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ABSTRACT

Deep-sea ecosystems of the Iberian margin have been widely impacted over the past decades, but the limited knowledge on their biodiversity and functioning limits our ability to contribute to their conservation. So far, in the Gulf of Cadiz, research has mostly been focused on megabenthic assemblages associated to mud volcanoes. However, several other geomorphological structures have remained widely unexplored. Here, by means of a quantitative analysis of 17 video transects conducted between 220 and 980 m depth, we investigated megabenthic assemblages associated to canyons, contouritic channels, contouritic furrows and open slopes. We report the presence of 8 different assemblages, segregated as a result of the different substrates and geomorphologic features. Megabenthic assemblages on hard substrates were characterized by mono or multispecific sponge assemblages. Soft bottoms hosted crinoid beds, pennatulacean meadows and fields of the gorgonian *Radicipes gracilis*. These results highlight the high diversity of megabenthic assemblages in deep-sea ecosystems of the Gulf of Cadiz and suggest that most of the geomorphological features that remained so far unexplored represent vulnerable marine ecosystems deserving protection and inclusion in future management plans.

1. Introduction

The deep sea is the largest biome on the planet covering more than 65% of Earth surface and hosting a multitude of unique ecosystems, whose biodiversity still remains largely undescribed (Danovaro et al., 2010; Ramirez-Llodra et al., 2010; Bolgan and Parmentier, 2020). The increased availability of image registering telepresence technologies, such as Remotely Operated Vehicles (ROVs), Autonomous Underwater Vehicles (AUVs), landers cabled observatories equipped with docked Internet Operated Technologies (IOVs) like crawlers, have allowed the exploration of deep-sea habitats over increasing spatial scales (Buhl--Mortensen and Mortensen, 2004; Mienis et al., 2012; Aguzzi et al., 2019; Simon-Lledó et al., 2019a). Consequently, in recent decades a great attention has been given to improve knowledge on the biodiversity and functioning of deep-sea ecosystems and their response to human impacts (e.g. Girard et al., 2019; Simon-Lledó et al., 2019b; Costa et al., 2020).

Since the second half of the XX century, deep-sea environments have been impacted by multiple human activities (e.g. bottom trawling, deep-sea mining, oil and gas extraction) that have caused long-lasting disturbances (Althaus et al., 2009; Vanreusel et al., 2016; Simon-Lledó

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et al., 2019b). However, among the different anthropogenic activities impacting the deep-sea, bottom trawling is the most widely distributed (Hiddink et al., 2017). In this regard, there is increasing evidence that bottom trawling has altered a large portion of the seafloor down to 1000 m depth, impairing biodiversity and ecosystem services (Pusceddu et al., 2014; Chiba et al., 2018). It is widely recognized that deep-sea benthic assemblages are generally composed of long-lived fragile organisms that present low resilience to impacts and slow recovering rates (Huvenne et al., 2016; Clark et al., 2019). For this reason many deep-sea megabenthic assemblages are considered Vulnerable Marine Ecosystems (VMEs) defined as ecosystems characterized by complex physical structures created by significant concentrations of biotic and abiotic features, which can host organisms that due to their life-history traits (e.g. slow growth rates, late maturity, low recruitment, long-live spans) are highly susceptible to anthropogenic activities, and that can be of high functional significance (e.g. feeding, nursery or rearing grounds) (FAO, 2009a,b). At international level, the necessity to preserve deep-sea VMEs from anthropogenic impacts, has led to the development of management and conservation measures to avoid their degradation (FAO, 2009a,b; United Nations, 2007). To reach this goal, several international strategies, such as the "International guideline for the management of the deep-sea fisheries in the high seas" were created (FAO, 2009a,b). Within the European Union's (EU) waters, large areas of the deep sea, hosting geomorphological features associated with high diversity and biomass such as submarine canyons, seamounts or island slopes, have recently been protected under the Habitat Directive (EC, 2020/96) as Sites of Community Importance of the Natura 2000 Network. The UN Agenda 2030 targets the preservation of at least 30% of the oceans by 2030, and to establish a coherent legislation and monitoring framework (COM/2020/380). The efficacy of any management and protection policy relies on the availability of accurate and comprehensive biological and ecological data, which are still lacking or insufficient for most deep-sea ecosystems (Danovaro et al., 2017, 2020).

The Gulf of Cádiz (GoC) (South-West Iberia) is not an exception, as it hosts a range of diversified deep-sea geomorphologies, such as mud volcanoes and pockmark fields spanning across the Moroccan, Portuguese and Spanish continental margins between 700 and 3000 m depth (Mazurenko et al., 2002). These habitats were discovered in the 90s and most benthic research has mainly focused on benthic communities occurring on mud volcanoes (Zeppilli et al., 2012; Rincón-Tomás et al., 2019; Rüggeberg and Foubert, 2019; Rueda et al., 2019; Sitjà et al., 2019; Urra et al., 2021). In 2016, an extension of 3177 km^2 of the Spanish margin hosting several mud volcanoes and pockmark fields was declared "Site of Community Importance" in the Natura 2000 network ("Mud Volcanoes of the Gulf of Cadiz" HD code ESZZ12002). However, other geomorphological structures, which represent potential suitable habitats for sessile habitat forming species remained unexplored. As a result, claims for their protection cannot be advanced. Considering that bottom trawling is widely extended across the local continental margin (Sobrino and Burgos, 2014) there is a pressing need to identify putative geomorphologies hosting VMEs by acquiring inventory of inhabiting sessile species, providing baseline knowledge for future management and conservation strategies.

In order to increase our bio-ecological knowledge on these deep-sea habitats, we investigated, by means of ROV image survey, the megafauna (organisms larger than 2 cm; e.g. Grinyó et al., 2018) in several unexplored geomorphologies (e.g., contouritic furrows, contouritic channels, canyons and muddy open slopes) of the Gulf of Cádiz. Thus, the aims of this study were to: (1) characterize the composition of megabenthic assemblages (defined as assemblages constituted by sessile and low motile invertebrates); (2) assess their geographic and bathymetric distribution; and (3) investigate the environmental factors that might influence megabenthic assemblage occurrence.

