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Deep-sea ROV video analysis used to characterize Vulnerable Marine Ecosystems at Gazul Mud Volcano (NE Atlantic)

Thesis submitted in partial fulfillment of the requirements for the Master degree
of Coastal Marine Biology and Ecology by:

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1. ABSTRACT

In today's marine research, a knowledge gap still remains in understanding deep-sea habitats and those animals that live in them. The occurrence and distribution of benthic organisms were investigated by assessing the Gazul Mud Volcano (GMV) ecosystems at ~ 400 - 500 m depth, in the Gulf of Cadiz (NE Atlantic, Spain). Scientific research still remains limited at this specific mud volcano, with regards to understanding the various types of it's deep-sea communities and quantifying the structuring species. Our study aims to fill these gaps and better understand the Vulnerable Marine Ecosystems (VMEs) located at Gazul. A total of 745 colonies / individuals were counted including 7 phyla and 46 Operational Taxonomic Units (OTUs) along a video transect recorded by a Remote Operated Vehicle (ROV). Important VME indicator organisms such as scleractinian CWCs *Madrepora oculata, Lophelia pertusa* (0.033 ind · m^{-2} combining both species)*,* the alcyonacean *Acanthogorgia* spp. (0.096 ind · m^{-2}) and hexactinellid sponge *Asconema setubalense* (0.020 ind · m^{-2} were observed at considerable densities. Certain commercially important species showed to be frequent (e.g. *E. cirrhosa and Scyliorhinus canicular*) and three different locations of fishing impacts were observed (e.g. trawling nets, fishing lines, debris). The distribution of CWCs and deep-sea sponges formed dense aggregations into four main clusters along the transect, which were dominated by different substrate and community composition. This study identifies different VMEs and/or VME indicator organisms at the GMV and provides detailed information that might help to implement future protection and conservation plans in the overlooked area.

2. INTRODUCTION

2.1 Vulnerable Marine Ecosystems (VMEs)

In the recent past, accessing the deep-sea has been a huge obstacle due to the absence of appropriate technology to sample and survey (Gage and Bett, 2007). Obtaining information and distribution patterns in deep-sea ecosystems remains a challenge due to the extreme conditions and locations inhabited by these organisms (Gage and Bett, 2007). Due to improvements within the last 10-15 years, in underwater technology as well as bottom-surveying techniques using autonomous underwater vehicles (AUV) and remotely operated vehicles (ROV), scientists nowadays have more opportunity to investigate accurate and quantitative studies on Vulnerable Marine Ecosystems (VMEs) in the deep (Gage and Bett., 2007; Corbera et al., 2019).

Vulnerable Marine Ecosystems are defined by FAO (Food and Agriculture Organization of the United Nations, 2015) as areas which, among others, may include unique features such as seamounts, hydrothermal vents, *etc*., that may be vulnerable to fishing impacts. These VME features promote the presence of highly diverse benthic habitats and communities (Rossi et al., 2017). Different VME habitats, comes with different VME indicator organisms and different vulnerabilites, defined as benthic taxa which indicate the (likely) occurrence of VMEs including species of corals and sponges (FAO, 2016). It is generally difficult to define exactly when an assemblage of organisms or a habitat should be considered a VME or simply a VME indicator organism. Some assemblages of key habitat-forming taxa such as CWCs and deep-sea sponges are also considered VMEs (FAO, 2015; International Council for the Exploration of the Sea (ICES), 2018) themselves and are of critical importance (Sweetman et al., 2017). For example, CWCs, such as *Madrepora oculata* and *Lophelia pertusa,* are responsible for building habitats that provide high structural heterogeneity and a complex mosaic of dwellings for multiple organisms and are therefore categorized as a VME (Henry and Roberts, 2007; Buhl-Mortensen et al., 2010, Chimienti et al., 2019). Both CWC and sponge communities provide important functions (Sweetman et al., 2017) for the ecosystem such as nutrient cycling (Thurber et al., 2014), protection (De la Torriente et al., 2018), enhancement of food availability (Davies et al, 2017; Guinan et al, 2009), carbon and nitrogen fixation *etc*. (Rossi et al., 2017). Beyond benthic invertebrates, VMEs also provide habitat and food for multiple fishes, many of commercial interest (D'Onghia 2019, D'Onghia et al, 2010; FAO, 2009; Buhl-Mortensen et al, 2010; Escobar and Johnson, 2011). Therefore, it is very urgent and important to study, protect and conserve these organisms and their habitats (Pham et al., 2015).

Cold-water corals may refer to both the order Scleratinia (stony, hard skeleton, colonial or solitary) and the organisms commonly known as gorgonians, which belong to the order Alcyonacea (colonial, stiff, branching skeleton; FAO, 2015), CWCs are also included in the Habitat Directive (Reef-1170, 92/43/ EU Environmental Law, 2017). In addition, coral gardens, deep-sea sponge aggregations and *Lophelia pertusa* reefs are all listed as threatened and/or declining species or habitats and are considered of interest for conservation by the OSPAR Commission (Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic) and other entities (e.g. Biocenosis of Deep-Sea Corals) (Escobar and Johsnson, 2011).

Some species or habitats may be more vulnerable than others, making it difficult to define precisely when an assemblage of organisms in an environment should be considered a VME (Andron et al., 2014). Furthermore, the habitats and organisms which form a VME could also be vulnerable to different human impacts in a number of ways (Sitjà et al., 2018, Rossi et al., 2017). Actions such as fishing (particularly trawling, long-line and bottom-nets), mining, dredging, *etc*., all pose a threat to the resilience of deep-sea VMEs (Sitjà et al., 2018, Rossi et al., 2017). A more efficient and precise way to recognize and identify VMEs is required in order to plan for protection and conservation measures, and to work on understanding the level of impact.

