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Driving forces that structure sublittoral macrobenthic communities in sandy beaches along environmental gradients

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ABSTRACT

Sandy beaches are very dynamic and changeable environments that present a wide variety of life forms, and, in some areas, high productivity. In the Gulf of Valencia (Western Mediterranean Sea, Spain) these areas have endured an economically important shellfishery of clams (*Donax trunculus* and *Chamelea gallina)*. In the Mediterranean, a sea with a notable oligotrophic character, rivers are particularly important because of the contributions of fresh water that carry nutrients and organic matter to coastal ecosystems. Consequently, close to the mouths of the rivers, it is possible to investigate the functions of physical, chemical and biological gradients on the macrofauna structuring of sandy beach communities. The objective of this study was to determine the response of sublittoral benthic communities to environmental variables in microtidal intermediate sandy beaches of the south of the Gulf of Valencia. Samples of benthic macrofauna, water and sediment were collected at 5 stations located in the sublittoral zone at different distances to freshwater sources, each one sampled at five depths, in three sampling campaigns. In general, the physicochemical and nutritional variables of water showed a spatial variation alongshore, and only a few variables (dissolved inorganic nitrogen, suspended solids and salinity) also had an across-shore variation. This variability was due to the different freshwater contributions. The sediment variables (mean grain size and organic matter) presented clear patterns related to depth. With respect to the macrofauna, positive relationships with depth were observed for the total density, density of bivalves, polychaetes, crustaceans and *C. gallina*, while *D. trunculus* showed the opposite pattern. The proximity to freshwater sources, favoured a greater abundance of organisms. Regarding temporal variation, two very different phytoplanktonic compositions between winter and summer could be distinguished, while, for the macrofauna, differences in abundance were observed with maximum values in summer. The influence of freshwater discharges on the phytoplankton primary production will have an influence on the abundance of macrobenthic fauna, but after a time lag. By means of CCA analyses, it was found that the communities of benthic macrofauna were strongly influenced by physical factors such as mean grain size, as well as the nutritional variables (organic matter in sediment and microalgae in the water column) and the distance to the freshwater discharges. The identification of these variables is crucial in order to develop an adequate coastal management, paying special attention to the anthropogenic activities that may be modifying the environment and, therefore will affect the biocoenoses.

Keywords: sandy-beach invertebrates; environmental factors; macrofaunal assemblages; microtidal sandy beaches

1. INTRODUCTION

Exposed sandy beaches are very dynamic systems in space and time, inhabited by biological communities that are fundamentally structured by physical drivers (Defeo and McLachlan, 2005). In most ecological systems, including those in the oceans, community structure arises from a complex interaction between biotic interactions and abiotic environmental factors (Blanchette et al., 2009; Cury et al., 2008). However, the ecological theory of sandy beaches establishes that physical characteristics control macrobenthic community structure on all beaches, except on the most dissipative ones (Ortega Cisneros et al., 2011).

Sandy beaches, despite their dynamism, harbour a wide variety of lifeforms, becoming very productive under dissipative conditions (Defeo and McLachlan, 2005; McLachlan, 1983; McLachlan, 2001; McLachlan and Defeo, 2018). Due to instability and complex environmental changes that sandy beaches undergo, fauna that inhabit them develop adaptations related to locomotion, sensory responses, nutrition, respiration and reproduction, among others (McLachlan and Defeo, 2018). Generally, in these ecosystems, molluscs, crustaceans and polychaetes are found as dominant groups of macrofauna, often constituting more than 90% of the species and biomass (Gray and Elliott, 2009; McLachlan and Defeo, 2018). At the meso-scale, variations of macrofauna communities can be observed both alongshore and across-shore (Defeo and McLachlan, 2005; McLachlan and Defeo, 2018). Distribution, abundance and richness of macrobenthic communities are conditioned by physical-chemical variables such as temperature, pH, salinity, dissolved oxygen, nutrients and those related to beach morphodynamics (slope, sediment particle size, hydrodynamism ...), as well as nutritional and biological factors (life cycles, mortality, depredation and competitiveness by resources, among others) (Brazeiro, 2001; Defeo and McLachlan, 2005; Hewitt et al., 1997; Lastra et al., 2006; Ortega Cisneros et al., 2011; Rodil et al., 2007). Authors such as Ortega Cisneros et al. (2011) highlighted the importance of combining physical, chemical and nutritional variables for studies on community structure. In most of the investigations carried out to explain patterns of community structure, only abiotic characteristics tend to be taken into account, but it would be more appropriate to also consider the potential biotic interactions (Defeo et al., 1997; Defeo and McLachlan, 2005; Hewitt et al., 1997; Rodil et al., 2012).

Sandy beaches have some physical features relevant to characterize it, such as sand particle size, type of wave action and breaker height, tide, beach face slope, intertidal and surf zone extend, number of bars, indices of beach type/state, beach type and beach state… Beach type is based on the contribution of waves and tides to the morphology of the beach and beach morphodynamic state can be defined principally by physical factors such as wave energy, tidal range and sand particle size (McLachlan, 2001; McLachlan et al., 2018). McLachlan et al. (2018) proposed a classification of sandy beaches being carried out in two steps: the first one is in order to determine the beach type (wave dominated, tide modified and tide dominated) and the second is to determine the beach state within the type (reflective, intermediate and dissipative). The physical factors that influence macrofauna community structure would primarily be sediment grain size, as well as coast slope and length, among other factors (Lastra et al., 2006; Lercari and Defeo, 2006; McLachlan, 1996; McLachlan and Dorvlo, 2005; Rodil and Lastra, 2004). There have also been several authors who have observed an influence of salinity on community abundance and richness, both decreasing in the areas closest to the freshwater contributions and during periods of greater precipitation (Lercari and Defeo, 2006; Lercari et al., 2002; Lozoya et al., 2010; Ortega Cisneros et al., 2011). In addition, other investigations highlighted the importance of the feeding of populations - organic matter in sediment or availability of food in the water column (Lastra et al., 2006; McLachlan and Defeo, 2018; Rodil et al., 2007).

River mouths are key locations for investigating the influence of physical, chemical and nutritional variables on benthic fauna. These environments are particularly important in oligotrophic seas such as the Mediterranean, which depends strongly on the contributions of fresh water that carry nutrients necessary for primary production, and organic matter which are sources of food for many organisms (Levinton, 2011; Ludwig et al., 2009; Schlacher and Connolly, 2009). On the Spanish Mediterranean coast, Pinedo et al. (1997) analysed the effect of precipitation, hydrographic factors, concentration of chlorophyll *a*, granulometry and organic matter in sediment, on the trophic groups of the macrobenthic community in the Bay of Blanes

(Northwestern Mediterranean Sea). In that study, the spatial distribution of the benthic macrofauna was explained by the depth, granulometry and organic matter, while the temporal distribution by the rainfall, chlorophyll *a* and organic matter in sediment, without any significant effect from temperature or salinity. In the Gulf of Valencia (Western Mediterranean Sea, Spain) few studies have been carried out in relation to determining the influence of environmental variables on benthic community structure, hence it is still a subject of investigation. Martí et al. (2007) analysed the temporal changes of bivalves and polychaetes from Cullera Bay (Western Mediterranean Sea), observing the influence of tourist pressure and water contributions in the shallowest area, while, in deeper areas, granulometry and climatic and meteorological conditions were more relevant. In these studies, it is important to take into account the repercussions that changes in the ecosystems may have on benthic communities, where species of socio-economic relevance can be found. In the case of the Gulf of Valencia, two species of bivalves, *Donax trunculus* and *Chamelea gallina*, have a high fishing interest but they have suffered notable declines in their populations in the last decade, leading to the closure of the fishery in June 2015 (Valencian Community Resolution of June 3, 2015).