2. Materials and methods

2.1. The study area

The GoC is located west of the Strait of Gibraltar, being enclosed by the South West Iberian and the North West African margin (Fig. 1). The geological history of the GoC is complex, having undergone rifting and compression since the Triassic (Maldonado et al., 1999). Its Iberian margin host mud volcanoes, salt diapirs, erosive furrows, canyons (in a depth range of 200–1300 m) and two large contouritic channels (in a depth range of 550–620 m and 660–750 m, respectively) (Medialdea et al., 2009; Mecho et al., 2020; Mulder et al., 2003; Hernández-Molina et al., 2014). These geological features have resulted from the complex interaction between the African and Eurasian tectonic plates and the abrasive actions of the Mediterranean Outflow Water (MOW) (Hernández-Molina et al., 2003; Medialdea et al., 2009). The MOW enters the GoC, spreading westward as multiple branches that progressively decrease in velocity, salinity and density forming a warm overflow (Baringer and Price, 1997, 1999; Serra et al., 2010).

2.2. Acquisition of video transects

Aboard the R/V Sarmiento de Gamboa, an Argus work class ROV was used to perform video transects with a frontal colour camera (Sony FCBH10 Argus RS Focus Zoom HDTV, 720 p resolution). Artificial lighting was provided by four 150W Argus RS HID, and four Halogen 250W DSPL lights. The ROV was equipped with two parallel laser beams that provided scale (50 cm) for posterior video analyses.

Seventeen video-dives were performed in different geomorphologic zones as defined by Hernández-Molina et al. (2014) and Mecho et al. (2020) (Table 1, Fig. 1): within an area of deep contouritic furrows; along two contouritic channels; on the open muddy slope; and finally, in two submarine canyons. During navigations the ROV was positioned between 1 and 1.5 m above the bottom, moving at a constant speed of 1.0 knots. ROV dives covered a surface of 0.1053 km^2 (see Table 1).

2.3. Video transect analyses

Quantitative video transect analysis was performed according to the methodology described in Grinyó et al. (2018), using the software VLC (version 2.2.8). When the ROV was stopped or moving in loops, sequences were not considered to avoid the overestimation of megabenthic organism. Where the ROV was too detached from the seafloor or when suspended sediments prevented a clear view of the seafloor, video sequences were considered unusable and discarded from analyses.

Megabenthic assemblages considered in this study were composed

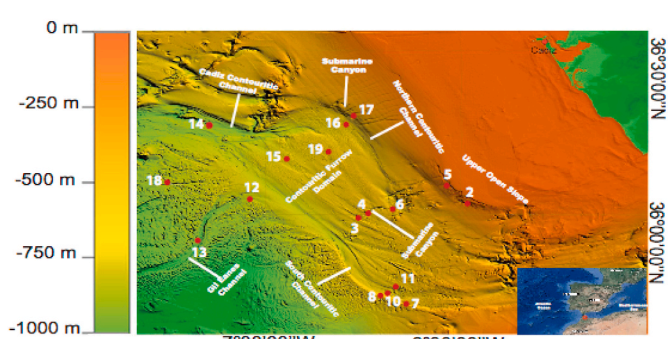

Fig. 1. Bathymetric map of the northern GoC and its location within the North Atlantic (red square), the dots represent the location of the video transects (2–19). Compilation of bathymetry from (Zitellini et al., 2009), GEBCO, CONTOURIBER projects (e.g., Hernández-Molina et al., 2014), and INDEMARES-CHICA and INDEMARES-LIFE by IEO (Instituto Español de Oceanografía).

Table 1
Metadata specifying dives (No.) characteristics in terms of starting date, latitude, longitude, averaged depth (m), video transect duration and area. Geomorphological zones are depicted as follows: UOS, upper open slope; F, Contouritic Furrow; CC, contouritic channel; and finally, C, Canyon.

Geomorphological zone	Transect Number	Date	Latitude	Longitude	Average depth (m)	Duration (hh:mm:ss)	Area (km^2)
UOS	2	March 09, 2014	36° 02.1931′N	6° 29.2622′W	222	03:45:02	0.0130
UOS	5	May 09, 2014	36° 05.4462′N	6° 32.4239′W	224	01:54:56	0.0039
C	3	April 09, 2014	35° 59.5952′N	6° 45.8666′W	660	06:23:19	0.0036
C	4	April 09, 2014	36° 00.4360′N	6° 44.2907′W	662	01:45:16	0.0004
C	6	May 09, 2014	36° 01.1509′N	6° 40.5630′W	626	04:58:03	0.0024
C	17	October 09, 2014	36° 17.7340′N	6° 46.7173′W	883	05:24:09	0.0285
CC	7	June 09, 2014	35° 43.9216′N	6° 38.2539′W	693	03:25:46	0.0137
CC	8	June 09, 2014	35° 45.2046′N	6° 42.0749′W	691	04:37:34	0.0039
CC	10	July 09, 2014	35° 45.8414′N	6° 41.4889′W	707	02:11:16	0.0011
CC	14	September 09, 2014	36° 16.2517′N	7° 08.0895′W	911	03:34:56	0.0007
CC	16	October 09, 2014	36° 16.1242′N	6° 47.5995′W	664	03:50:46	0.0076
F	11	July 09, 2014	35° 47.1166′N	6° 39.9556′W	633	03:21:59	0.0012
F	12	August 09, 2014	36° 03.1527′N	7° 02.0464′W	798	03:30:02	0.0036
F	13	August 09, 2014	35° 55.4302′N	7° 10.0469′W	978	03:52:20	0.0016
F	15	September 09, 2014	36° 09.7459′N	6° 56.8595′W	742	04:26:30	0.0058
F	18	November 09, 2014	36° 06.1391′N	7° 14.8768′W	891	02:59:07	0.0140
F	19	November 09, 2014	36° 11.5924′N	6° 50.3949′W	638	02:01:19	0.0003

by both sessile and low motile invertebrates, such as sponges and echinoderms, respectively (Grinyó et al., 2018). Each megabenthic organism was assigned a time code, which posteriorly was linked to a position following Gori et al. (2012). Megabenthic organisms were classified to the lowest taxonomic level following previous faunistic studies from the area (Sobrino et al., 2000; Silva et al., 2011) and by the trawl survey Program "Arrastre Región Sur-Atlántica" of the Spanish Institute of Oceanography (Sobrin and Burgos, 2014). The position of all megabenthic organisms observed was defined by the time elapsed since the beginning of the video transect to the crossing of the laser beams by the organism (see details in Santín et al., 2018).

