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Submarine outfall effect on subtidal macrobenthic communities in a southwestern Atlantic coastal city

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A B S T R A C T

Submarine outfalls are an effective alternative for the final discharge of wastewater. The aim was to evaluate the subtidal macrobenthic community's responses and the changes in bottom sedimentary dynamics due to submarine outfall (SO) location. Sampling stages were: before SO (B_{SO}), after SO (A_{SO}) and after treatment plant (A_{EDAR}). Sampling sites were determined at different distances from the coastline (coastal, oceanic, and reference) on both sides of the pipe (North and South). Species shifts (from tolerant to sensitive) were observed along with a decrease in organic matter in the A_{EDAR} Stage. There were changes in the sedimentary dynamic with sediment accumulation on the South side of the SO (finest sediments) and erosion on the North side (coarsest sediments) in the A_{SO} and A_{EDAR} Stages. Species turnover was higher than nesting in all stages. Functional trait analysis allowed the identification of temporal variations in benthic communities. The body size, development mode, feeding mode, habit, adult mobility and tolerance to pollution were useful functional traits to detect changes through Stages (B_{SO}, A_{SO}, and A_{EDAR}). Biotic indices classified the sites as slightly disturbed, indicating a slight improvement in the A_{EDAR} Stage.

1. Introduction

Coastal cities are affected by a combination of industrial and domestic pollution. This exerts significant pressure on the environment and the organisms that inhabit it, compromising the health of these ecosystems and the health of their services [1]. For this reason, the discharge of untreated wastewater alters the physicochemical properties of coastal waters and can be polluted the marine environment [2,3]. Submarine outfalls (SO) are an efficient alternative for wastewater discharge due to their high dispersal capacity in the marine environment through dilution. This capacity lies in the energy available in the marine environment due to the action of ocean currents in the dispersion of effluents, the availability of dissolved oxygen, and because it is a hostile environment for the survival of microorganisms [4].

According to the literature, there are more than 500 coastal or ocean outfalls discharging wastewater to the sea worldwide [5] and

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more than 130 SO with a length greater than 500 m in Latin America [6]. Puente and Diaz [7] evaluated the effects of 40 SO distributed worldwide on the surrounding macrobenthic invertebrates, and found that the probability of a significant impact on the community was much lower than expected by the Pearson-Rosenberg model (describing the response of benthos to a gradient of organic enrichment). However, there are no long-term studies in the literature that evaluate the effects of a SO on the subtidal benthic community, considering the periods before and after SO construction and start-up.

From 1989 until December 2014, wastewater from the city of Mar del Plata was discharged directly into the sea with pre-treatment which carried out screening and aeration of the sewage liquid [8]. In 2008, the construction of the SO began, starting with the incorporation of the breakwaters (North and South) and a mooring front. On December 2014, the SO of Mar del Plata city was officially inaugurated. The SO consists of a 2 m diameter, 4 km long pipe, plus a 540 m diffuser section with 130 nozzles (or diffusers), allowing up to 9 m^3/sec. of pre-treated sewage effluent to be discharged directly onto the subtidal. In addition, this facility was complemented by the execution of the new Wastewater Treatment Plant (EDAR, its acronym in Spanish) officially inaugurated on August 2018, which replaced the old pre-treatment plant. The EDAR plant performs a primary treatment of wastewater. A few studies were conducted in subtidal benthic communities in front of this locality; the first was conducted with semi-quantitative data and in depths greater than 12 m [9]. To have baseline data about the benthic communities in the zone where the submarine outfall will be constructed (around 11 m depth) another study also with semi-quantitative data was performed [10]. Finally, Elías et al. [11,12] carried out a quantitative sampling in the area that would be affected by the future submarine outfall to have baseline data.

The EDAR was designed for global coverage of 1,800,000 inhabitants, it performs the extraction and management of solids, sands, fats, and oils from the sewage effluent, before being disposed of by the submarine outfall in the sea. The plant includes rotary screens, solids conveyor screws, sand classifiers/washers, and solids compactors. All this is complemented by a desander-degreaser with a grease-sweeping bridge and sand extraction and a biological filtering system useful for separating the wet chambers from the closed dry enclosures, ultimately facilitating the preservation of air quality [13].

The effects of human-induced pollution can be assessed indirectly from the responses of the biota. In this context, ecological indicators are ecosystem elements, processes, or properties that represent environmental conditions that cannot be measured directly for technical or logistical reasons [14–17]. They are commonly used to provide synoptic information on the state and integrity of ecosystems [18,19]. These indicators are chosen because they are easy to measure, susceptible to a certain type of impact, and respond to stress unambiguously and predictably [15,20]. The use of benthic invertebrates as indicators of environmental quality and condition has several advantages: due to their low or no mobility, they are more susceptible to local physical and chemical disturbances; furthermore, the benthic associations they constitute include diverse species that exhibit different degrees of tolerance to stress. Indicators integrate recent disturbance history, which may not be detected in other biological compartments, such as pelagic communities [16,18,19,21,22]. Environmental quality indicators can be divided into three broad categories: (1) characteristic or indicator species, (2) univariate indices (e.g., abundance, density, etc.), and (3) multimetric indices (e.g., richness, diversity, etc.) [22,23].

Traditional studies based on uni- or multivariate analysis and taxonomic indices have been used to study species-environment relationships [24–30]. These studies may have accurately described the community, but they fail to capture the causal mechanism underlying the species-environment relationship [31,32]. An important tool to describe the behavior of an ecosystem in response to disturbance is functional diversity [25–28]. Functional diversity is the component of diversity that influences dynamics, stability, productivity, nutrient balance, and other aspects of ecosystem functioning [33], allowing assessment of how organisms affect ecosystem properties/processes [34] and which environmental factors and perturbations shape the diversity and distribution of functional traits of the assemblage over space and time [35]. Therefore, to provide a complete description of the response of ecosystem functioning to environmental factors and gradients, the taxonomic analysis must be combined with functional analysis [24,29,36].

Previous studies on the subtidal macrobenthic communities in the city of Mar del Plata [10–12], showed the presence of indicator organisms such as the polychaetes *Caulleriella trispina*, *Glycera* sp., *Owenia* sp., *Prionospio* sp., the tanaidacean *Monokalliapseudes schubarti*, the gastropod *Notocochlis isabelleana*, the amphipod *Melita* sp., among others. In addition, these studies showed that there is a predominance of small fauna and a great spatial heterogeneity, principally related to the hydrodynamic and sedimentological conditions of the area.

The coastal area of the city of Mar del Plata is one of the sectors of the province of Buenos Aires where the processes and effects of coastal erosion are present. Erosion is principally originated by storms (called "sudestada" due to the origin of the winds of the southeast) and the successive obstructions of the sedimentary dynamics caused by urbanization and coastal defense works [37]. A construction site of the magnitude of the SO, resting on the shallow seabed, would be affected by sandstorms and the differential loading of accumulated/eroded sand on both sides because interrupted the flow of transported sediments by the littoral current [38]. Storms at sea can impact the ocean floor to depths of more than 20 m [12,39,40], while the Mar del Plata SO has its deepest point at 11 m. Thus, the placement of the SO would modify the normal (South to North) flow of coastal waters causing significant changes in the sediment dynamics of the area, increasing the progressive accumulation of sediment on the South side of the outfall and erosion on the North side, leading to changes in the structure and function of the benthic communities. Therefore, the present study aims to assess the response of the macrobenthic community to sewage pollution and variation in bottom sediment dynamics in three periods before SO, after SO, and after EDAR start-up. This objective is approached through alpha diversity (based on richness, evenness, and diversity); beta diversity (divided into turnover and nesting) and functional diversity (based on biological traits and functional diversity indices). The ecological status is also assessed using the AMBI and M-AMBI environmental quality indices.

2. Material and methods

2.1. Study area

The study was carried out in front of the city of Mar del Plata (38° S, 57° 33″ W), Province of Buenos Aires, Argentina (Fig. 1), from the 5 m isobath to approximately 14 m depth. Winds are predominantly from the west, southwest in winter and northwest in summer. The climate is typically temperate marine, with regular rainfall (850 mm/year). A strong and constant coastal current, from South to North, affects the coast, as well as winter storms from the South-southeast [38]. Oceanographically, the area in front of the city of Mar del Plata is characterized by the presence of continental shelf residual waters, with temperatures between 8 and 21 °C and salinities of 33.5 and 33.8 [41]. Biogeographically, the region is temperate-warm and transitional between the Subantarctic region (Patagonia) and the Subtropical region (southern Brazil) [42].

The sea bottom in front of the city of Mar del Plata is characterized by fine to very fine sandy sediments near the coast and medium and coarse sediments around the discharge, with a variable proportion of shell remains. The sediment distribution pattern is patchy, due to tidal currents and frequent storms in the fall and winter months [11,38].

2.2. Sampling design and field and laboratory routines

Samples were taken at 3 different Stages: before SO (B_{SO} = April/May 1999), after SO (A_{SO} = April/May 2018) and after EDAR start-up (A_{EDAR} = December 2018). In 1999, a baseline study was carried out, taking 49 stations distributed regularly from shore to 5 km offshore and 3 km at each side of the hypothetical SO [see 11 for more details]. For this study, a subset of stations was selected to coincide with the 2018 stations to use as Factor before SO. From here and in the rest of the text the Stages will be referred to as B_{SO} (before SO), A_{SO} (after SO), and A_{EDAR} (after EDAR). Sampling stations were distributed in two zones: a) coastal (600 m from the coast) and b) oceanic (4,000 m) (discharge of SO) on each side of SO (North and South). In addition, two reference stations (R) were located on both sides of the SO (Fig. 1).

Samplings were carried out on board of ARA Luisito fishery training ship. Macrobenthic macrofauna and sediments samples were collected with Van Veen (0.05 m^2) and Day (0.1 m^2) grabs in 1999 and 2018, respectively. At every sampling Stage (B_{SO}, A_{SO}, A_{EDAR}), 4 to 8 (minimum-maximum) replicates were collected at each sampling station, depending on the climatic and meteorological conditions.

In addition, a sediment sample was collected with the same grab used for macrobenthic macrofauna. The half-sediment sample was kept in a plastic bag for laboratory determination of the organic matter content. The other half was kept for analysis of granulometry. The determination of organic matter content in the sediment samples was performed through two different methods: the titration

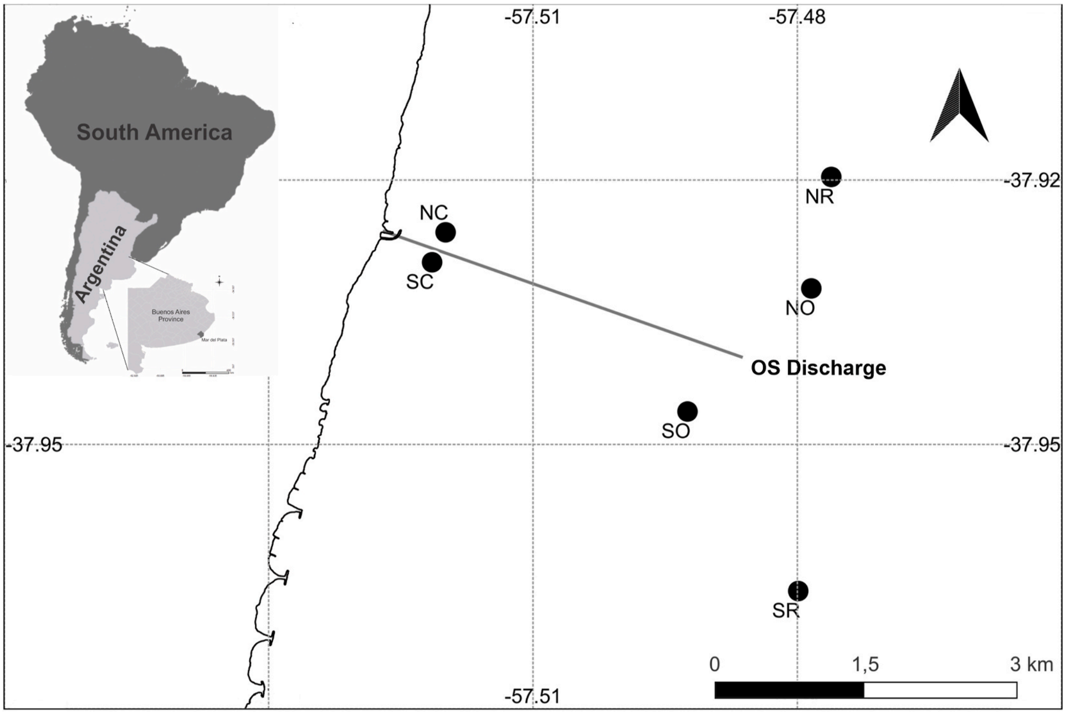

Fig. 1. Location of the Mar del Plata submarine outfall in the Province of Buenos Aires, Argentina. Location of the sampling stations: SC (South-coastal); NC (North-coastal); SO (South-oceanic); NO (North-oceanic); SR (South-reference) and NR (North-reference).

method [43] in 1999 and the loss on ignition (LOI) method, burning the dry sediment for 4 h at 550 °C [44] in 2018. For granulometry, the processing of the sediment samples (by dry sieving) was carried out at the laboratory of the *Instituto de Geología de Costas y del Cuaternario* of Mar del Plata. The sands were classified from very fine to very coarse according to granulometric parameters in the table of phi units (-$\log_2$ of the particle diameter) [38]. Biological samples were sieved on board (0.5 mm) and the retained material (macrobenthos) was fixed in a 7% neutralized formalin solution. Once in the laboratory, each sieved sample was separated and the organisms were identified through a stereomicroscope to the lowest possible taxonomic level. This laboratory work was carried out in the Laboratory of *Bioindicadores Bentónicos* of *Instituto de Investigaciones Marinas y Costeras* (IIMyC).