2.2 Factors controlling the occurrence and distribution of VMEs

The occurrence and distribution of VMEs and associated species depend on a number of factors; such as the type of substrate for settlement (Henry & Roberts, 2007; Buhl-Mortensen et al, 2010), a highly dynamic environment (Willig et al., 2003) which provides enough food supply (Levin et al, 2001) for the growth of CWCs and sponges, *etc.* Some studies suggest that CWC occurence among VMEs are correlated with a specific substrate type and geomorphology of the bottom that is characterized by strong slopes and irregularities (Guinan et al, 2009). Other studies in the NE Atlantic (Porcupine Seabight, Le Danois Bank seamount, Aviles Canyon and the Galicia Bank seamount) state that water masses and associated physical parameters (e.g. temperature and salinity; De Mol et al, 2005; Van Rooij et al, 2010; Sánchez et al, 2014) can positively influence VMEs occurrence, for both sponges (Sitjà et al., 2014) and CWCs (Dullo et al., 2008). However, more investigation is needed on understanding how water mass characteristics control the occurrence and distribution of benthic fauna, specifically on deep-sea features.

2.3 The Gazul Mud Volcano (Gulf of Cádiz, Spain). A seafloor feature that harbours VMEs

Among all the different types of VMEs, underwater gas seepage and fluid flows on the sea bottom are too included (FAO, 2019). The Gulf of Cádiz (GoC) is located off Spain's southern continental margin, and is known for the presence of one of the most extensive gas seepage areas in the NE Atlantic (Medialdea et al., 2009; Vanreusel et al., 2009; León et al., 2007), which originated several large sea bottom features called mud volcanoes (MV). Mud volcanoes are vents formed in the earth's surface with escaping gas and vapour causing mud to boil and overflow creating a conical shape mound around the vent (León et al., 2012). The GoC houses more than 50 MV, all of various size and shape (Rueda et al., 2016). These features often present unique characteristics, such as high current speeds and a high rate of nutrient transport (Palanques et al, 2006; Canals et al, 2009) and harbour VMEs or VME indicator organisms, like CWCs and deep-sea sponges (Gardner, 2001; Pinheiro et al, 2003; León et al, 2007; Medialdea et al, 2009; Palomino et al, 2016). The physical environment of MVs, specifically in this study, help to provide high food availability to benthic suspension feeders like CWCs and sponges (Palanques et al, 2006). Due to the particular location of the GoC, near the Strait of Gibraltar, in the Mediterranean - Atlantic transitional area, where water mass exchange take place (Rueda et al., 2016; Sitjà et al., 2018), this area is of particular importance for biodiversity and connectivity.

The Gazul Mud Volcano (GMV) is the shallowest MV ever explored in the GoC (Rueda et al., 2016). Major components at GMV are the varied substratum on the bottom, from soft to hard sediment and the many unique formations. These unique structures, known as hydrogen-derived authigenic carbonates (HDACs), provide a source of new hard substrate for the colonization of deep-sea fauna (Léon et al., 2007). These features may be in forms of cylindrical chimneys, slabs or crusts and attract demersal fauna and increase overall benthic biodiversity (León et al, 2007; Rueda et al, 2012; Palomino et al, 2016). Amongst GMV, some VMEs have been observed; CWCs and sponge communities have been found colonized on various substrate types and mainly existing in small patches of reef-building corals (Rueda et al, 2016). Regarding protectiong and conservation of VMEs at GMV, studies state that trawling fisheries in the vicinity of GMV may negatively impact the benthic communities and the associated fauna (Rueda et al., 2016). Furthermore, additional knowledge focused on the effects of environmental factors like hydrodynamic conditions would be beneficial for eventual research and planning of VMEs in the GoC (Álvarez-Pérez et al. 2005; Wienberg et al. 2009; Corbera et al. 2019). It is clear we need to improve our understanding on what environmental and human factors are driving differences in distribution of VMEs, by improving our knowledge in quantitative data and comprehensively understanding these ecosystems. Such information is essential for more effective protection plans in the future.

2.4 Aims and objectives

In the present study, we had two main objectives; (1) to characterize the composition of deep-sea megabenthic communities at GMV, (2) to try to understand distribution patterns, and the influence of different environmental factors in the occurrence, density and distribution of the structuring benthic species. We hypothesized that GMV acts as a host to different VMEs with varying biodiversity due to associated factors such as substrate type. More specifically, we aim to (1.1) characterize the habitats, communities and taxonomic composition. (1.2) Provide for the first time, values of densities for the most relevant and abundant taxa and (2.1) describe the different VME communities, understand influential factors (e.g. substrate) as well as the conservation status and the observed impacts (e.g. fishing). Our results will help to improve the knowledge on deep-sea VMEs and will help to inform stakeholders in the development of management plans and conservation actions.

3. MATERIALS AND METHODS

3.1 Research area

The study area is located in the Spanish waters of the GoC (NE Atlantic Ocean). Gazul Mud Volcano is located about 33 nm away from Cadiz, Spain at 36°24'N and 6°56'W (Fig 1; Rueda et al, 2016). Gazul Mud Volcano presents a South – Southwest / North – Northeast orientation with depths ranging between 393 and 475 m (Rueda et al, 2016). The GMV has an oval shaped morphology (Fig. 2), with an approximate diameter of 1 km (Santana et al., 2018).

