1 Deep-sea habitat characterization using acoustic data and underwater imagery in Gazul mud

2 volcano (Gulf of Cádiz, NE Atlantic)

- 3 Javier Urra^a*, Desirée Palomino^a, Pablo Lozano^a, Emilio González-García^a, Carlos Farias^b, Ángel
- 4 Mateo-Ramírez^a, Luis Miguel Fernández-Salas^b, Nieves López-González^a, Yolanda Vila^b,
- 5 Covadonga Orejas^c, Patricia Puerta^c, Jesús Rivera^d, Lea-Anne Henry^e, José L Rueda^a
- 6 ^aCentro Oceanográfico de Málaga, Instituto Español de Oceanografía, Puerto Pesquero S/N,
- 7 29640 Fuengirola, Málaga, Spain. *Corresponding author: javier.urra@ieo.es
- 8 bCentro Oceanográfico de Cádiz, Instituto Español de Oceanografía, Muelle Pesquero S/N,
- 9 11006 Cádiz, Spain
- 10 ^cCentro Oceanográfico de Baleares, Instituto Español de Oceanografía, Muelle de Poniente,
- 11 S/N, 07015 Palma de Mallorca, Spain
- 12 d'Centro Oceanográfico de Madrid, Instituto Español de Oceanografía, Calle del Corazón de
- 13 María, 8, 28002 Madrid, Spain
- ^eSchool of GeoSciences, Grant Institute, The University of Edinburgh, Edinburgh EH9 3FE, United
- 15 Kingdom

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Abstract

- 17 Gazul is the shallowest mud volcano (MV) within the Shallow Field of Fluid Expulsion (SFFE) of
- 18 the northeastern Gulf of Cádiz (NE Atlantic; 300-1200 m depth). The SFFE represents an
- important geo- and biodiversity area that was designated as a Site of Community Importance
- 20 under the European Habitats Directive in 2014. In this study, geological features, habitats and
- 21 associated biodiversity, as well as anthropogenic impacts, were characterized at the Gazul MV
- 22 from underwater imagery and multibeam bathymetry. Multivariate methods using the Bray-
- 23 Curtis similarity index identified six main habitats, each of which harbored a characteristic
- faunal assemblage that included: (1) sandy ripple bottoms typified by the actiniarian Actinauge
- 25 richardi; (2) sandy, muddy, coarse sand and bioclastic bottoms dominated by the solitary coral
- 26 Flabellum chunii; (3) coarse sand and bioclastic bottoms, together with soft sediments covered
- 27 by scattered methane-derived authigenic carbonates (MDACs) (mixed bottoms), characterized
- 28 by the echinoid Cidaris cidaris; (4) hard bottoms comprising MDACs dominated by a wide
- 29 variety of sponges and gorgonians; (5) coral-rubble bottoms typified by the presence of
- 30 colonial scleractinian communities dominated by *Madrepora oculata*; and (6) mixed bottoms
- 31 characterized by the presence of a styelid ascidian. Slope and water depth were the main
- 32 factors explaining assemblages' distribution, which was also supported by the presence of
- 33 MDACs such as slabs, crusts and chimneys on the seafloor, as well as by the geomorphologic
- 34 diversity of Gazul MV. The results highlight Gazul MV as an eco-biologically important area
- 35 harboring different vulnerable marine ecosystem (VME) elements with indicator taxa such as
- 36 scleractinians, sponges, gorgonians and black corals. ROV images revealed abandoned or lost
- 37 fishing gears and marine debris on the seafloor, indicating anthropogenic impacts in Gazul MV
- 38 and adjacent areas. Indeed trawling fisheries activities have also been detected in Vessel
- 39 Monitoring System datasets. A fishery restricted area is recommended in Gazul MV due to the
- 40 occurrence of diverse VMEs and species included in different conservation directives and
- 41 conventions.
- 42 Keywords: benthic communities, conservation, deep-sea habitats, mud volcano, ROV,
- 43 vulnerable marine ecosystem

1. Introduction

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Submarine elevations (e.g. seamounts, mounds, diapiric ridges, mud volcanoes) are considered exceptional seafloor features harboring distinct benthic and demersal associated communities and habitats (Samadi et al., 2007; Ramos et al., 2016; Rueda et al., 2016; Corbera et al., 2019), which can even promote large pelagic biodiversity in the open ocean (Morato et al., 2006; Holland and Grubbs, 2007; Litvinov, 2007). There is a strong link between deep-sea benthic communities at submarine elevations with local bottom current and substrate types (Van Rooij et al., 2010). In areas with strong bottom currents, erosion and sediment transport are promoted and, in some cases, hard substrata are exhumed and colonized by suspension feeders (Van Rooij et al., 2010; Gasser et al., 2011). In those areas, associated benthic communities are usually dominated and structured by slow-growing and long-living organisms, mainly suspension feeders (e.g. colonial scleractinians such as the so called white corals, gorgonians and sponges), which benefit from a local high productivity derived from moundinduced upwelling and the enhanced currents around them (Genin et al., 1986; Roden, 1986; Rogers, 1994; Richer de Forges et al., 2000; Samadi et al., 2007). These organisms provide three-dimensionally complex habitats (sometimes known as "marine animal forests"; Rossi et al., 2017; Wienberg and Titschack 2017) for a large number of vagile invertebrates and demersal fish, and they only occur in specific locations (Richer de Forges et al., 2000; Samadi et al., 2007; Altuna, 2013). These habitat-forming organisms are important features of the deep sea, recently classified as vulnerable marine ecosystems (VMEs hereinafter), and are very sensitive and vulnerable to fishing activities, particularly to bottom contact gears (Koslow et al., 2001; Clark and Koslow, 2007).

Ecosystems on submarine elevations are at the forefront of international concerns and negotiations, and discussed by worldwide organizations, policy makers and scientific researchers in order to improve the sustainable use of resources as well as the protection of the associated biodiversity in VMEs in the high seas (Gjerde and Breide, 2003; WWF/IUCN, 2004; United Nation, 2007; FAO, 2009). Nevertheless, the level of scientific knowledge of deep-sea habitats is still limited (Koslow et al., 2015). Scientific data regarding the description of VMEs and associated communities are crucial for establishing management strategies in order to protect ecologically important deep-sea areas. Regarding deep hard bottoms, which are difficult to study using classical extractive methods without destroying ecosystem components, the development of non-invasive techniques (e.g. underwater cameras, remotely operated vehicles (ROVs hereinafter), multibeam echosounder systems) has enabled the direct visual inspection of the seafloor characteristics and the megafauna, allowing the identification,

mapping and quantitative study of different habitats (e.g. Orejas et al., 2009; Howell et al., 2010; Bo et al., 2011; Sánchez et al., 2014; Taviani et al., 2015; Ramos et al., 2016; Lim et al., 2017; van den Beld et al., 2017; de la Torriente et al., 2018; Price et al., 2019). Underwater images and videos have largely increased our knowledge on deep-sea VMEs, fostering management and conservation plans (WWF/IUCN, 2004; Ramirez Llodra and Billett, 2006), and providing precise information for the assessment of the environmental status for several indicators of the European Marine Strategy Framework Directive (MSFD, 2008/56/EC) concerning the conservation of natural habitats (Kazanidis et al., 2020).

Over the last few decades, commercial fisheries shifted effort and expanded into the deep-sea following fisheries overexploitation on global continental shelves (Morato et al., 2006; Norse et al., 2011; Costello et al., 2012; FAO, 2018). This shift in exploitable fishing depths is leading to potentially long-term impacts including biodiversity loss in VMEs (Pitcher et al., 2010). Within southern Europe, there is detailed and extended information regarding deep-sea VMEs for the western and central Mediterranean basin (e.g. D'Onghia, 2019; Puig and Gili, 2019; Rueda et al., 2019; Gulf of Lions: Orejas et al., 2009; Gori et al., 2013; Aymà et al., 2019; off Cape Santa Maria di Leuca: Tursi et al., 2004; Taviani et al., 2005; Mastrototaro et al., 2010; D'Onghia et al., 2011; Strait of Sicily: Zibrowius and Taviani, 2005; Schembri et al., 2007; Freiwald et al., 2009; Taviani et al., 2009), whereas other areas are less studied, as for instance the southern Iberian continental margin (Díaz-del-Río, 2014; de la Torriente et al., 2018; Corbera et al., 2019; Rueda et al., accepted).

