Amphipoda	<i>Ampelisca brevicornis</i>	3.1 \pm 1.1	183.2 \pm 57.5	369.4 \pm 101.2	0.1 \pm 0.1	0	0.1 \pm 0.1
Amphipoda	<i>Bathyporeia elegans</i>	0	34.5 \pm 12.4	7.1 \pm 3.6	0	0	0
Amphipoda	<i>Leptocheirus pectinatus</i>	0	0	0	0.1 \pm 0.1	0	0
Amphipoda	<i>Pontocrates arenarius</i>	0	0	0.1 \pm 0.1	0	0	0.3 \pm 0.3
Amphipoda	<i>Urothoe marina</i>	0	14.0 \pm 7.5	4.0 \pm 2.1	0	0	0
Amphipoda	<i>Urothoe pulchella</i>	0	3.3 \pm 2.6	1.8 \pm 1.2	0	0	0
Cnidaria	<i>Anemona</i> sp.	0	0.1 \pm 0.1	0	0	0	0
Cumacea	<i>Iphinoe canariensis</i>	0	2.9 \pm 1.9	4.6 \pm 2.9	0	0	0
Decapoda	<i>Anapagurus laevis</i>	0	0.1 \pm 0.1	0.1 \pm 0.1	0.1	0	0
Decapoda	<i>Liocarcinus zariquieyi</i>	0	0.2 \pm 0.2	0	0	0	0
Decapoda	<i>Processa canaliculata</i>	0	0.1 \pm 0.1	0	0	0	0
Decapoda	<i>Upogebia pusilla</i>	0	0.1 \pm 0.1	0.1 \pm 0.1	0	0	0
Misidacea	<i>Gastrosaccus sanctus</i>	0	0.1 \pm 0.1	0.4 \pm 0.3	0.1 \pm 0.1	0	0
Decapoda	<i>Callinassa tyrrhena</i>	0	0	0	0	0.2 \pm 0.2	0
Decapoda	<i>Pagurus anachoretus</i>	0	0	0	0	0.1 \pm 0.1	0
Isopoda	<i>Anthurus gracilis</i>	0	0	0	0	0	0.1 \pm 0.1
Isopoda	<i>Eurydice pulchra</i>	0	0.1 \pm 0.1	0	0	0	0
Mollusca	<i>Abra alba</i>	0.3 \pm 0.3	0	0	0	0	0
Mollusca	<i>Acanthocardia tuberculata</i>	0	0.1 \pm 0.1	0.3 \pm 0.2	0	0	0
Mollusca	<i>Bela ornata</i>	0.1 \pm 0.1	0.6 \pm 0.5	0.1 \pm 0.1	0	0.2 \pm 0.2	3.2 \pm 1.8
Mollusca	<i>Conus guanche</i>	0	0	0	0	0.1 \pm 0.1	0.1 \pm 0.1
Mollusca	<i>Donax venustus</i>	0	0	0.1 \pm 0.1	0	0	0
Mollusca	<i>Ervilia castanea</i>	0	0	0	0	0.4 \pm 0.4	1.0 \pm 0.9
Mollusca	<i>Gregariella subclavata</i>	0	0	0	0.2 \pm 0.2	0	0
Mollusca	<i>Irus irus</i>	0	0	0	0	0.3 \pm 0.3	0
Mollusca	<i>Linga adansonii</i>	0	0.9 \pm 0.7	0.1 \pm 0.1	0	0.1 \pm 0.1	0.2 \pm 0.2
Mollusca	<i>Loripes lacteus</i>	0	0.2 \pm 0.1	0.2 \pm 0.2	0	0.8 \pm 0.6	0
Mollusca	<i>Mactra glabrata</i>	0	0.4 \pm 0.3	0.2 \pm 0.2	0.1 \pm 0.1	0	0
Mollusca	<i>Nassarius cuvieri</i>	0	0	0	0	0.3 \pm 0.3	0.1 \pm 0.1
Mollusca	<i>Natica furva</i>	0	0	0	0	0	0.1 \pm 0.1
Mollusca	<i>Olivella oteroi</i>	0	0.1 \pm 0.1	0	0	1.3 \pm 1.1	0.3 \pm 0.2
Mollusca	<i>Parvicardium scriptum</i>	0	0	0	0.1 \pm 0.1	0	0
Mollusca	<i>Spisula subtruncata</i>	0	0	0	0	0	0.1 \pm 0.1
Mollusca	<i>Tellina incarnata</i>	0	0.1 \pm 0.1	0	0	0	0
Mollusca	<i>Tricolia pullus canarica</i>	0	0	0	0	0	0.1 \pm 0.1
Mollusca	<i>Turbonilla lactea</i>	0	0	0	0	0.1 \pm 0.1	0
Mollusca	<i>Venerupis senegalensis</i>	0	0	0	0.2 \pm 0.2	0.2 \pm 0.2	0.3 \pm 0.3
Nemertea	<i>Nemertea</i> sp1	0	0	0.8 \pm 0.4	0.6 \pm 0.5	0.8 \pm 0.5	0.8 \pm 0.7