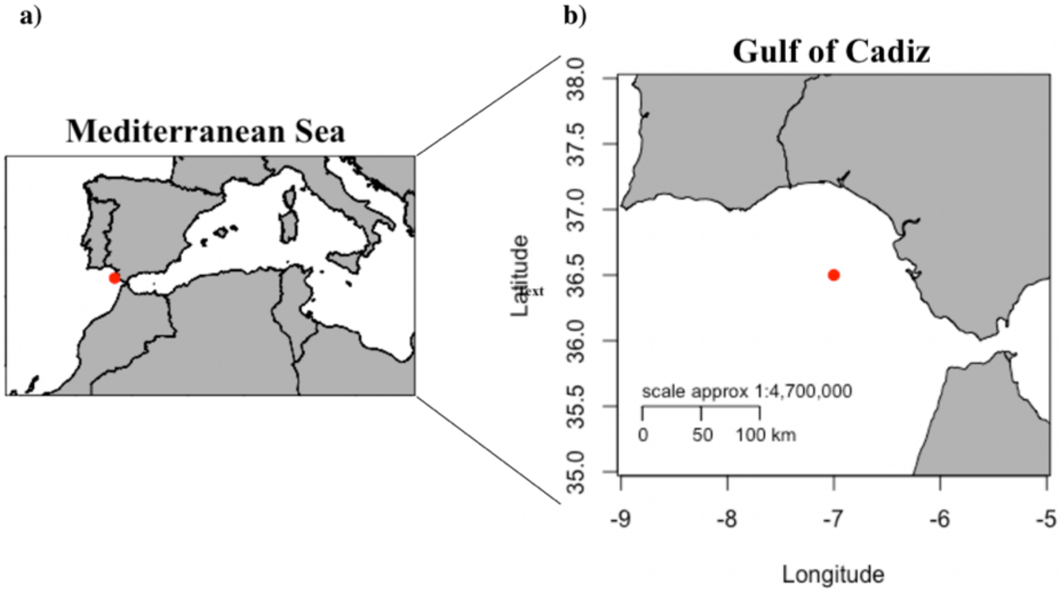

*Figure 1. Map showing the study area and location of the Gazul Mud Volcano (red dot) in **a)** the Mediterannean Sea and in, **b)** the Gulf of Cadiz.*

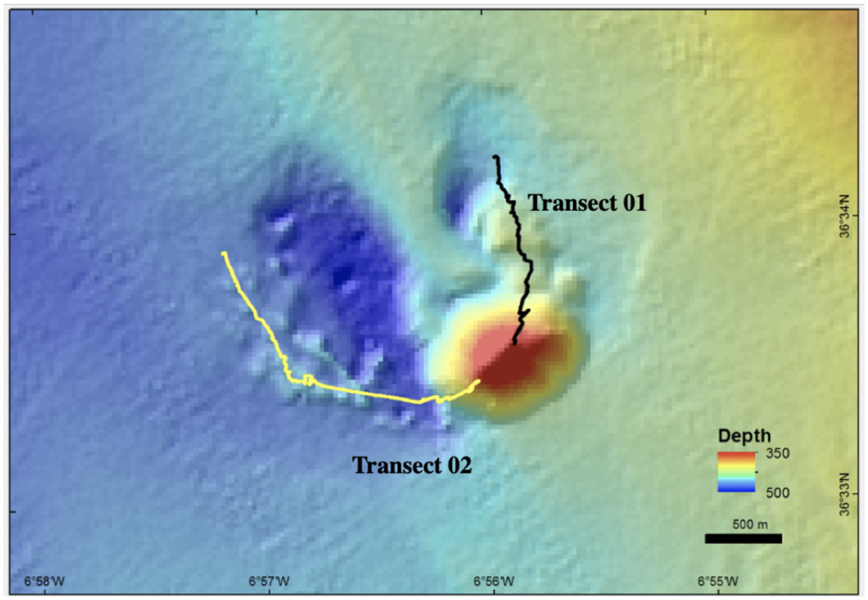

__Figure 2__. Bathymetry map showing the two video transects performed with a Remotely Operated Vehicle at Gazul Mud Volcano during the MEDWAVES cruise (Source: Jesus Rivera, ATLAS project).

Moreover, the region of GoC is oceanographically complex, as it is highly influenced by two different water masses (Fig. 3; Sánchez-Leal et al., 2017). A two-layer flow covers the GMV with both the Mediterranean Outflow Water (MOW) mass in a dense bottom-current and the North Atlantic Central Water (NACW) mass in the upper water column (Sánchez-Leal et al., 2017).