On sandy beaches, the sublittoral area is characterised by saturated sand, strong wave energy, bottom currents and a bed mobile (Brazeiro and Defeo, 1996; McLachlan and Defeo, 2018). In this area we can find the surf zone, the transition zone and the outer turbulent zone. In the outer turbulent zone the bottom becomes more stable and a rich macrofauna develops, with greater richness and biomass. This decreases in the surf zone as it is a turbulent zone with a less stable seabed (McLachlan and Defeo, 2018). At greater depths the abundance and diversity of benthic invertebrates increases (Barros et al., 2002). The zonation of the macrobenthonic species responds to the strong physical gradients of the beach, mainly due to the individual response of each species to abiotic variables and also to intra and interspecific competition, especially in more dissipative beaches (Defeo and McLachlan., 2005; Defeo et al., 2003; McLachlan and Defeo, 2018; McLachlan and Dorvlo 2005; Ortega Cisneros et al., 2011). In addition, freshwater discharges to the sea generate modifications in the sandy beach ecosystems, due to the contribution of nutrients, organic matter and particulate matter, as well as geomorphology (Cantera et al., 1994; Levinton, 2011; Gillanders and Kingsford, 2002; Ludwig et al., 2009; Schlacher and Connolly, 2009) affecting the distribution of macrofauna (Bergamino et al., 2009). Variations in freshwater discharges have an impact on benthic habitats. Nutrient enrichment of coastal waters is linked to high densities of some invertebrates such as crustaceans (Gillanders and Kingsford, 2002; Loneragan and Bunn, 1999).

Therefore, we suggest that in addition to the physical characteristics of the environment, the contributions of fresh water to the sea can condition the distribution and abundance of the benthic macrofauna of the sublittoral area of sandy beaches. We hypothesized that sandy beaches influenced by freshwater discharges may present a greater abundance and richness of species due to the variation of environmental variables along the coast. To determine whether the macrobenthic community responds to different physical, chemical and nutritional factors in the sublittoral zone of wave dominated intermediate sandy beaches, three sampling campaigns were carried out at five sampling along-shore stations, each one sampled at five depths, and located at different distances to freshwater sources in Gandia (Western Mediterranean Sea, Spain). To address this aim, the objectives were: (1) to characterize the temporal and spatial dynamics of the macrobenthic community structure in the sublittoral area; (2) to determine the temporal and spatial patterns for physical, chemical and nutritional variables; and (3) to determine which physical, chemical and nutritional variables were most influential in describing changes in the macrobenthic community structure.

2. MATERIAL AND METHODS

2.1. Study area

The study area is located on the microtidal wave dominated intermediate sandy beaches of the Gulf of Valencia (Western Mediterranean Sea, Spain) (Fig. 1) close to: i) the Serpis River mouth, which is the main river that provides fresh water to this area and it is artificially regulated by a complex system of weirs and irrigation channels that provide fresh water to irrigated crops of the Safor County; ii) the Gandia Port mouth, that receives waters from the San Nicolas ravine, a 14 km long watercourse of which the flow relies strongly on torrential rainfalls and only in its last

1.5 km the flow is continuous, due to the input of fresh water draining the Safor Wetland (Sebastiá et al., 2012) and; iii) the submarine outfall of the wastewater treatment plant (WWTP) of Gandia that discharges wastewater at 17 m depth and at 1900 m from the coast, in front of the harbour. Climatically, this area is characterized by its water scarcity in the dry season (summer) and abundant and brief rainfall in the wet season (autumn) (Garófano-Gómez et al., 2009). The annual precipitation of the zone shows values over 800 mm (Miró et al., 2018). Morphodynamically, the beaches of the zone were classified as intermediate exposed sandy beaches by Escrivá (2013).

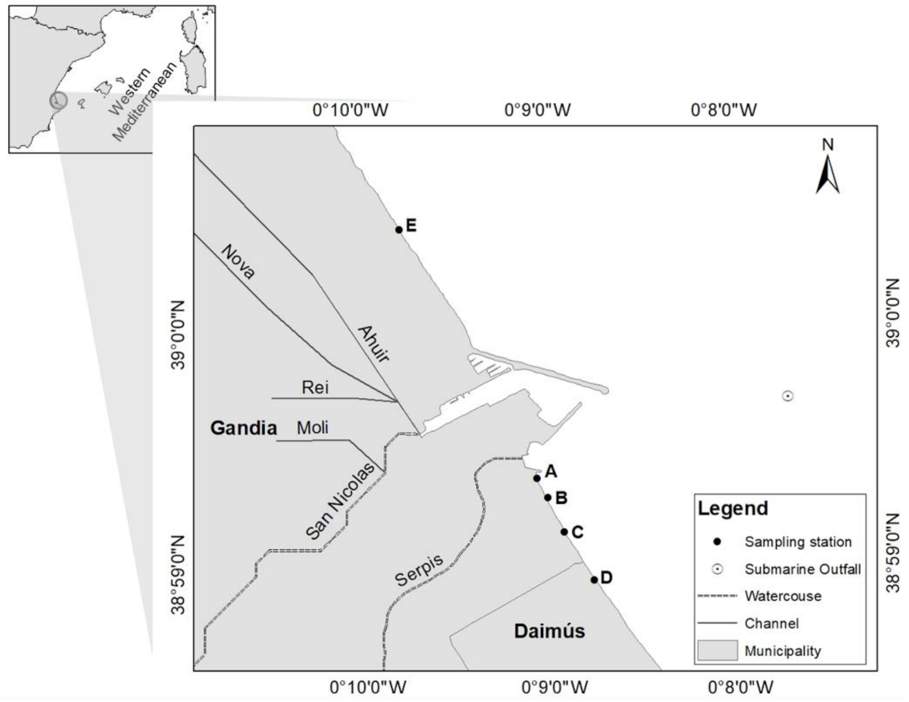


Fig. 1. Study area in the Gulf of Valencia (Western Mediterranean Sea, Spain) indicating the position of the five sampling stations (from A to E).

2.2. Sampling design

Three sampling campaigns were carried out in the sublittoral zone: the first one (July 2013) from 30th July to 5th August 2013; the second (February 2014) between 3rd and 14th February 2014 and the third (December 2014) between 12th and 17th December 2014.

In each campaign, samples of water, sediment and benthic macrofauna were obtained at five sampling stations: four of them located to the south of the mouth of the Serpis River at 50 (A), 200 (B), 500 (C) and 1000 (D) m away - presumably influenced by freshwater contributions from both the river and the Port of Gandia, since the dominant currents move from north to south

(CEDEX, 1997; Millot, 1999) - and a fifth sampling station at 1000 (E) m distance north of the mouth, away from the influence of fluvial inputs (Fig. 1).

At each station, transects were established perpendicular to the coastline, taking samples in the sublittoral zone at five different depths (0.5, 1, 2, 3 and 4 m): at 0.5 m in the zone between the low tide line and the area where the waves break, at the break point (1 m), and at 2, 3 and 4 m in the transition and outer turbulent zone.

2.3. Environmental and nutritional variables analysis

2.3.1. River flow and precipitation

The Serpis flow and rainfall in the area of influence were obtained from the Automatic Hydrological Information System (SAIH) of the Júcar Hydrographic Confederation (CHJ). The values of the flow and precipitation were gathered from the Assut d'En Carròs meteorological and gauging station, located about 10 km from the estuary, as it is the closest station to the mouth of the river where the information is available.

2.3.2. Water variables

In order to analyse the physicochemical characteristics of the water, samples were taken at each sampling station and depth. To do this, a Niskin oceanographic bottle was used to collect water from 50 cm above the seabed.