Nemertea	<i>Nemertea</i> sp2.	0	0	0.1 \pm 0.1	0	0	0
Oligochaeta	<i>Grania</i> sp.	3.8 \pm 2.5	0	0	4.8 \pm 1.2	5.4 \pm 2.3	32.4 \pm 13.9
Oligochaeta	<i>Tubificidae</i> sp1.	2.5 \pm 1.3	0.1 \pm 0.1	0	0	0	0
Ostracoda	<i>Cypridina mediterranea</i>	0.3 \pm 0.1	0.5 \pm 0.2	1.0 \pm 0.3	0.3 \pm 0.2	0	0.1 \pm 0.1
Ostracoda	<i>Cypridina norvergica</i>	0.1 \pm 0.1	0.1 \pm 0.1	1.1 \pm 0.9	0.1 \pm 0.1	0.1 \pm 0.1	0.6 \pm 0.5
Polychaeta	<i>Aonides oxycephala</i>	0.1 \pm 0.1	0.1 \pm 0.1	0	0.7 \pm 0.2	0.6 \pm 0.3	0
Polychaeta	<i>Aponuphis bilineata</i>	0.3 \pm 0.2	0.2 \pm 0.2	0.3 \pm 0.2	0.1 \pm 0.1	0	0
Polychaeta	<i>Capitomastus minimus</i>	0	0	0	1.4 \pm 1.1	0.6 \pm 0.5	0
Polychaeta	<i>Demonax brachychona</i>	0	0	0.3 \pm 0.2	0	0	0
Polychaeta	<i>Desdemona</i> sp.	0	0	0	0.8 \pm 0.5	3.2 \pm 1.9	0.7 \pm 0.6
Polychaeta	<i>Euclymene palermitana</i>	0	0	0	0.1 \pm 0.1	0.1 \pm 0.1	0
Polychaeta	<i>Glycera dayi</i>	0.3 \pm 0.1	0	0	0	0.1 \pm 0.1	0.2 \pm 0.2
Polychaeta	<i>Glycera</i> sp.	0.1 \pm 0.1	0	0	0	0	0
Polychaeta	<i>Lumbrineris cingulata</i>	0.1 \pm 0.1	0	0	0	0.1 \pm 0.1	0
Polychaeta	<i>Maldanidae</i> sp1	0.1 \pm 0.1	0.2 \pm 0.2	0.6 \pm 0.4	0.1 \pm 0.1	0	0
Polychaeta	<i>Nematonereis unicornis</i>	0.1 \pm 0.1	0.1 \pm 0.1	0	0	0	0
Polychaeta	<i>Nephtys caeca</i>	0.4 \pm 0.2	0.7 \pm 0.5	2.3 \pm 1.6	0	0	0
Polychaeta	<i>Nephtys cirrosa</i>	0	0	0	0.7 \pm 0.5	0.1 \pm 0.1	0
Polychaeta	<i>Onuphis eremita</i>	0	0	0.1	0	0	0
Polychaeta	<i>Owenia fusiformis</i>	0	0	0.2 \pm 0.1	0	0	0
Polychaeta	<i>Periquesta canariensis</i>	0.5 \pm 0.4	0.9 \pm 0.7	0.4 \pm 0.3	0.7 \pm 0.6	0.3 \pm 0.2	0.1 \pm 0.1
Polychaeta	<i>Pista maculata</i>	0	0	0	0	0.1 \pm 0.1	0
Polychaeta	<i>Poecilochaetus serpens</i>	0	0.1 \pm 0.1	0.1 \pm 0.1	0	0	0
Polychaeta	<i>Prionospio steenstrupii</i>	0	0.2 \pm 0.2	0.1 \pm 0.1	0	0	0.1 \pm 0.1
Polychaeta	<i>Schistomeringos albomaculata</i>	0.3 \pm 0.3	0	0	0.2 \pm 0.2	0.1 \pm 0.1	0
Polychaeta	<i>Scolecopsis squamata</i>	0	0	0	0	0.1 \pm 0.1	0
Polychaeta	<i>Scoloplos (Leodamas)</i> sp.	1.6 \pm 1.2	1.8 \pm 1.4	1.0 \pm 0.9	0	0	0
Polychaeta	<i>Scoloplos armiger</i>	0.6 \pm 0.5	2.9 \pm 1.9	0.4 \pm 0.3	1.8 \pm 1.3	0.1 \pm 0.1	0
Polychaeta	<i>Sigalion squamatum</i>	0	1.1 \pm 0.9	0.7 \pm 0.3	0.3 \pm 0.2	0	0
Polychaeta	<i>Spio filicornis</i>	0.9 \pm 0.7	0.1 \pm 0.1	0.1 \pm 0.1	0	0	0.1 \pm 0.1
Polychaeta	<i>Streptosyllis bidentata</i>	0	0	0	0.1 \pm 0.1	0	0.4 \pm 0.3
Sipuncula	<i>Sipunculus nudus</i>	0	0	0	0	0.1	0
Tanaidacea	<i>Apseudes talpa</i>	0.1 \pm 0.1	0.1 \pm 0.1	0	0	0	0
Tanaidacea	<i>Leptocheilia dubia</i>	0	0.1 \pm 0.1	0	0	0	0