Moreover, data on the water column (pH and dissolved oxygen) were collected in each station with a multiparametric equipment, AQUAREAD AP-5000. In addition, water samples for microbiological analysis (*Enterococcus* concentration, NMP/100 ml) were collected, but only for the A_{so} and A_{EDAR} Stages. The determination of *Enterococcus* concentration was carried out by the laboratory of the *Universidad Tecnológica Nacional* (UTN).

2.3. Data analysis

2.3.1. Multivariate analysis

Density (n° individuals/m^2) was calculated for each unit sample, due to the different samplers used.

nMDS (non-metric multidimensional scaling) ordination plot was raised out to visualize differences in the species assemblage according to Stage (B_{SO}, A_{SO}, A_{EDAR}) and Distance to the coast (coastal, oceanic, and reference). For this purpose, the similarity matrix was calculated according to the Bray-Curtis index after a 4th-root transformation [45]. Moreover, two ANOSIM (analysis of similarities) [46] analyses were performed to determine if there are differences in the composition of the species assemblage for each factor (Stage, Distance, and Side). A two-way crossed ANOSIM test was used to examine the differences between the factors Stage (B_{SO}, A_{SO}, A_{EDAR}) and Distance (coastal, oceanic, and reference). Another ANOSIM was performed with Stages A_{SO} and A_{EDAR} (due to no presence of the pipeline in Stage B_{SO}) between Distance (coastal, oceanic and reference) and Side (North and South). The relative contribution of the species to the dissimilarity of the groups was analyzed with a SIMPER analysis (Similarity Percentage Analysis). All analyses were performed with R statistical software (v4.1.0) [47].

2.3.2. Alpha diversity

Alpha diversity metrics such us richness (S), Pielou evenness (J') [48], and Shannon-Wiener diversity (H') [49] were calculated for each case (unit sample) from a density data matrix. Community parameters were calculated with the DIVERSE routine of R statistical software (v4.1.0) [47]. The spatial and temporal variability of the above community parameters was assessed by two two-way ANOVA, previously the data were transformed to $\log_{10}$. An ANOVA was performed with the following fixed factors; Stage with three levels (B_{SO}, A_{SO}, and A_{EDAR}) and Distance with three levels (i.e., coastal, oceanic, and reference). For another ANOVA, the following fixed factors were considered: Side with two levels (i.e., North and South) and Distance with three levels (coastal, oceanic, and reference). For the latter, Stage B_{SO} was excluded because there was no presence of the pipeline. When assumptions of normality and homogeneity could not be met, the non-parametric Kruskal-Wallis test was applied. ANOVA and Kruskal-Wallis analyses were performed using R statistical software (v4.1.0) [47].

Table 1
List of biological traits and respective categories.

Biological traits	Trait categories	Labels
Maximum size	Very small (<1 cm)	SVS
	Small (1–3 cm)	SS
	Medium (>3 cm)	SM
	Large (>10 cm)	SL
Development mode	Indirect	ID
	Direct	DD
Feeding mode	Deposit-feeder	FD
	Filter/suspension feeder	FF
	Opportunist/scavenger	FO
	Grazer	FG
	Predator	FP
Living habit	Burrow dweller	HB
	Attached	HA
	Tube dweller	HT
	Free living	HF
Relative adult mobility	None	MN
	Low	ML
	Medium	MM
	High	MH
Tolerance to pollution	1st order opportunistic	V
	2nd order opportunistic	IV
	Tolerant	III
	Indifferent	II
	Sensitive	I

2.3.3. Beta diversity partitioning

Beta diversity (β) and its components (turnover and nestedness, based on Simpson's dissimilarity) were evaluated according to the dissimilarities detected between the different Distances (coastal, oceanic, and reference sites) during the three Stages (B_{SO}, A_{SO}, A_{EDAR}). Furthermore, β and its components (turnover and nestedness) were evaluated according to Distance from the coast (coastal, oceanic, and reference stations) and North or South Side, during two Stages (A_{SO} and A_{EDAR}). Sørensen's dissimilarity index was used as a measure of overall β and was calculated for each pair of stations, considered comparisons of the species composition present at each station within each Stage. β analyses were performed using R statistical software (v4.1.0) [47].

2.3.4. Biological traits analysis (BTA)

In order to conduct a biological traits analysis (BTA), a bibliographic survey was performed to assign trait information to the identified species. The BTA was used to evaluate the variation of the composition of macrofaunal functional traits between the Stages (B_{so}, A_{so}, A_{EDAR}), the Distance (oceanic, coastal and reference) and the Side (North and South). Only the more representative species obtained from the SIMPER analysis were used to perform the BTA (60% cumulative contribution) [50,51]. Six functional traits was selected, divided into several categories that best represent aspects of the life history, morphology, and behavior of each species: maximum size, feeding mode, relative adult mobility, developmental mode, life habitat, and tolerance to pollution (Table 1). Biological and functional trait information has been collected from a variety of sources: identification guides, scientific journals, research papers and reports, including their appendices, and web databases such as MarLIN BIOTIC – Biological Traits Information Catalogue: http://www.marlin.ac.uk/biotic/, WORMS – World Register of Marine Species: http://www.marinespecies.or0 and SeaLifeBase: http://www.sealifebase.org.

When reliable information was not available, expert opinion and data from the closest phylogenetically related species were used, followed by the 'fuzzy coding' method, where each taxon was classified according to its affinity to different modes (i.e. trait categories) of functional traits [52]. Each functional trait modality was scored between 0 and 3. No affinity for a trait was coded as 0 and complete affinity as 3. Scores for each trait were assigned taking into account the adult form of the species. The trait scores for each taxon were multiplied by their abundance in each sample and then summed to obtain a matrix of the total frequency of each trait category per sample. The total frequency of each trait category was analyzed using two Principal Component Analyses (PCA). In these analyses, the relationship between sampling stations with the factors Stage and Distance, and on the other hand, the relationship with the factors Distance and Side, but only in Stages after SO (A_{SO} and A_{EDAR}), was explored for each biological trait. Sampling units from the same sampling station were averaged to facilitate understanding of the plot generated. The analysis was performed with the statistical software R (v4.1.0) [47].

2.3.5. Functional diversity analysis

To assess the different components of the functional diversity (FD), the following indices were calculated: functional richness (FRic), functional evenness (FEve), functional divergence (FDiv) and functional dispersion (FDis) [53,54]. These indices were selected as they provide adequate and complementary information on functional diversity components for different assemblages [54,55]. Functional diversity indices were calculated based on a matrix of Jaccard distances of species functional traits with the FDiversity software [56]. All metrics were calculated per replicate. To evaluate the spatial and temporal differences in the functional indices two analysis of variance (two-way ANOVA) was performed. A two-way ANOVA was developed considering the Stages and Distance as fixed factors and their interaction. Another two-way ANOVA was made using the Distance and Side as fixed factors and their interaction. The Shapiro-Wilk test was done to evaluate the normality of the data and Cochran's test was used to test the homogeneity of variances. When the data didn't meet the assumptions even after transformations, a non-parametric test was used [57]. The acceptable level of statistical significance used was $p < 0.05$. All analyses were performed using R statistical software (v4.1.0) [47].

2.3.6. Environmental variables

Environmental variables data were compared in a table.

2.3.7. Ecological status

To assess the environmental status of the study area in each Stage (B_{SO}, A_{SO}, and A_{EDAR}), two biotic indices were used: AMBI and M-AMBI. The index values were calculated using the software available on AZTI's webpage (http://ambi.azti.es). The AMBI index is based on the percentage of the abundance of five ecological groups according to their sensitivity to organic pollution, already listed in the software [58,59]. Most of the species found in this study are listed in the AMBI software package. However, the assignment of some species such as *Balanus* sp., *Cyrtograpsus altimanus*, *Eucallista purpurata*, *Mactra isabelleana*, *Notocochlis isabelleana* among others, was based on local studies [10–12,60]. The M-AMBI index was calculated by the factorial analysis of AMBI, richness (as the number of taxa), and Shannon–Wiener diversity values [61–63]. The two evaluated indices have different ranges and scales to determine the environmental quality of the studied sites. Indices values were calculated for each replicate, and their ecological status was therefore attributed as High, Good, Moderate, Poor, and Bad according to these differing scales.

3. Results

A total of 125 taxa were identified and quantified in this study, 49 of which belongs to Phylum Annelida, Class Polychaeta and 41 to Phylum Arthropoda. Several taxa of mollusks and smaller numbers of nemerteans, nematodes, cnidarians, echinoderms, and chordates were also found. The full list of identified taxa is included in the Supplementary Material (Table S1). Of the total number of identified taxa (N = 125), 64% were main taxa, 32% were rare taxa (density ≤100 ind. m^2) and 4% singletons taxa (density ≤10 ind. m^2). A total of 63 species (or taxa) were not present in the B_{SO} and appeared during the A_{SO} and A_{EDAR}, while only 30 taxa were present earlier (B_{SO}) and disappeared towards the A_{EDAR} Stage. On the other hand, 20 taxa were present only in the A_{so} Stage and 15 taxa only in the A_{EDAR} Stage (see Table S1).

3.1. Multivariate analysis

In the nMDS non-multidimensional space, two groups are observed, one belonging to Stage B_{SO} and another belonging to the Stage after the SO (A_{SO} and A_{EDAR}). In addition, other subgroups can be observed: the coastal zones before the outfall (B_{SO}), the Reference sites after the SO (A_{SO}) and the EDAR plant (A_{EDAR}) (Fig. 2).

Analysis of similarity (ANOSIM) showed significant differences both between Stages (Global R = 0.6; $p = 0.001$; 9999 permutations) and between Distance (R = 0.44; $p = 0.001$). Pairwise comparisons showed significant differences between all Stages (B_{SO} *vs.* A_{SO}: R = 0.69; $p = 0.001$; B_{SO} *vs.* A_{EDAR}: R = 0.70, $p = 0.001$; A_{SO} *vs.* A_{EDAR}: R = 0.41, $p = 0.005$; coastal vs. oceanic: R = 0.58, $p = 0.001$; coastal *vs.* reference: R = 0.60, $p = 0.001$), but not for oceanic vs. reference (R = 0.00, $p = 0.405$).

The SIMPER routine identified which species were responsible for the differences between Stages at different Distances from the coast (Tables S2 and S3). Species contributions to these differences between Stage and Distance were <20%. Between the Stage B_{SO} and A_{SO} in the coastal group, the differences are mainly given by the species *Prionospio* sp., *Magelona* sp., and *Monokalliapseudes schubarti*, showing higher abundance towards Stage A_{SO}. The oceanic group showed differences between both Stages (B_{SO} *vs.* A_{SO}) for the species *Axiothella* sp. with a higher abundance towards Stage A_{SO} (Supplementary Material, Table S2).

The species that most contributed to the differences within Stage B_{SO} between the coastal and oceanic groups were one species of the Spionidae, *Bathyporeia* sp., and *Notocochlis isabelleana*, the latter being more abundant in the coastal group (Table S2). In contrast, the species that most contributed to the differentiation between the coastal and oceanic groups in Stage A_{SO} were *Axiothella* sp., *Prionospio* sp., and *Magelona* sp., being the first more abundant in the oceanic group and the other two in the coastal group. In Stage A_{EDAR}, *Prionospio* sp., and *Magelona* sp. were also more abundant in the coastal group, but in the oceanic group, the species that most contributed was *Eucallista purpurata* (Table S2). Finally, the species that most contributed to the differentiation between Stages A_{SO} and A_{EDAR} in the oceanic group were *Axiothella* sp., *Prionospio* sp., and *E. purpurata*. The first two species having the highest abundance towards Stage A_{SO} and *E. purpurata* having the highest mean abundance towards Stage A_{EDAR} (Table S2).