In situ, temperature and salinity were measured using the multiparametric probe Multi 340i WTW. In the laboratory, the following variables were analysed: photosynthetic pigments, suspended solids, total phosphorus and dissolved nutrients (ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}) and silicate ($Si(OH)_4$). The dissolved nutrients were analysed using the methods proposed by Aminot and Chaussepied (1983) and adapted by Baumgarten et al. (2010). Dissolved inorganic nitrogen (DIN) was calculated as the sum of: ammonium, nitrite and nitrate. To determine total phosphorus (TP), digestions of unfiltered samples were made according to Valderrama (1981) before its determination using the same method for PO_4^{3-}. The photosynthetic pigments (alloxanthin, chlorophyll *a* (Chl_*a*), chlorophyll *b* (Chl_*b*), fucoxanthin, lutein, neoxanthin, peridinin, prasinoxanthin, violoxanthin, zeaxanthin, 19'butanoyloxyfucoxanthin, 19'hexanoyloxyfucoxanthin) were analysed using the highperformance liquid chromatography (HPLC) technique. The HPLC method employed was that proposed by Wright et al. (1991), recommended by the Scientific Committee on Oceanic Research (SCOR), and slightly modified as per Hooker et al. (2000). Suspended solids were analysed according to APHA (2012).

2.3.3. Sediment variables

At each sampling station, sediment samples were taken at each of the depths. At each point a sample was collected using a Ponar dredge. A granulometric analysis (Shepard, 1954) was conducted on each sample in triplicate and the mean grain size was calculated (Friedman and Sanders, 1978). Furthermore, the concentration of organic matter (OM) was analysed in triplicate, employing the method proposed by Pusceddu et al. (2004).

2.4. Macrobenthic community analysis

At each point, sediment samples were taken in triplicate to reduce heterogeneity in the distribution of macrofauna in the sediments, using a Ponar dredge (with an area of 0.059 m^2). The samples were sieved through a 500 µm mesh to remove excess sand and retain the benthic macrofauna as per the methodology proposed by Castelli et al. (2004). Subsequently, the organisms were submerged in a 7 % magnesium chloride solution as an anaesthetic and preserved in 10 % buffered formaldehyde. In the laboratory, the remaining sediment was removed manually, and the specimens were identified to the lowest taxonomic level possible and counted. In addition, the organism density was calculated as the number of individuals per square meter (ind m^{-2}).

2.5. Data analyses

Data analyses were conducted through a range of statistical analysis. To determine the spatial and temporal influence of the different variables, ANOVA multifactorial tests were done and a Tukey post hoc test done when a significant result was found. These analyses were carried out for each variable using as factors “Time”, “Depth” and “Station”. In addition, the interaction among factors was also analysed. Those variables that did not follow a normal distribution were transformed by their natural logarithm. In addition, a cluster analysis was done to investigate similarity in macrobenthic composition of the different points and sampling campaigns, using the Ward method and Manhattan distance. These tests were carried out using the software package

Statgraphics Centurion XVII. Finally, a canonical correspondence analysis (CCA) was conducted in order to determine the most influential physical, chemical and nutritional variables in the macrobenthic community, taking into account those species that were found in at least 20 % of the sampling points (Rodil et al., 2006). Furthermore, a second CCA was carried out on the species richness, using all the species at each sampling point. For these analyses, the CANOCO 5 software was used. The statistical significance of the relationships was evaluated using Monte Carlo permutation tests with a manual forward selection procedure.

3. RESULTS

3.1. River flow and precipitation

The annual precipitation obtained from the nearest meteorological station to the study area (Assut d'En Carròs) was 471 and 258 L m^{-2} for the years 2013 and 2014 respectively. These values showed precipitation clearly lower than what is usual in the area (Miró et al., 2018). During the 30 days prior to the beginning of the first sampling campaign (July 2013), there was no discharge of water from the river, and the cumulative monthly rainfall was low (2 mm). In contrast, in February 2014 and December 2014, the river had a mean flow of 1 m^3 s^{-1} and 0.8 m^3 s^{-1} during each prior month respectively, and the cumulative monthly precipitation was 12.4 and 96.2 mm.

3.2. Environmental and nutritional variables

Table 1 shows the minimum and maximum values of the main environmental variables, for each station and sampling campaign, as well as the depths at which they were obtained. Variables that had significant statistical differences between stations (Table S1) were salinity (p = 0.0015), which showed lower values at the station located 1000 m north of the mouth (E) and 200 m south (B) and SS with highest values at station E and the two stations located immediately south of the freshwater sources (A and B). With respect to nutrients, DIN (p = 0.0154) and $Si(OH)_4$ (p = 0.0058) also showed significant effects, with higher concentrations at station B which present an inverse pattern to salinity. Regarding pigments that showed significant differences, Chl_*b* (p = 0.0015) and 19'hexanoyloxyfucoxanthin (p < 0.0001) showed the highest values at the two stations furthest away from freshwater sources (E and D), while for peridinin (p < 0.0001) and zeaxanthin (p = 0.0432) E and D were the ones with the lowest concentration. Furthermore, in sediments, the mean grain size ($p = 0.0043$) showed statistical differences with lower values in the transects closest to the freshwater contributions (A, B and C) and E.

The variables that presented significant statistical effects with respect to the depth in water were: salinity ($p = 0.0007$), increasing with depth, while SS ($p = 0.0175$) and DIN ($p = 0.0001$) showed the opposite pattern than salinity, with highest values in the shallowest areas (0.5 and 1 m). With respect to sediment variables, both mean grain size ($p < 0.0001$) and OM ($p < 0.0001$) showed a significant effect, with clear patterns of variation with depth, decreasing mean grain size and increasing OM when depth increases.

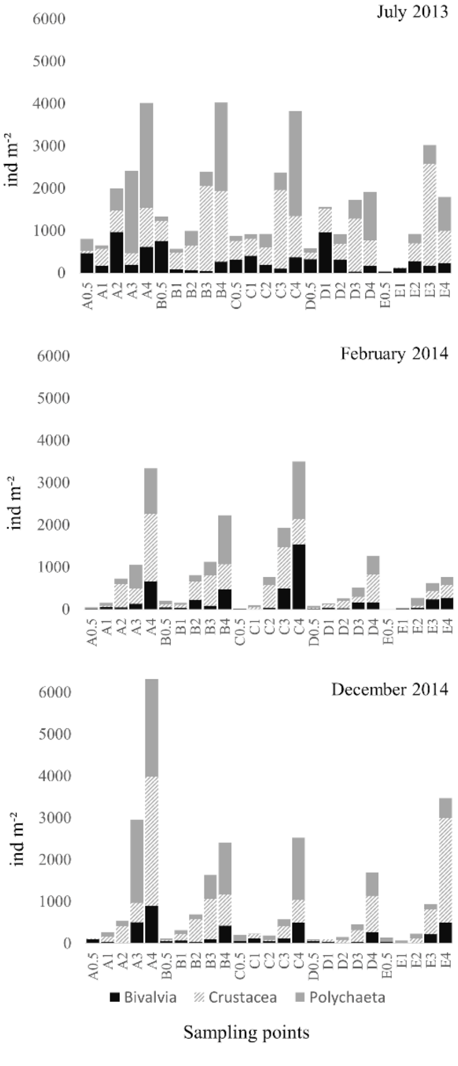
Table 1. Minimum and maximum values of the environmental variables at each station and sampling campaign, the depths at which they were found are shown in brackets. Temp.: temperature; Allox.: alloxanthin; Perid.: peridinin; 19’hexan.: 19’hexanoyloxyfucoxanthin; MGS: mean grain size.