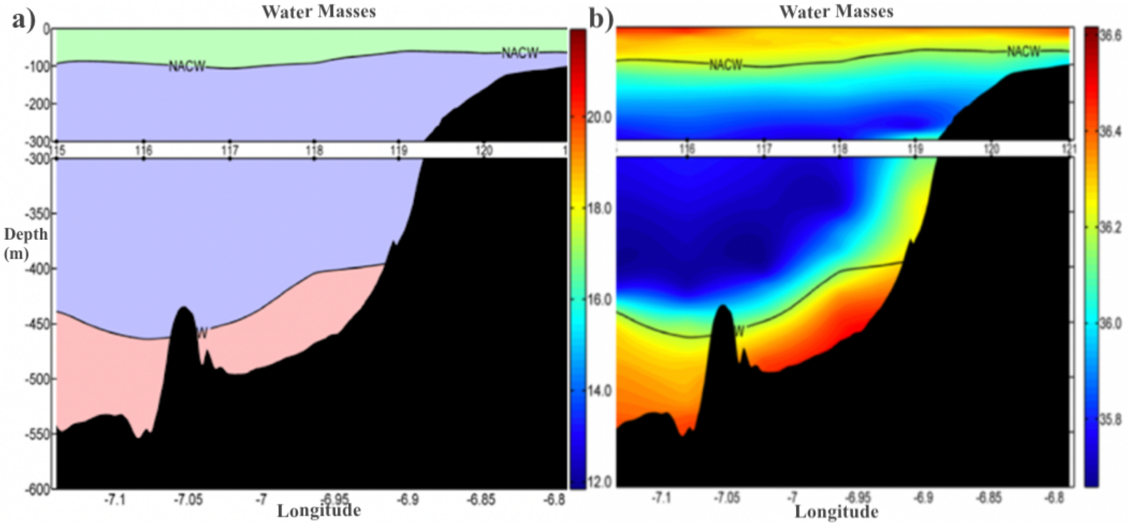

__Figure 3__.The two main water masses at Gazul Mud Volcano and their associated conditions; __a)__ Water masses: North Atlantic Cold Water (NACW) in purple and Mediterranean Outflow Water (MOW) in pink, also surface waters in green, plotted with depth (m), and __b)__ Water masses NACW and MOW plotted with temperature (ºC; left colour gradient) and salinity (psu; right colour gradient). (Source: Angela Mosquera, MEDWAVES-IEO, ATLAS project).

This outflow results in an impressive channel system (Sánchez-Leal et al., 2017) surrounding the GMV and develops a unique benthic environment on the Spanish continental margin (Rueda et al., 2012). Due to the enhancement of sediment accumulation from the double layer flow, the afore mentioned HDAC features are continuously produced on the seabed floor (Fig 4; Rueda et al., 2012). The leading factor influencing the rate of sediment accumulation is; MOW circulation, which greatly contributes to the transport and deposition of sediments, to seabed erosion and to the formation of seabed structures (Rueda et al., 2012). Overall, such geomorphological alterations due to high current speeds at GMV significantly influences the presence and occurrence of various types of deep-sea habitats and VMEs (Hernández-Molina et al. 2016, Pinheiro et al. 2003, Rueda et al. 2016).

3.2 Sampling

The study was conducted during the MEDWAVES cruise (Orejas et al., 2017) as part of the H2020 European project ATLAS (*A Trans-Atlantic assessment and Deep-Water Ecosystem-Based Spatial Management Plan for Europe*; https://www.eu-atlas.org/).

Figure 4. *Hydrogen-Derived Authigenic Carbonates (HDCA) in form of mud chimneys found on the muddy slopes of Gazul Mud Volcano at approximately 460 m depth (Source: MEDWAVES, ATLAS project).*

The cruise took place between September 22nd to October 26th, 2016 on the Spanish research vessel 'Sarmiento de Gamboa'. Sampling was done by conducting video transects with the use of the ROV 'Liropus' (Super Mohawk from Sub Atlantic). The previously acquired multi-beam bathymetry from the study area (Luis Miguel Fernández Salas, IEO, project LIFE+ INDEMARES CHICA) was used to select the locations to conduct the ROV video transects (Fig. 2). The ROV video transects were chosen as a sampling technique to gather detailed quantitative data on the spatial distribution and density of the organisms at a small spatial scale, as well as to reduce damage to organisms and minimize invasiveness. The ROV collected data by moving parallel along the seabed floor while recording both photographic and video footage. The ROV was equipped with still and motion cameras (*Kongsberg*), as well as navigational devices used for different purposes (Orejas et al., 2017). An HD video-camera was used for obtaining high quality images to use for video analysis and the identification of Operational Taxonomic Units (OTUs; which include species, genus or other taxonomic levels and morphotypes). A flash and Sealite Spheres were also attached for illumination requirements as complete darkness in the deep-sea dominates (Orejas et al., 2017). Laser beams spaced 10 cm apart were used as a scale to determine sampling area. The lasers were located in the middle of the video recording scope which made it useful for comparison and measurement purposes. Positioning of the deep-sea submersible on the seafloor was obtained using HYPACK software. The ROV was also equipped with a CTD sensor (conductivity, temperature, pressure) that gathered temperature and salinity data of the water masses bathing the benthic communities at every second of ROV video recording. Two independent ROV video transects were completed at GMV (Fig. 2), although the present investigation focuses solely on only one of the two video transects. Our studied video transect 01 was linearly oriented North to South, ending at the middle of the summit of the GMV, lasting approximately 1.5 km in length, starting at its deepest point (457 m) becoming shallower towards the end of the video transect and towards the summit (393 m) of the MV.

3.3 Video analysis

The analysis of the video transect was carried out with the OFOP software (Ocean Floor Observation Protocol). Video footage was analyzed in three steps; (1) to determine the useful footage for quantitative data analysis, (2) to characterize the substrate, and (3) to identify and count densities of the different organisms (e.g. OTU) and of other features (e.g. fishing remains, mud chimneys, *etc.*; Gori et al., 2011).

1) To ensure standardization of the obtained data, terms were given to each data set and to each type of sequence observed. The term *quantitative data* refers only to the sequences of the footage where the ROV navigated in a linear transect. The term *linear transect* refers to only the useful sequences from the recorded footage that were inside the 1 m sampling frame. These include sequences with 1) good visibility, 2) consistent distance to the bottom, 3) straight and linear movement of the ROV, and 4) laser beams turned on. Lastly, if the data was not part of the linear transect, and was annotated outside of the 1m sampling frame, the observations were placed in another data set and was given the term *out-sampling frame data*. Overall, the term *video transect* refers to all of the footage recorded from the ROV.

After categorization of different video sequences, the subsequent characterization of (2) the substrate type, and (3) the benthic community composition was analyzed in the linear transect sequences only. Annotations on either substrate or OTUs were made along the horizontal laser pointer field, within a 1.0 m sampling frame (Fig. 5). The selection of the sampling width was aimed to increase accuracy of identification as it was sometimes difficult to identify towards the edges of the frame (Fig. 5).