2.4. Data processing

2.4.1. Megabenthic organism's occupancy and abundance

Each dive was split into 5 m^2 segments defined as sampling units, to measure megabenthic fauna occupancy (i.e. number of sampling units where a species is present), abundance (i.e. the number of individuals per sampling unit), and to assess megabenthic assemblage composition. This unit splitting was carried out following other image-based studies addressing megabenthic assemblage composition in deep-sea environments (e.g. Grinyó et al., 2018; Enrichetti et al., 2019).

Sampling units were characterized by quantifying the number of megabenthic organisms of each identified species (density = number of individuals per m^2) and the coverage percentage of each substrate and slope category. Seabed substrate type was classified into four categories based on an adaptation of the Wentworth scale (Santín et al., 2018) according to practices followed by other deep-sea megabenthic studies: silts, sands and gravels, rock and sediment covered rocks. Additionally, sampling units were also characterized based on the geomorphological feature in which they were find: canyons, contouritic channels, contouritic furrows and open slopes.

The multivariate matrix of species was analyzed using a 50-50 multivariate analysis of variance (Manova) procedure (Langsrud, 2002) a generalized multivariate Anova method based on principal component analysis (PCA) on standardized data. Manova was conducted in order to observe significant differences among the "Dominant substrate", "Geomorphology" and "Depth" factors (and their interaction) considering the Species matrix. Adjusted p-values were conducted on a rotation test based on 99999 simulated datasets. The contribution of variables was extracted for each rotation test (Infantino et al., 2016; Violino et al., 2020).

2.4.2. Sessile megabenthic assemblage composition

Variation in their composition were identified by means of a non-metric Multi-Dimensional Scaling (nMDS), species abundance data were square root transformed and distance between pairs of samples were calculated using a Bray-Curtis dissimilarity matrix. Adonis permutation multivariate analysis of variance and subsequent pairwise tests were used to test for significant differences among assemblages. The nMDS and Adonis test were performed using the R-language function *metaMDS* and *Adonis*, available in the *vegan* library of the R software platform (Oksanen et al., 2016). Rare taxa, defined as species occurring 3 times or less, were excluded from the nMDS analysis (Simon-Lledó et al., 2019b).

The Indicator Value (IndVal) index was used to determine which taxa were representative from each assemblage. That index allows to identify combinations of species that allow to discriminate a group of samples from other samples in the analysis (Jordà Molina et al., 2019). IndVal measures were computed using the function *indval* included in the *labdsv* package (Roberts, 2013).

3. Results

3.1. Megabenthic assemblage composition and distribution

A total of 351 sampling units (i.e. dives 5 m^{-2} segments) presented megabenthic organisms. Overall, 14,925 individuals were spotted and classified, resulting in the identification of 26 species, 6 classes and 3 Phyla (Fig. 2, Supplementary material 1).

Eight sessile megabenthic assemblages could be visually identified in the nMDS plots (Fig. 3). The first axis segregated assemblages, occurring on soft sediments from those occurring on hard substrates (i.e. sand and gravels, rock and partially sediment covered rock) (Fig. 3a), being this difference matched at the level of sampling unit coverage percentages (Fig. 3b). Permutation multivariate analysis and subsequent pair wise test revealed that all eight assemblages significantly differed from each other ($p < 0.001$).

Assemblage A was monospecific, being represented by the sponge *Pheronema carpenteri* (Figs. 3a, 5a and 5b, Table 2). This assemblage occurred on muddy and partially silted rocks (Fig. 3a and b) on canyons and contouritic furrows (Fig. 3c and d) between 600-650 m and 850–900 m depth respectively (Fig. 3e and f).

Assemblage B was mostly found on hard substrates (81%) (Fig. 3a and b), in canyons and contouritic channels (45% and 36% of sampling units, respectively) (Fig. 3c, and d), between 600 and 750 m depth (Fig. 3e and f). This assemblage was characterized by several sponge species (Table 2, Fig. 5c) and was widely distributed across the study area (Fig. 4). Sponges were the most diverse (13 species) and abundant group (96% of all organisms) reaching maximum densities of 109 ind. m^{-2} (30 ± 24 ind. m^{-2}, average ± SD). The fan shaped sponge *Pachastrella monilifera* formed dense aggregations reaching densities of