		July 2013					February 2014					December 2014				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
		Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.	Min.-Max.
Temp.	ºC	26.1-26.8	25.6-26.3	24.6-25.6	24.1-25.1	25.7-26.7	11.6-12.6	12.5-13.5	12.4-12.7	12.9-13.7	11.2-12.1	14.1-14.9	14.2-15.1	15.2-16.3	15.5-16.3	13.7-16.3
Salinity		37.6-37.8	37.3-37.5	37.6-37.8	37.3-37.6	37.1-37.5	36.7-37.1	36.8-37.0	36.8-37.1	37.0-37.2	36.7-37.0	37.5-37.6	37.3-37.6	37.1-37.7	37.5-37.6	37.2-37.8
Depth	m	(3-2;4)	(2;3-0.5;1)	(0.5-1)	(1-2;3;4)	(0.5;1-4)	(2-4)	(4-2)	(0.5;4- 1;2;3)	(0.5;2-1;4)	(0.5-2)	(0.5;2;4-1;3)	(0.5-3;4)	(0.5-3)	(0.5;1-2;3;4)	(0.5-4)
SS	mg L^{-1}	9.0-15.8	10.5-18.3	7.9-15.8	8.0-16.5	8.2-13.8	8.4-13.8	6.6-10.0	6.3-9.4	7.0-9.8	9.0-14.8	12.7-19.5	10.5-20.7	8.8-16.0	10.2-13.8	11.2-53.5
Depth	m	(2-1)	(3-2)	(0.5-2)	(3-2)	(2-1)	(2-1)	(1;3-4)	(4-0.5)	(2;4-1)	(3-0.5)	(0.5-2)	(3-0.5)	(3-0.5)	(1-0.5)	(2-0.5)
DIN	µM	0.1-3.0	3.5-6.6	0.3-5.8	0.3-6.4	0.1-3.3	3.2-4.6	3.2-5.5	3.7-4.7	1.8-4.1	3.0-4.2	3.7-6.6	4.6-7.7	2.8-10.0	3.7-6.8	2.0-4.9
Depth	m	(4-3)	(4-1)	(4-1)	(3-0.5)	(4-2)	(1-4)	(4-2)	(4-0.5)	(4-2)	(4-3)	(1-3)	(4-1)	(3-0.5)	(2-1)	(3;4-0.5)
Si(OH)$_4$	µM	3.3-3.9	5.1-6.8	2.3-4.0	2.3-6.2	2.9-5.0	0.9-3.6	0.9-6.8	1.7-5.2	1.2-2.4	0.8-1.3	4.5-5.5	4.1-8.2	3.5-10.6	4.0-5.7	3.0-4.34
Depth	m	(2-1)	(3-2)	(4-0.5)	(3-0.5)	(4-1)	(1-2)	(4-0.5)	(1-4)	(1-2)	(1-2)	(2-0.5)	(4-0.5)	(4-0.5)	(3-1)	(0.5-2)
TP	µM	0.18-0.26	0.23-0.38	0.20-0.29	0.11-0.23	0.14-0.23	0.03-0.26	0.12-0.14	0.03-0.18	0.07-0.13	0.12-0.19	0.12-0.24	0.09-0.19	0.16-0.26	0.22-0.36	0.17-0.61
Depth	m	(4-2)	(1-4)	(3;4-0.5;1)	(4-0.5)	(0.5-2)	(0.5-3)	(0.5;3-4)	(0.5-1)	(4-0.5)	(2-1)	(1-3)	(2-0.5)	(2;3-0.5;1)	(0.5-4)	(4-1)
Chl_*a*	µg L^{-1}	0.123-0.245	0.186-0.232	0.062-0.146	0.114-0.219	0.130-0.370	0.170-0.295	0.217-0.369	0.263-0.367	0.331-0.530	0.281-0.550	0.151-0.353	0.003-0.229	0.108-0.237	0.003-0.174	0.107-0.141
Depth	m	(3-2)	(1-2)	(4-1)	(3-1)	(0.5-4)	(4-1;3)	(0.5-3)	(2;4-1)	(0.5-1)	(2-4)	(2-0.5)	(0.5-3)	(2-0.5)	(0.5-1)	(1-2)
Allox.	µg L^{-1}	0.027-0.063	0.068-0.102	0.015-0.091	0.036-0.131	0.045-0.113	0.059-0.113	0.059-0.161	0.137-0.311	0.212-0.508	0.105-0.192	0.010-0.029	<0.003-0.016	0.007-0.021	<0.003-0.017	0.003-0.009
Depth	m	(3-2)	(2-0.5)	(4-1)	(3;1)	(0.5-4)	(4-3)	(2-0.5)	(4-0.5)	(3-1)	(1-3)	(2-0.5)	(0.5;4-2)	(2-0.5)	(0.5;2-1)	(1-2;4)
Perid.	µg L^{-1}	<0.03-0.16	0.11-0.13	<0.03-0.05	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Depth	m	(0.5;1-2)	(3-0.5)	(1-0.5)												
19’hexan.	µg L^{-1}	<0.01-0.06	0.03-0.14	<0.01-0.14	0.12-0.28	0.15-0.24	<0.01-0.07	<0.01	<0.01	0.03-0.30	<0.01-0.29	<0.01-0.01	<0.01	<0.01	<0.01	<0.01
Depth	m	(2;3;4-1)	(1-0.5)	(4-1)	(3-1)	(3-1)	(1;2;3;4-0.5)			(2-3)	(0.5;3-2)	(1;2;3;4-0.5)				
MGS	mm	0.14-0.24	0.15-0.25	0.16-0.26	0.17-0.23	0.16-0.20	0.14-0.25	0.18-0.23	0.16-0.32	0.18-0.28	0.18-0.23	0.13-0.27	0.15-0.28	0.15-0.24	0.17-0.27	0.17-0.23
Depth	m	(4-0.5)	(4-0.5)	(4-2)	(4-2)	(2;4-0.5)	(4-1)	(3;4-0.5)	(4-0.5)	(3;4-2)	(2;4-0.5)	(4-0.5;1)	(4-0.5)	(3-1)	(4-0.5)	(4-1)
OM	%	0.31-1.34	0.75-1.09	0.50-0.86	0.49-0.95	0.36-0.61	0.71-1.27	0.53-0.97	0.40-1.28	0.26-1.06	0.49-0.84	0.06-0.75	0.01-1.05	0.38-1.25	0.15-0.88	0.37-0.77
Depth	m	(1-4)	(1-3)	(2-4)	(2-3)	(1-0.5)	(0.5-4)	(1-2)	(0.5-3)	(2-3)	(4-1)	(1-0.5)	(1-4)	(0.5-4)	(0.5-3)	(0.5-3)

Regarding temporal variation of the environmental variables (Table S1), the highest temperatures were observed in July 2013, coinciding with the summer period, and the lowest in February 2014, showing significant statistical differences between the three campaigns ($p < 0.0001$). In the case of salinity, the differences were found between February 2014, with lower values, and the other two sampling campaigns ($p < 0.0001$). Some nutrients also showed significant differences between sampling campaigns. The highest DIN values were observed in February 2014 and December 2014 sampling campaigns ($p < 0.0001$). However, $Si(OH)_4$ and the variable TP (both $p < 0.0001$) showed the same pattern with higher concentrations in December 2014 and July 2013. The SS ($p < 0.0001$) displayed significant differences between the three sampling campaigns, with maximums in December 2014. Finally, all the photosynthetic pigments displayed significant differences between sampling campaigns. The pigments that showed significant differences between February 2014 and the remaining sampling campaigns were Chl_*a* ($p < 0.0001$), Chl_*b* ($p < 0.0001$), fucoxanthin ($p < 0.0001$), neoxanthin ($p = 0.0001$), peridinin ($p < 0.0001$) and prasinoxanthin ($p < 0.0001$) presenting in all cases the highest values in February 2014. In the case of alloxanthin ($p < 0.0001$), violaxanthin ($p < 0.0001$), 19'butanoyloxyfucoxanthin ($p < 0.0001$) and 19'hexanoyloxyfucoxanthin ($p < 0.0001$), there were differences between the three sampling campaigns, also with higher values in February 2014, except 19'hexanoyloxyfucoxanthin which showed higher concentrations in July 2013. Finally, lutein had significant differences ($p = 0.0135$) between February 2014 and December 2014, and zeaxanthin ($p = 0.001$) between July 2013 and December 2014, with higher concentrations in July 2013. In sediments, only OM showed significant differences between February 2014 and December 2014. In the different water variables analysed, there were significant interactions between factors. The most frequent interaction was between station and sampling campaign for the variables temperature, salinity, SS, DIN, TP, alloxanthin, Chl_*a*, Chl_*b*, fucoxanthin, peridinin, 19'butanoyloxyfucoxanthin and 19'hexanoyloxyfucoxanthin (Table S1), while for the variables of sediment the interactions were mainly between depth and station. The sediment variables showed a greater similarity between the different depths in the transect less influenced by the contribution of fresh water (E).