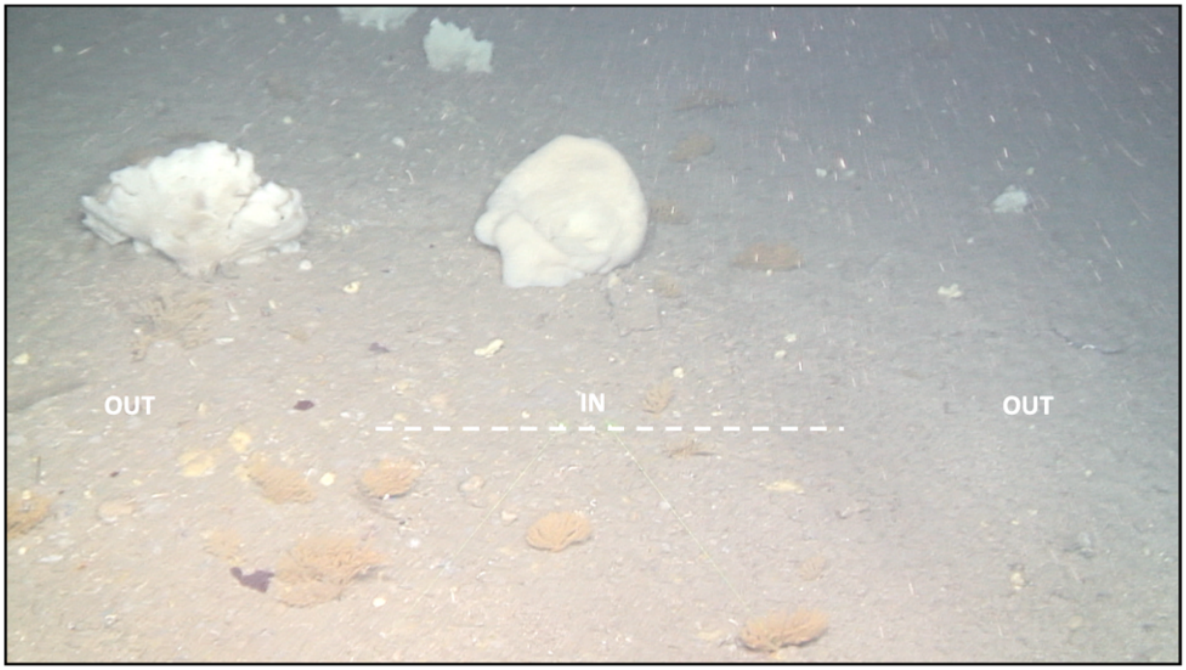

Figure 5. *Framework of the video processing method. IN represents the one meter sampling frame (within the dash line) used to characterize the substrate and count the Operational Taxonomic Units used for quantitative analysis. OUT represents the outside of the sampling frame where any data collected was used for Out-Sampling Frame Data analysis only.*

2) The characterization of substrate type was made based on previous studies (Van den Beld et al., 2017; Santana Bernaldo de Quirós, 2018). Substrates were categorized as either mud, sand, detritic, soft flagstone, hard flagstone or rock (Figure 6). Mud (Fig. 6a) was characterized by its bare and soft appearance, and by the presence of holes made by animals. Sand (Fig. 6b) frequently formed distinctive ripples and lines embedded in the seafloor. When the ROV hit sand off the seabed, it was more visible to see smaller, separated particles and its rate to descend during suspension was much faster than mud. The detritic sediment (Fig. 6c) was determined by the appearance of sand with thicker grains and particles, as well as coral rubble and carbonate debris. Hydroden-Derived Authigenic Carbonate features such as soft and hard flagstone were identified by the layer of compact sediment in different degrees.