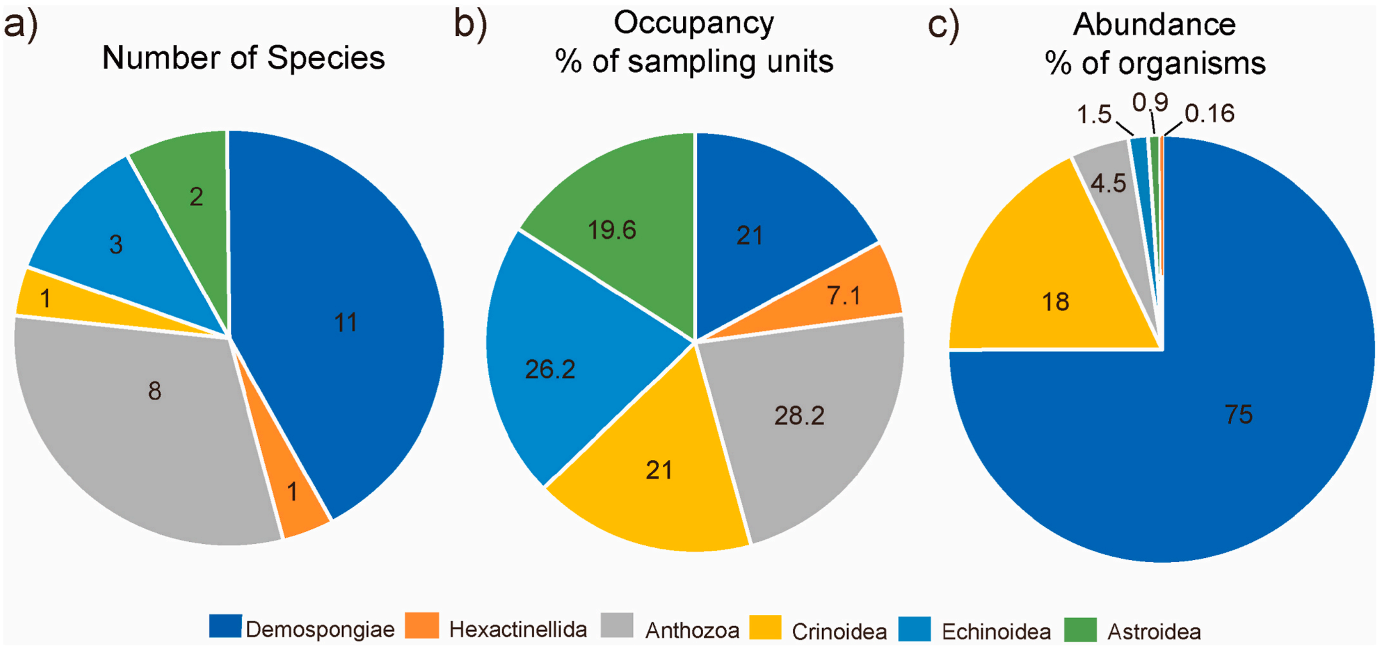

Fig. 2. a) Megabenthic species richness. b) Occupancy (number of sampling units were a species is present); c) abundance (number of organisms).

37 ind. m^{-2} (7.3 ± 7 ind. m^{-2}, average ± SD) (Fig. 5d). In this assemblage, we also reported the presence of a cidarid sea urchin that formed aggregations reaching densities of 6 ind. m^{-2} (2.6 ± 2 ind. m^{-2}, average ± SD).

Assemblage C mainly occurred on rocky substrates on canyons and contouritic channels (58% and 42% of sampling units, respectively) (Fig. 3a, d and 4) between 600 and 750 m depth (Fig. 3c and d). It is characterized by the presence of the cold-water corals *Madrepora oculata* and *Dendrophyllia cornigera* (Fig. 5e and f, Table 2). Both species occurred as isolated colonies reaching densities of 14 colonies m^{-2} and 3 colonies m^{-2} (1.5 ± 0.83 ind. m^{-2}, average ± SD), respectively. In this assemblage, we also reported the presence of the asteroid *Peltaster placenta* (Fig. 5g) that reached densities of 7 ind. m^{-2} (2 ± 1 ind. m^{-2}, average ± SD).

Assemblage D was solely constituted by the crinoid *Leptometra celtica* (Fig. 5i, Table 2) which reached densities of 130 ind. m^{-2} (10 ± 33 ind. m^{-2}, average ± SD). This assemblage was widely distributed (Fig. 4), occurring on silted grounds (Fig. 3a and b) on contouritic furrows and open slope (34% and 57% of sampling units, respectively) environments (Fig. 3d and e) between 200 and 750 m depth.

Assemblage E occurred on silted grounds (Fig. 3a and b) on contouritic channels and contouritic furrows (75% and 20% of sampling units, respectively) (Fig. 3c and d) between 600 and 650 m depth (Fig. 3e and f and 4). This assemblage was entirely formed by an unidentified ceriantid species (Fig. 5h, Table 2) that reached maximum densities of 9 ind. m^{-2} (1.2 ± 1.9 ind. m^{-2}, average ± SD).

Assemblage F was highly localized on silted grounds on canyons and contouritic furrow environments (95% and 5% of sampling units, respectively) between 600 and 650 m depth (Figs. 3 and 4). Assemblage F was exclusively formed by the pennatulacean *Kophobelemnon stelliferum* (Fig. 5j, Table 2) reaching densities of 14 col. m^{-2} (6 ± 5.5 col. m^{-2}, average ± SD).

Assemblage G was highly localized occurring on silted grounds on canyon and contouritic furrow environments (93% and 7% of sampling units, respectively) between 600 and 650 m depth (Figs. 3 and 4). This assemblage was entirely formed by the pennatulacean *Funiculina quadrangularis* (Fig. 5k, Table 2) that reached densities of 2.4 col. m^{-2} (1.3 ± 1.6 col m^{-2}, average ± SD).

Assemblage H was highly localized on silted grounds on contouritic furrow and canyon environments (75% and 25% of sampling units, respectively) mostly occurring between 850 and 900 m depth (Figs. 3 and 4). It was characterized by the presence of the asteroid *Tethyaster subinermis* and the octocoral *Radicipes gracilis* (Figs. 5l, m, Table 2) that reached densities of 38 col. m^{-2} (18 ± 14 col. m^{-2}, average ± SD) and 2 ind. m^{-2} (0.24 ± 0.4 ind. m^{-2}, average ± SD), respectively.

The MANOVA (50-50 MANOVA procedure) reported significant differences ($p < 0.001$) among the 'Dominant substrate', 'Geomorphology' and "Depth" (Table 3).

4. Discussion

4.1. General considerations

The analysis, combining ROV surveys with accurate image analysis allowed to identify 8 different megafaunal assemblages in deep-sea ecosystem of the Gulf of Cadiz. These assemblages were segregated due to substrate type and geomorphological features. These findings support the results of previous investigations demonstrating that specific seabed features characterised by high 3D complexity, such as seamounts, canyon and mud volcanoes, support benthic assemblages with high biodiversity and organism abundance (Olu-Le Roy et al., 2004; Stocks, 2004; Vetter and Dayton, 1999). Megabenthic assemblages described in the present study are of high ecological interest, since they are mainly characterized by habitat-forming species that can increase biodiversity at a local level. Consequently, the areas hosting these assemblages should be considered for potential protection management plans in order to preserve their biodiversity and the ecosystem services they might provide.