In the other ANOSIM, significant differences were only observed within the Distance factor (Global R = 0.06, $p = 0.001$). The

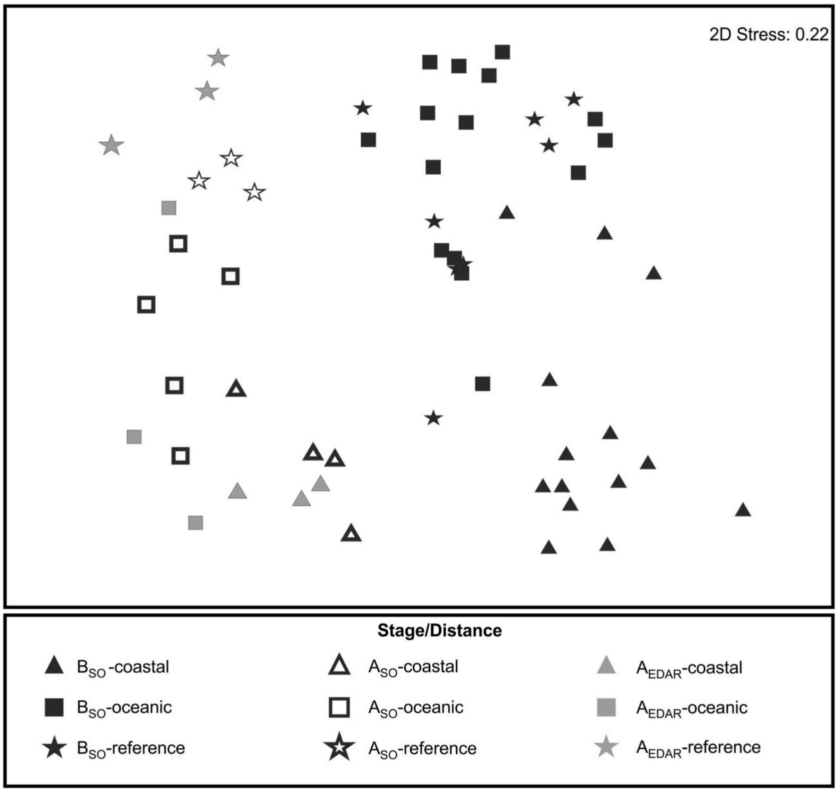

Fig. 2. Non-Multidimensional ranking method (nMDS) of the sample units considering the factor Distance to the shore (coastal, oceanic, and reference site) and Stages (before SO (B_{SO}), after SO (A_{SO}) and after EDAR plant (A_{EDAR})).

species that most contributed to the dissimilarity between the coastal and oceanic zones in the Stages A_{SO} and A_{EDAR} were *Prionospio* sp., *Magelona* sp., and *Axiothella* sp., are the first two most abundant in the coastal group and the last one in the oceanic group. No significant differences were found between the North and South sides (Global R = 0.19; $p = 0.380$). However, the SIMPER routine showed different species contributions on both sides of the SO (Table S3). The species *Melita* sp., and *E. purpurata* have a higher contribution to the South side of the SO, whereas, on the North side, *Prionospio* sp., *Magelona* sp., and *Axiothella* sp. have a greater contribution to the dissimilarity in the species assemblage (Supplementary Material, Table S3).

3.2. Alpha diversity

For the richness, the ANOVA detected a significant interaction between the factors Stage and Distance (F = 2.87, $p = 0.03$). This variable was lower during Stage B_{SO} and A_{EDAR} in all three zones (coastal, oceanic, and reference site), compared to Stage A_{SO}, where richness increased at all three distances (Fig. 3A). Richness was also higher in the oceanic zones B_{SO} and A_{EDAR} compared to the reference stations. On the other hand, richness showed relatively lower values in coastal and oceanic areas compared to the reference site, at A_{SO} Stage (Fig. 3A). For the diversity variable, the ANOVA detected a significant interaction between Stage and Distance factors

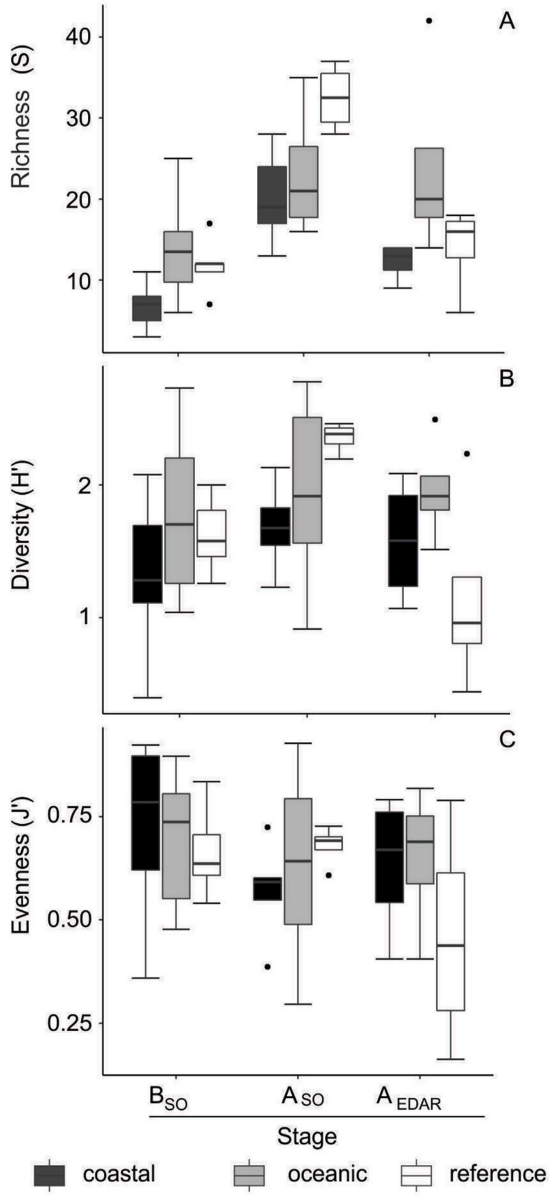

Fig. 3. Spatio-temporal variation of (A) richness (S), (B) diversity (H′), and (C) evenness (J′) according to Distance from the coast (coastal, oceanic, and reference) in each Stage: before the SO (B_{SO}), after the SO (A_{SO}), and after start-up of the EDAR plant (A_{EDAR}).

(F = 2.59, $p = 0.04$). At Stage B_{SO}, diversity was lower in the coastal zones compared to the A_{SO} and A_{EDAR} Stages. The same pattern is observed for the oceanic zones (Fig. 3B). Finally, for the variable evenness, the Kruskal Wallis Pielou's index (J′) test also showed significant differences between the three Stages (H = 7.11; $p = 0.02$); showing a decrease during Stages A_{SO} and A_{EDAR} (Fig. 3C). However, it did not show significant differences concerning Distances (J' = 1.72; $p = 0.63$).

Regarding the ANOVA performed with the Side and Distance factors, the richness had significant differences for the factor Side (F = 7.3790; $p = 0.01$) being higher on the North side than on the South side after SO (Fig. 4); however, the ANOVA did not detect significant differences for the factor Distance (F = 2.24; $p = 0.12$). On the other hand, for evenness, the ANOVA did not detect significant differences for either of the two factors Side (F = 0.46; $p = 0.50$) and Distance (F = 0.25; $p = 0.76$); finally, for diversity, the Kruskal Wallis test did not detect significant differences for either of the two factors, Side (H = 0.01; $p = 0.92$) and Distance (H = 1.83; $p = 0.39$).

3.3. Beta diversity

A similar trend in β was observed between B_{SO} and A_{SO} according to Distance from the coast (oceanic, coastal, and reference site). Higher values of β were observed in oceanic zones (OB_{SO}-OA_{SO}) and reference sites (RB_{SO}-RA_{SO}) (β = 0.57 and β = 0.58, respectively), while low values of β were observed in oceanic zones (OA_{SO}-OA_{EDAR} and reference sites (RA_{SO}-RA_{EDAR}) during Stages A_{SO} and A_{EDAR} (β = 0.40 and β = 0.45, respectively) (Fig. 5a). As for the components resulting from the beta diversity partitioning, species turnover dominated over nestedness in the different Stages (B_{SO}, A_{SO}, and A_{EDAR}). The highest values of species turnover were observed in the oceanic zone between Stages B_{SO} and A_{SO} (OB_{SO}-OA_{SO}) and the highest values of nestedness in the coastal zone and reference sites (CA_{SO}-CA_{EDAR}) between Stages A_{SO} and A_{EDAR} (Fig. 5a).

On the other hand, β components on both Sides of the SO between A_{SO} and A_{EDAR} presented different patterns (Fig. 5b). The highest values of nestedness and lowest values of turnover were recorded in the coastal zones (CNA_{SO}-CNA_{EDAR}) and reference sites (RNA_{SO}-RNA_{EDAR}) on North side. An opposite pattern was found in the coastal zones (CSA_{SO} -CSA_{EDAR}) and reference sites (RSA_{SO} -RSA_{EDAR}) on South side. The oceanic zones presented a similar pattern on both Sides of the outfall (North and South) in terms of the different components of β in Stages A_{SO} and A_{EDAR} (Fig. 5b).

3.4. Biological traits analysis

The PCA considering functional traits showed a cumulative percentage of variance explained by the two main axes of 76% (46.8% and 29.2% for axes 1 and 2, respectively) (Fig. 6). A separation between the A_{so} Stage and the other Stages (B_{SO}, and A_{EDAR}) is observed. The composition of assemblage traits in the A_{so} Stage was represented by medium-sized organisms, low mobility, indirect development, and tube and burrow dwellers. In terms of pollution tolerance, second-order opportunistic and pollution indifferent organisms were also associated with the A_{SO} Stage. In the other PCA, axis 1 showed a separation between the North and South stations and axis 2 showed a separation of the A_{EDAR} and A_{SO} Stages (44.6% and 35.2% for axes 1 and 2, respectively) in terms of the composition of the functional traits of the assemblage (Fig. 7). The A_{SO} Stage was mostly influenced by small sizes and filter-feeders/suspenders, and no mobility or sessile. In contrast, the A_{EDAR} Stage was characterised by very small organisms, free-living, mobile (high mobility), surface deposit feeders, and predators, direct mode of development, and disturbance sensitive species. On the other hand, no pattern was observed in relation to the Distance factor in any of the PCAs.

3.5. Functional diversity indices

Only FRic showed significant differences between Stages (Table 2); being higher in B_{so} than in A_{so} and A_{EDAR} (Fig. 8). Indeed, the FEve, FDiv, and FDis indices showed no significant differences between the factors or their interaction (Table 2). On the other hand, the

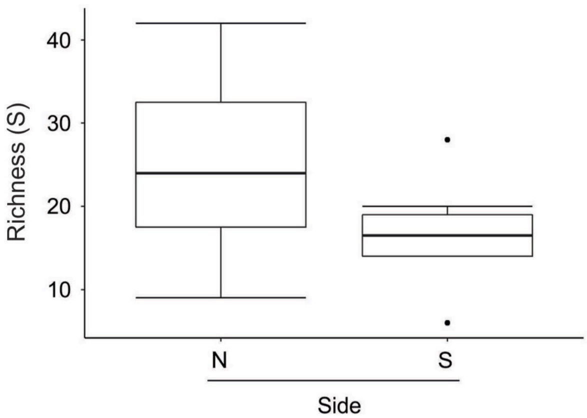

Fig. 4. Spatial variation of the richness (S) on both sides of the submarine outfall (N = North, S = South) at A_{SO} and A_{EDAR} Stages.

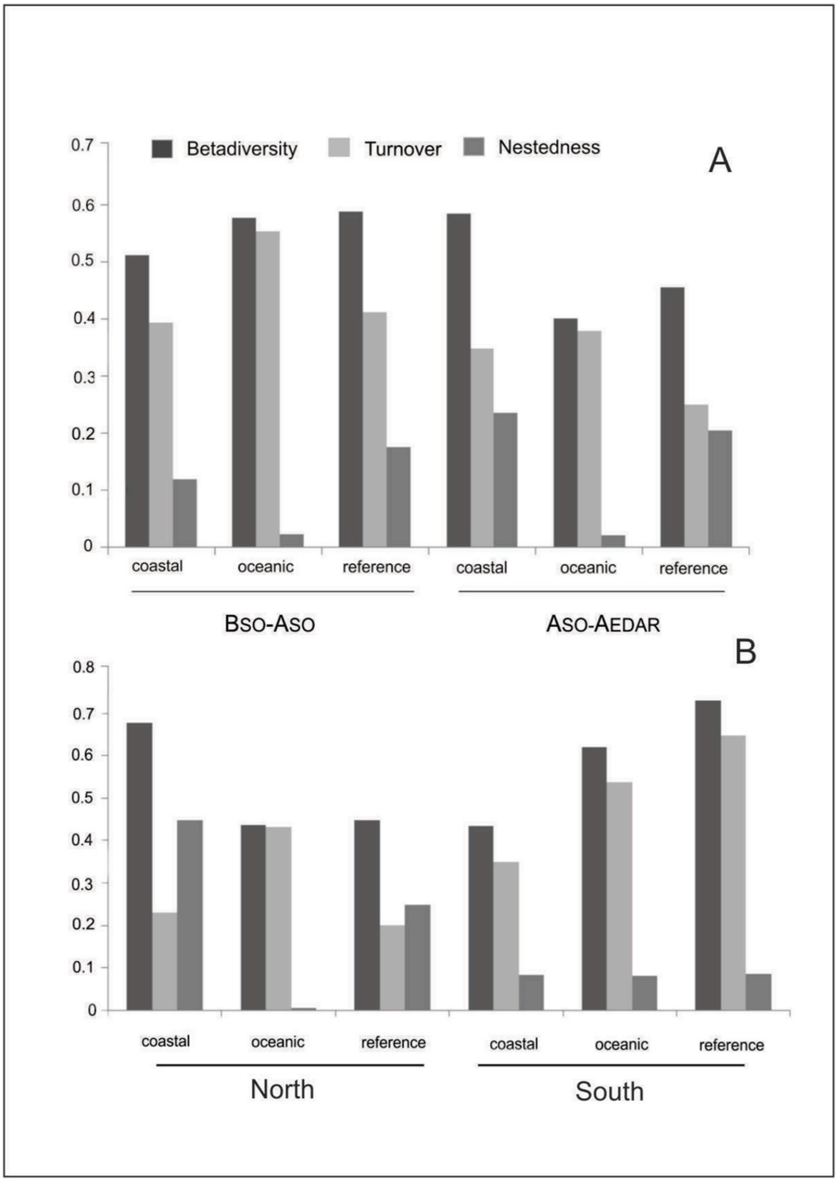

Fig. 5. Beta diversity (β) partitioned into turnover and nestedness components according to (A) Distance from the coastline (coastal, oceanic, and reference site) during the three Stages (before outfall (B_{SO}), after outfall (A_{SO}), after start-up of the EDAR (A_{EDAR})) and (B) Distance from the coastline (coastal, oceanic, and reference site) and both Sides of the outfall (North and South) between after submarine outfall (A_{SO}) and after EDAR plant (A_{EDAR}) Stages.

two-way ANOVA performed with the factors Distance and Side for FRic showed significant differences only for the factor Side, with no interaction between the factors (Table 2). The FRic showed higher values on the South side than on the North side (Fig. 9). However, the FEve, FDiv, and FDis indices showed no significant differences between the factors or their interaction (Table 2).