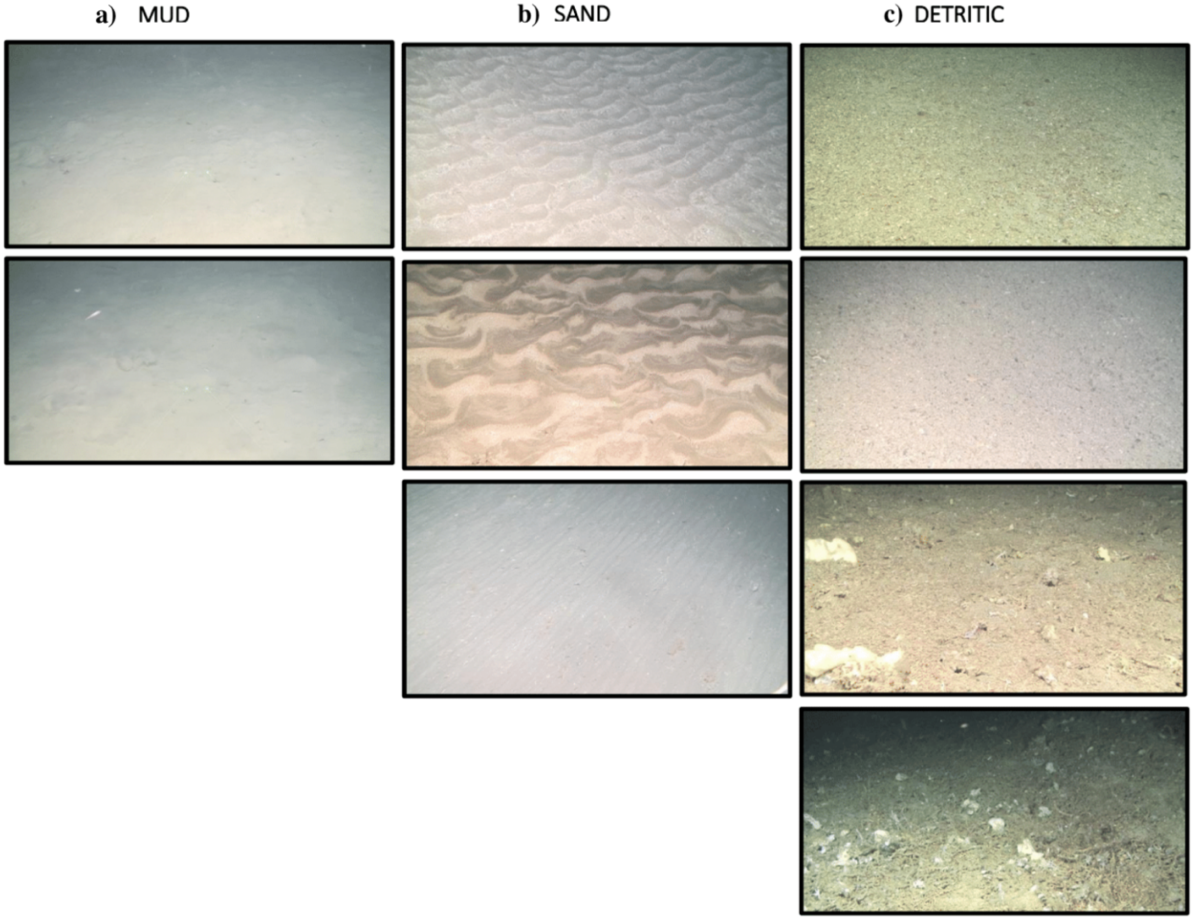

Figure 6. *Classification of the different substrate types used in Gazul Mud Volcano. The images encompass different substrates recorded during the MEDWAVES survey (Source: MEDWAVES, ATLAS project).*

The major difference was that soft flagstone (Fig. 6d) contained a smaller and thinner crust of compact sediment, whereas hard flagstone (Fig. 6e) contained a thicker, platform of compact sediment with cracks and slabs. Rock (Fig. 6e) was characterized by its large, boulder morphology and occasionally, a thin layer of sediment might cover the rock surface. Sometimes the presence of specific organisms helped in determining that a particular substrate was present, as different taxa require different substrate types. A maximum of two different substrate types were annotated at any location within the sampling frame, as either primary or secondary. When two substrates co-occurred, the primary substrate was assumed to cover > 60% of the seafloor, while the rest covered by 40% of the secondary substrate type.