4.2. Megafaunal assemblages

The *Pheronema* assemblage (see assemblage A in Fig. 2) was monospecific and characterized by the presence of the hexactinellid sponge *Pheronema carpenteri*. A common north Atlantic species (Reiswig and Champagne, 1995), *P. carpenteri* is known to form dense aggregations on its areas of occurrence (Rice et al., 1990). Due to their vulnerability to trawling and other anthropogenic impacts, *P. carpenteri* has been described as an indicator for VMEs (Vieira et al., 2020). This species is

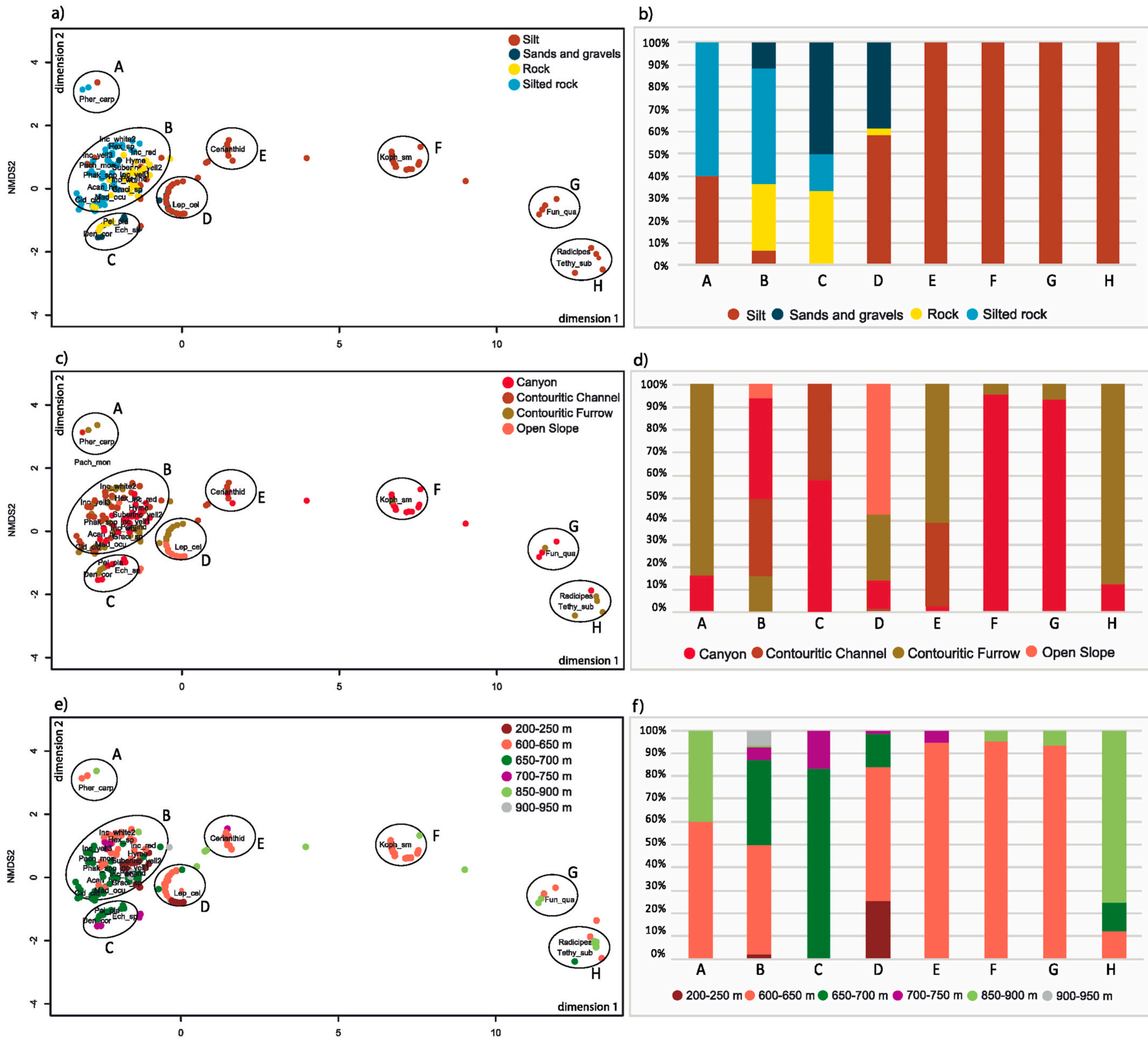

Fig. 3. Non-metric Multi-Dimensional Scaling (nMDS) outputs plots. A stress estimate of 0.019 was obtained. Sampling units containing megabenthic organisms (n = 351) are ordered considering a) substrate type, c) geomorphological features d) and depth interval. Column charts represent each assemblage sampling unit percentage covered by a certain b) substrate type or d) occurring on a certain geomorphological feature and e) depth range interval. Species labels are as follows: Aca_hir (*Acanthogorgia hirsuta*); Cer (Cerianthid); Cid_cid (*Cidaris cidaris*); Den_cor (*Dendrophyllia cornigera*); Ech_sp (*Echinus* sp.); Fun_qua (*Funiculina quadrangularis*); Gra_sp (*Gracilechinus* sp.); Hex_sp (*Hexadella* sp.); Hym (*Hymedesmia* sp.); Inc_red (Incrusting red sponge); Inc_whi (Incrusting white sponge); Inc_white2 (Incrusting white sponge 2); Inc_yell1 (Incrusting yellow sponge 1); Inc_yell2 (Incrusting yellow sponge 2); Inc_yell3 (Incrusting yellow sponge 3); Kop_ste (*Kophobelemnon stelliferum*); Lep_cel (*Leptometra celtica*); Mad_ocu (*Madrepora oculata*); Pac_mon (*Pachastrella monilifera*); Pel_pla (*Peltaster placenta*); Pha_spp. (*Phakellia* spp.); Phe_car (*Pheronema carpenteri*); Por_ind (Unidentified Porifera); Rad_gra (*Radicipes gracilis*) Sub_sp. (cf. *Suberites* spp.); and finally, Tet_sub (*Tethyaster subinermis*).

prevalent in the investigated area of the GoC and has been repeatedly reported in both sides of the GoC since the late XIX century (Kent, 1870, 1871; Arnesen, 1920; Topsent, 1928; Boury-Esnault et al., 1994; Barthel et al., 1996; Sitjà et al., 2019). Nevertheless, its presence across the area is not homogeneous (Boury-Esnault et al., 1994), with the large aggregations towards the Moroccan margin (Barthel et al., 1996). In the studied area, the assemblage was mainly found in silty rocky outcrops along contouritic furrows, yet while this was the dominant substrate in 9 of the 18 transects studied, the *Pheronema* assemblage only occurred in less than 100 linear meters on a single transect (no. 6), with just two additional solitary individuals recorded on muddy grounds in canyons. *P. carpenteri* is also known to possess a 'weak' anchoring system, which would limit its occurrence in areas with strong bottom currents (White, 2003). Furthermore, it has been hypothesized that *P. carpenteri* grounds thrive not just in areas with low bottom currents, but at the same time located near areas with strong bottom current and internal wave breaking phenomena, thus benefiting from the organic matter resuspension (Rice et al., 1990). These rather specific requirements would thus explain the patchy pattern of the *Pheronema* assemblage in the studied area, being nearly absent from most of it, but with dense aggregations when encountered.