3.6. Environmental variables

Comparative results regarding the composition of the grain sizes showed a predominance of fine to very fine sediments in the coastal zone and coarser sediments in the oceanic zones and reference stations (Fig. 10). In addition, finer sediments were more abundant on the southern side of the outfall and coarser sediments on the northern side (Fig. 10).

The data of the environmental variables are shown in a table (Table 3). Organic matter (OM) values in the sediments decreased in the A_{EDAR} Stage. In addition, a progressive decrease in OM was observed in A_{SO} and A_{EDAR}, in the oceanic zone, and on the South side (Table 3).

The pH values decreased in Stage A_{EDAR} compared to Stage B_{SO} in the oceanic zones and reference stations and on the South Side of

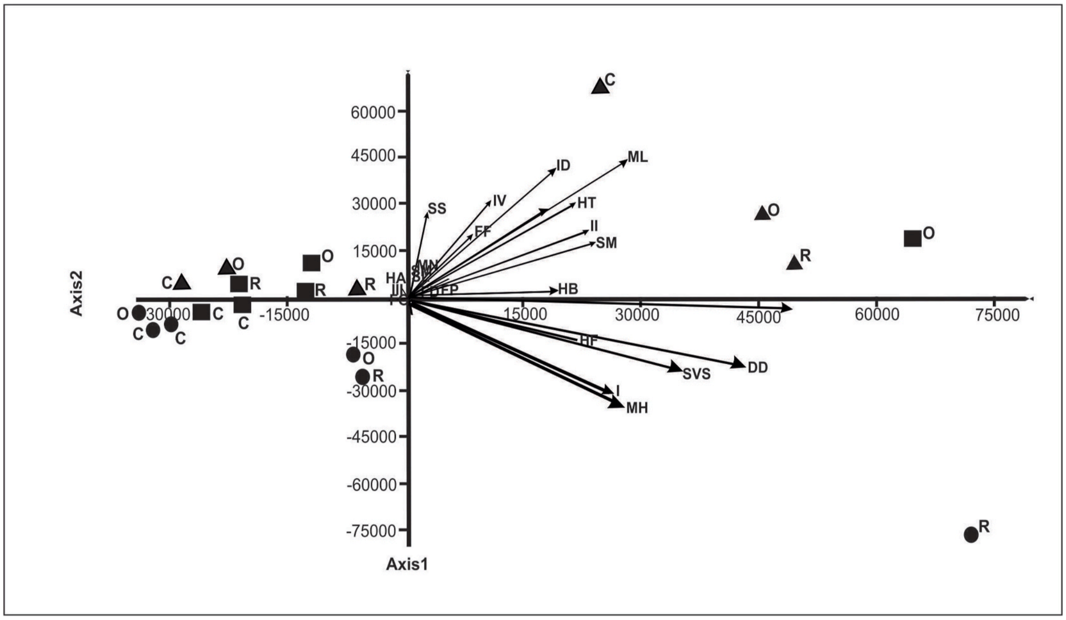

Fig. 6. Principal Component Analysis (PCA) depicting the variability in assemblage trait modalities composition across Stages and Distance. Refer Table 1 for the full list of functional trait modalities. Squares: represent the B_{so} Stage, Triangles: represent the A_{so} Stage and circles: represent the A_{EDAR} Stage. C: coastal, O: oceanic, R: reference.

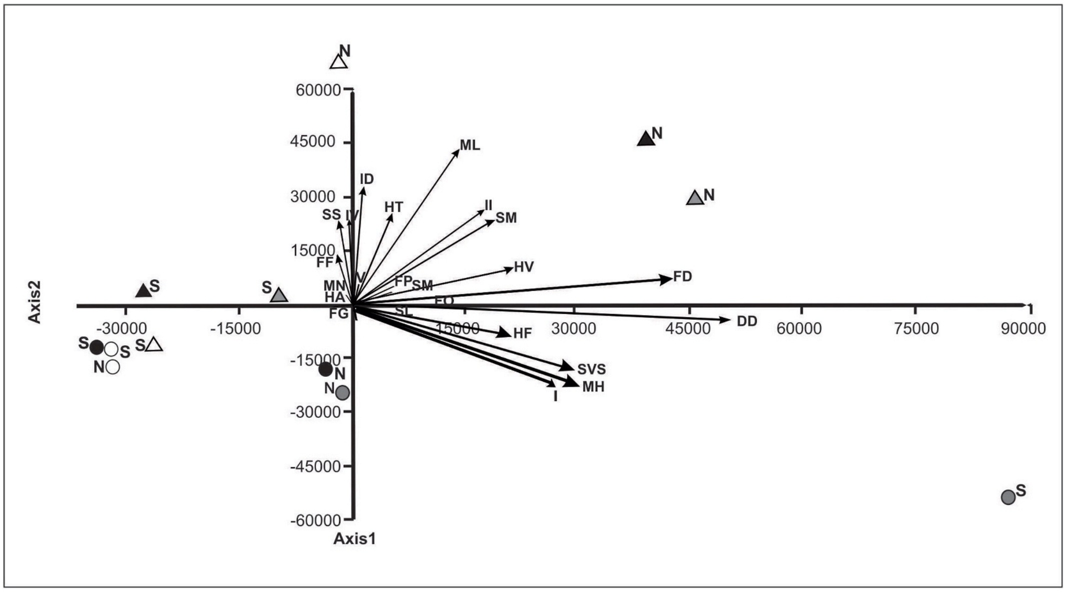

Fig. 7. Principal Component Analysis (PCA) depicting the variability in assemblage trait modalities composition across Stages (A_{so} and A_{EDAR}) and Side (North and South). Triangles: represent the A_{so} Stage and circles represent the A_{EDAR} Stage. White: coastal, black: oceanic and grey: references, N: North, S: South.

the outfall. Dissolved oxygen (DO) values decreased in Stage A_{EDAR} compared to Stage B_{SO} in all zones except the coastal zone, where it remained generally constant.

Enterococcus concentration (NMP/ml) was lower in Stage A_{EDAR} compared to Stage A_{SO} in the reference stations and the coastal zones (Table 3).

Table 2
Analysis of variance (two-way Anova or kruskal Wallis) comparing the mean values of the functional diversity indices (functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), and functional dispersion (FDis)), and the factors stage ($B_{SO,}$ $A_{SO,}$ and A_{EDAR}), Distance (coastal, oceanic, and references), and side (North and south).df: degrees of freedom; p: significance level, *indicates significant p values (<0.05).

	df	H	*p*		df	H	*p*
FRic				**FRic**			
Stage	2	31.08	0.00*	Distance	2	3.58	0.16
Distance	2	2.02	0.36	Side	1	4.88	0.02*
	df	**H**	***p***		**df**	**F**	***p***
FEve				**FEve**			
Stage	2	0.88	0.64	Distance	2	0.17	0.84
Distance	2	0.02	0.99	Side	1	1.12	0.30
				Distance*Side	2	0.53	0.60
	df	**F**	***p***		**df**	**F**	***p***
FDiv				**FDiv**			
Stage	2	0.16	0.85	Distance	2	0.60	0.55
Distance	2	0.36	0.70	Side	1	0.42	0.52
Stage*Distance	4	0.20	0.94	Distance*Side	2	2.93	0.07
FDis	**df**	**F**	***p***	**FDis**	**df**	**F**	***p***
Stage	2	0.78	0.46	Distance	2	0.30	0.74
Distance	2	0.06	0.93	Side	1	0.00	0.98
Stage*Distance	4	2.11	0.09	Distance*Side	2	1.19	0.32

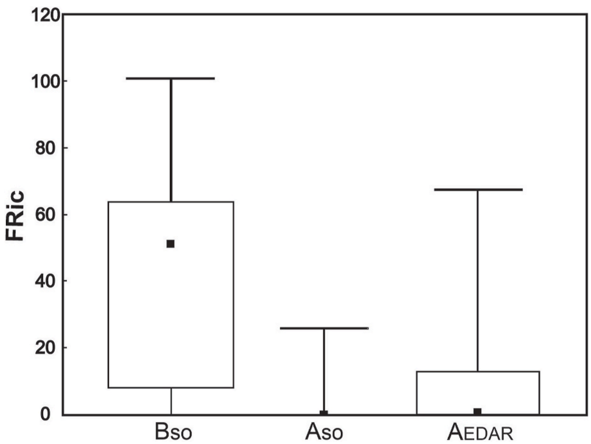

Fig. 8. Median values of FRic (Functional Richness) for each study Stage: before the SO (B_{SO}), after the SO (A_{SO}), and after start-up of the EDAR plant (A_{EDAR}). The point indicates the median value, the boxes the 25%–75% percentiles and the whiskers indicate the Min-Max values.

3.7. Ecological status

The mean values of the environmental quality indices (AMBI and M-AMBI) showed a similar ecological status in each Stage (B_{SO}, A_{SO}, A_{EDAR}), being the three Stages slightly disturbed. In addition, the oceanic zones in the three Stages presented a high environmental quality, and the coastal zones and the reference stations after the EDAR plant (A_{EDAR}) are almost undisturbed (Table 4).

4. Discussion

This is the first study to address the response of subtidal macrobenthos to the effect of construction and functioning of the submarine outfall (SO) and the EDAR treatment plant of residual waters, considering both the physical effect (barrier generated by the pipeline) and the change in environmental effect (organic enrichment). The structure of the subtidal benthic community changed between the different studies Stages (B_{SO}, A_{SO}, and A_{EDAR}). A change of species was also observed (from tolerant to sensitive to organic enrichment) mainly due to the decrease in the organic matter values after the EDAR start-up. On the other hand, confirming our hypothesis, there were changes in the sedimentary dynamics since the SO was built, due to sediment accumulation on the South side of the SO (finest sediments) and erosion on the North side (coarsest sediments). In addition, this is the first work to investigate some aspects of functional diversity (BTA and functional diversity indices) of soft-bottom macrobenthic assemblages around the Mar del Plata SO, Argentina. Functional trait analysis allowed the identification of temporal variations in benthic communities through Stages (B_{SO}, A_{SO}, and A_{EDAR}). Macrobenthic trait composition showed different species' responses to different pollution scenarios (Stages).

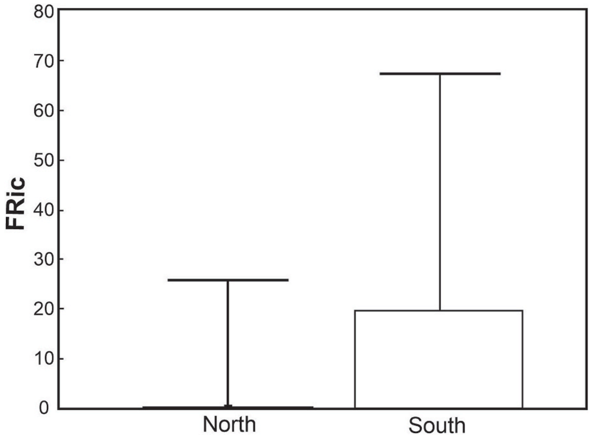

Fig. 9. Median values of FRic (Functional Richness) for each side of the submarine outfall (N = North, S = South) at A_{SO} and A_{EDAR} Stages. The point indicates the median value, the boxes the 25%–75% percentiles and the whiskers indicate the Min-Max values.