from the seabed were taken with sterilized bottles and pore water salinity was determined by a LF-95/SET conductivitymeter.

2.3. Identification of macrofauna

Samples were preserved in a 10% seawater formaldehyde solution, and subsequently decanted through a 0.5 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 with a LEICA DMLB microscope equipped with Nomarski interference.

2.4. Statistical analysis

Differences in macrofaunal assemblage structure sampled at different distances from the brine discharge point (i.e. distance: 0, 15 and 30 m) through both sampling dates (May 2008 vs. January 2009) were tested by means of a permutational MANOVA (PERMANOVA [4]) that included the factors: 'Distance' (fixed factor) and 'Year' (random factor, orthogonal to 'Distance'). Permutation-based ANOVA, using the same model but in a univariate context, tested for differences in total macrofaunal abundance and the abundance of the most abundant taxa (the amphipod *Ampelisca brevicornis*, the oligochaetes *Grania* sp. and Tubificidae sp1 and the polychaete *Scoloplos armiger*). Multivariate data were square root transformed to down-weight the most abundant taxa, and multivariate and univariate analyses were based on Bray–Curtis dissimilarities and Euclidean

distances, respectively. P-values were calculated from 4999 unrestricted permutations of the raw data. Despite variances remained heterogeneous in all univariate cases, we avoided an increase in a type I error by reducing the α value to a 0.01 level [40]; ANOVA is robust to such departures for balanced studies. Pairwise comparisons (using 4999 permutations) were used, when appropriate, to resolve differences among levels of significant factors. Rarefaction curves were calculated to compare patterns of both species abundance and species richness [22,39] located at different distances from the brine discharge point.