While *Pheronema* occurred in monospecific assemblages, the rest of the observed sponges occurred altogether in the same assemblage, which was dominated by fan shaped sponges of the genus *Pachastrella*

Table 2
Taxa characterizing each megabenthic assemblage based on their IndVal values.

Assemblage	Species	IndVal
A	*Pheronema carpenteri*	1.000
B	Incrusting white sponge	0.984
	Incrusting yellow sponge	0.956
	Acanthogorgia hirsuta	0.948
	Pachastrella monilifera	0.795
	cf. *Suberites* spp.	0.685
C	*Dendrophyllia cornigera*	1.000
	Madrepora oculata	0.441
D	*Leptometra celtica*	1.000
E	Ceriantid	1.000
F	*Kophobelemnon stelliferum*	0.883
G	*Funiculina quadrangularis*	0.441
H	*Radicipes gracilis*	0.875
Tethyaster subinermis	0.496	

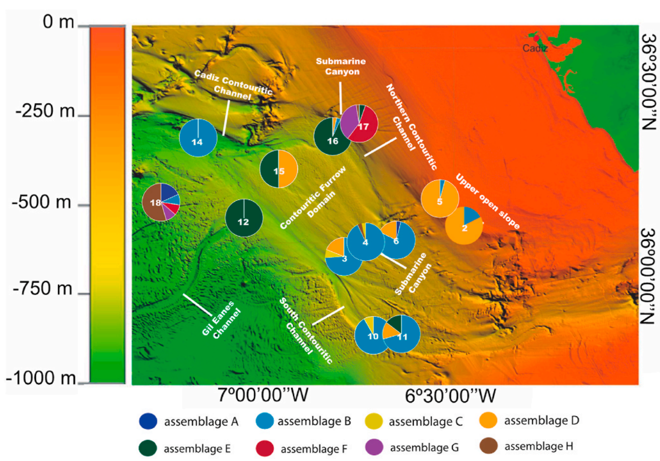

Fig. 4. Spatial distribution of megabenthic assemblages in the study area. Pie charts represent the percentage of sampling units occupied by a certain assemblage within each transect. White numbers within each pie chart represent the transect number. Transects 7, 8, 9, 13 and 19 are not represented as no sessile megabenthic organisms were observed.

(see assemblage B in Fig. 2). In contrast to the *Pheronema* assemblage, which was mainly limited to contouritic furrows, this assemblage appeared to be rather limited by the presence of hard substrates indistinctly of the geomorphological features that constituted it. Fan-shaped sponges-dominated assemblages are common across the Atlanto-Mediterranean region (Sitjà and Maldonado, 2014; Ramos et al., 2016; Santín et al., 2018, 2019; Busch et al., 2021), where they can play a paramount role as ecosystem engineers (Bo et al., 2012). Nevertheless, while widespread, species composition among those fan shaped sponge assemblages (mainly dominated by species of the genera *Pachastrella*, *Vulcanella*, *Poecillastra* and/or *Phakellia*) widely varies between areas, with its distribution and composition appearing to be mostly influenced by water currents and constrained by species-specific habitat requirements (Bo et al., 2012; Santín et al., 2019). Concurrently, *Phakellia* spp. individuals were also identified in the assemblage, but their abundances were considerably lower than those of *Pachastrella*. *Phakellia* spp. appears as a thin sheet attached to the substrate by a peduncle and might be more susceptible to strong currents and other destructive events than *Pachastrella,* which is a far more robust sponge, with a wider attachment area to the substrate (Santín et al., 2019). *Pachastrella* individuals were growing perpendicular to the prevailing water current, a common trait observed in benthic sessile fauna such as gorgonians (Buhl-Mortensen and Mortensen, 2005) and most likely intended to facilitate or maximize the volume of water passing through the sponge. Furthermore, in some locations sediments plumes were observed trailing the sponges, giving clear visual evidence of effects these sponges might have on their surroundings (Fig. 5d). Large sponges can indeed alter the environment around them, including the local water circulation and the recycling of nutrients. In addition, the altered benthic boundary layer can locally enhance biodiversity and functioning of the system (Maldonado et al., 2017). The assemblages dominated by *Pachastrella* were the most diverse, with dense populations of unidentified encrusting Porifera and a stalked, sub-globular sponge (tentatively identified as cf. *Suberites* spp.). Moreover, while some sub-globular stalked sponges have been reported to form dense beds (Ríos et al., 2018), they are commonly reported alongside fan-shaped sponges across the Atlantic-Mediterranean region (Sitjà and Maldonado, 2014; Santín et al., 2018). These beds are hypothesized to benefit from the turbidity that fan-shaped sponges create around their bodies (Bo et al., 2012; Santín et al., 2019).

In assemblage B, we also reported the presence of aggregations of a cidarid echinoid in silted rocky areas of contouritic channels, with lower density respect to what has been reported for the Bay of Biscay (Stevenson et al., 2015). Cidarid aggregation have also been observed in the Galicia Bank (Serrano et al., 2017). It has been suggested that Cidarid aggregations respond to feeding and reproduction needs (Stevenson et al., 2015). On the GoC Cidarid dominated assemblages have been observed on soft sediment environments on the proximities of the Gazul mud volcano (Urra et al., 2021). However, in this environment cidarids were sparsely distributed (Urra et al., 2021).

Assemblage C was dominated by the cold-water corals *Madrepora oculata* and *Dendrophyllia cornigera* (Fig. 3), which was highly localized on a 30 m^2 rocky outcrop. As previously observed on CWC assemblages in other areas of the GoC, *M. oculata* was the dominant scleractinian species (88% of observed colonies) followed by *D. cornigera* (12% of observed colonies) (Rueda et al., 2016). *M. oculata* colonies reached densities of 14 col m^{-2} resembling previous observations on mud volcanoes in the GoC (Rueda et al., 2016; Urra et al., 2021). However, unlike other areas of the Gulf, here no coral rubble was observed (Rueda et al., 2016) perhaps indicating that this area was recently colonized by these CWC species. *D. cornigera* mainly occurred as solitary colonies sparsely distributed across this assemblage. This trend is consistent with previous observations conducted in mud volcanoes in the Gulf of Cadiz, (Urra et al., 2021), Atlantic seamounts (Ramos et al., 2016) and Mediterranean submarine canyons and slopes (Orejas et al., 2009; Grinyó et al., 2018).