3.3. Macrobenthic community

In Fig. 2 the abundance of bivalve, crustacean and polychaete can be observed for each station, depth and sampling campaign. Regarding the difference between stations (Table S2) total density ($p < 0.0001$), and the density of bivalves ($p = 0.0252$), crustaceans ($p < 0.0001$), polychaetes ($p = 0.0069$) and *D. trunculus* ($p = 0.0039$) showed a significant statistical effect between sampling stations, with higher values generally at those stations closer to the freshwater inputs (A, B and C). As regards differences between depths, all the variables of fauna presented changes with depth. The total density ($p < 0.0001$), and the density of bivalves ($p < 0.0001$), crustaceans ($p < 0.0001$), polychaetes ($p < 0.0001$) and *C. gallina* ($p < 0.0001$) showed increases with depth, while *D. trunculus* ($p < 0.0001$) had lower values at the deepest points.


July 2013

February 2014

December 2014

Fig. 2. Abundance (ind m^{-2}) of main classes of macrofauna (bivalvia, crustacea and polychaeta) for each station and depth in each sampling campaign.

With respect to temporal variation of fauna (Table S2), total density ($p < 0.0001$), bivalve density ($p = 0.0001$), crustacea ($p < 0.0001$), polychaeta ($p = 0.0090$) and *D. trunculus* ($p < 0.0001$) densities presented significant differences between July 2013 and the other two sampling campaigns, with higher values in July 2013. The density of *Chamelea gallina* also showed significant differences ($p = 0.0153$) but only between July 2013 and December 2014, with higher values again in July 2013. With the exception of the density of polychaetes and *C. gallina*, an interaction between depth and sampling campaign was observed for the different fauna groups (Table S2). In general, it was observed that the effect of the sampling campaign was more decisive in the shallowest areas, while at greater depth the temporality had a lesser influence on the densities of the different groups.

In general, the most abundant species of Bivalvia in the shallowest sampled areas was *D. trunculus*. At greater depths, 2-4 m, *D. trunculus* was replaced by *Donax semistriatus*, *Mactra stultorum* and *Loripes orbiculatus*. Regarding polychaetes, *Scolelepis squamata* predominated in the shallower area, while at 2 m depth, *Magelona johnstoni*, *S. squamata*, and *Glycera sp* were the most abundant. At deeper points (3 and 4 m) *Magelona mirabilis*, *M. johnstoni*, *Paradoneis armata* and *Sigalion mathildae* were the most abundant. In the case of crustaceans, *Pontocrates arenarius* was the predominant at 0.5-1 m along with *Urothoe grimaldii*. *Siphonoecetes sabatieri* and *Bathyporeia elegans* became the most abundant crustaceans from 2 m depth, and in some cases *Apseudopsis bacescui*.

The dendrogram obtained from the cluster analysis showed a separation of the sampling points in three clusters when a cut-off point of 60 000 was selected (Fig. 3). When carrying out the hierarchical analysis with Ward's method, we could see a greater dissimilarity of the 4 m deep points of the transects A, B and C with respect to the rest of the sampling points. When establishing a cut-off at 60 000, which was considered the most appropriate, we can see a clear separation in three clusters. The first two were grouped according to depth, regardless of the sampling campaign. The first one comprised the points from 0.5 and 1 m depth while the second those from the intermediate and deepest zone (from 2 to 4 m). Finally, a third cluster, which showed a greater dissimilarity, was obtained where only the points of 4 m from the stations closest to the freshwater contributions (A, B and C) were grouped (including all three sampling campaigns). The different clusters highlighted the influence of bathymetry on the macrobenthic community structure, although the third one also incorporated the proximity to the freshwater contributions.

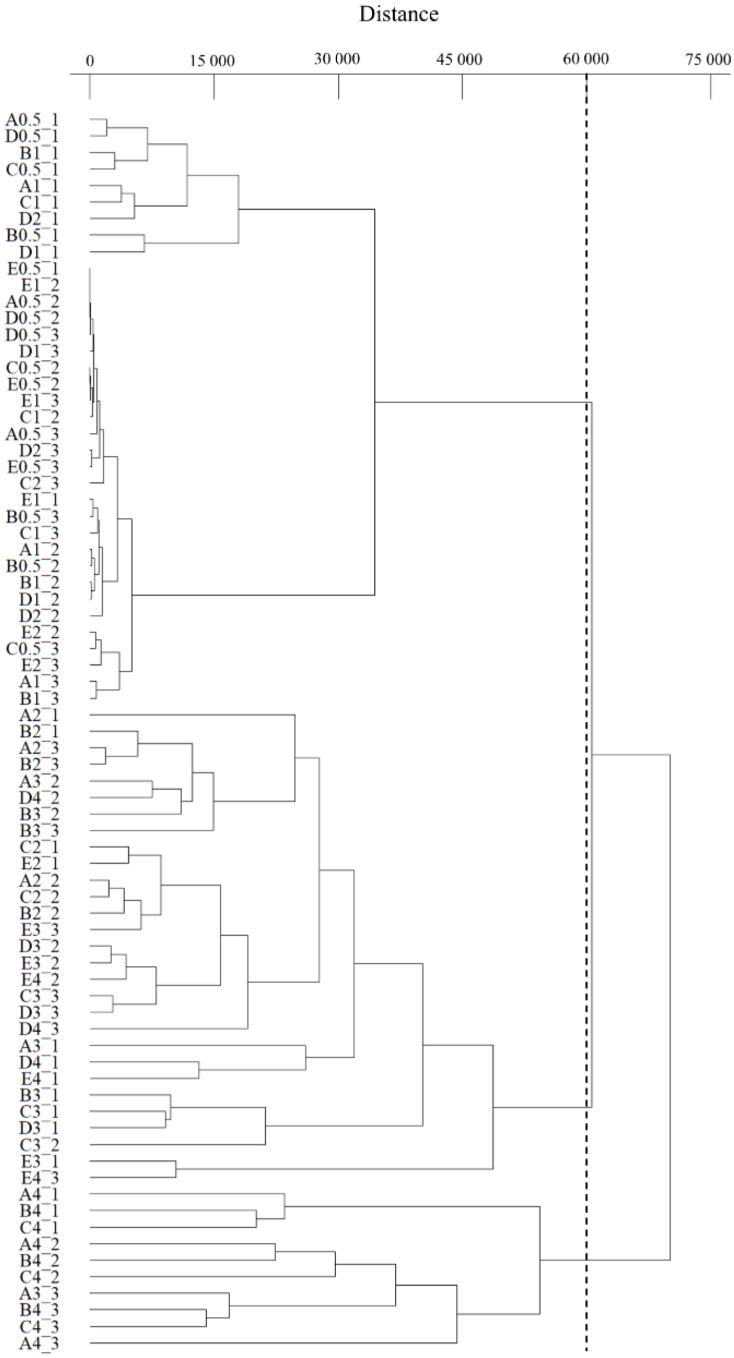


Fig. 3. Dendrogram resulting from the cluster analysis of the macrobenthic fauna density, in which presents the three clusters obtained using a cut-off at 60 000. The labels show the sampling station (letter), depth (first number) and sampling campaign (second number; 1: July 2013; 2: February 2014; 3: December 2014).