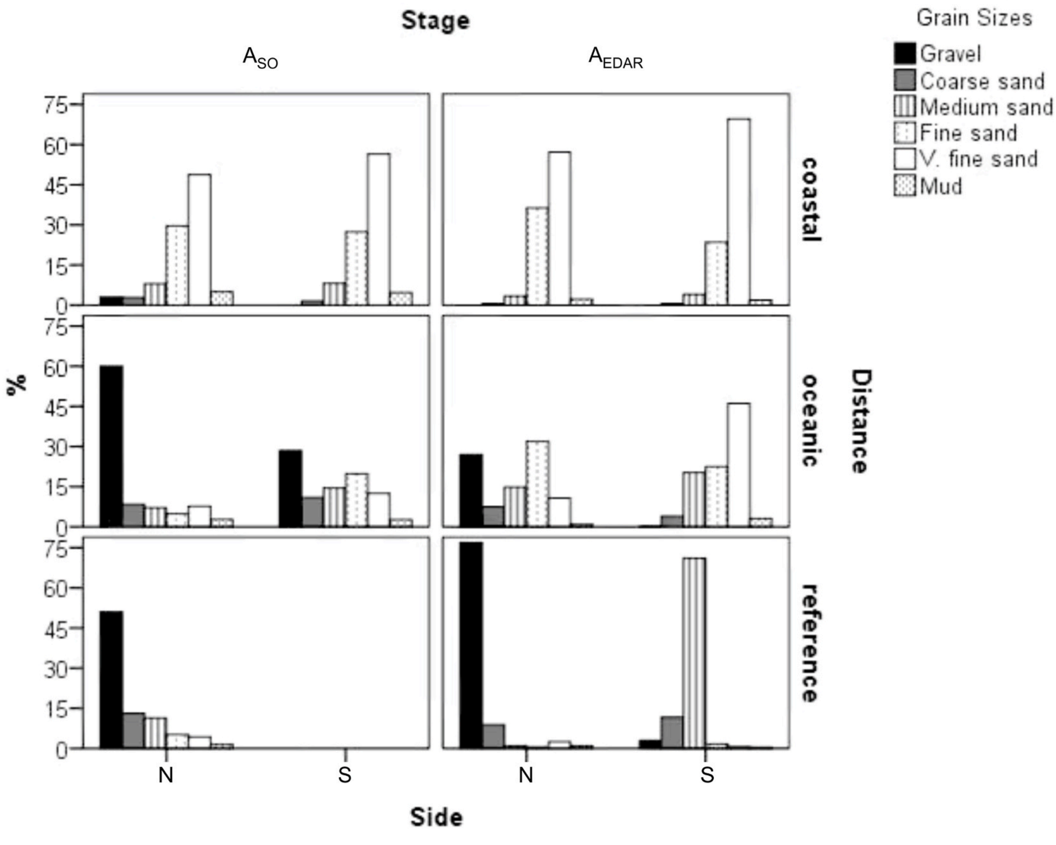

Fig. 10. Sediments grain sizes according to the Distance from the coastline, both sides of the submarine outfall and the Stages A_{SO} and A_{EDAR}.

The obtained results are relevant to know how the quality of the marine environment changes (through the response of organisms and the sediments) after investing in an efficient alternative for wastewater disposal (such as SO).

According to our results, the subtidal benthic community presented a clear temporal separation between B_{SO} and A_{SO}. The differences between the two phases were given by the relative abundances of *Prionospio* sp., *Axiothella* sp., and *Magelona* sp. In B_{SO}, *Prionospio* sp. was absent but reached a greater abundance after A_{SO}, like *Axiothella* sp. Even if the polychaete *Prionospio* sp. is an indicator of organic enrichment in subtidal areas [64], *Axiothella* sp., is an indicator of the opposite because maldanids are generally found in sediments with low organic matter content [58,65–68]. This could be explained by the patched distribution of benthic

Table 3
Data of the environmental variables in the 3 sampling stages on both sides of the submarine outfall. Method 1: the titration method (Walkley and Black, 1934), and Method 2: loss on ignition (LOI) (Heiri et al., 2001). OM (Organic Matter) is expressed in percentage (%), DO (Dissolved Oxygen) in mg/l and Enterococcus concentration (NMP/100 ml).

		Distance	Ph	OM		DO	Enterococcus concentration
B_{SO}		coastal	8.78	Method 1	1.87	9.74	
	N	oceanic	8.95	Method 1	1.36	11.24	
		reference	8.85	Method 1	1.3	11.04	
		coastal	8.79	Method 1	1.71	11.12	
	S	oceanic	8.67	Method 1	1.22	11.71	
		reference	8.72	Method 1	1.07	11.83	
A_{SO}		coastal		Method 2	0.95		1400
	N	oceanic		Method 2	1.12		1100
		reference		Method 2	1.17		1700
		coastal		Method 2	0.96		11000
	S	oceanic		Method 2	1.05		1500
		reference		Method 2	0.47		800
A_{EDAR}		coastal	8.43	Method 2	0.57	9.01	90
	N	oceanic	8.45	Method 2	0.43	9.21	3000
		reference	8.51	Method 2	0.97	9.26	400
		coastal	8.50	Method 2	0.59	9.10	80
	S	oceanic	8.25	Method 2	0.53	9.24	14000
		reference	8.13	Method 2	0.26	9.18	800

Table 4
Values of AMBI and M-AMBI for each stage according to the distance from the coast and both sides of the SO.

	Side	Distance	AMBI	Status (AMBI)	M-AMBI	Status (AMBI)
		coastal	2.08	Slightly disturbed	0.72	Good
	North	oceanic	2.01	Slightly disturbed	0.83	High
		reference	1.94	Slightly disturbed	0.67	Good
B_{SO}						
		coastal	1.47	Slightly disturbed	0.59	Good
	South	oceanic	1.84	Slightly disturbed	0.73	Good
		reference	2.74	Slightly disturbed	0.56	Good
		coastal	2.31	Slightly disturbed	0.69	Good
	North	oceanic	1.75	Slightly disturbed	0.77	Good
		reference	1.64	Slightly disturbed	0.91	High
A_{SO}						
		coastal	1.34	Slightly disturbed	0.73	Good
	South	oceanic	2.47	Slightly disturbed	0.69	Good
		reference	1.57	Slightly disturbed	0.76	Good
		coastal	1.04	Undisturbed	0.64	Good
	North	oceanic	2.06	Slightly disturbed	0.74	Good
		reference	1.06	Undisturbed	0.67	Good
A_{EDAR}						
		coastal	1.44	Slightly disturbed	0.64	Good
	South	oceanic	1.16	Slightly disturbed	0.71	Good
		reference	0.29	Undisturbed	0.55	Good

communities on Mar del Plata shores. The area is affected by periodical storms coming from the southeast [69] that produce disturbed bottoms, and maintained a benthic community in earlier Stages of succession [12]. The oceanic zone around the diffusers section of the SO is subject to natural disturbance by storms, plus organic inputs from residual wastes. Therefore, the increasing organic enrichment from residual wastes is minimized by the periodic irruption of storms. These storms, which occur throughout the year, are more frequent and intense in autumn-winter and have the effect of sweeping the seabed, thus maintaining good environmental quality.

In addition, the results showed differences in the macrobenthic assemblage between the coastal areas before (B_{SO}) and after (A_{SO} and A_{EDAR}), probably due to the direct intertidal sewage discharge that took place at that moment. This difference was due to the high abundance of indicator species of organic enrichment such as *Prionospio* sp., *Magelona* sp., *Monokalliapseudes schubarti,* and species of family Cirratulidae. Although in the B_{SO} Stage, wastewater discharge occurred in the coastal zone, the highest abundance of indicator species occurs, paradoxically, in the A_{SO} Stage. This unusual behavior could be because the coastal zone was strongly affected by the environmental change induced by the SO, promoting the sedimentation of fine and very fine sands together with particulate organic material possibly from the sewage treatment plant by-pass process (Elías, pers. obs.). In general, a bypass usually occurs in: periods of heavy rainfall when the effluent exceeds the capacity of the submarine emitter, diversion of the effluent due to overflow, clogging of the pipe or diffuser, or cleaning and maintenance [70,71]. In the past, when the sewage discharge was intertidal (B_{SO}), the effect of cleaning and maintenance of the pre-treatment plant showed impoverishment of both the environment and the benthic intertidal fauna [72], just as it would take place with the current bypass process.

Previous studies in the study area found that *M. schubarti* is associated with *Prionospio* sp. and suggested that *M. schubarti* is an opportunistic species due to its high fecundity and rapid growth [73,74]. *M. schubarti* was present in the area during 1999, reaching high densities (3–1600 ind. m^{-2}) in 25 of the 49 sampling stations [11], but it is absent in 2018. In the salt marshes of *Spartina* these species have an opportunistic behavior, but rotate periods of high abundance with subsequent vanishing [75]. This could be the reason for the absence, together with the reduction of organic matter input due to the functioning of the SO.

Cirratulidae are considered tolerant due to their high abundance in organically enriched sites [76–78]. Studies off the coasts of Indonesia [79] and Brazil [80] at sites of tourism, oil and industrial waste dumping found the family Cirratulidae to be an indicator of disturbed sites. BTA represented groups with different combinations of trait modalities associated with each Stage. Thus, functional traits such as small to medium-sized, low mobility, filter feeder, indirect development, tube-dweller, second-order opportunistic and pollution-indifferent organisms were associated with the Stage A_{SO}, linked to a patched distribution of benthic communities and periodical storms (as it was previously mentioned). Body size is a key trait in benthic studies; larger organisms have a greater ability to modify the sediment environment and increase oxygen levels, thereby increasing the rate of decomposition of organic matter [81]. Mobility, in turn, plays a fundamental role in access to resources [82]. Low-mobility species are better adapted to disturbed habitats [83]. In contrast, filter-feeding organisms tend to dominate in areas with high nutrient levels, while carnivores dominate in areas with low nutrient levels [29,84]. Developmental mode reflects the adaptability of organisms to environmental conditions [85,86]. Organisms with planktonic larvae will have a higher dispersal capacity and lower risk of extinction than organisms with other developmental modes [85,87]. These traits are associated with the polychaetes *Prionospio* sp., and *Magelona* sp., and the peracarid crustacean *M. schubarti*, showing higher abundance towards Stage A_{SO}. Tube-dwelling polychaetes can play an important role in denitrification, helping maintain nutrient cycling and ensuring sediment stabilization, which favors larval settlement [88,89].

The main objective of the EDAR residual waters treatment plant is to remove the maximum amount of organic matter (OM) from the sewage effluent and gradually reduce the OM discharged to the sea [13]. The results of this study indicate that this goal was reached because a decrease in OM in sediment in both oceanic and coastal zones (see Table 3) was observed after the construction and EDAR start-up. This decrease in OM during A_{EDAR} Stage was also reflected in the benthic community, finding species like the bivalve *Eucallista purpurata* and the amphipod belonging to Phoxocephalopsidae in the oceanic zone. Case studies in Latin America (Colombia, Mexico, and Argentina) used bivalves as bioindicators in environmental quality monitoring programs because of their ecological characteristics such as presence in a great diversity of habitats, sedentary lifestyle, potential behavioral, physiological responses to habitat modifications and pollution [90–92]. Likewise, amphipods are also considered susceptible to organic pollution, even with greater sensitivity than polychaetes in places with low wastewater flows [93]. Due to this response, the polychaetes/amphipods ratio is an environmental quality index [94,95] as high ratios indicate good conditions, while low ratios indicate poor conditions.

On the other hand, the BTA results also showed that the A_{EDAR} Stage was characterised by very small organisms, free-living, mobile (high mobility), surface deposit feeders and direct developmental mode. Body size, feeding-mode, habitat, and indicator-role are useful proxies to detect changes in environmental conditions in organically enriched habitats [96]. Surface deposit feeder trait modality is associated with impacted sites [26,97,98]. However, the difference in feeding mode between A_{SO} and A_{EDAR} Stages could be related to the change in sediment type (coarse to finer sediment), as changes in sediment allow the establishment of species with different feeding habits [25,99]. Nevertheless, other trait modality, such as the low tolerance to pollution (sensitive organisms) associated with the A_{EDAR} Stage was observed. This trait could be related to the species *E. purpurata,* and *Axiothella* sp. that were the most abundant after the operation of the outfall (A_{SO} and A_{EDAR}). This diversity of functionality could indicate a shift towards a relatively good environmental status [100].

A finding to highlight was the presence of *Polygordius* sp. during the A_{SO} and A_{EDAR} Stages. The occurrence of this species coincides with the changes in sedimentary dynamics previously mentioned (finest sediments on the South side and coarsest sediments on the North side). Martins et al. [101] found *Polygordius* sp. in coarse sand habitats, and Ramey et al. [102] related coarse sand habitats to reproductive mode, finding that planktonic larvae are more successful in this sediment type. Organic enrichment reduces richness and increases the densities and numbers of a few opportunistic species and their associated biomass [76]. According to our results, the richness (S) and the diversity (H') of the subtidal benthic community in the coastal zones at B_{SO} Stage were lower compared to the rest of the Stages. This may be because in these zones (coastal zones) the disturbance is greater as a consequence of coastal processes like waves, winds, sea level variations, and currents; in addition to the discharge of wastewater directly onto the coastal zone at that time (B_{SO} Stage). On the other hand, the oceanic zones (close to the discharge) showed a higher richness and diversity during A_{SO} and A_{EDAR} Stages, which could be due to the new type of discharge after the start-up of the EDAR plant. A deep subsurface discharge would affect the macrofaunal assemblages less than direct surface effluents, increasing richness and minimizing environmental degradation [103]. Similarly, in the reference sites, an increase in richness was evidenced in B_{SO} Stage, and at the same time, the reference sites at A_{SO} and A_{EDAR} Stages are similar in terms of richness. This indicates that after the EDAR started operating, the conditions began to resemble those before the SO installation. Finally, a clear increase in dominant species was observed from B_{SO} to A_{EDAR} Stages, as Pielou's evenness index (J') decreased. This may be due to an increase in the reproduction of opportunistic species such as *Prionospio* sp. at A_{SO}, which led to a decrease in the number of species. Regarding β, the highest values of β were observed between B_{SO} and A_{SO} Stages, and the lowest values were observed between A_{SO} and $A_{EDAR.}$ Species distribution patterns across Stages could differ in response to differences in the degree of anthropogenic impact (4 km pipeline at the study site) [104] or the discharge of hundreds of liters of wastewater each day [105].