The European LIFE+ INDEMARES Project was carried out in Spanish waters with the main aim of providing the necessary scientific information to establish a network of deep-sea areas of biological interest for conservation purposes. One of the study cases was the Shallow Field of Fluid Expulsion (SFFE hereinafter) (Díaz-del-Río, 2014), which is located on the upper and middle slope of the Gulf of Cádiz (GoC hereinafter) continental margin, between 300 and 1200 m water depth. This area presents an important number of mud diapirs and mud volcanoes resulting from massive plastic material movements and actively extruding material, respectively, from sub-seafloor layers to the surface due to density differences (Díaz-del-Río et al., 2003; Pinheiro et al., 2003; Medialdea et al., 2009), which is why the area is also known as the mud volcanoes of the GoC (MVGoC hereinafter). This geomorphological diversity is enriched by the presence of fields of methane-derived authigenic carbonates (MDACs hereinafter) exhumed by bottom currents erosive processes, which have triggered a diversification of habitat types and biological communities (Greinert et al., 2001).

Traditionally, soft bottoms across the MVGoC have been exploited by multispecific bottom trawl fleets, with the Norway lobster Nephrops norvegicus (Linnaeus, 1758) as one of the main target species due to its high economic value (Jiménez et al., 2004; Vila et al., 2015). The MVGoC were integrated into the European Natura 2000 network as a Site of Community Importance (SCI-ESZ 12002 "Volcanes de fango del Golfo de Cádiz") due to the presence of (1) five habitat subtypes within "1180 Submarine structures made by leaking gases", and (2) nine habitat subtypes within "1170 Reefs", both categories included within Annex I of the Habitats Directive of the European Union (Council Directive 92/43/EEC) (Díaz-del-Río, 2014). Furthermore, highly fragile keystone epibenthic species related to the OSPAR (Oslo and Paris Commissions) habitats list (https://www.ospar.org/work-areas/bdc/species-habitats) such as "coral gardens" and "deep-sea sponge aggregations" are also present in the MVGoC (Rueda et al., 2016). Nevertheless, despite the increasing interest on deep-sea ecosystems, few studies have been focused on the associated benthic fauna of MVGoC (Delgado et al., 2013; Rueda et al., 2016; Lozano et al., 2020a, b), most of them studying the distinct fauna associated with fluid migration and seepage (Oliver et al., 2011; Rueda et al., 2012; Cunha et al., 2013; Rodrigues et al., 2013), while some others analyzed the spatial and temporal distribution of cold-water corals (Taviani et al., 1991; Wienberg et al., 2009).

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The aims of the present study were: (i) to identify and describe the main habitats and megabenthic assemblages occurring on Gazul MV and surrounding areas, (ii) to map their distribution using a backscatter raster in order to get an interpretative habitat map, and (iii) to analyze the potential influence of seafloor characteristics (obtained using acoustic systems) on the distribution of these habitats. This information will improve the knowledge on the biological and ecological characteristics of this specific area of the GoC, as well as of a recently declared SCI.

2. Materials and Methods

2.1. Study area

The geomorphology of the GoC is largely shaped by tectonics and diapiric processes related to a complex geodynamic evolution of the continental margin (Medialdea et al., 2009). These processes provide escape pathways for over-pressured sediments and fluids from deeper layers to the seafloor surface, and favor the formation of characteristics seafloor structures such as MVs (Pinheiro et al., 2006; Fernández-Puga et. al., 2007). On the upper and middle slope of the Spanish continental margin (300-730 m depth), the SFFE is characterized by the presence of two main diapiric ridges (DR hereinafter), the Guadalquivir DR and the Cádiz DR (Somoza et al., 2003; Fernández-Puga et al., 2007); two main contourite channels with sinuous morphology, the Gusano and Huelva channels (García et al., 2009); and several MVs and mud diapirs (Díaz-del-Río, 2014; Palomino et al., 2016).

Gazul is one of the four MVs that have been confirmed in the SFFE (Palomino et al., 2016). It is located in the northeastern part of the SFFE and to the north of the Cádiz DR (Fig. 1). This MV has its summit at 363 m depth (relief: 107 m) and a diameter of 1200 m. According to Palomino et al. (2016), this MV has a subcircular base and an asymmetrical bathymetric profile, with flanks of different lengths and oriented NE–SW. It presents two prolongations running NW–SE that are interpreted as 20 to 40 m height outcrops of muddy materials, surrounded by two depressions (depth: 15 and 20 m below adjacent bottoms; length: 2.1 and 2.3 km, respectively) located north and northwest of the MV main edifice (Fig. 1C). There are also isolated and grouped mounds about 55 m wide occurring to the west of the western depression, at depths between 460 and 480 m and oriented NW–SE (Fig. 1C). The summit of Gazul MV is characterized by a thin veneer of hemipelagic muddy sediments overlying greenish grey-mud breccia facies with abundant bioclasts and MDACs; the area of the mounds presents MDACs, mainly slabs and chimneys; finally, sediments of the depressions are coarser and composed of sand and bioclastic gravel (Palomino et al., 2016).

The pattern of oceanographic circulation in the GoC is controlled by the exchange of water masses through the Strait of Gibraltar, with the surficial North Atlantic Central Water flowing into the Alboran Sea and the deeper Mediterranean Outflow Water (MOW hereinafter) flowing out to the Atlantic Ocean (Lacombe and Lizeray, 1959; Ochoa and Bray, 1991; Sánchez-Leal et al., 2017) (Fig. 1A). The MOW, which shows high salinity (38.45 psu) and temperature (12.9 °C), sweeps along the southwestern Iberian margin as a seafloor bottom current between 300 m and 1200 m water depth, flowing to the north and west due to the

Coriolis deflection, and splitting into two main branches, the Mediterranean Upper Core and the Mediterranean Lower Core (Madelain, 1970; Ambar and Howe, 1979; Sánchez-Leal et al., 2017). The present study area is under the influence of the Mediterranean Upper Core (Fig. 1A), with bottom currents reaching a maximum speed of 0.3–0.5 m·s⁻¹ along the contourite channels, in contrast to the minimum speeds of between 0.01–0.1 m·s⁻¹ observed between the diapiric ridges (Díaz-del-Río, 2014; Sánchez-Leal et al., 2017). This MOW branch exerts a great influence on the bottoms of this area as it circulates in contact with the friction surface of the seabed, resulting in diverse morphological features, both depositional and erosional (Hernández-Molina et al., 2003; García et al., 2009; Sánchez-Leal et al., 2017; Lozano et al., 2020b).

2.2. Video sampling design and video data collection

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Underwater video transects have been recorded in three expeditions conducted at 3 sites on Gazul MV (the summit, northern flank and southern flank) and also at 4 sites from the surrounding areas (the eastern and western adjacent areas, and the western and central depressions) between 2012 and 2016 (Fig. 1C). The first expedition was carried out within the LIFE+ INDEMARES/CHICA project on board the Research Vessel (RV) Ramón Margalef (CHICA-0412 expedition); during this expedition three ROV video transects (total recording time of 6h 20min) were performed using the ROV LIROPUS 2000 (model Super Mohawk II; equipped with a high definition Kongsberg video camera, a Kongsberg tooling camera and three frontal flash LED Matrix). The second survey was carried out within the ISUNEPCA project on board the RV Ángeles Alvariño (ISUNEPCA-0616 expedition); during this expedition three Remotely Operated Towed Vehicle (ROTV) video transects (2h 55min) were performed using the ROTV TST-HORUS (equipped with two Full HD video cameras which have an angle of inclination of 45° in relation to the ground, and a unique frontal flash LED Matrix). The third survey was carried out within the H2020 ATLAS project on board the RV Sarmiento de Gamboa (MEDWAVES expedition); during this expedition two ROV video transects (13h 31min) were performed using the ROV LIROPUS 2000. Underwater transects of all three expeditions were georeferenced with a transponder that allowed the position of the images to be precisely determined relative to the RV vessel along each transect. Additionally, both the ROV and the ROTV were equipped with two parallel laser beams providing either a 10 cm (ROV) or a 7.5 cm spatial scale (ROTV).