3.4. Correlations between community structure and environmental variables

Due to the fact that both depth (as seen in the cluster analysis, Fig. 3) and temperature (linked to species life cycle) are the most relevant factors which could mask the influence of any other variables, both were discarded in order to conduct the CCA. The ordination biplot for the major species of macrofauna obtained using CCA showed the species distribution, whose proximity indicates species often occurring together, along with environmental variables. The two axes explained 27.12 % of the variability. Species such as *U. grimaldii*, *S. squamata*, *Gastrosaccus sp*., *D. trunculus*, *Schistomysis assimilis* and *P. arenarius* showed a high positive correlation with the variable mean grain size. These species are also positively influenced by the pigments 19'hexanoyloxyfucoxanthin and, to a lesser extent, by alloxanthin as well as DIN. Furthermore, the polychaetes species, specifically *M. mirabilis*, *Prionospio pygmaea*, *P. armata* and *Nephtys hombergii*, showed a positive correlation with OM, as well as peridinin and DIN, and negative when the distance to freshwater contributions increases. The numbers of *Scoloplos sp*., *D. semistriatus* and *Glycera sp*. were the least explained by the environmental variables since their positions in the CCA model were close to the origin.

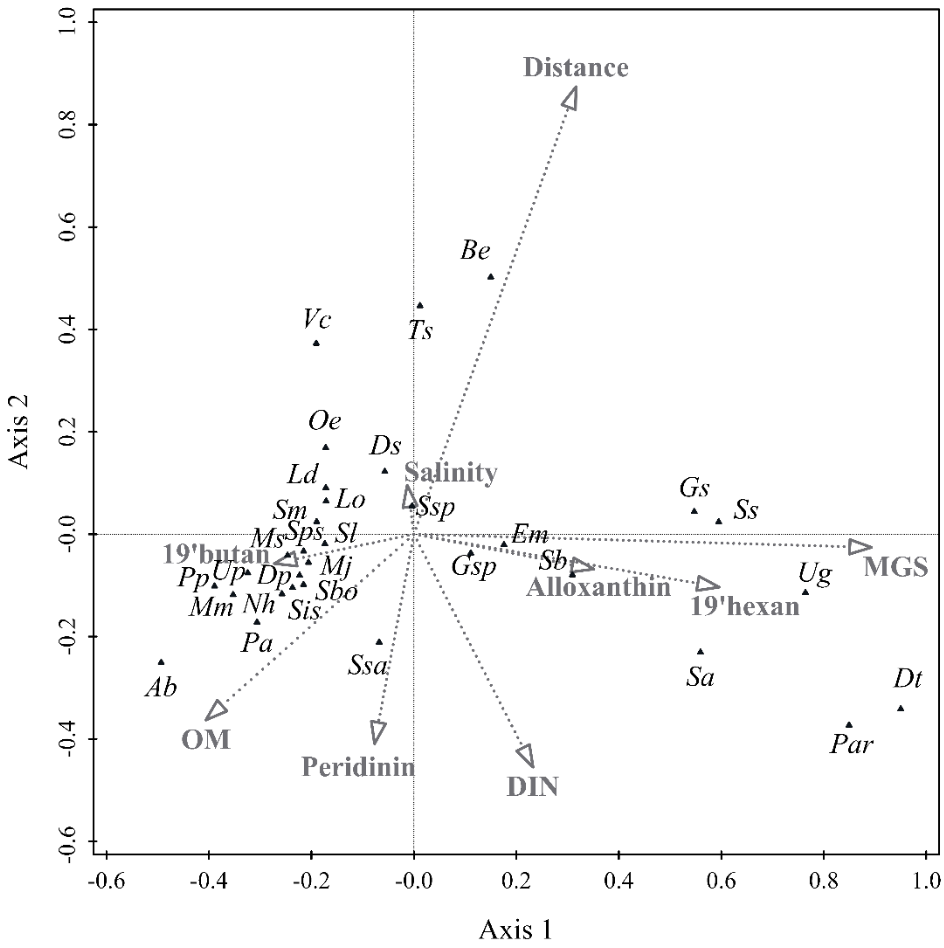


Fig. 4. CCA ordination diagram between the densities of species (triangles) and environmental variables (arrows). The length of the arrows indicates the strength of the variable in that dimensionality of the solution, and they point in the direction of the gradient. DIN: dissolved inorganic nitrogen; MGS: mean grain size; OM: organic matter; 19’butan: 19’butanoyloxyfucoxanthin; 19’hexan: 19’hexanoyloxyfucoxanthin. *Ab: Apseudopsis bacescui; Be: Bathyporeia elegans; Dp: Diogenes pugilator; Ds: Donax semistriatus; Dt: Donax trunculus; Em: Echinocardium mediterraneum; Gs: Gastrosaccus sp.; Gsp: Glycera sp.; Lo: Loripes orbiculatus; Ld: Lucinella divaricata; Ms: Mactra stultorum; Mj: Magelona johnstoni; Mm: Magelona mirabilis; Nh: Nephtys hombergii; Oe: Onuphis eremita; Pa: Paradoneis armata; Par: Pontocrates arenarius; Pp: Prionospio pygmaea; Sa: Schistomysis assimilis; Sb: Scolelepis bonnieri; Ssp: Scoloplos sp.; Ss: Scolelepis squamata; Sis: Sigambra sp.; Sm: Sigalion mathildae; Ssa: Siphonoecetes sabatieri; Sbo: Spiophanes bombyx; Sps: Spio sp.; Sl: Sthenelais limícola; Ts: Tylos cf. sardous; Ug: Urothoe grimaldii; Up: Urothoe poseidonis; Vc: Venus casina.*

In addition, a CCA ordination diagram between the species richness and the environmental variables can be observed in figure 5. In general, those points that presented lower richness were located at lower depths, relating to the mean grain size and the pigment 19'hexanoyloxyfucoxanthin. However, those points that showed higher values of richness were located at greater depths, mainly at 3 and 4 m, generally with positive correlations with OM and 19'butanoyloxyfucoxanthin, and negative correlation when the distance to the freshwater sources increases.