The functional richness index (FRic) detected temporal and spatial changes, which were significantly higher at the B_{so} Stage and on the South side. FRic is considered to be a sensitive predictor of disturbance, as it decreases at high levels of disturbance due to the filtering out of species [31,106]. Low functional richness indicates that some of the potentially available resources are not being used and could lead to reduced productivity of an ecosystem [107]. No differences in FEve, FDiv, and FDis were found between Stages (B_{SO}, A_{SO}, and A_{EDAR}), distance (coastal, oceanic, and reference), and side (North and South).

The processes that structure communities are key to explaining the functioning of ecosystems (by determining the degree of redundancy) and the maintenance of biodiversity (through patterns of coexistence) [108–110]. The environmental filter hypothesis suggests that coexisting species are more similar to each other because environmental conditions act as a filter, allowing only some traits to persist [111]. Therefore, if environmental filters structure the community, the most abundant species are expected to have similar niches that allow them to tolerate the conditions imposed by the filter [107,110,111].

A community can changes in taxonomic diversity and remain stable in functional diversity [36]. In this study, the environmental filter had an effect at the taxonomic level, but not had an effect at the functional level. An increase in taxonomic richness and a decrease in functional richness at the A_{SO} Stage was detected butdid not affect functional diversity (FEve, FDiv, and FDis) (Supplementary Material, Table S4). If the increase in richness does not affect functional diversity, it can be concluded that species are functionally redundant and therefore the change in taxonomic richness would not affect ecosystem functioning [110].

β partitioning can provide additional information on the underlying causes of variability in biotic community composition compared to total β alone [112]. The components of β are generally opposite, and there is a trade-off between nestedness and species turnover in response to different intensities of environmental impact [113]. In general, species turnover was the process that most drove the differences in species assemblages in the different Stages, which is in agreement with other marine benthic studies that observed this pattern in environments with high environmental stress [114–117]. Between B_{SO} and A_{SO} Stages, opportunistic and/or tolerant species were dominant, such as *Prionospio* sp., *Magelona* sp., and Cirratulidae (r-strategists) which coincides with the highest values of species turnover. In the opposite case, between A_{SO} and A_{EDAR} Stages, the process that most drove the differences in species assemblages was nestedness. The latter is in agreement with an increase in richness towards A_{EDAR} and the gain of species sensitive to organic enrichment in sediments, such as *Melita* sp. and *Axiothella* sp.

The β components on both sides of the outfall (North and South) differed. The highest nestedness values were found in the coastal zones on the North side of the SO, while the highest values of turnover of species were found on the South side. The difference in β components on both sides of the outfall could be the result of environmental filtering, which is an important factor in areas characterized by high abiotic stress [118–120]. Environmental filters can drive both turnover and the nestedness components of β, but the relative importance of each element depends on the spatial scale of investigation [120]. Specifically, the placement of a 4 km pipe resting on a seabed causes a change in sediment type (finer sediments on the South side and coarser sediments on the North side). Changes in the sediments (sediment composition, grain size) or sediment-associated variables (total organic matter, TOM) act as environmental filters that determine macrobenthic community structure by significantly influencing β components [101,105].

In general, the AMBI and M-AMBI indices were good descriptors of ecological conditions in the study area. Although M-AMBI gave the highest category of ecological quality at some sites, both indices showed the same pattern. The indices classified the zones (coastal, oceanic, and reference) in the three Stages (B_{SO}, A_{SO}, A_{EDAR}) as "slightly disturbed" (AMBI) and with good and high environmental quality (M-AMBI). Studies on macrobenthic communities near a wastewater treatment plant classify such communities as "moderately disturbed" [121] while in this study the communities presented less disturbance, especially after the EDAR. The sum of the sewage discharge and the hydrodynamics could dilute the effects of the pollution, normalizing the organic content of the sediments and consequently getting a better ecological state of the site. This fact would be accompanied by the presence of species susceptible to organic enrichment, such as the bivalve *Eucallista purpurata* and the amphipod *Melita* sp. Likewise, the sewage treatment plant has proven to be beneficial for environmental quality, as both environmental and biological indicators show improvements.

The Mar del Plata disposal area benefits from high turbulence generated by high hydrodynamics. The management decision to implement disposal of wastewater through 130 nozzles and at a depth of 11 m has delivered an ecological benefit as illustrated by the current study. Adding primary treatment to the wastewater (EDAR) has helped to improve the quality of the discharge and minimize the effect on the near field (less than 500 m).

5. Conclusions

The three sampling Stages (B_{SO}, A_{SO}, and A_{EDAR}) presented clear differences in the subtidal community structure generated by the new wastewater discharge (at 4 km from the coast) through the SO. These differences are due to mainly species turnover, the species opportunistic and/or tolerant to organic enrichment (Spionidae, Cirratulidae, *M. schubarti*, *Prionospio* sp., *Magelona* sp.) dominated at B_{SO} and A_{SO} Stages coinciding with the increase in the OM content in the sediment. After the start-up of the EDAR plant (A_{EDAR}), environmental conditions showed a slight improvement with the appearance of sensitive's species to organic enrichment (*Melita* sp., *Eucallista purpurata*), together with an increase in richness and a decrease in OM. Also, there was a change in the sedimentary dynamics of the study area at the A_{SO} Stage, finding finer sediment (fine sand) on the South side and coarser sediment (coarse sand) on the North side. It was concluded that the SO and the EDAR start-up had an effect at the taxonomic level (structure and composition), but not at the functional level. The increase in richness would not affect functional diversity and therefore would not affect ecosystem functioning.

Author contribution statement

Graciela Verónica Cuello; Maria Andrea Saracho Bottero; Jaubet María Lourdes: Conceived and designed the experiments;

Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Griselda Valeria Garaffo: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Rodolfo Elias: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] J.M. Barragán, M. de Andrés, Expansión urbana en las áreas litorales de América Latina y Caribe, Rev. Geogr. Norte Gd. 64 (2016) 129–149, https://doi.org/10.4067/S0718-34022016000200009.

[2] A.C. Teodoro, W. Duleba, S. Gubitoso, S.M. Prada, C.C. Lamparelli, J.E. Bevilacqua, Analysis of foraminifera assemblages and sediment geochemical properties to characterise the environment near Araçá and Saco da Capela domestic sewage submarine outfalls of São Sebastião Channel, São Paulo State, Brazil. Mar. Pollut. Bull. 60 (4) (2010), 536553, https://doi.org/10.1016/j.marpolbul.2009.11.011.

[3] B. Lapointe, K. Thacker, C. Hanson, L. Getten, Sewage pollution in Negril, Jamaica: effects on nutrition and ecology of coral reef macroalgae, Chin. J. Oceanol. Limnol. 29 (2011) 775–789. https://10.1007/s00343-011-0506-8.

[4] R.C. Feitosa, Emissários submarinos de esgotos como alternativa à minimização de riscos à saúde humana e ambiental, Ciênc. saúde colet. 22 (6) (2017) 2037, https://doi.org/10.1590/1413-81232017226.15522016. –2048.

[5] IAHR/IWA, Joint Committee on Marine Outfall Systems, Outfall Database, 2014.

[6] D.K. S Jaquetti, Proposta de Avaliação de Impacto Ambiental de Emissários Submarinos. Tesis de Licenciatura, Universidade Tecnológica Federal do Paraná, 2012.

[7] A. Puente, R.J. Diaz, Response of benthos to ocean outfall discharges: does a general pattern exist? Mar. Pollut. Bull. 101 (1) (2015) 174–181, https://doi.org/10.1016/j.marpolbul.2015.11.002.

[8] M. Scagliola, A.P. Comino, P. Roberts, D. Botelho, The history of the Mar del Plata outfall system: a tale worth telling, Hydrolink 4 (2020) (2006) 120–125.

[9] S.R. Olivier, R. Bastida, M.R. Torti, Las comunidades bentónicas de los alrededores de Mar del Plata, Actas del IV Congreso Latinoamericano de Zoología, (Caracas, Venezuela), 1968, pp. 559–594.

[10] R. Elías, C.S. Bremec, E.A. Vallarino, Polychaetes from a southwestern shallow shelf Atlantic area (Argentina, 38° S) affected by sewage discharge, Rev. Chil. Hist. Nat. 74 (3) (2001) 523–531, https://doi.org/10.4067/S0716-078X2001000300003.

[11] R. Elías, E.A. Vallarino, M. Scagliola, F.I. Isla, Macrobenthic distribution patterns at a sewage disposal site in the inner shelf off Mar del Plata (SW Atlantic), J. Coast Res. 20 (4) (2004) 1176–1182.

[12] R. Elías, J.R. Palacios, M.S. Rivero, E.A. Vallarino, Short-term responses to sewage discharge and storms of subtidal sand-bottom macrozoobenthic assemblages off Mar del Plata City, Argentina (SW Atlantic), J. Sea Res. 53 (2005) 231–242.

[13] OSMGP, Obras Sanitarias municipalidad de General pueyrredón. http://www.osmgp.gov.ar/osse/2022, 2022. February, 2022.

[14] V. Carignan, M.-A. Villard, Selecting indicator species to monitor ecological integrity: a review, Environ. Monit. Assess. 78 (1) (2002) 45–61. https://10.1023/A:1016136723584.

[15] P.J. Goodsell, A.J. Underwood, M.G. Chapman, Evidence necessary for taxa to be reliable indicators of environmental conditions or impacts, Mar. Pollut. Bull. 58 (3) (2009) 323–331, https://doi.org/10.1016/j.marpolbul.2008.10.011.

[16] Á. Borja, S.B. Bricker, D.M. Dauer, N.T. Demetriades, J.G. Ferreira, A.T. Forbes, P. Hutchings, X. Jia, R. Kenchington, J.C. Marques, C. Zhu, Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide, Mar. Pollut. Bull. 56 (9) (2008) 1519–1537, https://doi.org/10.1016/j.marpolbul.2008.07.005.

[17] Á. Borja, Q. Basset, S. Bricker, J.C. Dauvin, M. Elliott, T. Harrison, J.C. Marques, S.B. Weisberg, R. West, Classifying ecological quality and integrity of estuaries, in: E. Wolanski, McLusky (Eds.), Treatise on Estuarine and Coastal Science, vol. 1, Academic Press, Waltham, 2011, pp. 125–162.

[18] F. Salas, C. Marcos, J.M. Neto, J. Patrício, A. Pérez-Ruzafa, J.C. Marques, User-friendly guide for using benthic ecological indicators in coastal and marine quality assessment, Ocean Coast Manag. 49 (2006) 308–331, https://doi.org/10.1016/j.ocecoaman.2006.03.001.

[19] J. Patrício, J.M. Neto, H. Teixeira, F. Salas, J.C. Marques, The robustness of ecological indicators to detect long-term changes in the systems, Mar. Environ. Res. 68 (1) (2009) 25–36, https://doi.org/10.1016/j.marenvres.2009.04.001.

[20] V.H. Dale, S.C. Beyeler, Challenges in the development and use of ecological indicators, Ind. Ecol. 1 (1) (2001) 3–10, https://doi.org/10.1016/S1470-160X(01)00003-6.

[21] R. M Warwick, K.R. Clarke, Increased variability as a symptom of stress in marine communities, J. Exp. Mar. Biol. Ecol. 172 (1–2) (1993) 215–226, https://doi.org/10.1016/0022-0981(93)90098-9.

[22] J.C. Dauvin, G. Bellan, D. Bellan-Santini, Benthic indicators: from subjectivity to objectivity – where is the line? Mar. Pollut. Bull. 60 (7) (2010) 947–953, https://doi.org/10.1016/j.marpolbul.2010.03.028.

[23] P. Muniz, N. Venturini, C.C. Martins, A.B. Munshi, F. García-Rodríguez, E. Brugnoli, A.L.L. Dauner, M.C. Bícego, J. García-Alonso, Integrated assessment of contaminants and monitoring of an urbanized temperate harbor (Montevideo, Uruguay): a 12-year comparison, Braz. J. Oceanogr. 63 (3) (2015) 311–330, https://doi.org/10.1590/S1679-87592015088506303.

[24] J. Bremner, S.I. Rogers, C.L.J. Frid, Matching biological traits to environmental conditions in marine benthic ecosystems, J. Mar. Syst. 60 (3–4) (2006) 302–316, https://doi.org/10.1016/j.jmarsys.2006.02.004.

[25] J.B. Gusmao, K.M. Brauko, B.K. Eriksson, P.C. Lana, Functional diversity of macrobenthic assemblages decreases in response to sewage discharges, Ecol. Indicat. 66 (2016) 65–75, https://doi.org/10.1016/j.ecolind.2016.01.003.

[26] G.V. Garaffo, M.L. Jaubet, E.N. Llanos, M.A. Saracho Bottero, R. Elías, Assessing functional diversity of microbenthic assemblages in sewage-affected intertidal shores, Int. Aquat. Res. 10 (2018) 333–347. https://10.1007/s40071-018-0211-8.

[27] G.V. Garaffo, E.N. Llanos, M.A. Saracho Bottero, E. Hines, R. Elías, M.L. Jaubet, Functional diversity on rocky shores of the SW Atlantic: sewage effluents influence and mask the effects of the latitudinal gradient, Mar. Ecol. Prog. Ser. 648 (2020) 39–49. https://10.3354/meps13441.