To visualize affinities in assemblage structure, an nm-MDS (non-metric multidimensional scaling) was carried out on square-rooted 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, [27]) tested whether variation in any of the measured abiotic variables significantly contributed to explain variation in the macrofaunal assemblage structure located at different distances from the brine discharge point. Multivariate multiple regression, using the DISTLM routine [4], then tested the significance of these relationships by fitting a linear model based on Bray–Curtis dissimilarities from square-rooted transformed abundance data. To retain variables with good explanatory power, as a result of collinearity among variables, the AIC routine was used as a selection criterion (the smaller the value the better the model, [27]). Analyses were based on a forward selection procedure. All multivariate procedures were carried out via the PRIMER v6 [11] and PERMANOVA + [5] statistical package.

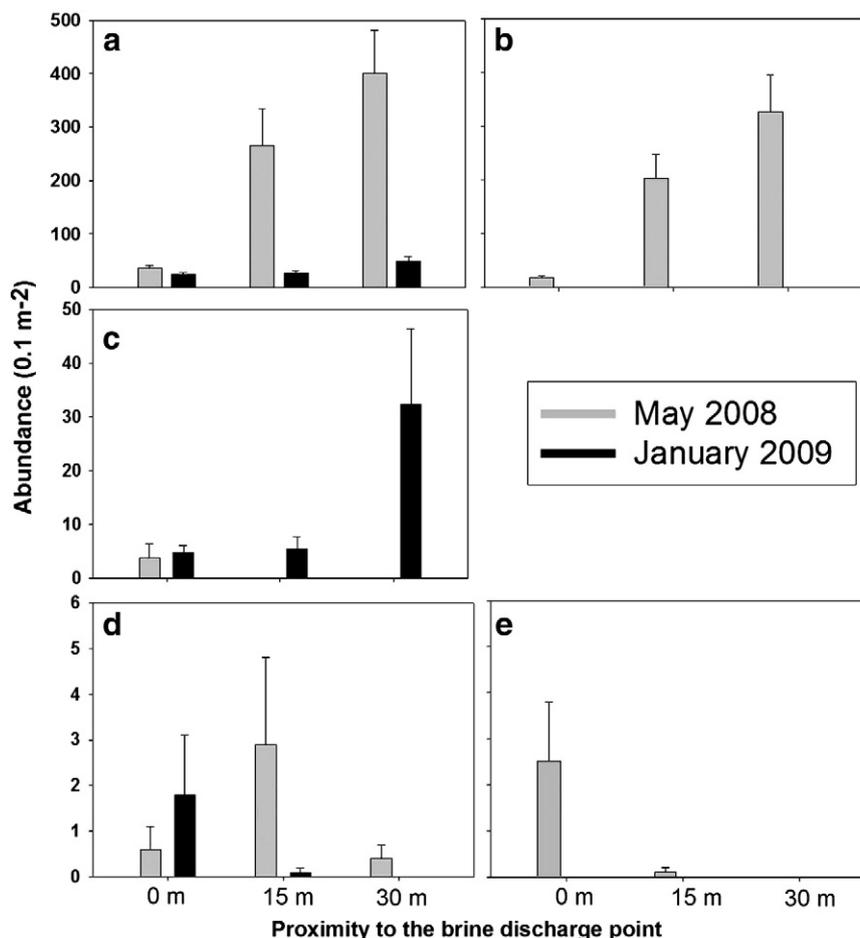


Fig. 2. (a) Overall macrofaunal abundance, and abundances of (b) *Ampelisca brevicornis*, (c) *Grania* sp., (d) *Scoloplos armiger*, and (e) Tubificidae sp1, located at different distances (0, 15 and 30 m) from the brine discharge point. Error bars are SE of means. Grey bars: May 2008; white bars: January 2009.

3. Results

A total of 6864 individuals were collected throughout the study, belonging to 13 broad taxonomic groups (Amphipoda, Cnidaria, Cumacea, Decapoda, Isopoda, Misidacea, Mollusca, Nemertea, Oligochaeta, Ostracoda, Polychaeta, Sipuncula and Tanaidacea) (Table 2). The most abundant groups were amphipods (5823 ind, 84.8% of the overall abundance), followed by oligochaetes (435 ind, 6.3%) and polychaetes (316 ind, 4.6%). In terms of species, the most abundant taxa were the amphipod *Ampelisca brevicornis* (5134 ind, 74.8% of the overall abundance), followed by the oligochaete *Grania* sp. (414 ind, 6%) and the amphipod *Bathyporeia elegans* (409 ind, 5.9%).