The bathymetric data of Gazul MV (and surrounding areas) used in this study were acquired during the ISUNEPCA project using a Kongsberg Simrad EM 710 multibeam echosounder (from 70 to 100 kHz) with a lower velocity, and processed with CARIS HIPS & SIPS

data processing software to produce a 5×5 m bathymetric grid model. Backscatter values and other seafloor predictors were also processed from swath bathymetry using the Geocoder algorithm of CARIS HIPS & SIPS software. In addition, a backscatter mosaic was created after the processing of bathymetric data with CARIS HIPS & SIPS software with a 3×3 m of spatial resolution and it was analyzed with the ArcGis v10.7.

The video footage (22h 46min of recording) was visually analyzed and split into oneminute video track units (N_t= 1366 video track units) for the geological and biological data acquisition, and named as samples hereinafter. These 1-min samples were treated as different samples and those with different substrate types were removed in order to avoid ambiguous classifications with species from different habitats. The video tracks that corresponded to sampling events (when the ROV is not moving), zooms or indistinct images were removed from the analysis, and only those 1-min video samples displaying continuous movement ROV tracks at similar low speeds (ROV: 12 m·min⁻¹; ROTV: 16 m·min⁻¹) and distance from the bottom (generally between 0.5 and 2.0 m) were included in the analyses. This methodology has been recently used in similar studies for identifying epibenthic habitats and spatial patterns of associated benthic assemblages (e.g. Beaman et al., 2016; de la Torriente et al., 2018). The interpretation of soft bottoms at Gazul MV in the video footage has been carried out using information regarding the nature and sediment texture obtained from previous works carried out in the LIFE+ INDEMARES project and MEDWAVES expedition (Díaz-del-Río, 2014; Palomino et al., 2016; Orejas, 2017). Finally, a total of 505 out of 1366 1-min video samples corresponding to a unique substrate type and covering similar characteristics (ROV speed and distance from the bottom) were included for the habitat identification analysis.

2.3. Biological and geological data sets

The abundance of identifiable and conspicuous epibenthic and demersal species was evaluated in each one of the 505 1-min video samples according to a semi-quantitative index coded as 1 (1 observation [i.e. individual/colony] per 1-min video sample), 2 (2-5 observations), 3 (5-30 observations), 4 (30-100 observations) and 5 (>100 observations). Several groups of morphologically similar species were used instead when species identification using video imagery was not possible in order to avoid taxonomic inconsistencies (see Table 1). This approach was applied mainly to sponges, which were very diverse and abundant in the area, but scarcely distinguishable from each other in the underwater images, being grouped as "large sponges", "small and digitate sponges" and "encrusting sponges". Other groups included small gorgonians and were referred to the "Bebryce group",

alcyonaceans and hydrozoans. Despite these taxonomic limitations, this approach gives an idea of the range of organisms that form part of the faunal communities identified in the samples. In addition, the density (in terms of individuals or colonies m⁻²) of these species (or species groups) was evaluated on specific video frames obtained from different 1-minute video samples by measuring the areas of the frames with the help of the scale provided by the laser beams. Some video-taxonomic determinations of epibenthic taxa were confirmed with specimens collected with benthic dredge and beam-trawl (sampling area ca. 300 m² and 2000 m², respectively, and targeting epifaunal and demersal organisms from sedimentary and non-sedimentary habitats) in previous LIFE+ INDEMARES/CHICA expeditions carried out before obtaining underwater imagery in Gazul MV (Díaz-del-Río, 2014; Palomino et al., 2016; Rueda et al., 2016; Sitjà et al., 2019).

Six different bottom types were defined in order to avoid uncertainties and to facilitate interpretation, including (1) coarse sand and bioclastic bottoms (CSBB); (2) sandy ripple bottoms (SRB); (3) sandy bottoms (SB); (4) hard bottoms comprising MDACs (i.e. bottoms dominated by large crusts or slabs partly covered by a thin layer of sediment) (HB); (5) coral-rubble bottoms (CRB); and (6) mixed bottoms (i.e. soft sediments covered by scattered MDACs) (MXB). Substrate types and seafloor micro-morphologies (e.g. ripples) as well as fluid venting related features (e.g. carbonate chimneys) were annotated in each 1-min video sample and, whenever possible, ground truthing by samples and available sedimentological and backscatter data obtained in different LIFE+ INDEMARES/CHICA expeditions at Gazul MV (Díazdel-Río, 2014; Palomino et al., 2016).

Data based on first-order derived statistics of bathymetric data were obtained using different extensions within ArcGIS v10.7. These seafloor predictors included slope (degrees of inclination), aspect (orientation of the seabed), backscatter (where high values correspond to hard substrates), profile curvature (topographic unevenness) and Bathymetric Position Index (BPI; it measures whether the area is on a topographic high (crest) or low (depression) relative to the surrounding area), as well as water depth as a position variable.

2.4. Anthropogenic activity indicators

Bottom trawling activity in Gazul MV and adjacent areas was obtained from Vessel Monitoring System (VMS) datasets for the period between 2006 and 2012, which were supplied by the *Secretaría General del Mar* of the Spanish government. These datasets included date, time, vessel registration number, vessel position and speed, and type of fishing gear (e.g. trawl, purse seine, etc.). Annual and average fishing efforts (as total trawled hours)

were calculated for a grid of the study area with 1 km² cell size. These analyses were carried out using the package VMStools in R software (Hintzen et al., 2012). Interpolation between positional signals was calculated in VMStools. Positional signals corresponding to fishing activity were defined by a speed range of 2 to 5 knots, as this is the usual speed for trawling vessels operating in the area. The results obtained were expressed in trawling hours and represented in a map with the software ArcGIS v10.7.

Additionally, the abundance and distribution of marine litter was analyzed by annotating its presence on each 1-min video sample. Litter was classified following the Master List of Categories of Litter Items given by the MSFD Technical Subgroup on Marine Litter (2013), which divided the marine litter into five main categories of material (plastics, metal, rubber, glass/ceramics, natural products). Additionally, marine litter was analyzed considering the type of item (e.g. fishing lines, cans, drink bottles), and their presence was evaluated through the relative frequency of occurrence of each type, estimated as the percentage of samples in which the litter was present. The potential impact of marine litter on benthic fauna was also evaluated following the classification of Angiolillo et al. (2015) (i.e. covering, abrasion, hanging, lying).

2.5. Data analyses

The total frequency of occurrence of each taxa and assemblage identified in the analyzed underwater imagery has been determined as the percentage of samples in which a particular taxa/assemblage is present, and it was expressed as a frequency index (%F). In the case of those taxa included in further analysis, the relative frequency of occurrence per bottom type was also considered.

Multivariate methods such as group-average sorting classification (CLUSTER) with the UPGMA agglomeration algorithm (Sneath and Sokal, 1973) and non-metric multidimensional scaling ordination (nMDS) using the Bray-Curtis similarity index were carried out independently on the same matrix of abundance values (i.e. as semi-quantitative ranks above mentioned). For these multivariate analyses, only those characteristic epibenthic taxa that provided a biological structure (potential habitat-forming species) and displayed a total frequency of occurrence in samples of more than 1% were considered, and referred to as habitat-typifying species (Table 1). These multivariate analyses were done for detecting spatial patterns and identifying epibenthic assemblages forming habitats on Gazul MV. To test for differences between the identified assemblages, a distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001; McArdle and Anderson, 2001) was

performed. This design included 2 fixed and orthogonal factors: substrate type (6 levels: coarse sand and bioclastic bottoms, sandy ripples bottoms, sandy and muddy bottoms, hard bottoms, coral-rubble bottoms, mixed bottoms) and sites (7 levels, 3 in the MV: summit, northern flank, southern flank; and 4 in the surrounding area: central depression, western depression, eastern adjacent area, western adjacent area). The PERMANOVA analysis was based on Bray-Curtis similarity on semi-quantitative data, using 999 random permutations. The identification of the main taxa characterizing each group (i.e. epibenthic assemblages) in the CLUSTER analysis was performed through a similarity percentage analysis (SIMPER) with a 90% cut-off for low contributions. All statistical analyses were carried out using PRIMER 6+ software (Clarke and Warwick, 2001).

Information regarding the bottom type observed along the video tracks and the epibenthic assemblages identified from multivariate analyses was superimposed on the backscatter mosaic, resulting in an interpretative habitat map obtained with the ArcGIS desktop software.