On silty grounds on canyons, contouritic furrows and open slope environments we encountered assemblage D which was chiefly characterized by the crinoid *Leptometra celtica* (see assemblage D in Fig. 3). *L. celtica* was one of the most abundant species representing 18% of all observed organisms (Fig. 2). This high abundance values resemble those observed on the Gazul mud volcano (80 km from the study area), were this species represented 17.23% of all observed organisms (González-García et al., 2020). Indeed, certain crinoid species are known to form dense aggregations, commonly referred as crinoid beds (Colloca et al., 2004; Grinyó et al., 2018). In the study area, *L. celtica* beds were mainly found between 200 and 650 m depth in contouritic furrow and slope environments, resembling previous observations on the Iberian margin (Fonseca et al., 2014). *L. celtica* beds have commonly been associated to bottom currents that provide regular food supply (Lavaleye et al., 2002). This, could suggest that contouritic furrows and slopes, where *L. celtica* beds occur are exposed to constant hydrodynamic process. Furthermore, crinoid beds of the genus *Leptometra* are known to sustain the presence of multiple motile species enhancing local biodiversity and acting as nursery and recruitment grounds for commercial fish species (Colloca et al., 2004).

Assemblage E occurred on silted grounds of contouritic channels and contouritic furrows and was chiefly constituted by cerianthid unidentified species. In the GoC, information regarding cerianthid assemblages have been reported to occur on non-active pockmarks (Somoza et al., 2021). In bathyal sediments in other areas of the North Atlantic or the

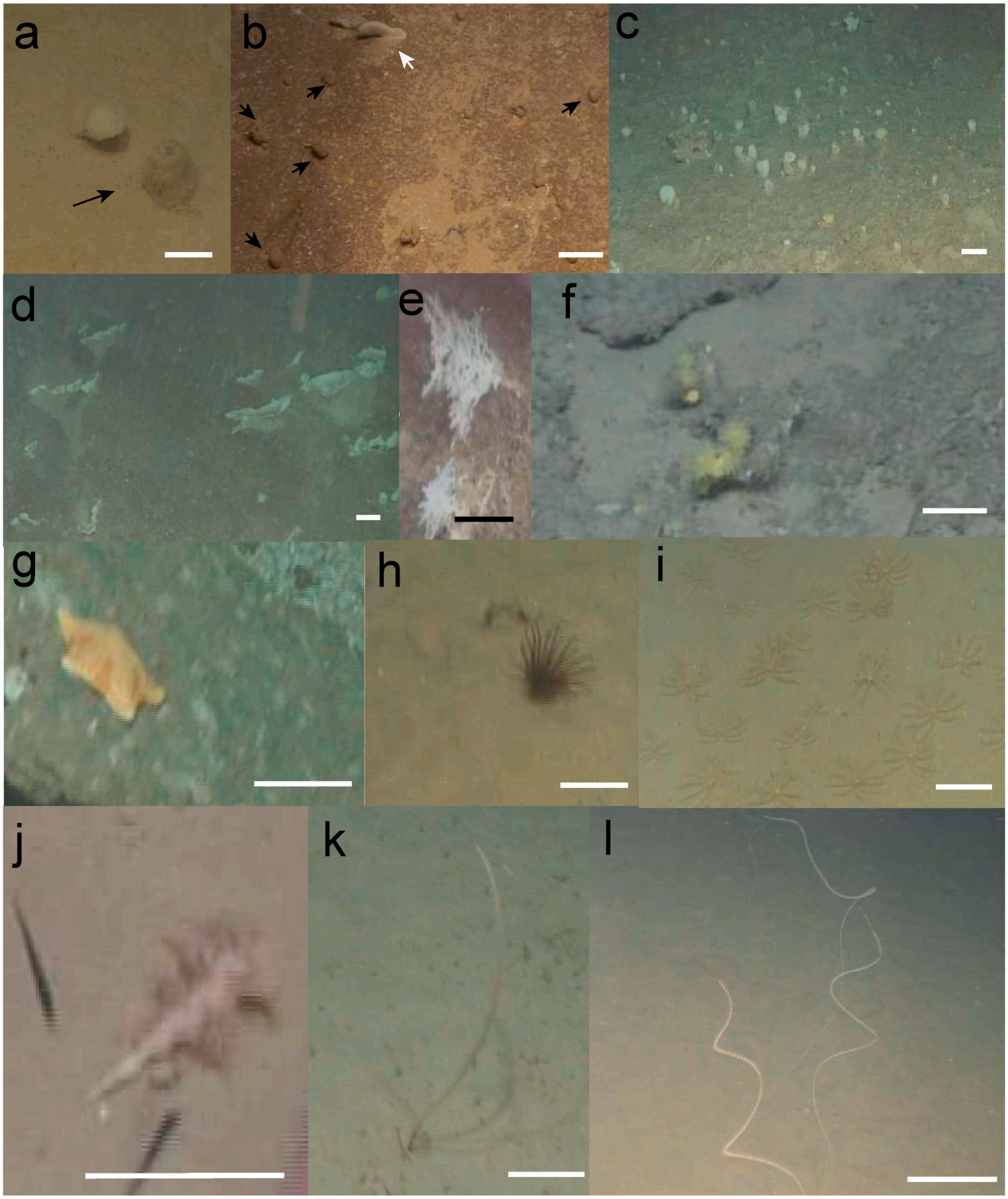

Fig. 5. Taxa characterizing each megafaunal assemblage a) *Pheronema carpenteri* (black arrow) covered by silty sediments and *Hyalonema* sp., b) *Pheronema carpenteri* (black arrows) aggregation on rocky substrate and individual of *Pachastrella monilifera* (white arrow), c) Multispecific sponge assemblage, d) *Pachastrella monilifera* aggregation with sediment accumulation down current, e) *Madrepora oculata* colonies, f) *Dendrophyllia cornigera* colonies, g) *Peltaster placenta*, h) unidentified ceriantihid i) *Leptometra celtica* bed, j) *Kophobelemnon stelliferum,* k) *Funiculina quadrangularis,* l) *Radicipes gracilis* (scale bar = 15 cm).