[28] M. Bon, J. Grall, J.B. Gusmao, M. Fajardo, C. Harrod, A.S. Pacheco, Functional changes in benthic macrofaunal communities along a natural gradient of hypoxia in an upwelling system, Mar. Pollut. Bull. 164 (2021), 112056, https://doi.org/10.1016/j.marpolbul.2021.112056.

[29] E.N. Llanos, M.A. Saracho Bottero, M. L Jaubet, R. Elías, G.V. Garaffo, Functional diversity in the intertidal macrobenthic community at sewage-affected shores from Southwestern Atlantic, Mar. Pollut. Bull. 157 (2020), 111365, https://doi.org/10.1016/j.marpolbul.2020.111365.

[30] N. Sahu, S. Haldar, Evaluation of benthic quality status and ecosystem functioning of soft bottom macrobenthos in the intertidal region with reference to Gulf of Khambhat, India, J. Sea Res. 189 (2022), 102273, https://doi.org/10.1016/j.seares.2022.102273.

[31] D. Mouillot, D.R. Bellwood, C. Baraloto, J. Chave, R. Galzin, M. Harmelin-Vivien, C.T. Paine, Rare species support vulnerable functions in high-diversity ecosystems, PLoS Biol. 11 (5) (2013), e1001569, https://doi.org/10.1371/journal.pbio.1001569.

[32] R.D. Stuart-Smith, A.E. Bates, J.S. Lefcheck, J.E. Duffy, S.C. Baker, R.J. Thomson, J.F. Stuart-Smith, N.A. Hill, S.J. Kininmonth, L. Airoldi, M.A. Becerro, S. J. Campbell, T.P. Dawson, S.A. Navarrete, G.A. Soler, E.M.A. Strain, T.J. Willis, G.J. Edgar, Integrating abundance and functional traits reveals new global hotspots of fish diversity, Nature 501 (7468) (2013) 539–542, https://doi.org/10.1038/nature12529.

[33] D. Tilman, Functional diversity, in: S.A. Levin (Ed.), Encyclopedia of Biodiversity, Academic Press, San Diego, USA, 2001, pp. 109–120.

[34] V. Gagic, I. Bartomeus, T. Jonsson, A. Taylor, C. Winqvist, C. Fischer, E.M. Slade, I. Steffan-Dewenter, M. Emmerson, S.G. Potts, T. Tscharntke, W. Weisser, R. Bommarco, Functional identity and diversity of animals predict ecosystem functioning better than species-based indices, Proc. R. Soc. B: Biol. Sci. 282 (1801) (2015), 20142620, https://doi.org/10.1098/rspb.2014.2620.

[35] M. Gerisch, V. Agostinelli, K. Henle, F. Dziock, More species, but all do the same: contrasting effects of flood disturbance on ground beetle functional and species diversity, Oikos 121 (4) (2012) 508–515. https://10.1111/j.1600-0706.2011.19749.x.

[36] S. Villéger, J.R. Miranda, D.F. Hernández, D. Mouillot, Contrasting changes in taxonomic vs. functional diversity of tropical fish communities after habitat degradation, Ecol. Appl. 20 (6) (2010) 1512–1522. https://10.1890/09-1310.1.

[37] J.M. Fernández, Dinámica costera, Red Mar del Plata Entre Todos, 2018, pp. 140–155.

[38] F.I. Isla, A. Ferrante, Corrientes, in: F.I. Isla (Ed.), Estudio del sector de plataforma receptor de la descarga cloacal de Camet, Mar del Plata, Facultad de Cs. Exactas y Naturales, UNMDP. Cap, vol. 5, 1997, pp. 63–116.

[39] W.P. Sousa, The role of disturbance in natural communities, Annu. Rev. Ecol. Systemat. 15 (1984) 353–391, https://doi.org/10.1146/annurev.es.15.110184.002033.

[40] M. Posey, W. Lindberg, T. Alphin, F. Vose, Influence of storm disturbance on an offshore benthic community, Bull. Mar. Sci. 59 (1996) 523–529.

[41] R.A. Guerrero, A.R. Piola, Masas de agua en la plataforma continental, in: E.E. Boschi (Ed.), El Mar Argentino Y sus Recursos Pesqueros I, INIDEP, Argentina, 1997, pp. 107–118.

[42] E.E. Boschi, Species of Decapod Crustaceans and Their Distribution in the American Marine Zoogeographic Provinces, vol. 13, Instituto Nacional de Investigación y Desarrollo Pesquero, 2000, p. 136.

[43] A. Walkley, I.A. Black, An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method, Soil Sci. 37 (1934) 29–37. https://10.1097/00010694-193401000-00003.

[44] O. Heiri, A.F. Lotter, G. Lemcke, Loss of ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results, J. Paleolimnol. 25 (2001) 101–110, https://doi.org/10.1023/A:1008119611481.

[45] K.R. Clarke, R.M. Warwick, Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2da edición, Plymouth Marine Laboratory, UK, 2001, p. 172.

[46] K.R. Clarke, Non-parametric multivariate analyses of changes in community structure, Aust. J. Ecol. 18 (1993) 117–143. https://10.1111/j.1442-9993.1993.tb00438.x.

[47] R. R Core Team, A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2021. URL, https://www.R-project.org/.

[48] E.C. Pielou, An Introduction to Mathematical Ecology, Wiley-Interscience, New York, 1969, p. 286. https://10.1126/science.169.3940.43-a.

[49] C.E. Shannon, W. Weaver, The Mathematical Theory of Communication, University Illinois Press, Urbana, Illinois, 1963.

[50] C. Munari, Benthic community and biological trait composition in respect to artificial coastal defence structures: a study case in the northern Adriatic Sea, Mar. Environ. Res. 90 (2013) 47–54, https://doi.org/10.1016/j.marenvres.2013.05.011.

[51] A.Z. Lacson, D. Piló, F. Pereira, A.N. Carvalho, J. Curdia, M. Caetano, T. Drago, M.N. Santos, M.B. Gaspar, A multimetric approach to evaluate offshore mussel aquaculture effects on the taxonomical and functional diversity of macrobenthic communities, Mar. Environ. Res. 151 (2019), 104774, https://doi.org/10.1016/j.marenvres.2019.104774.

[52] F. Chevenet, S. Dolédec, D. Chessel, A fuzzy coding approach for the analysis of long-term ecological data, Freshw. Biol. 31 (1994) 295–309. https://10.1111/j.1365-2427.1994.tb01742.x.

[53] S. Villéger, N.W.H. Mason, D. Mouillot, New multidimensional functional diversity indices for a multifaceted framework in functional ecology, Ecol. 89 (2008), 22902301. https://10.1890/07-1206.1.

[54] E. Laliberte, P. Legendre, A distance-based framework for measuring functional diversity from multiple traits, Ecol. 91 (1) (2010) 299–305. https://10.1890/08-2244.1.

[55] A. Schuldt, H. Bruelheide, W. Durka, S.G. Michalski, O. Purschke, T. Assmann, Tree diversity promotes functional dissimilarity and maintains functional richness despite species loss in predator assemblages, Oecologia 174 (2014) 533–543. https://10.1007/s00442-013-2790-9.
[56] J.A. Di Rienzo, F. Casanoves, L. Pla, FDiversity, Version 2008, Cordoba, Argentina, 2008. Link, www.fdiversity.nucleodiversus.org.
[57] J. Zar, Biostatistical Analysis, fourth ed., Prentice Hall, 1999, pp. 65–89.
[58] Á. Borja, J. Franco, V. Pérez, A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments, Mar. Pollut. Bull. 40 (12) (2000) 1100–1114, https://doi.org/10.1016/S0025-326X(00)00061-8.
[59] Á. Borja, I. Muxika, J. Franco, The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts, Mar. Pollut. Bull. 46 (7) (2003) 835–845, https://doi.org/10.1016/S0025-326X(03)00090-0.
[60] R. Elías, C.S. Bremec, D. Giberto, M.C. Gravina, L. Schejter, Estudio faunístico de las comunidades bentónicas infaunales de El Rincón, Resultados de la campaña CC 14//00, Informe Técnico Interno INIDEP 8 (2001) 1–11.
[61] Á. Borja, J. Franco, V. Valencia, J. Bald, I. Muxika, M.J. Belzunce, O. Solaun, Implementation of the European water framework directive from the Basque country (northern Spain): a methodological approach, Mar. Pollut. Bull. 48 (3–4) (2004) 209–218, https://doi.org/10.1016/j.marpolbul.2003.12.001.
[62] J. Bald, Á. Borja, I. Muxika, J. Franco, V. Valencia, Assessing reference conditions and physicochemical status according to the European Water Framework Directive: a case-study from the Basque country (Northern Spain), Mar. Pollut. Bull. 50 (2005) 1508–1522, https://doi.org/10.1016/j.marpolbul.2005.06.019.
[63] I. Muxika, Á. Borja, J. Bald, Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive, Mar. Pollut. Bull. 55 (2007) 16–29, https://doi.org/10.1016/j.marpolbul.2006.05.025.
[64] L.A. Levin, Polychaetes as environmental indicators: response to low oxygen and organic enrichment, Bull. Mar. Sci. 67 (1) (2000) 668. https://10.1016/j.marpolbul.2005.08.003.
[65] K.D. Stolzenbach, E.E. Adams (Eds.), Contaminated Sediments in Boston Harbor, MIT Sea Grant College Program, Marine Center for Coastal Processes, Cambridge, MA, 1998, p. 170.
[66] B. Holte, Possible ecological effects of maldanid (Annelida, Polychaeta) 'superdominance' in a small sill system in northern Norway, Ofelia 55 (1) (2001) 69–75. https://10.1080/00785236.2001.10409474.
[67] D.J. Gillett, S.B. Weisberg, T. Grayson, A. Hamilton, V. Hansen, E.W. Leppo, M.C. Pelletier, Á. Borja, D.C. Cadien, D. Dauer, R. Diaz, M. Dutch, J.L. Hyland, M. Kellogg, P.F. Larsen, J.S. Levinton, R. Llansó, L.L. Lovell, P.A. Montagna, D. Pasko, C.A. Phillips, C. Rakocinski, J.A. Ranasinghe, D.M. Sanger, H. Teixeira, R.F. Van Dolah, R.G. Velarde, K.I. Welch, Effect of ecological group classification schemes on performance of the AMBI benthic index in US coastal waters, Ecol. Indicat. 50 (2015) 99–107, https://doi.org/10.1016/j.ecolind.2014.11.005.
[68] P.A. Montagna, J.G. Baguley, C. Cooksey, J.L. Hyland, Persistent impacts to the deep soft-bottom benthos one year after the Deepwater Horizon event, Integrated Environ. Assess. Manag. 13 (2) (2017) 342–351. https://10.1002/ieam.1791.
[69] M. Manolidis, J.A. Alvarez, Grandes tormentas en la zona costera marplatense entre 1980–1992, Centro Oceanográfico Buenos Aires, Ser. Cien. Tec. 5 (1994) 1–33.
[70] N.A. Philip, T.R. Pritchard, Australia's first deepwater sewage outfalls: design considerations and environmental performance monitoring, Mar. Pollut. Bull. 33 (7–12) (1996) 140–146, https://doi.org/10.1016/S0025-326X(96)00165-8.
[71] A. Mendonça, M.Á. Losada, M.T. Reis, M.G. Neves, Risk assessment in submarine outfall projects: the case of Portugal, J. Environ. Manag. 116 (2013) 186–195, https://doi.org/10.1016/j.jenvman.2012.12.003.
[72] R. Elías, M.S. Rivero, M.A. Sánchez, M.L. Jaubet, E.A. Vallarino, Do treatments of sewage plants really work? The intertidal mussels' community of the southwestern Atlantic shore (38°S, 57°W) as a case study, Rev. Biol. Mar. Oceanogr. 44 (2) (2009) 357–368, https://doi.org/10.4067/S0718-19572009000200009.
[73] F.P.P. Leite, A. Turra, E.C.F. Souza, Population biology and distribution of the tanaid *Kalliapseudes schubarti* Mañé-Garzon, 1949, in an intertidal flat in southeastern Brazil, Braz, J. Biol. 63 (2003) 469–479, https://doi.org/10.1590/S1519-69842003000300013.
[74] R. Elías, M.S. Campodonico, E.A. Vallarino, First record of *Kalliapseudes schubarti* mane-garzon, 1949 Crustacea tanaidacea peracaridae in argentinean waters, Neotropica 47 (2001) 97–99.
[75] P. Lana, C. Guiss, Influence of *Spartina alterniflora* on structure and temporal variability of macrobenthic associations in a tidal flat of Paranagua Bay (southeastern Brazil), Mar. Ecol. Prog. Ser. 73 (1991) 231–244. https://10.3354/meps073231.
[76] T.H. Pearson, R. Rosenberg, Macrobenthic succession in relation to organic enrichment and pollution of the marine environment, Oceanogr. Mar. Biol. Annu. Rev. 16 (1978) 229–311.
[77] R. Elías, M.S. Rivero, E.A. Vallarino, Sewage impact on the composition and distribution of Polychaeta associated to intertidal mussel beds of the Mar del Plata rocky shore, Argentina, Iheringia, Ser. Zool. 93 (3) (2003) 309–318. https://10.1590/S0073-47212003000300009.
[78] R. Elías, M.S. Rivero, A new species of Cirratulidae (Polychaeta) with characteristics of three genera, and a key to the known species around Mar del Plata (south-western Atlantic), J. Mar. Biol. Assoc. U. K. 91 (7) (2011) 1529–1535, https://doi.org/10.1017/S0025315410002146.
[79] S.W. Pawhestri, J.W. Hidayat, S.P. Putro, Assessment of water quality using macrobenthos as bioindicator and its application on abundance-biomass comparison (ABC) curves, Int. J. Eng. Sci. 8 (2) (2015) 84–87. https://dpi.org/10.12777/ijse.8.2.
[80] P. Muniz, A.M.S. Pires, Polychaete associations in a subtropical environment (sao sebastiao channel, Brazil): a structural analysis, Mar. Ecol. 21 (2) (2000) 145–160, https://doi.org/10.1046/j.1439-0485.2000.00696.x.
[81] S.K. J Cochrane, T.H. Pearson, M. Greenacre, J. Costelloe, I.H. Ellingsen, S. Dahle, B. Gulliksen, Benthic fauna and functional traits along a Polar Front transect in the Barents Sea–Advancing tools for ecosystem-scale assessments, J. Mar. Syst. 94 (2012) 204–217, https://doi.org/10.1016/j.jmarsys.2011.12.001.
[82] E.K. Hinchey, L.C. Schaffner, C.C. Hoar, B.W. Vogt, L.P. Batte, Responses of estuarine benthic invertebrates to sediment burial: the importance of mobility and adaptation, Hydrobiologia 556 (2006) 85–98.
[83] P.A. Jumars, K. Fauchald, in: B.C. Coull (Ed.), Between-community Contrasts in Successful Polychaete Feeding Strategies, Ecol. Mar. Bentos, University of South Carolina Press, Columbia. SC, 1977, pp. 1–15.
[84] V. Pandey, D.K. Jha, P.S. Kumar, J. Santhanakumar, S. Venkatnarayanan, J.P.P. Jebakumar, G. Dharani, Effect of multiple stressors on the functional traits of sub-tidal macrobenthic fauna: a case study of the southeast coast of India, Mar. Pollut. Bull. 175 (2022), 113355, https://doi.org/10.1016/j.marpolbul.2022.113355.
[85] D. Paganelli, A. Marchini, A. Occhipinti-Ambrogi, Functional structure of marine benthic assemblages using biological traits analysis (BTA): a study along the emilia-romagna coastline (Italy, north-west adriatic sea), Estuar. Coast Shelf Sci. 96 (2012) 245–256, https://doi.org/10.1016/j.ecss.2011.11.014.
[86] O. Beauchard, H. Veríssimo, A.M. Queirós, P.M.J. Herman, The use of multiple biological traits in marine community ecology and its potential in ecological indicator development, Ecol. Indicat. 76 (2017) 81–96, https://doi.org/10.1016/j.ecolind.2017.01.011.
[87] D. McHugh, P.P. Fong, Do life history traits account for diversity of polychaete annelids? Invertebr. Biol. 121 (4) (2002) 325–338, https://doi.org/10.1111/j.1744-7410.2002.tb00133.x.
[88] T.A. O'Meara, J.E. Hewitt, S.F. Thrush, E.J. Douglas, A.M. Lohrer, Denitrification and the role of macrofauna across estuarine gradients in nutrient and sediment loading, Estuar. Coast 43 (2020) 1394–1405, https://doi.org/10.1007/s12237020-00728-x.
[89] C. Van Colen, F. Montserrat, M. Vincx, P.M.J. Herman, T. Ysebaert, S. Degraer, Macrobenthic recovery from hypoxia in an estuarine tidal mudflat, Mar. Ecol. Prog. Ser. 372 (2008) 31–42, https://doi.org/10.3354/meps07640.
[90] E.R. Baqueiro-Cárdenas, L. Borabe, C.G. Goldaracena-Islas, J. Rodríguez-Navarro, Mollusks and pollution. A review, Rev. Mex. Biodivers. 78 (2) (2007), https://doi.org/10.22201/ib.20078706e.2007.002.293, 1S–7S.
[91] M.N. Gil, E. Giarratano, V. Barros, A. Bortolus, J.O. Codignotto, R.D. Schenke, G.M.E. Góngora, G. Lovrich, A.J. Monti, M. Pascual, A.L. Rivas, A. Tagliorette, Southern Argentina: the patagonian continental shelf, in: World Seas: an Environmental Evaluation, vol. I, Europe, the Americas and West Africa, Elsevier, 2019, pp. 783–811, https://doi.org/10.1016/B978-0-12-805068-2.00040-1.
[92] I.A. E Contreras-Almazo, M. Gonzalez-Renteria, Bivalvos, organismos modelo en el biomonitoreo del riesgo ecotoxicológico de los antinflamatorios no esteroideos (AINE) para los ecosistemas acuáticos, Ecosistemas 2167 (2022), https://doi.org/10.7818/ECOS.2167.