Finally, the potential effect of seafloor variables in the spatial distribution of assemblages was determined using canonical correspondence analysis (CCA), previously applied in other works using underwater imagery (e.g. Sánchez et al., 2014; Gunton et al., 2015; de la Torriente et al. 2018; Grinyó et al., 2018). The set of variables used in the CCA analysis included backscatter, slope, aspect, BPI and profile curvature, as well as the location of sites in terms of depth (m). The statistical significance of the effect of each variable was tested by a Monte Carlo permutation test. Prior to this, the seafloor variables were screened for correlations in order to avoid collinearity using a Spearman correlation analysis. These multivariate analyses were executed using the software CANOCO 4.5.

3. Results

3.1. Bottom types

Two major substratum types were identified at Gazul MV. Hard substrates showed higher backscatter values (mean values ranging between -16.72 and -18.73 dB) and are located in areas with greater slopes (mean slope: 9.13±0.4 degrees), whereas soft substrates showed lower backscatter values (between -20.64 and -23.33 dB) and were located in generally flat surfaces (mean slope: 3.04±0.2 degrees).

CSBB were observed in 23.6% of the samples (n= 119 samples), mainly located in the western depression with many shell remains (mostly belonging to the pectinid bivalve Pseudamussium peslutrae and to the brachiopod Gryphus vitreus) and echinoid spines (Cidaris cidaris), with the eastern mounds containing similar bioclasts (Fig. 2). Sinusoidal and partly bifurcated SRB were locally restricted to a flat surface in the western adjacent area (17.2 % of the samples) with the lowest backscatter values, whereas homogeneous SMB were observed in the central depression (6.8 %) (Fig. 2). SB were also detected close to the summit area of the MV and along the southwestern flank, in the latter displaying some bioturbation (6.7 %) (Fig. 2). HB dominated by MDACs, mainly slabs, was mainly found on the eastern mounds as well as on the southwestern flank (17.2%) and displayed the highest backscatter values (Fig. 2). In HB located close to the eastern mounds, a chimney forest was observed with some chimneys still showing an erected position (Fig. 2). These chimneys displayed lengths ranging between 50 and 100 cm and maximum diameters between 10 and 20 cm, and some of them were colonized by large sponges. CRB mostly composed of Madrepora oculata remains were usually found on the northern flank of the MV (16.8%), a steep area with steep slopes and high BPI values (Fig. 2). Finally, MXB (16 %) were widespread throughout the study area (Fig. 2).

3.2. Epibenthic assemblages and habitats

A total of 51 taxa (42 epibenthic taxa and 9 fishes) were identified in the samples (Table 1) including the actinarians *Actinauge richardi* and *Peachia* sp., the scleractinians *Flabellum chunii*, *Dendrophyllia cornigera*, *Madrepora oculata* and *Lophelia pertusa*, the gorgonians *Acanthogorgia hirsuta* and *Callogorgia verticillata*, the antipatharians *Antipathella subpinnata* and *Leiopathes glaberrima*, a styelid ascidian (probably belonging to the genus *Polycarpa*), the poriferan *Asconema setubalense* (hexactinellid) and the echinoderm *Cidaris cidaris* (echinoid). Small gorgonians (*Bebryce* group) were mostly represented by *Bebryce mollis* and *Swiftia dubia*; large sponges included *Poecillastra compressa*, *Petrosia crassa*,

Phakellia robusta and Pachastrella sp.; Axinella vellerea was among the small and digitate sponges; encrusting sponges would include Jaspis sp. (likely J. incrustans) whereas alcyonaceans were mainly represented by Paralcyonium spinulosum; finally, Polyplumaria flabellata was one of the most frequently observed hydrozoans.

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The best represented groups characterizing epibenthic assemblages forming habitats were cnidarians (17 taxa) and echinoderms (12 taxa) (Table 1) as well as sponges, but in the latter case they were aggregated into groups of morphologically similar species. Large sponges and the echinoid *C. cidaris*, followed by the gorgonian *A. hirsuta* and the scleractinians *F. chunii* and *M. oculata*, were the most frequent habitat-typifying species observed in the study area (Table 1). Finally, a total of 9 ichthyofauna taxa were also identified, with the blackbelly rosefish *Helicolenus dactylopterus* being the most representative fish species identified in Gazul MV and surrounding areas and observed mostly on MXB, HB and CRB.

CLUSTER analyses split the samples into two main clusters at a level of 95.93% of dissimilarity (Fig. 3A) and in a similar way of Fig. 2E. Bottom type seems to have a role in the clustering, since the first cluster grouped samples mainly located on soft bottoms (corresponding to soft bottom assemblages), whereas the second cluster was characterized by samples mainly located on hard and mixed bottoms (corresponding to hard and mixed bottom assemblages). The first cluster was further divided into three groups: Group IA (57% similarity within the group) with samples collected on SRB, group IB (50% similarity) comprised mainly samples of CSBB but also of SB, and group IC (40% similarity) grouped mainly samples of CSBB but also of MXB (Fig. 3A). The second cluster grouped samples of MXB and hard bottoms that included rocky and CRB, which formed one large group clustering at 24% similarity that was divided into group IIA (36% similarity) with samples mainly located on bottoms with MDACs but also on MXB, and group IIB (37% similarity) comprising samples mostly located on CRB, and one small group (IIC) that corresponded to samples mostly located on MXB (Fig. 3A). Assemblages obtained by means of CLUSTER analysis are also consistent in the twodimensional MDS plot. This revealed a good data fit in the ordination of all samples since raw stress (a measure of the goodness-of-fit of the representation of the true similarity matrix values by the ordination according to Clarke (1993)) was 0.08, with a segregation of samples according to faunal composition and structure, which also responded to bottom type (Fig. 3B). Table 2 shows faunal differences between clusters (interpreted as different assemblages conforming habitats) highlighted by the SIMPER analysis. This analysis revealed that differences between assemblages on soft and hard-mixed bottoms were mostly due to the

presence and/or higher abundance of *F. chunii*, *A. richardi*, *C. cidaris* and *Peachia* sp. on soft bottoms, and of large and small-digitate sponges, *A. hirsuta*, *M. oculata*, hydrozoans and *A. setubalense* on hard-mixed bottoms (Average dissimilarity= 94.99%).

 Differences in the composition and structure of faunal assemblages between bottom types and sites were detected by the PERMANOVA analysis (Table 3). Pairwise comparisons revealed that differences were consistently significant among all bottom types (p< 0.001, for all cases) and sites (p< 0.001, for all cases). The largest differences among bottom types were detected between assemblages of CRB and those of SRB (Pairwise test: t= 20.802; Average dissimilarity: 1.79%), and among sites, between assemblages at the northern flank of the MV and those located at the western adjacent area (Pairwise test: t= 13.578; Average dissimilarity: 94.53%). Overall, soft bottoms presented a significantly lower number of taxa than hard and mixed bottoms (Mann-Whitney: U= 6725.5; p< 0.001), with the lowest value observed in SB (12 taxa) and the highest one in MXB (32 taxa); on the other hand, CRB displayed the highest mean number of taxa per sample (5.3±0.2 taxa; mean±SE), whereas the lowest values was observed in CSBB (1.5±01 taxa).

PERMANOVA also showed, for some assemblages, significant differences regarding different bottom types and/or sites. For instance, hard bottom assemblage IIB (corresponding to CRB with colonial scleractinians, see below) displayed a significantly different structure considering both factors, being more distinct for site than for bottom-type (PERMANOVA: factor bottom type, Pseudo-F: 6.186, p < 0.001; factor site, Pseudo-F: 18.641, p < 0.001). In this case, pairwise comparisons after PERMANOVA revealed that the largest average dissimilarity was detected between the MV summit and the northern flank (SIMPER: 63%) due to higher abundances of A. subpinnata, L. glaberrima, M. oculata and large sponges in the northern flank. In the case of the widely distributed soft bottom assemblage IB (corresponding to a F. chunii assemblage, see below), differences were only significant for site (PERMANOVA: factor bottom type, Pseudo-F: 0.634, p = 0.499; factor site, Pseudo-F: 15.626, p < 0.001), with the highest average dissimilarity (SIMPER: 82.37%) observed between the western depression and the southern flank of the MV due to the higher abundance of F. chunii and the presence of C. cidaris in the western depression, and the presence of Peachia sp. in the southern flank.