Differences in total macrofaunal abundance located at different distances from the brine discharge point were inconsistent between successive years (Fig. 2a, ANOVA: “Distance×Year”, $F=8.06$, $P=0.0016$, Table 3). The magnitude of these differences in total abundance with distance away from the brine discharge point was more accentuated in May 2008 than in January 2009 (Fig. 2a). Pairwise comparisons (Table 3) showed that macrofaunal abundances were higher at 15 m and 30 m away from the brine discharge point than at 0 m in May 2008, but not in January 2009. The most abundant species, the amphipod *Ampelisca brevicornis*, showed a similar pattern of abundance located at different distances from the brine discharge point as total macrofaunal abundance (Fig. 2b). Significantly higher abundances of *A. brevicornis* were observed in May 2008, but not in January 2009 (pairwise comparisons, Table 3), at 15 and 30 m away from the brine discharge point than at 0 m. The oligochaete *Grania* sp. (Fig. 2c) showed higher abundances at 30 m away from the brine discharge point than at 0 and 15 m in January 2009, but not in May 2008 (pairwise comparisons, Table 3). The polychaete *Scoloplos armiger* (Fig. 2d) showed higher abundances at 15 m than at 0 m and 30 m away from the brine discharge point in May 2008, while abundances were higher at 0 m than at 15 m and 30 m away from the brine discharge point in January 2009 (pairwise comparisons, Table 3). The oligochaete Tubificidae sp1 (Fig. 2e) showed higher abundances at 0 m than at 15 m and 30 m away from the brine discharge point exclusively in May 2008 (pairwise comparisons, Table 3).

Differences in macrofaunal assemblage structure located at different distances from the brine discharge point were inconsistent between successive years (PERMANOVA, “Distance×Year”, $F=6.09$, $P=0.0002$, Table 3). Proximity to the brine discharge point influenced patterns in macrofaunal assemblage structure in May 2008: assemblages at 0 m were different than assemblages at 15 and 30 m away from the brine discharge point, but assemblages at 15 m were not different than assemblages at 30 m away (Fig. 3; pairwise comparisons, Table 3). Proximity to the brine discharge point also influenced patterns in macrofaunal assemblage structure in January

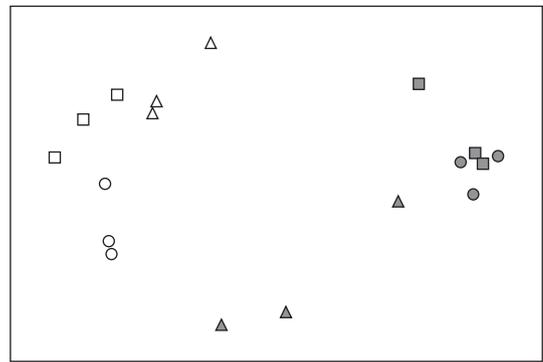


Fig. 3. Ordination plot (nm-MDS, stress = 0.14) showing similarities in macrofaunal assemblage structure located at different distances (0, 15 and 30 m) from the brine discharge point. Centroids for each distance are plotted. Triangles: 0 m; squares: 15 m; circles: 30 m. Grey: May 2008; white: January 2009.

2009: assemblages at 0 m were different than assemblages at 15 and 30 m away from the brine discharge point and assemblages at 15 m were different than assemblages at 30 m away (Fig. 3; pairwise comparisons, Table 3).

The macrofaunal species density (i.e. number of species per area) was higher at 15 and 30 m than at 0 m away from the brine discharge point (Fig. 4a). However, species richness (i.e. number of species per number of individuals) was higher at 0 m than at 15 and 30 m away from the brine discharge point (Fig. 4b), as a result of the higher abundance of individuals observed at 15 and 30 m relative to 0 m away from the brine discharge point (Fig. 2a).