[93] J.A. de-la-Ossa-Carretero, Y. Del-Pilar-Ruso, F. Giménez-Casalduero, J.L. Sánchez-Lizaso, J.C. Dauvin, Sensitivity of amphipods to sewage pollution, Estuar. Coast Shelf Sci. 96 (2012) 129–138, https://doi.org/10.1016/j.ecss.2011.10.020.

[94] L. Gómez Gesteira, J.C. Dauvin, Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities, Mar. Pollut. Bull. 40 (11) (2000) 1017–1027, https://doi.org/10.1016/S0025-326X(00)00046-1.

[95] J.C. Dauvin, T. Ruellet, Polychaete/amphipod ratio revisited, Mar. Pollut. Bull. 55 (1–6) (2007) 215–224, https://doi.org/10.1016/j.marpolbul.2006.08.045.

[96] M.E. Pedelacq, G.V. Garaffo, E.N. Llanos, N. Venturini, P. Muniz, Pollution has negative effects on macrozoobenthic trait diversity in a large subtropical estuary, Mar. Pollut. Bull. 184 (2022), 114101, https://doi.org/10.1016/j.marpolbul.2022.114101.

[97] D. Piló, R. Ben-Hamadouc, F. Pereira, A. Corzo, M.B. Gaspar, S. Carvalho, A. Carriço, P. Pereira, How functional traits of estuarine macrobenthic assemblages respond to metal contamination? Ecol. Indicat. 71 (2016) 645–659, https://doi.org/10.1016/j.ecolind.2016.07.019.

[98] H. Selck, A.W. Decho, V.E. Forbes, Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete Capitella species I, Environ. Toxicol. Chem. 18 (1999) 1289–1297, https://doi.org/10.1002/etc.5620180631.

[99] R. Rosenberg, Marine benthic faunal successional stages and related sedimentary activity, Sci. Mar. 65 (S2) (2001) 107–119, https://doi.org/10.3989/scimar.2001.65s2107.

[100] Y. Rao, L. Cai, X. Chen, X. Zhou, S. Fu, H. Huang, Responses of functional traits of macrobenthic communities to human activities in Daya Bay (A subtropical semi-Enclosed Bay), China, Front. Environ. Sci. 9 (2021), 766580, https://doi.org/10.3389/fenvs.2021.766580.

[101] R. Martins, L. Sampaio, A.M. Rodrigues, V. Quintino, Soft-bottom Portuguese continental shelf polychaetes: diversity and distribution, J. Mar. Syst. 123–124 (2013) 41–54, https://doi.org/10.1016/j.jmarsys.2013.04.008.

[102] P.A. Ramey, D. Fiege, B.S. Leander, A new species of *Polygordius* (Polychaeta: polygordiidae): from the inner continental shelf and in bays and harbours of the north-eastern United States, J. Mar. Biol. Assoc. U. K. 86 (5) (2006) 1025–1034, https://doi.org/10.1017/S0025315406014007.

[103] L. Bigot, C. Conand, J.M. Amouroux, P. Frouin, H. Bruggemann, A. Grémare, Effects of industrial outfalls on tropical macrobenthic sediment communities in Reunion Island (Southwest Indian Ocean), Mar. Pollut. Bull. 52 (8) (2006) 865–880, https://doi.org/10.1016/j.marpolbul.2005.11.021.

[104] J.B. Socolar, J.J. Gilroy, W.E. Kunin, D.P. Edwards, How should beta-diversity inform biodiversity conservation? Trends Ecol. Evol. 31 (1) (2016) 67–80, https://doi.org/10.1016/j.tree.2015.11.005.

[105] J. Dong, X. Sun, Q. Zhan, Y. Zhang, X. Zhang, Patterns and drivers of beta diversity of subtidal macrobenthos community on the eastern coast of Laizhou Bay, Biodivers. Sci. 30 (3) (2022), 21388, https://doi.org/10.17520/biods.2021388.

[106] W.K. Cornwell, D.W. Schwilk, D.D. Ackerly, A trait-based test for habitat filtering: convex hull volume, Ecology 87 (6) (2006) 1465–1471, https://doi.org/10.1890/0012-9658 (2006)87[1465:ATTFHF]2.0.CO;2.

[107] N.W.H. Mason, D. Mouillot, W.G. Lee, J.B. Wilson, Functional richness, functional evenness and functional divergence: the primary components of functional diversity, Oikos 111 (2005) 112–118. https://10.1111/j.0030-1299.2005.13886.x.

[108] M. Nyström, Redundancy and response diversity of functional groups: implications for the resilience of coral reefs, AMBIO A J. Hum. Environ. 35 (1) (2006) 30–35, https://doi.org/10.1579/0044-7447-35.1.30.

[109] N.W. H Mason, C. Lanoiselée, D. Mouillot, J.B. Wilson, C. Argillier, Does niche overlap control relative abundance in French lacustrine fish communities? A new method incorporating functional traits, J. Anim. Ecol. 77 (2008) 661–669, https://doi.org/10.1111/j.1365-2656.2008.01379.x.

[110] F. Córdova-Tapia, L. Zambrano, La diversidad funcional en la ecología de comunidades, Ecosistemas 24 (3) (2015) 78–87. https://10.7818/ECOS.2015.24-3.10.

[111] M. Zobel, The relative role of species pools in determining plant species richness. An alternative explanation of species coexistence? Trends Ecol. Evol. 12 (7) (1997) 266–269. https://10.1016/S0169-5347(97)01096-3.

[112] J. Soininen, J. Heino, J. Wang, Un metanálisis de los componentes de anidamiento y rotación de la diversidad beta en organismos y ecosistemas, Ecología global y biogeografía 27 (1) (2018) 96–109, https://doi.org/10.1111/geb.12660.

[[113] C. Gutiérrez-Cánovas, A. Millán, J. Velasco, I.P. Vaughan, S.J. Ormerod, Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms: beta diversity along natural and anthropogenic stress gradients, Global Ecol. Biogeogr. 22 (7) (2013) 796–805, https://doi.org/10.1111/geb.12060.

[114] Z. Alsaffar, J. Cúrdia, Á. Borja, X. Irigoien, S. Carvalho, Consistent variability in beta-diversity patterns contrasts with changes in alpha-diversity along an onshore to offshore environmental gradient: the case of Red Sea soft-bottom macrobenthos, Mar. Biodivers. 49 (1) (2019) 247–262, https://doi.org/10.1007/s12526-017-0791-3.

[115] A.B. Jöst, M. Yasuhara, C. Wei, H. Okahashi, A. Ostmann, P. Martínez Arbizu, B. Mamo, J. Svavarsson, S. Brix, North Atlantic Gateway: test bed of deep-sea macroecological patterns, J. Biogeogr. 46 (9) (2019) 2056–2066, https://doi.org/10.1111/jbi.13632.

[116] D. Piló, A.N. Carvalho, F. Pereira, H.E. Coelho, M.B. Gaspar, Evaluation of macrobenthic community responses to dredging through a multimetric approach: effective or apparent recovery? Ecol. Indicat. 96 (2019) 656–668, https://doi.org/10.1016/j.ecolind.2018.08.064.

[117] M. L Moraitis, I. Karakassis, Assessing large-scale macrobenthic community shifts in the Aegean Sea using novel beta diversity modelling methods, Ramifications on environmental assessment, Sci. Total Environ. 734 (2020), 139504, https://doi.org/10.1016/j.scitotenv.2020.139504.

[118] B. Sommer, P.L. Harrison, M. Beger, J.M. Pandolfi, Trait-mediated environmental filtering drives assembly at biogeographic transition zones, Ecol. 95 (4) (2014) 1000–1009.

[119] J. Heino, T. Nokela, J. Soininen, M. Tolkkinen, L. Virtanen, R. Virtanen, Elements of metacommunity structure and community environment relationships in stream organisms, Freshw. Biol. 60 (2015) 973–988. https://10.1111/fwb.12556.

[120] A. Menegotto, C.S. Dambros, S.A. Netto, The scale-dependent effect of environmental filters on species turnover and nestedness in an estuarine benthic community, Ecol. 100 (7) (2019), e02721. https://10.1002/ecy.2721.

[121] D. Piló, F. Leitão, R. Ben-Hamadou, P. Range, M. Chícharo, L. Chícharo, Macrobenthic response to sewage discharges in confined areas from coastal lagoons: implication on the ecological quality status, Vie Milieu 61 (2) (2011) 107–118.