Regarding multivariate analyses based on both biological and seafloor characteristics, six different assemblages were detected (Fig. 3), with three main soft bottom assemblages identified as:

i) Soft bottoms dominated by the actiniarian *Actinauge richardi* (group IA): this assemblage was found on SRB of the western adjacent area, where it was observed in 85 samples (17.6 %F). It is characterized by *A. richardi* (semi-quantitative index [SQI] values: 2-4), which reached densities up to 25 individuals per square meter (indiv. m⁻²) (Fig. 4A-C); secondary characteristic species included *Peachia*-like actinarians (SQI values: 1-2), which were observed in high densities in some samples, the echinoid *C. cidaris* (SQI values: 1-2) and isolated individuals of the solitary scleractinian *F. chunii*.

- ii) Soft bottoms dominated by the scleractinian *Flabellum chunii* (group IB): this assemblage was mainly found on CSBB, being recorded in 128 samples (25.3 %F), and at a lower extent on SMB. This is the most frequent assemblage found on soft bottoms of Gazul MV and surrounding areas (50.2 %F). Its characteristic species is the solitary coral *F. chunii* (Fig. 4D-G), which showed densities between 1 and 3 indiv. m⁻² (SQI values: 2-3). Nevertheless, a highly dense aggregation was observed at the western depression, with densities up to 10 indiv. m⁻² (SQI values: 4-5) (Fig. 4F). Characteristic species include *Peachia*-like actinarians, which were observed on SMB of the central depression, and were locally very abundant (SQI values: 3) (up to 4 indiv.·m⁻²), with *C. cidaris* mainly occurring at the western depression (SQI values: 1-2), and with isolated individuals of the polychaete *Hyalinoecia tubicola* (Fig. 4H).
 - iii) Soft bottoms dominated by the echinoid *Cidaris cidaris* (group IC): the sea urchin *C. cidaris* was widely distributed (SQI values: 1-2), mainly observed on soft bottoms (52.7%), but also on hard (27.3%) and mixed (20%) ones. It was found to be a locally dominant species on several soft bottoms of the study area, characterizing this assemblage in 7.5% of the samples. Density of *C. cidaris* did not usually exceed 2 indiv. m⁻² (Fig. 4I-J), and it was accompanied by *F. chunii* in CSBB, isolated pennatulaceans (e.g. *Funiculina quadrangularis*) in SMB, and by the decapod *Munida intermedia* in MXB.
 - Three main assemblages were identified on CRB and on hard bottoms (Fig. 3), mainly conformed by MDACs and sometimes intermixed with soft bottoms:
 - iv) Hard and mixed bottoms dominated by sponges and gorgonians aggregations (group IIA): this assemblage included (1) aggregations of sponges, which were observed colonizing MXB and hard substrates in 84 samples (16.6 %F). Characteristic species, such as the demospongians *Phakellia* spp. and *Poecillastra compressa* among others, constitute the large sponges group (SQI values: 2-3) (Fig. 5A), recorded in 127 samples and reaching densities up to 12 colonies per square meter (col. m⁻²), together with large individuals of the hexactinellid *Asconema setubalense* (SQI values: 2-3) (40 samples; Fig. 5C), and encrusting and digitate

sponges (SQI values: 2) (22 samples; up to 23 col. m⁻²). The (2) gorgonian aggregations (<1 %F) 455 456 were observed on MXB and dominated mainly by Acanthogorgia hirsuta (SQI values: 2) (up to 15 col. m⁻²; Fig. 5D) and the *Bebryce*-group (SQI values: 1), which were sometimes 457 458 accompanied by other gorgonians such as the large Callogorgia verticillata (Fig. 5E), as well as 459 by isolated large sponges and C. cidaris. The (3) mixed sponge-gorgonian aggregations were observed in 79 samples (15.6 %F), mainly on hard bottoms of the mounds located in the 460 eastern adjacent area. A mixed and dense aggregation of A. setubalense (up to 4 col. m⁻²) and 461 A. hirsuta (up to 10 col. m⁻²) was found in those mounds. Sponge aggregations and mixed 462 463 sponge-gorgonian aggregations also included other typifying species such as C. verticillata (SQI 464 values: 1), small gorgonians (mostly Bebryce mollis and Swiftia dubia; Fig. 5B) (SQI values: 2-3), 465 different echinoderms such asteroids (e.g. Chaetaster longipes), crinoids (e.g. Leptometra 466 phalangium) and echinoids (e.g. C. cidaris, Gracilechinus acutus) (SQI values: 1, respectively), 467 the decapod M. intermedia (SQI values: 2) and the octopus Eledone cirrhosa (SQI values: 1). 468 v) Coral-rubble bottoms dominated by colonial scleractinians (group IIB): this assemblage is 469 associated with CRB occurring at the northern flank and close to the summit of Gazul MV. It is 470 characterized by the white coral species Madrepora oculata (SQI values: 2-4) (96 samples; 471 density up to 10 col. m⁻²), Lophelia pertusa (SQI values: 1-2) (16 samples; up to 1 col. m⁻²) and Dendrophyllia cornigera (SQI values: 1-2) (14 samples; up to 3 col. m⁻²), representing 16.8% of 472 473 the samples (Fig 5F-H). In addition, other typical species include the antipatharians (black corals) Antipathella subpinnata (up to 6 col. m⁻²) and Leiopathes glaberrima (up to 4 col. m⁻²), 474 475 highly branched hydrozoans (e.g. Polyplumaria flabellata), large sponges (up to 6 ind. m⁻²) (SQI 476 values: 2-3, respectively), encrusting and small-digitate sponges (SQI values: 2-3), gorgonians 477 (e.g. A. hirsuta, B. mollis), as well as abundant bright-orange ophiurids (Ophiothrix), the 478 echinoids G. acutus, Echinus melo and C. cidaris, and the decapod M. intermedia (SQI values: 1-479 2, respectively). 480 vi) Mixed bottoms dominated by styelid ascidian aggregations (group IIC): this assemblage is 481 strongly associated with slabs located on bottoms with sand ripples of the western adjacent 482 area of the Gazul MV. This assemblage showed a low frequency of occurrence (<1 %F), and 483 was characterized by the presence of a dominant styelid ascidian (probably belonging to the genus *Polycarpa*) (SQI values: 4-5), which reached densities up to 50-70 ind. m⁻² (Fig. 5I-J). 484 485 These ascidians were accompanied by B. mollis colonies (SQI values: 2-3), crinoids, the 486 decapods M. intermedia and Bathynectes maravigna, yellowish encrusting sponges and large 487 colonies of the polychaete Filograna implexa (SQI values: 1-2, respectively). Ascidians were

western adjacent area, intermixed with aggregations of *A. hirsuta* (up to 15 col. m⁻²) and large white sponges (up to 3 ind. m⁻²).

3.3. Effects of seafloor variables on the epibenthic assemblages

The multivariate CCA ordination analysis of the characteristic species generated an ordination biplot with the first two axes explaining 16.4% of variance of the species data, and which accounted for 84.4% of the variation of the species and the seafloor variables relationship (Table 4). A Monte Carlo test indicates that all canonical axes together were highly significant (p < 0.001; 999 permutations under full model). Marginal effects (fit of the first CCA axis with each variable entered separately) determined by the forward selection procedure of CANOCO were highest for slope, depth and backscatter. Regarding conditional effects (additional fit as each variable is added to the model), the analysis highlighted slope, backscatter and BPI. Figure 6 shows the CCA biplot based on CCA axes 1 and 2, with slope and depth negatively correlated with axis 1, whereas axis 2 was positively correlated with backscatter (Table 4). Seafloor profile curvature made the lowest contribution to axis building, showing low discriminatory power. CCA sample discrimination and grouping were consistent with the groups obtained from the cluster and ordination analysis (Fig. 3).

3.4. Anthropogenic activity indicators at Gazul MV and surrounding areas

Bottom trawling represented the main type of commercial fishing technique in the area; however, it must be considered that the authors could not obtain information on recreational or artisanal fishing that could occasionally occur at Gazul MV. Bottom trawling effort (total number of hours of trawling per km² for the period 2006-2012) showed the highest values in the upper slope of the continental margin (depths ranging between 130-530 m). Trawling effort decreased in intensity between 400-500 m depth, with the lowest effort values observed around Gazul MV. No signs of bottom trawling were observed on the seabed. Effort increased towards the westernmost part of the SCI "Volcanes de fango del Golfo de Cádiz" (depths ranging between 500-600 m) and around Anastasya MV, where trawling activity is focused on the Norway lobster (Nephrops norvegicus) (Fig. 7).