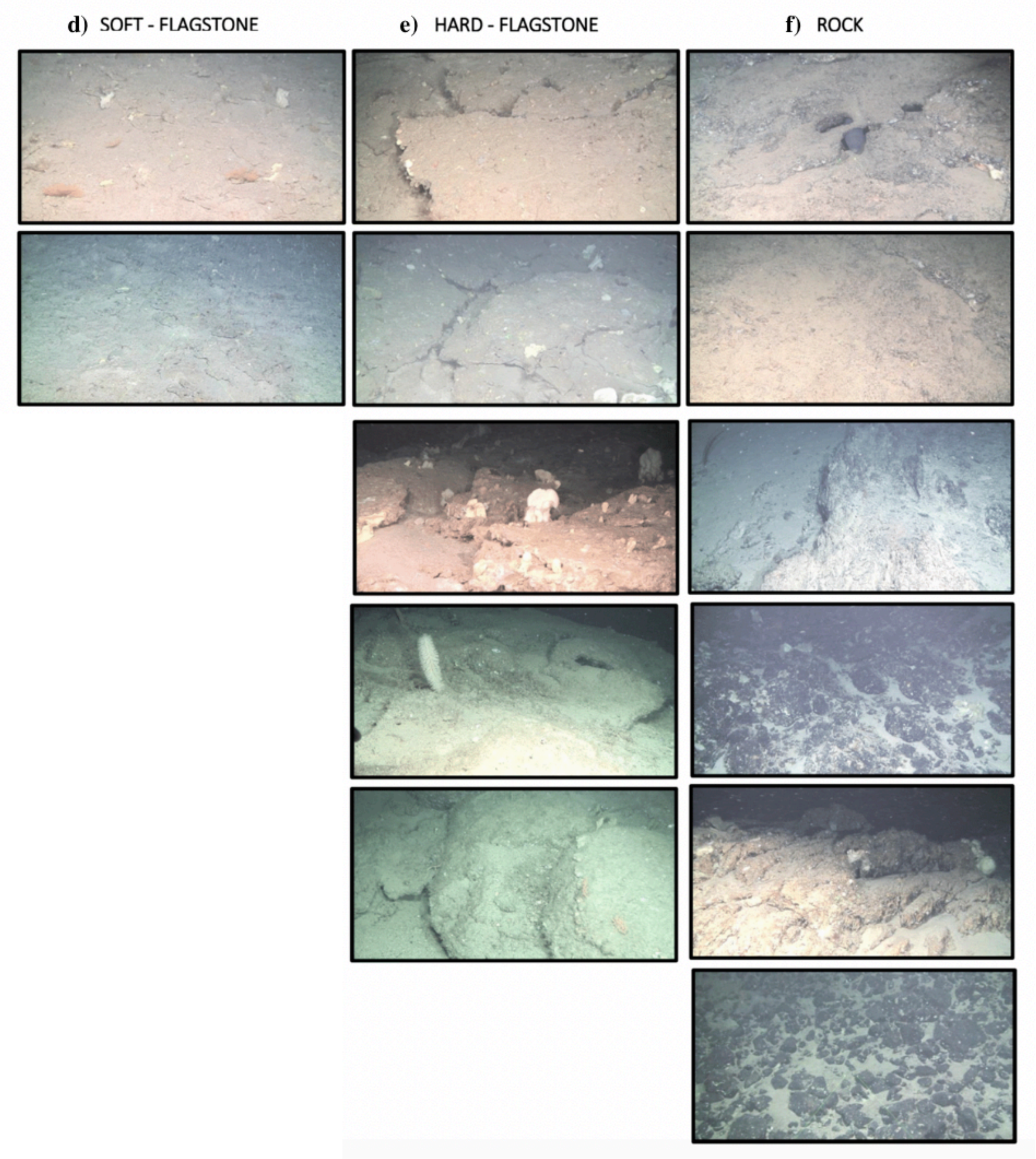

Figure 6. (continue) *Classification of the different substrate types used in Gazul Mud Volcano. The images encompass different substrates recorded during the MEDWAVES survey (Source: MEDWAVES, ATLAS project).*

3) Prior to the processing of linear transect sequences for quantitative analyses of the fauna, we identified and compiled a list of all observed OTUs (Annex 1). Organisms were identified to the lowest possible taxonomic level or given a general morphotype within a group or class of organisms, forming the OTUs . Further, in the specific case of Porifera, the taxonomical classification using solely images can be very difficult and in many cases, impossible. In those and other similar cases, assistance on taxonomic identification was required and obtained from literature, online research, colleagues and experts in the field. When morphotype names were given, general descriptions were used; for example two different reef-building species: *M. oculata* and *L. pertusa,* recorded at GMV were categorized together in an OTU termed 'White Corals' (Annex 3), due to differentiation difficulty, since colonies of both species commonly intermingled.

Only organisms larger than 5cm were counted within the 1 m sampling frame (Fig. 5) for quantitative data analysis. A 5 cm minimum limit was used to reduce any bias from visual error, such as missing records or misidentification of any OTU. Any footage that did not meet the requirements to be included in the linear transect was termed *non-useful* and was not used in the data analysis. Many sequences did not meet the criterion due to poor image quality or navigational purposes, such as; sampling errors, stopped at the bottom for photography, bad visibility, erratic movement, moving side to side, or laser-beams off.

3.4 Fishing activity in the area

The only sign of anthropogenic impacts documented in the video transect derived from human sources. Impacts were observed and thus counted in the analyzed video transect at GMV. The presence of fishing activities and other debris such as man-made trash, rests of fishing nets, plastics, lines, debris, etc. were recorded, along with location and depth.

4. RESULTS

4.1 Selection of useful video footage for quantitative analyses

The total amount of linear transect sequences used for quantitative data was 7.2% of the total video transect (Fig. 7), encompassing approximately 809.7 m distance navigated. With that being said, 92.8% of the video transect was non-useful for this type of data analysis and was not included as part of the quantitative data analysis. The leading cause for non-useful sequence types was due to bad visibility (33.9%). Bad visibility arose from a number of reasons; an excessive amount of marine snow (e.g. organic material falling from the upper water column to the deep ocean), or when the ROV hit bottom and formed a cloud of mud and/or sand. In addition, when the ROV is simply not moving (stopped to sample or capture a panoramic image) the sequences could neither be used.