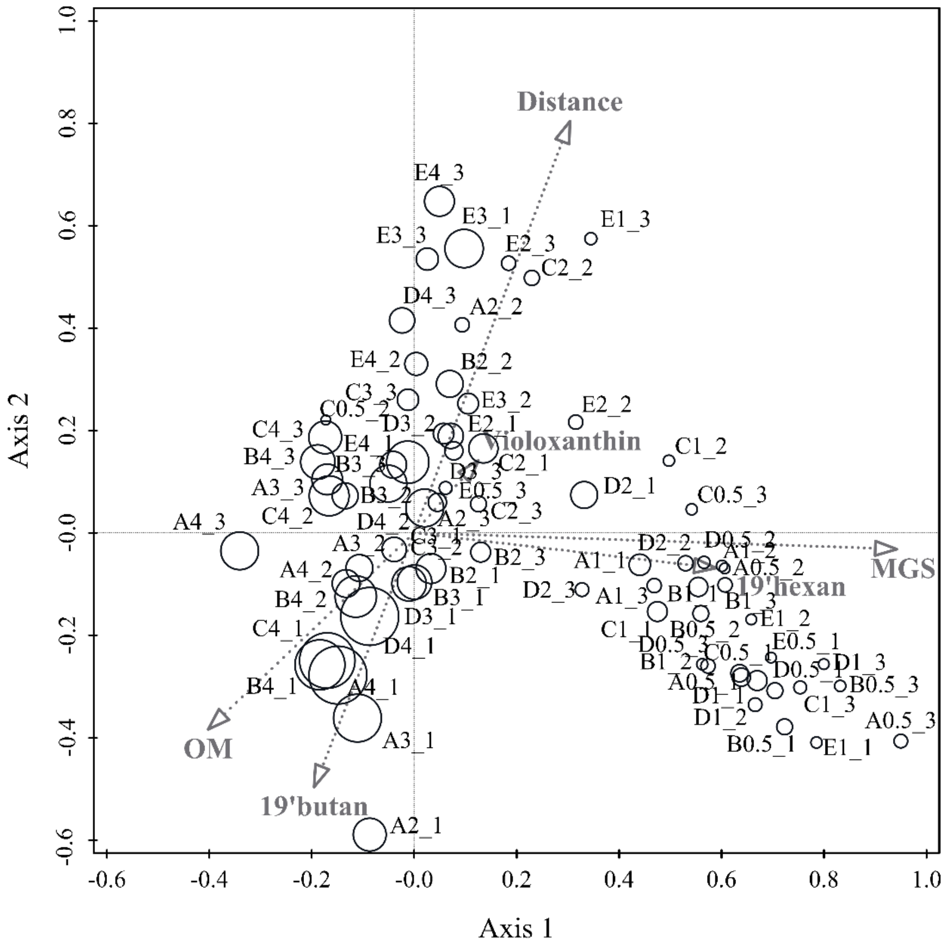


Fig. 5. CCA ordination diagram between species richness (circles) and environmental variables (arrows). Circle size varies to reflect the value of richness: the greater the size, the larger the richness. The labels show the sampling station (letter), depth (first number) and sampling campaign (second number; 1: July 2013; 2: February 2014; 3: December 2014). Abbreviations were defined in the caption of Figure 4.

4. DISCUSSION

McLachlan (2001) proposes physical control (wave energy, tidal range and sand particle size) as one of the paradigms that explain macro-scale variations of macrobenthic communities in the intertidal zone of sandy beaches. However, biological factors (including food availability, competition and predation) are considered to play a role in the structuring of macrofaunal communities at the meso-scale only in physically benign habitats, such as undisturbed dissipative beaches. In extremely microtidal environments (< 30 cm), the intertidal zone is so narrow that the shallower sublittoral zone is that which harbours a community, subject to instability and continuous changes due to the action of wave breaks. Other studies have also observed the influence of freshwater contributions to the sea on fauna (Gillanders and Kingsford, 2002; Lercari and Defeo, 2006; Ortega Cisneros et al., 2011). It is important to determine whether the sublittoral macroinfaunal community that inhabits dynamic beaches (intermediate morphodynamic type), such as those of the study area, responds significantly to the physical environment at the meso-scale, as predicted by the theory, as well as the contributions of fresh water.

The most abundant macrofauna classes in the study area were molluscs, crustaceans and polychaetes, as what happens in a generalised way on sandy beaches (Martí et al., 2007; McLachlan and Defeo, 2018). We could see that the densities of these groups, along with the total density, *C. gallina* density, *D. trunculus* density and richness across the shore were influenced by depth. By means of the cluster analysis, three groupings by similarity were observed, including in the first group the fauna from the shallowest points (0.5 and 1 m), another with the fauna between 2-4 m deep, and finally a third with the 4 m deep points of the transects closest to the freshwater supply (A, B and C). The shallowest area presents more dynamic and turbulent conditions, with less diversity and abundance of species (Janssen and Mulder, 2005; McLachlan and Defeo, 2018). While in deeper areas, sediments present a greater stability allowing the development of richer and denser macrofauna, showing a greater similarity between the points from 2 to 4 m (second group). The cluster analysis of the abundance of macrobenthic species thus showed the clear zonation that species present across shore. The increase in abundance and diversity of benthic invertebrates in deep waters is a pattern that occurs in a generalized way (Barros et al., 2002; McLachlan and Defeo, 2018). The bathymetric zonation of the fauna in the present study is linked to the mean grain size of the sediment and the percentage of OM, which coincides with what was described by Lercari and Defeo (2006), since these variables also presented a clear dependence on depth. The smallest sand particle size, observed at the deepest points of the study zone, is related to higher densities of macrofauna and richness (McLachlan and Defeo, 2018; McLachlan and Dorvlo, 2005; Rodil et al., 2006). The greater concentration of OM in deeper zones is a consequence of sedimentation and accumulation on the seabed of both finer geological materials and the majority of the particulate organic matter in this area. This pattern in the physicochemical variables of sediment was also observed by Ramón (1993) and Martí et al. (2007) in an area located just north of our study zone. In the Mediterranean, the bivalve *D. trunculus* is characterized by a higher density of individuals at depths between 0.5 and 2 m (Gaspar et al., 2002; Ramón et al., 1995), a pattern also observed in the present study. As depth increases, another species of the same genus, *D. semistriatus*, becomes more abundant (Ansell and Lagardère, 1980; Martí et al., 2007). This distribution also coincides with the general scheme of zonation across sandy shores in the sublittoral proposed by McLachlan and Defeo (2018), in which the bivalves of the genus *Donax* can be found in the inner turbulent zone and in the transition zone. These bivalves are also accompanied by spionids polychaetes (*S. squamata*) and amphipods (*P. arenarius and U. grimaldii*) in the surf zone. The abundance of *S. sabatieri* at greater depth can be linked, on the one hand, to the interaction with other species (Bigot et al., 2006), but also to the availability of sedimentary detritus that serve as food (Navarro-Barranco et al., 2013) as what happens with the increase in depth. Both the polychaetes *M. mirabilis*, *M. johnstoni*, *P. armata* and *S. mathildae*, and the crustacean *A. bacescui* were found at greater depths, where the higher content of organic matter is found, most of them being surface deposit feeders (Fauchald and Jumars, 1979; Jumars et al., 2015). Deposit feeders dominate more mature ecosystems, with low hydrodynamism, where the content of organic matter and clay is high (Aneiros et al., 2018). *C. gallina*, although not one of the most abundant species, was found in higher densities at the deepest points of the study area similar with the bathymetric range observed by Moschino and Marin (2006).