The first two axes from the db-RDA explained a ca. 47.8% of the total variation in macrofaunal assemblage structure (Fig. 5). In the biplot of the first two db-RDA axes, the percentage of very fine sands was positively correlated with the first axis, which accumulated up to ca. 36% of total variability in macrofaunal assemblage structure. The second axis of the db-RDA was positively correlated with the salinity (Fig. 5). In turn, these two abiotic variables were selected as those mostly contributing to explain variation in macrofaunal assemblage structure (sequential tests in the multivariate multiple regression, Table 4), as a result of strong colinearity among several abiotic variables, principally among the percentages of the different granulometric fractions. Collections located 0 m away from the brine discharge point had similar particle size distribution irrespective of the sampling date (May 2008 vs. January 2009, Fig. 5). However, collections at 15 and 30 m away from the brine discharge point had different particle size distribution between both sampling dates (Fig. 5). These results indicate that, although variation in salinity located at different distances to the brine discharge point was a relevant driver

Table 3

Results of multi- and univariate ANOVA testing for differences in macrofaunal (multivariate) assemblage structure, overall macrofaunal abundance, and *Ampelisca brevicornis*, *Grania* sp., Tubificidae sp1 and *Scoloplos armiger* abundances located at different distances to the brine discharge point ('Distance', fixed factor) through years ('Year' random factor, orthogonal to 'Distance'). Significant differences ($p<0.01$) are highlighted in bold. Results of pairwise comparisons between distances away from the brine discharge point for each year are included as a result of a significant interaction between 'Distance' and 'Year'.

Source of variation	Assemblage structure				Overall macrofaunal abundance			<i>A. brevicornis</i> abundance			<i>Grania</i> sp. abundance			Tubificidae sp1 abundance			<i>S. armiger</i> abundance		
	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Distance (D)	2	11086	0.92	0.5764	338.5	1.88	0.341	298.74	0.99	0.4949	13.47	0.54	0.6538	1.91	1.00	0.5000	2.14	0.30	0.7616
Year (Y)	1	47837	24.23	0.0002	821.54	36.90	0.0002	1435.1	64.1	0.0002	100.39	58.97	0.0002	1.91	9.71	0.0004	1.57	5.12	0.0262
D×Y	2	12035	6.09	0.0002	179.54	8.06	0.0016	300.14	13.4	0.0002	24.57	14.93	0.0002	1.91	9.71	0.0002	7.09	23.05	0.0002
Residual	48	1974.3			22.26			22.38			1.70			0.91			0.30		
May 2008				0 ≠ 15			0 ≠ 15			0 ≠ 15			0 = 15			0 ≠ 15			0 ≠ 15
				0 ≠ 30			0 ≠ 30			0 ≠ 30			0 = 30			0 ≠ 30			0 = 30
				15 = 30			15 = 30			15 = 30			15 = 30			15 = 30			15 = 30
January 2009				0 ≠ 15			0 = 15			0 = 15			0 = 15			0 = 15			0 ≠ 15
				0 ≠ 30			0 ≠ 30			0 = 30			0 ≠ 30		n/a	0 ≠ 30			0 ≠ 30
				15 ≠ 30			15 ≠ 30			15 = 30			15 ≠ 30			15 = 30			15 = 30