A total of 28 marine litter items were recorded in the samples, including mostly artificial polymer materials (85.2 %), but also glass (14.8 %). Overall and regarding type of item, fishing gears represented the dominant source of debris (63.0 %) (Fig. 8A-F), including fishing lines and nets. Ropes were occasionally found, representing 3.7 % of the samples. Finally, plastic and glass were observed in 18.5 % and 14.8 % of the samples, mostly as bottles (Fig.

8G). The highest occurrence of marine litter, mostly fishing lines, was observed in the western adjacent area (42.9% of the total observed) followed by the western depression (25%) and the northern flank (14.3%).

More than half of marine litter items (61.5%) were observed in contact with sessile invertebrates, probably causing potential abrasion on large sponges, gorgonians and/or ascidians on mixed bottoms (56.2%) (Fig. 8A); on colonial scleractinians, black corals and large sponges on coral-rubble bottoms (25%) (Fig. 8B-C); and on *Actinauge* individuals on soft bottoms (18.8%). A small piece of net was observed snagged on a MDAC covering partly a *Madrepora* colony (Fig. 8F). The remaining portion of debris (plastic, bottles) was recorded lying on soft bottoms (60%) (Fig. 8G), and up to three lost fishing lines were observed hanging in hard substrates on mixed bottoms (40%), in both cases without producing any apparent injury to sessile organisms.

Discussion

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Substrate and habitat heterogeneity

Based on the new observations made in this study, Gazul is an example of a MV with latent/dormant conditions (León et al. 2007). Gazul MV presents a high availability of MDACs (e.g. tabular-shape chimneys, slab-shaped pavements and crusts) on the seafloor (Palomino et al., 2016; this study). MDACs were formed within the sediments during past seepage activity, and exhumed and exposed to seawater as a consequence of erosive processes by strong bottom currents, resulting in a high structural complexity of the seabed (Cunha et al., 2002; León et al., 2007; Magalhães et al., 2012; Viola et al., 2014; Sánchez-Leal et al., 2017). The chimney forest reported in this study for Gazul MV represents one of the very few examples of columnar carbonates fields in vertical position found in European waters (Angeletti et al., 2015), and to the best of our knowledge, it is the first reported for the GoC, which complements other observations on chimney fields of the GoC (Díaz-del-Rio et al., 2003; Somoza et al., 2003; Magalhães et al., 2012). The identified epibenthic assemblages forming habitats associated with soft and hard bottoms of Gazul MV and surrounding areas showed a patchy mosaic distribution with spatial differences, with hard bottoms of the MV edifice containing the most biodiverse assemblages. Several authors have observed that the existence of different habitat types in submarine elevations (e.g. seamounts) are promoted by the presence of diverse substrate types and

geomorphological structures occurring at different depths (McClain and Lundsten, 2015; Du

Preez et al., 2016; de la Torriente et al., 2018). In a similar way, the high geomorphological variability and diversity of bottom types of Gazul MV, as well as the different exposition to bottom currents (Palomino et al., 2016), promotes the existence of biodiverse and vulnerable faunal communities dominated by sessile slow growing suspension and filter feeding macroorganisms such as deep-water corals and sponges, which potentially benefit from moderatestrong bottom currents (Fernández-Salas et al., 2012) and from an enrichment process promoted by land-based runoff of important rivers such as the Guadalquivir (García-Isarch et al., 2006). On the other hand, the diversity of soft bottoms around Gazul MV promotes a colonization by a variety of species, some of them with a high commercial value (e.g. Norway lobster), enriching the associated benthic communities when compared to other soft bottoms within the GoC (Fernández-Zambrano, 2010; Lozano et al., 2020a). Each assemblage at Gazul was preferentially associated with a specific bottom type and a combination of seafloor characteristics, with slope, backscatter and depth as the most important factors determining the distribution of habitats and assemblages. These results are similar to the observations made by de la Torriente et al. (2018) when studying the distribution of epibenthic communities at the Seco de los Olivos Seamount (also known as Chella Bank) (western Mediterranean Sea), as well as by other authors at different submarine elevations and depth ranges (McClain and Lundsten, 2015; Bernardino et al., 2016; Du Preez et al., 2016). The presence of hard structures (e.g. MDACs) increase the habitat complexity of the MV edifice and those of the central and western depressions, when compared to the adjacent bottoms, representing a major factor influencing the distribution of the benthic communities (Cunha et al., 2002, 2009; Rueda et al., 2016). Similar patterns of spatial differentiation have been found in coral mounds and adjacent bottoms without colonial scleractinians that are generally composed by a lower number of species (Henry and Roberts, 2007), as well as in those communities of seamounts when compared to the impoverished adjacent bottoms (Danovaro et al., 2010). Bottom complexity further increases once MDACs on the seafloor are colonized by organisms usually presenting complex three-dimensional structures (e.g. colonial scleractinians, gorgonians, sponges) (Jensen et al., 1992; Díaz-del-Río et al., 2003; León et al., 2007; Buhl-Mortensen et al., 2010; Levin et al., 2015; Wienberg and Titschack, 2017), promoting a diversification of microhabitat types that are used by numerous species, enriching the associated faunal communities. These habitat-forming organisms are in some cases also responsible for generating the substrate, shelter and food for other benthic species (Buhl-Mortensen et al., 2010; Rossi et al., 2017), thus increasing the differences with the adjacent

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soft bottoms, as it has been observed in the present study. In this line, for instance Henry and Roberts (2007), as well as Buhl-Mortensen et al. (2016), proposed that coral communities offer high vertical habitat heterogeneity (living/dead coral, rubble, sediment-clogged framework, etc.) and thus high biodiversity, which foster a characteristic reef fauna. At Gazul MV, the species previously reported that may benefit from habitat-forming species included the polychaete Eunice norvegica (Linnaeus, 1767) (associated with the scleractinian Madrepora oculata), the gastropods Emarginula spp. and triphorids that feed on sponges, and solenogastres, epitonids and ovulids that are generally associated with gorgonians, actinaria and scleractinians of Gazul MV (Rueda et al., 2016; Utrilla et al., 2020).

Soft bottom assemblages

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- Three main epibenthic assemblages were identified in the soft bottoms of Gazul MV and surrounding areas, being characterized by deposit feeders such as solitary hard (Flabellum chunii) and soft cnidarians (Actinauge richardi), as well as by cidarid echinoids (Cidaris cidaris). Similar species were reported by Fernández-Zambrano (2010) as dominant ones in adjacent sedimentary habitats around Gazul MV from samples collected with a combination of gears (beam-trawl, box-core and shipek grab), mostly contributing to differences between faunal assemblages inhabiting adjacent bottoms in comparison to those of the MV.
- Trophic and oceanographic drivers control the spatial distribution of A. richardi populations, which were observed in the western adjacent area of Gazul MV, an area with strong bottom currents reflected by the presence of a sandy ripples field. This sea anemone is a passive suspension feeder that inhabits muddy or sandy bottoms, always offshore (BIOTIC, 2019). Hormathiid actinarians (Hexacorallia) such as A. richardi have been observed dominating faunal communities on circalittoral and bathyal sedimentary bottoms of the Cantabrian Sea (e.g. Avilés canyon), GoC and Mediterranean Sea (e.g. Llanes canyon) (Aguilar et al., 2009; Ruiz-Pico et al., 2010; Rueda et al., 2016; Lozano et al., 2020a), and represents a key taxa typifying deep-sea sedimentary assemblages.
- In contrast, the solitary scleractinian F. chunii presents a certain structuring character in sedimentary habitats, being locally abundant in specific soft bottoms of the GoC, as well as of the Galicia Bank (NW Spain) and of the Conception Bank (Canary Islands) (Almón et al., 2010; de la Torriente et al., 2014; Díaz-del-Río, 2014).
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 - Finally, the dominant echinoderm C. cidaris showed a patchy distribution in Gazul MV; it occurs mainly in soft bottoms, but also in hard and mixed bottoms. Similar observations were made by Delgado et al. (2013) in soft bottoms of the SFFE of the GoC from otter-trawl samples,

where *C. cidaris* represented one of the dominant species throughout the year, relating its patchy distribution to sediment characteristics.