Mediterranean Sea, cerianhtids have been described to form assemblages resembling the once encountered in the study area (Sánchez et al., 2014; Davies et al., 2015; Grinyó et al., 2020). Cerianthid tubes provide habitat to a wide range of associated species acting as local biodiversity hotspots (Ceriello et al., 2020). Since cerianhtids are characterized by a long-life span and an upright and fragile structures, certain cerianhtids species have been identified as highly sensitive to bottom trawling (Pommer et al., 2016). Consequently, ceriantid assemblages have been proposed as a VME by the Northeast Atlantic Fisheries Commission (NEAFC recommendation 19:2014) and by the General Fisheries Commission for the Mediterranean VME's working group (Report WGVME, 2018).

Both *Kophobelemnon stelliferum* and *Funiculina quadrangularis* monospecific assemblages were found on silted grounds in canyon environments (see assemblage F and G, respectively in Fig. 3). In the adjacent Alboran Sea both species have also been reported to form monospecific assemblages in bathyal muds in continental slopes (Grinyó et al., 2020). In the GoC both species have been reported to occur on soft sediments in diapiric rises and mud volcanoes jointly characterizing multispecific megabenthic assemblages along with the sponge *Thenea muricata*

Table 3
MANOVA results based on species samples.

Source	DF	exVarSS	nPC	nBu	exVarPC	exVarBu	*p*-Value
Dominant substrate	3	0.042	19	7	0.910	1	0.000
Geomorphology	3	0.0	19	8	0.909	1	0.000
Depth	5	0.027	18	8	0.890	1	0.000
Error	339	0.843					

DF – Degrees of Freedom; exVarSS – explained variances based on sums of squares; nPC – number of principal components used for testing; nBu – number of principal components used as buffer components; exVarPC – variance explained by nPC components; exVarBU – variance explained by (nPC + nBU) components; p-Value – the result from 50 to 50 MANOVA testing.

(Palomino et al., 2016; González-García et al., 2020). *K. stelliferum* and *F. quadrangularis* reached similar densities to those reported on bathyal sediments in other areas of the GoC and in the Mediterranean Sea (Pérès and Picard, 1964; Mastrototaro et al., 2013; Palomino et al., 2016). Both species abundances are of relevance as they have been associated to increase local diversity (De Clippele et al., 2015; Grinyó et al., 2020). Indeed, several decapod (e.g. *Munida* spp. and unidentified pandalid shrimp) and fish species (e.g. *Coelorhinchus coelorhinchus*, *Hoplosthetus mediterraneus),* including commercial ones (*Merluccius merluccius*) were associated with 57% of *K. stelliferum* and 60% of *F. quadrangularis* colonies, confirming that both species are habitat-forming species that may provide refuge or feeding grounds for motile species (De Clippele et al., 2015).

The gorgonian *Radicipes gracilis* and the asteroid *Tethyaster subinermis* were abundant on the silt sediments in both contouritic furrows and canyon areas and characterized Assemblage H (see Fig. 3l). Both species have been previously reported in the GoC in mud volcano areas (Rueda et al., 2016). *Radicipes* is widely distributed on sedimentary environments across the North and central Atlantic (Buhl-Mortensen et al., 2015; Cordeiro et al., 2017). In some areas, such as the Norwegian margin *Radicipes* colonies have been described to form extensive dens meadows (Gonzalez-Mirelis and Buhl-Mortensen, 2015). Similarly, *Radicipes gracilis* assemblages in the study area reached high densities of 38 col. m^{-2} (18 ± 14 col. m^{-2}, average ± SD). In the Japanese continental margin, *Radicipes* colonies were reported to provide habitat for brittle starts (Fujita and Ohta, 1988; Horikoshi et al., 1990). Although this association was not observed in the study area, the richness of vagile species associated to *Radicipes* assemblages was two times higher than the one encountered in surrounding soft sediments (7 species). This could indicate that *R. gracilis* can locally increase diversity, although this assemblage was highly localized GoC (35 m^2) and thus further research is advised to confirm this trend.

Overall, megabenthic assemblages in the study area resembled those occurring both on hard substrates and soft sediment environments on mud volcanoes on the GoC. However, it should be noted that both hard and soft sediment environments on mud volcanoes hosted a higher sessile species diversity than the once reported in the study area (Urra et al., 2021).

Additionally, the described assemblages were mainly found between 600 and 750 m depth (Fig. 3e and f), contrasting with previous research on the study area which reported these assemblages in shallower environments (Rueda et al., 2016; González-García et al., 2020; Urra et al., 2021). These differences likely derived from the fact that previous research has mainly been developed on mud volcanoes located below 650 m depth (González-García et al., 2020; Urra et al., 2021). Therefore, the present study expands the current knowledge on megafauna assemblages geographic and bathimetric distribution in the GoC (De Mol et al., 2012; Rueda et al., 2016).

4.3. Habitat conservation

Our study provides new insights about megabenthic assemblages on the GoC in different geomorphological areas evidencing the presence of assemblages that represent Vulnerable Marine Ecosystems, thus advocating for their protection through the institution of off-shore Marine Protected Areas (MPAs). This could implement the Natura 2000 Network of off-shore MPAs recently instituted thanks to the LIFE project INDEMARES (http://www.indemares.es/en/project/description) carried out by several institutions/universities, which successfully identified ten new off-shore Sites for Community Importance. Our data support the need for further studies in this area to better understand the ecological value of its ecosystems, for geomorphologies not studied before such as submarine canyons or contouritic furrows.

5. Conclusions

In the present study we reported the evidence for a high heterogeneity of deep-sea habitats in the GoC, most of them characterised by high diversity. In particular, we found:

- Eight megafaunal assemblages segregated by substrate and geomorphological features.
- Dominance of monospecific and multispecific sponge assemblages on hard substrates.
- Crinoid beds, pennatulacean and *Radicipes gracilis* assemblages dominated soft sediments of all geomorphologic features.
- Submarine canyons where the geomorphological feature hosting the highest megabenthic assemblage diversity.
- These fragile assemblages are part of VMEs, and thus are threatened by bottom contact gears. For this reason we propose to include these ecosystems in future management plans for their protection.

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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