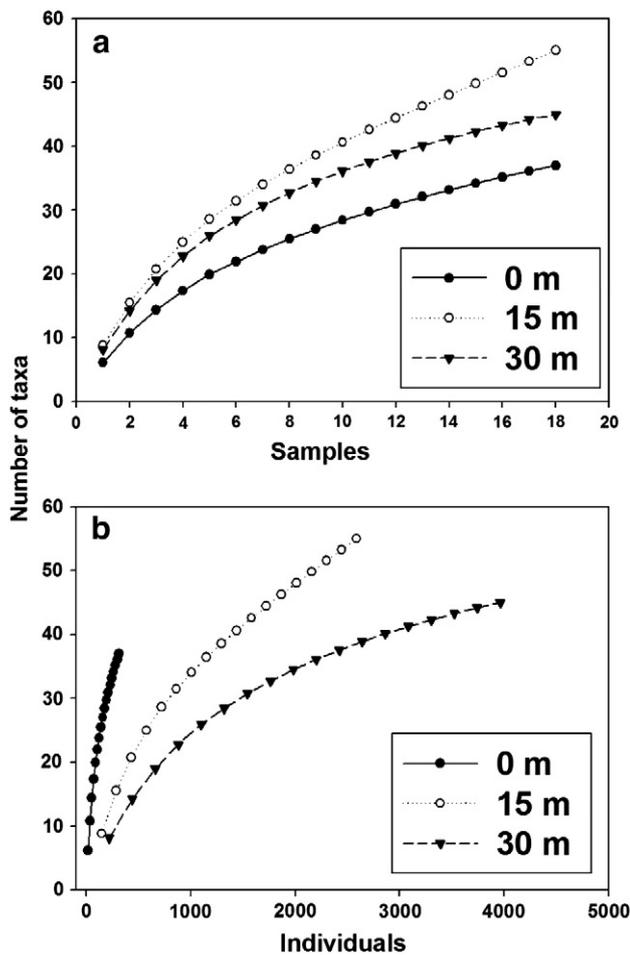


Fig. 4. (a) Patterns of species density and (b) species richness of macrofauna located at different distances from the brine discharge point (0, 15 and 30 m). Data from both sampling dates (May 2008 and January 2009) were pooled.

explaining patterns in assemblage structure, variation in sediment particle size distribution explained a large portion of the variability in the biota located at different distances from the brine discharge point.

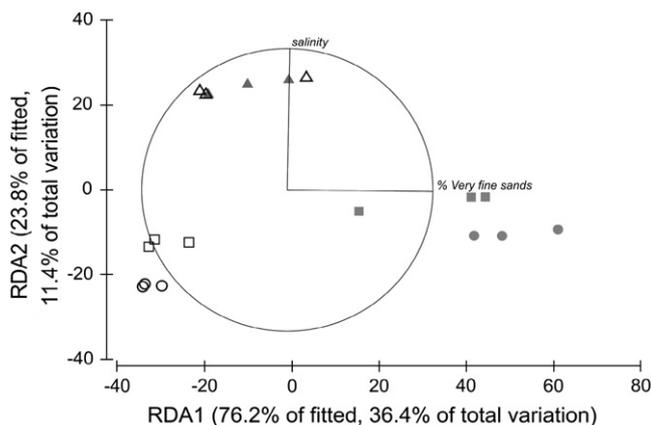


Fig. 5. Distance-based redundancy analysis (db-RDA) biplot of first and second axes relating those abiotic variables that affected significantly (see Table 4) the assemblage structure of macrofauna located at different distances to the brine discharge point. Centroids for each distance are plotted. Triangles: 0 m; squares: 15 m; circles: 30 m. Grey: May 2008; white: January 2009.

Table 4

Results of multivariate multiple regression testing the relationship between the measured set of abiotic variables (Table 1) and the macrofaunal assemblage structure. To retain variables with explanatory power, the AIC procedure was chosen as model selection criterion (sequential tests, [27]).

Variable	AIC	SS (trace)	Pseudo-F	P	Proportion of explained variation
+ % Very fine sands	139.15	18749	9.1404	0.0004	0.3636
+ Salinity	137.58	5913.4	3.2966	0.0006	0.1146

4. Discussion

Our results have demonstrated that soft-bottom macrobenthic assemblages are influenced by proximity to the brine discharge point associated with Las Burras desalination plant: significant differences were found in terms of abundance, assemblage structure and diversity of macrobenthic fauna. Despite shifts in particle size distribution might also have affected these results, this outcome highlights the possible effect of a change in salinity, from 45 at 0 m to 38–36 at 15 and 30 m away from the brine discharge point, respectively, as a mechanism explaining the observed patterns. Therefore, the driving effect on macrofauna was considerably localized (<30 m). An increase in salt concentration immediately adjacent to any brine outfall may result in a dehydration of cells, a decrease of turgor pressure and, ultimately, death of larvae and young marine invertebrates [17]. This conclusion would be straightforward without potential confounding effects caused by any environmental driver that could co-vary with proximity from the brine discharge point. Our analyses, however, identified a relevant change in the particle size distribution of samples collected at 15 and 30 m away from the brine discharge point between successive years. Hence, this fact may mask ultimate interpretations about the importance of brine disposal into recipient soft-bottom biological assemblages. Indeed, disentangling the separate contributions of both a change in salinity and particle size distribution located at different distances from the brine discharge point remains untested.