Faunal assemblages inhabiting soft bottoms around MVs are not as biodiverse as those associated with MVs themselves due to the lower structural complexity of habitats (Fisher et al., 2007; Cunha et al., 2009; Levin et al., 2015; Rueda et al., 2016). Nevertheless, according to Delgado et al. (2013), soft bottoms of the MVGoC presents species richness values that are higher to those reported in northern Spain and the Alboran Sea at similar depths, and this could be related to the geographical location of the GoC, in the Atlantic-Mediterranean and subtropical-template transition (i.e. confluence of fauna). Indeed, the oceanographic context of the GoC is complex and characterized by a surficial Atlantic Inflow Water (AIW) current towards the Atlantic Ocean (Sánchez-Leal et al., 2017). These water masses may transport larvae and organisms from the Mediterranean and the Atlantic, promoting a biological confluence in the GoC that could result in high biodiversity in those areas with a high benthic complexity that can harbor species associated with different substrates as detected at the Gazul MV.

Hard bottom assemblages

Hard bottoms of Gazul MV showed a heterogeneous distribution, being represented by MDAC formations and by coral-rubble, in both cases increasing the complexity of soft bottoms and promoting a mixture of hard- and soft-bottoms fauna. The exhumation of MDACs are favored by moderate-strong bottom currents that at the same time promotes a continuous availability of food particles, which supports complex faunal communities mainly composed of filter and suspension feeders (Hovland, 2008). Live colonies of the white coral Madrepora oculata, intermixed with some dispersed colonies of other colonial scleractinians such as Dendrophyllia cornigera and the less frequent Lophelia pertusa, were observed associated with coral-rubble bottoms of the northern flank and close the summit of Gazul MV. This represents one of the few records of live colonies of these colonial scleractinians in the GoC (Rueda et al., 2016), which seems to represent an unsuitable area for these species after the past glacial periods (Wienberg et al., 2009; Van Rooij et al., 2011). Nevertheless, densities of M. oculata colonies in Gazul MV are occasionally as high as in other areas of the Mediterranean Sea (up to 10 colonies m⁻²) (Orejas et al., 2009). In several studies dealing with scleractinians of the GoC and adjacent areas, abundant coral graveyards related to massive declines after glacial periods have been found but no live colonies could be detected (Wienberg et al., 2009; Wienberg and Titschack, 2017; Hebbeln et al., 2019). Recently, carbonate mounds with abundant remains of

M. oculata have also been found in the Pen Duick Escarpment in the Moroccan margin, but no living colonies of this coral could also be detected (Van Rooij et al., 2011). In the Strait of Gibraltar, remains of this species were found between 33 and 443 m depth, especially in one particular area with mounds, but no clear indication was given on the presence of live colonies by the authors (Álvarez-Pérez et al., 2005). In the Mediterranean Sea, live scleractinian colonies (mainly of L. pertusa and M. oculata, but also Dendrophyllia spp. and Desmophyllum dianthus) have been found in specific areas such as the southern Adriatic Sea, Santa Maria di Leuca, the Corsica Channel and the Strait of Sicily in the central Mediterranean (Schembri et al., 2007; Vertino et al., 2010; Rossi et al., 2017; Angeletti et al., 2019; D'Onghia, 2019; Lo lacono et al., 2019; Rueda et al., 2019; Angeletti et al., 2020), and Cap de Creus canyon and some Alboran Sea seamounts and banks in the western Mediterranean (Orejas et al., 2009; Pardo et al., 2011; Gori et al., 2013; Lo lacono et al., 2014; de la Torriente et al., 2018; Aymà et al., 2019; Corbera et al., 2019; Puig and Gili, 2019; Rueda et al., 2019). Further deep-sea exploration with the use of non-invasive methods such as the ones used here (e.g. ROV, ROTV) will allow the discovery and documentation of new white coral sites, as well as to increase the information about their associated communities with the combination of sampling methods targetting endofauna, epifauna and demersal fauna.

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The widespread presence of colonial scleractinians and antipatharians in the northern flank and close to the summit of the MV is related to the characteristics of the substrate (i.e. the presence of coral-rubble bottoms, which gives them a preferable elevated position), as well as potentially to the enhanced availability of food. This could be linked to the high-speed flow promoted by the interaction between bottom currents and topography (Palomino et al., 2016), which benefit these and other organisms (White et al., 2005, Dorschel et al., 2007, Mienis et al., 2007, Orejas et al., 2009, Sánchez et al., 2014), as observed for other filter-feeding assemblages inhabiting similar mud volcanoes of the GoC (Fernández-Salas et al., 2012; Lozano et al., 2020a, b). On the other hand, although high reflectivity values may indicate the presence of hard substrates (e.g. coral-rubble) on the southern-southeastern flank of the MV (see Fig. 2), potential lateral sedimentation by dragging along the slope would cause the sediment to be deposited on this flank of the MV, resulting in the scarce occurrence of hard formations on the seafloor. A high sediment supply is necessary to guarantee the fast infilling of the coral framework, being entrapped within it and providing a stabilizing effect, but it also increases sediment accumulation (Wienberg and Titschack, 2017; Hebbeln et al., 2019). This is probably the cause of the scarce presence of colonial scleractinians on this flank of the MV, which is characterized by sandy and muddy bottoms colonized by solitary corals and cidaroid echinoids. Similar observations were made by Orejas et al. (2009) in the Cap de Creus canyon, linking the scarcity of colonial scleractinians on the northern flank of the canyon to a prevailing depositional regime with high sediment accumulation rates.

Besides colonial scleractinians, many suspension-feeding species are also favored by the environmental conditions at Gazul MV, including large deep-sea sponges (90% of the biomass collected with different sampling gears; see Rueda et al., 2016, Sitjà et al., 2019; e.g. Asconema setubalense, Pachastrella sp., Poecillastra compressa, Petrosia crassa), several species of gorgonians (e.g. Swiftia dubia, Bebryce mollis, Acanthogorgia hirsuta) and black corals (Antipathella subpinnata and Leiopathes glaberrima), among other macro-organisms. These species have been observed colonizing both exposed MDACs and those with moderate charges of fine sediments located in the MV and adjacent mounds. Regarding this, Gazul MV represents a conical edifice that reaches 100 meters above adjacent bottoms, which may induce turbulent hydrodynamics due to the interaction between the predominant northwestern wards current and the MV that provides organic particles and removes sediments that may smother sessile organisms as those abovementioned. Moreover, these organisms are probably benefited by productive local upwellings and mass and energy inputs from land-based runoff (e.g. Guadalquivir river) that occur in this area as also found along the Portuguese coasts of the GoC (García-Isarch et al., 2006; García-Lafuente and Ruiz, 2007; Echevarría et al., 2009). In addition, Vázquez et al. (2009) and Bruno et al. (2013) explained how large-amplitude internal waves generated on the lee side of Camarinal sill induce a suction of chlorophyll-rich water masses from the coastal margins (e.g. Cape Trafalgar, see Sala et al., 2018) toward the central zones of the channel (i.e. towards the pathway of the MOW).

Deep-sea sponges are one of the dominant taxa at Gazul MV, with some species capable of forming dense local aggregations, e.g. the sponges *A. setubalense*, *P. compressa* and *P. crassa*. This was also observed by Sitjà et al. (2019), who studied the sponge fauna of the whole MV field of the northern GoC from samples collected using a 2 m-wide beam trawl. These authors highlighted the presence of a 'micro-aggregation' of 71 individuals of the carnivorous sponge *Lycopodina hypogea* (Vacelet and Boury-Esnault, 1996) on a flattened carbonate boulder of 35 cm² collected from Gazul MV, this species being rarely found in such high densities. The sponge faunal list provided by Sitjà et al. (2019) for the MVGoC, with three sponge species previously documented in the Mediterranean Sea and recorded in the Atlantic Ocean for the first time (*Geodia anceps* (Vosmaer, 1894), *Coelosphaera (Histodermion) cryosi* (Boury-Esnault, Pansini and Uriz, 1994) and *Petrosia (Petrosia) raphida* (Boury-Esnault, Pansini and Uriz, 1994)), would support the idea that the natural export of Mediterranean deep-sea benthos by the

MOW might be more important than previously believed. Moreover, Sitjà et al. (under review) found that the deep-sea sponge fauna of the GoC showed closer affinity with that of the western Mediterranean than with the fauna of the northeastern Atlantic. According to the authors, this pattern clearly illustrates the importance of the MOW in transporting components of the Mediterranean deep-sea sponge communities towards the bathyal communities of the GoC.