When comparing the samples obtained at 4 m depth, the stations closest to the contributions from the Serpis River (A, B and C) were those which showed a higher abundance of polychaetes which coincides with the higher levels of OM and lower mean grain size. The habitual conditions in this zone were adequate for the development of polychaetes, mainly surface deposit feeders and some carnivores which feed on small invertebrates (Fauchald and Jumars, 1979; Jumars et al., 2015). This was reflected in the cluster analysis which distinguished a third group that included the samples taken at 4 m depth from the stations closest to the contributions of fresh water (A, B and C), which showed, in all the sampling campaigns, a higher abundance of polychaetes than the 4 m samples from stations D and E (figure 2). Hydrodynamism has a great influence on abundance and biomass of deposit feeders and suspension feeders in macrobenthic communities, due to their food supply depending largely on water velocity (Zhang et al., 2015). The food in the turbulent zone is in suspended form, therefore, suspension feeders dominate this area, excluding the establishment of deposit feeders (Wildish, 1977). In the deepest zone, where the sedimentation of particulate material takes place, a gradient of deposit feeders from the closest areas to the freshwater sources to the furthest ones, could be observed. When analysing alongshore species distribution patterns, it should be noted that the contributions from the drainage channels from the Safor Wetland that end up in Gandia Port, as well as from the overflow channel of the Gandia WWTP that discharges near the Serpis mouth, had a clear influence at the stations located nearest to the south of the mouth - as dominant currents come from the North (Sebastiá and Rodilla, 2013). These discharges provide fine sediments, organic matter, as well as the necessary nutrients to favour primary production. On the other hand, the lower salinity at station E, the furthest from surface water contributions, could be due to the diffuse contributions of groundwater discharges that the area presents from the wetland (Sebastiá et al., 2012). Stations A, B and C generally presented the highest values of fauna density, in addition to the highest concentrations of nutrients, pigments such as peridinin and zeaxanthin related to dinoflagellates, cyanophytes and prochlorophytes (Vidussi et al., 2001), as well as smaller mean grain size. Higher macrofauna density in areas with smaller mean grain size has already been observed in other studies (Lastra et al., 2006; McLachlan, 1996; McLachlan and Dorvlo, 2005; Rodil and Lastra, 2004). Numerous publications have observed negative influences of contributions of fresh water to the sea on the benthic macrofauna, mainly due to the abrupt decrease in salinity (Lercari and Defeo, 1999; Lercari and Defeo, 2006; Lercari et al, 2002; Lozoya et al., 2010; Ortega Cisneros et al., 2011), however, this was not observed in the study area. Therefore, in the case of the Mediterranean Sea, which is an oligotrophic system (Ludwig et al., 2009), freshwater contributions benefit the macrofauna community, by providing the necessary nutrients for primary production, organic matter and fine sediments.

Regarding temporary variations, the highest abundance of the different groups of macrofauna (bivalves, polychaeta and crustaceans) along with the total density and *D. trunculus* and *C. gallina* densities, were obtained in the summer sampling campaign (July 2013) which presented the highest temperature. This type of seasonal variation was described by McLachlan and Defeo (2018) as a habitual pattern due to the displacement of organisms that occurs in winter when the water temperature falls or the deeper burial during stormy weather. The seasonality of *D. trunculus* was also associated with increases in temperature by authors such as Manca Zeichen et al. (2002) and Neuberger-Cywiak et al. (1989). However, in addition, it was possible to notice a relationship with the availability of food. In the case of species of commercial interest, both *D. trunculus* and *C. gallina* are filter feeders that feed on particulate material suspended in water (Romanelli et al., 2009; McLachlan and Defeo, 2018). The population size is closely related to the quantity and quality of the particulate material (Lastra et al., 2006; Lee et al., 2019). In late winter (February 2014), when the salinity and temperature were lower, the highest concentrations of the majority of the photosynthetic pigments analysed were found, and therefore of the abundance of phytoplankton (Table S1), which coincides with the general model described in the area of study by authors such as Gadea et al. (2013) and Sospedra (2014). In contrast, two variables, to some extent related to plankton such as suspended solids (SS) and total phosphorus (TP), showed higher levels in the summer and autumn campaigns. Phytoplanktonic pigments, being associated with certain algae divisions, reveal, although partially, the composition of the phytoplanktonic population. Based on the works of Jeffrey and Vesk (1997), Vidussi et al. (2001), Gallardo (2004) and Brewin et al. (2010), the phytoplanktonic pigments found allowed us to distinguish very different phytoplanktonic compositions between late winter and summer. At the end of winter, the pigments indicated the presence of green algae (chlorophyceae, prasinophyceae and euglenophyta), cyanophytes, cryptophytes, prymnesophyceae and chrysophyceae, but mainly diatoms, concordant with Garmendia et al. (2011), Levinton (2011) and Sebastiá and Rodilla (2013) who indicated that diatoms dominate during the end of winter and spring. However, in the summer campaign the pigments revealed greater abundances of dinoflagellates and cyanobacteria, as observed by Sebastiá et al. (2013) in the study area during the summer, which is consistent with the general patterns described in temperate zones (Levinton, 2011). The SS includes both organic particles (organisms and non-living matter) and inorganic particles, and the TP comprises dissolved and particulate forms, the latter including phosphorus present in organisms, dead organic matter and adsorbed on particles. This would indicate that in summer and autumn, the detritus and inorganic particles were the dominant components of these variables instead of phytoplanktonic organisms. Therefore, it would seem that the greater biomass of diatoms in late winter - early spring triggers two processes: an increase in the abundance of secondary producers that link their life cycle to a greater food availability (Zhang et al., 2015) and, furthermore, a higher level of detritus due to death and sedimentation of both primary and secondary producers. Therefore, in the water column, microalgae could determine the population dynamics of the benthic macrofauna, enriching it when food availability is higher (Lastra et al., 2006; McLachlan and Defeo, 2018; Rodil et al., 2007). The sublittoral zonation pattern described by McLachlan and Defeo (2018) proposes that diatoms predominate in the most turbulent areas. In this same area, Wildish (1977) and Brown et al. (1989) establish the dominance of suspension feeders, excluding the establishment of deposit feeders. In addition, Herman et al. (1999) suggest that the benthic community integrates the environmental influences of a place for a relatively long period of time, so that the increases in the planktonic system would be reflected in the benthic with some time lag. However, phytoplankton increases in late winter-early spring do generate a faster increase in the zooplankton community (Rodrigues et al., 2019), which also implies the presence of meroplankton that will subsequently result in recruits for the benthic community (Marcus and Boero, 1998) and will also contribute to the increase in detritus available after the spring and autumn production peaks (Soetaert et al., 2000). The increase in detritus during the summer campaign could encourage the presence of many species of polychaetes, mysids etc that feed on them. The deposition of detritus occurs mainly at greater depth, the conditions of this area being suitable mainly for the settlement of surface deposit feeder polychaetes (Fauchald and Jumars, 1979; Jumars et al., 2015).

The CCA showed an influence of the sediment variables (mean grain size and OM), microalgae in the water column, and the distance to the freshwater sources on the macrobenthic community. Most species were negatively influenced by the increase in mean grain size. This influence of granulometry on organisms was also observed by different authors (Lastra et al., 2006; Lercari and Defeo, 2006; McLachlan, 1996; McLachlan and Dorvlo, 2005; Rodil and Lastra, 2004). The importance of granulometry on the benthic macrofauna is a key point to consider in areas where beach nourishment occurs, as is the case of the beaches near our study area. It is important to select an adequate sediment to preserve species that can be susceptible to habitat modifications such is the case of *D. trunculus* (De la Huz et al., 2002; La Valle et al., 2011). In addition, the OM in sediment was another important factor in the CCA. Increases of organic matter in sediments facilitate the richness and abundance of many species (Lercari and Defeo, 2006). Finally, the distance to sources of fresh water, encouraged the greater abundance of polychaete deposit feeders, conditioned by the contributions of finer sediments and organic matter at the deepest points of transects A, B and C.

Our results show that in the shallower sublittoral zone, which includes the surf zone, the benthic macrofauna communities are strongly influenced by physical factors associated with the beach profile and hydrodynamics, i.e the granulometric parameters. However, in the zone beyond the breaking area, where the turbulence is lower, the importance of nutritional variables, i.e. the organic matter in the sediment and the dynamics of phytoplanktonic groups, increases. In addition, the distance to the freshwater discharges presented an influence on the fauna. This highlights the importance of considering both physical and nutritional variables in the study of sublittoral benthic communities in micro-tidal zones, agreeing with the paradigms described by McLachlan (2001) and Ortega Cisneros et al. (2011). The identification of these key variables is crucial to develop adequate coastal management. Anthropogenic activities in coastal areas such as coastal structures, beach nourishment, dredging, freshwater discharges, etc through modifying the characteristics of the environment (hydrodynamism, beach profile, granulometry and nutritional sources) will affect the biocoenoses. This could have consequences on the food web, as well as socio-economic repercussions when affecting species of high fishing interest.

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