Differences in both assemblage structure and overall abundance patterns located at different distances from the brine discharge point were accentuated in May 2008. Soft bottoms at both 15 and 30 m away from the brine discharge point were constituted by a diverse and abundant macrofaunal assemblage, typical of subtidal soft bottoms dominated by fine sands in the study area [24]. In January 2009, however, sediments at 15 and 30 m away from the brine discharge point were predominantly dominated by coarse grained fractions, which probably helped to explain the decrease in overall abundances relative to those registered in May 2008. The degree of sand permeability and organic content is high when the particle size distribution is dominated by fine sediments. Soft-bottom fauna is then spatially limited to the upper cm of the sediments and mainly composed by numerous, small-sized taxa [29]. In contrast, where soft bottoms have a low quantity of fine sediments, the high oxygenation of the sand promote macrofaunal assemblages dominated by a few species that drive the assemblage structure [28]. Such a change in sediment composition between successive years, coupled with a slight increase in the sediment organic matter, may help out to explain the large abundances of the oligochaete *Grania* sp. at 15 m and 30 m away from the brine discharge point in January 2009. These granulometric changes between May 2008 and January 2009 might have been likely caused by differences in the magnitude of physical mechanisms, such as differences in swell height, that typically occur in the study region between summer (e.g. May 2008) and winter (e.g. January 2009), a period of often ca. 2–3 m swells, [3]).

The amphipod *Ampelisca brevicornis* dominated, in terms of overall abundance, the macrobenthic assemblage at 15 and 30 m away from the brine discharge point. This species is a common macrofaunal element of subtidal soft-bottoms across the Canarian Archipelago,

where it typically lives on soft bottoms dominated by fine sands with low organic content [36]. The large difference in the abundance of this species between successive years (May 2008 vs. January 2009) may be explained by a change in the particle size distribution, from fine-to-very fine sands in May 2008 to coarse and medium sands in January 2009 at 15 and 30 m away from the brine discharge point. Similar changes in the abundance of this crustacean were reported previously, as a result of shifts in the particle size distribution of sandy substrates [12].

The effect of brine discharges over recipient macrobenthic communities may change depending on the specific ecological peculiarities of each taxonomic group. In the Mediterranean, brine discharges have caused a replacement of natural macrobenthic assemblages dominated by polychaetes, crustaceans and molluscs, to an assemblage overwhelmingly dominated by macrofaunal-sized nematodes [14]. In our study, oligochaetes showed higher abundances at 0 m relative to both 15 and 30 m away from the brine discharge point in the first sampling date (May 2008). This fact could be explained by the large resilience of oligochaetes to environmental perturbations, such as brine [21], and their trophic affinities, mainly scavengers and detritivorous taxa, that allow oligochaetes to take advantage of decaying organisms [16,20].

In the study region, the environmental effects of brine discharges over native subtidal vegetation on shallow soft bottoms (*Cymodocea nodosa* and *Caulerpa* spp. meadows) have been previously observed to distances up to 20 m from the discharge point [33]. [37] showed a significant decrease of meiofaunal abundances immediately adjacent to a brine outfall, with an increase in overall meiofaunal abundances at 15 and 30 m away from the discharged point. Moreover, [37] observed that shifts on grain size composition could largely drive the meiofaunal community structure. Our results also showed that proximity to a brine disposal point may alter the ecological pattern of macrofauna within the same spatial range (0–30 m). Therefore, the effects of brine disposal on biological, recipient, assemblages seem to be localised, with a high degree of inter-annual differences in the study area. A change in salinity, coupled with a source of natural variability (e.g. sedimentary composition), seemed as a likely explanation to account for the observed ecological pattern.

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