Anthropogenic impact

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The most active commercial fishing fleet operating around Gazul MV is the trawling fleet, which represents a multi-species fishery spanning a wide geographic area, with the main target species including the Norway lobster and the deep-water rose shrimp, as well as other accompanying species such as the European hake (Merluccius merluccius (Linnaeus, 1758)) and the monkfish (Lophius spp.), among others (González-García et al., 2020). The greatest fishing effort throughout the historical dataset is located where the fishing grounds for the Norway lobster and for the deep-water rose shrimp overlap, matching spatially with the geographical location of these fishing grounds according to Ramos et al. (1996). Shallow areas of the upper continental shelf of the GoC are suitable for some commercial species that prefer muddy bottoms, such as in the case of the Norway lobster, concentrating a great part of the fishing activity carried out in the GoC. Contrary to this, the trawling activity around Gazul MV is low mostly due to the presence of MDACs and moderate and high near-bottom current speed that may promote the presence of coarser sediments that are not ideal for the Norway lobster and that may make the trawling operations very difficult (González-García et al., 2020). Another important commercial fishing fleet operating in the area, but with a low impact on benthic habitats, are represented by that using purse seine nets along the continental shelf down to 100 m depth and targeting mainly the European anchovy Engraulis encrasicolus (Linnaeus, 1758) and the European pilchard Sardina pilchardus (Walbaum, 1792) (Silva et al., 2012). Finally, commercial bottom longline fishing is carried out mostly in the Strait of Gibraltar area targeting mainly the blackspot seabream Pagellus bogaraveo (Brünnich, 1768) (Silva et al., 2012).

In this study, the occurrence of marine litter is mainly caused by fishing gears such as lost nylon long lines, representing more than half of all debris found at Gazul MV. Bottom longline fishing activity obtained from VMS datasets for the evaluation of the Marine Strategy Framework Directive shows that this fleet operates mostly off Trafalgar Cape, but also at some isolated locations of the northern Gulf between Cádiz and Matalascañas, in all cases on the

continental shelf (Silva et al., 2012). These areas are geographically distant from Gazul MV, and thus the longlines observed in the images would come from remote locations, being swept away by strong bottom currents in this area of the GoC. Another explanation could be that these lost longlines come from recreational fishing taking place around Gazul MV, where longlines get stuck in hard substrates. Boats engaged in recreational fishing are not required to carry any location system or to track their catches in fishing logbooks, so it is very difficult to track their fishing activity within the GoC. Similar observations have been made in other deepsea areas of southern Europe characterized by intense fishing activities, such as those in the Tyrrhenian Sea (NW Mediterranean Sea) (Bo et al., 2014; Angiolillo et al., 2015). Although the abundance of marine litter recorded at Gazul MV is relatively low, a nylon line takes up to six hundred years to decompose (Bollmann et al., 2010). Therefore, lost or abandoned fishing gears such as lines and nets can potentially have a high impact on benthic communities dominated by large and branched scleractinians, gorgonians, black corals and sponges, which are easily snagged, covered and/or abraded by derelict gears as they can move laterally across the seabed by the action of strong bottom currents (Ragnarsson et al., 2017). Besides this direct damage, injured specimens can suffer epibiotic colonization by opportunistic fastgrowing species (e.g. hydroids, or polychaetes) that can lead to the death of colony portions or even the entire colony (Mistri, 1994). In those areas heavily impacted by fishing and lost fishing lines, potential effects include the decrease of the coverage of habitat-forming species and lastly the diversity and abundance of associated invertebrates (Bo et al., 2014; Fabri et al., 2014), which could modify the structure and functioning of deep-sea ecosystems. Future adequate management actions to be developed within the Site of Community Importance "Volcanes de fango del Golfo de Cádiz" could take advantage of the results presented here, as they provide a quantitative assessment of marine litter and its distribution at Gazul MV, as well as a baseline for future monitoring efforts. Similarly, this information can be used as a first step for setting the targets of Descriptor 10 (marine litter) within the Marine Strategy Framework Directive. Further monitoring of potential recreational fishing activity should be implemented in the area together with trawling restrictions for the commercial trawling fleet.

Habitat & Biodiversity Conservation

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Gazul MV, as well as other fluid venting submarine structures of the GoC, represents a clear example of Habitat 1180 (Submarine structures made by leaking gases) under the Habitat Directive 92/43/EEC (European Commission DG Environment, 2013) considering its geological past and the presence of mud breccia and MDACs (Palomino et al., 2016). Regarding the composition and structure of the faunal assemblages identified in this and other more general

studies (Rueda et al., 2016; Lozano et al., 2020a), Gazul MV may also represent an example of Habitat 1170 (Reefs) due to the presence of large quantities of MDACs colonized by deep-sea sponges (e.g. A. setubalense, P. crasa, Phakellia spp., among others) with some colonial scleractinians (e.g. M. oculata), black corals (A. subpinnata, L. glaberrima) and gorgonian species (e.g. A. hirsuta, S. pallida, among others). Habitats found in Gazul MV are also similar to those included in other conventions such as OSPAR (Convention for the protection of the Marine Environment of the North-East Atlantic) under the names "Coral gardens" or "Deepsea sponge aggregations" (OSPAR Commission, 2008); furthermore, the VMEs documented in Gazul MV have been recently included in the VME database from ICES (https://www.ices.dk/marine-data/data-portals/Pages/vulnerable-marine-ecosystems.aspx). Gazul MV is located close to the coast at shallower depths than other MVs of the northern GoC, and is therefore one of the most vulnerable MVs in Spanish waters to human activities (Palomino et al., 2016). Bottom-trawl fisheries may have already caused significant impacts to benthic communities, as detected in the abundant remains of nets, ropes and wires documented in the underwater images. Taking into account the Marine Strategy Framework Directive (adopted in June 2008) that aims to more effectively protect the marine environment across Europe, bottom trawl fisheries should be banned in the vicinity of the Gazul MV as this ecosystem represents a small but locally significant area within the GoC with high ecological values due to the presence of several ecologically important VMEs (three species of reef framework-forming corals, coral gardens including solitary scleractinians, gorgonians and black corals, as well as deep-sea sponge aggregations) that seem to be rare in other areas of the GoC (Cunha et al., 2009; Rueda et al., 2016). Moreover, trawling activity in Gazul MV is already relatively low compared to that around other MVs and adjacent bottoms as documented in this study, and this surely has benefited the establishment and persistence of populations of very slow-growing and structurally complex organisms. In this sense, the potential socioeconomic impact to bottom trawling after closuring this area to fisheries is expected to be very low according to González-García et al. (2020). As commented previously, other fisheries operating in the northern GoC are concentrated on areas far from Gazul MV such as along the continental shelf (down to 100 m depth) (e.g. purse seine nets) and close to the Strait of Gibraltar (e.g. longlines), and also would not be compromised by closure of the Gazul area. Finally, some biological and ecological attributes of Gazul MV (e.g. presence of MDACs colonized by colonial scleractinians and antipatharians, sponges and black corals, among other fauna; high biodiversity, including endemism species, for some faunal groups such as molluscs

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(Utrilla et al. 2020) fit several criteria under the Convention on Biological Diversity for an

Ecologically or Biologically Significant marine Area (EBSA) (e.g. uniqueness or rarity; importance for threatened, endangered or declining species and/or habitats; vulnerability, fragility, sensitivity or slow recovery; biological diversity). In addition to Gazul MV, other areas of the GoC also have high diversity of benthic habitats including carbonate mounds, submarine canyons, channels and escarpments shaped by tectonic activity and erosion, MVs) (Díaz-del-Río et al., 2006; Medialdea et al., 2009; Palomino et al., 2016), which could contribute to the future potential nomination of a wider EBSA in the southern Iberian Peninsula that would include the connected areas of the Alboran Sea, the Strait of Gibraltar and the GoC.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

852 Authors Contributions

JU and JLR designed the structure of the manuscript. JU and DP coordinated and wrote the manuscript. All authors were substantially involved in the literature search and/or writing of the different sections of the manuscript, contributed to the final product in significant ways, and/or provided approval for publication. Oceanographic expeditions' chiefs were LMFS (CHICA-0412), YV (ISUNEPCA-0616) and CO (MEDWAVES).

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