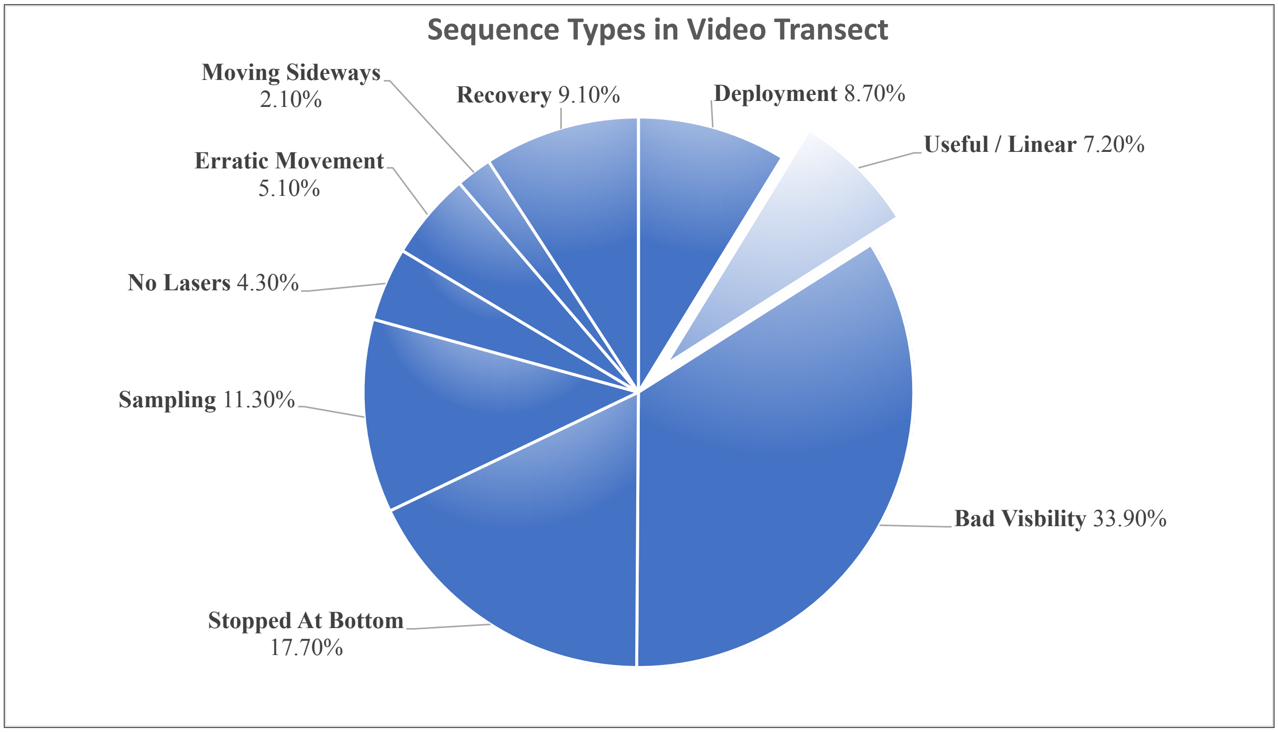

Figure 7. *The different sequence types (in percentage) identified along the remote operated vehicle video transect at Gazul Mud Volcano (see Figure 1 for location of the transect). The light blue slice represents the linear transect sequences whereas the dark blue slices represent the non-useful sequences.*

4.2 Substrate characteristics

Throughout the linear transect sequences, substrate type consisted of approximately 90% of detritic sediments with soft flagstone as the remainder 10% (Fig. 8). With regards to the distance navigated in the linear transect (Fig. 8), the first 168 m were dominated by detritic substrate until the 319 m mark. Soft flagstone began to take over between 319 m and 723 m, followed by hard flagstone between 429 m to 485 m navigated. At the end of the linear transect, detritic was the dominant substrate with mud and rock as a secondary substrate between 1434 m to 1564 m and then rock present again at 1944 m (Fig. 8). Blanks refer to any sequences that were non-useful and therefore not part of the linear transect.

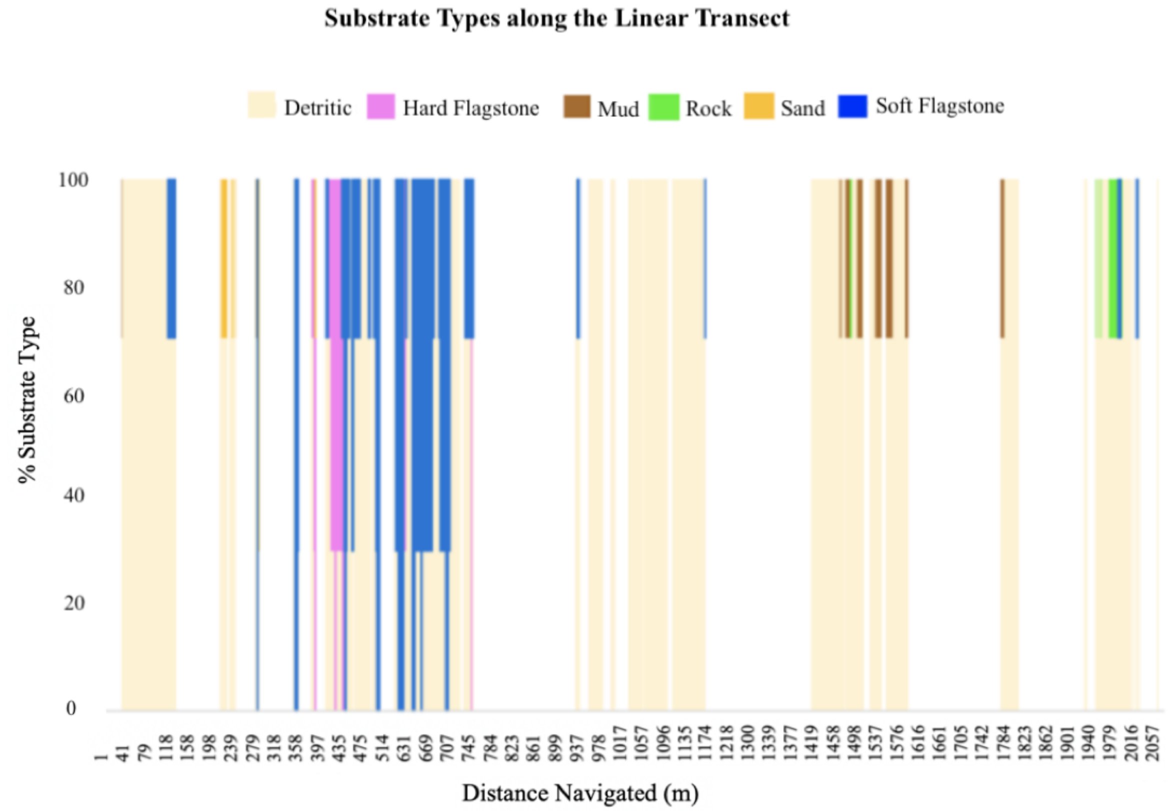

***Figure 8**. The different substrate types (in percentage) along the distance navigated (m) in the video transect in Gazul Mud Volcano. Blanks represents non-useful sequences.*

4.3 Total occurrence and abundance of megabenthic organisms

A total of 745 organisms, belonging to 46 OTUs (Annex 1), were recorded in the linear transect, belonging to the quantitative data set (Fig. 9a). Overall, the most abundant OTUs (Fig. 9a,b; from both quantitative and out-sampling frame data sets) were Cnidaria with 411 organisms total, followed by Porifera with 243 organisms total and Echinodermata with 50 organisms total. The quantitative data abundance plot and the out-sampling frame data abundance plot (Fig. 9) showed very similar counts and groups in all OTUs. The most frequent and abundant OTU was *Acanthogorgia* spp., which represented almost one third of the total fauna observed and 26% of the total number of cnidaria recorded from the linear transect (belonging to the quantitative data set). The second most abundant OTU was *F. chunii* (12%) and the third most abundant OTU was 'White Coral', with 85 colonies / individuals (11%).

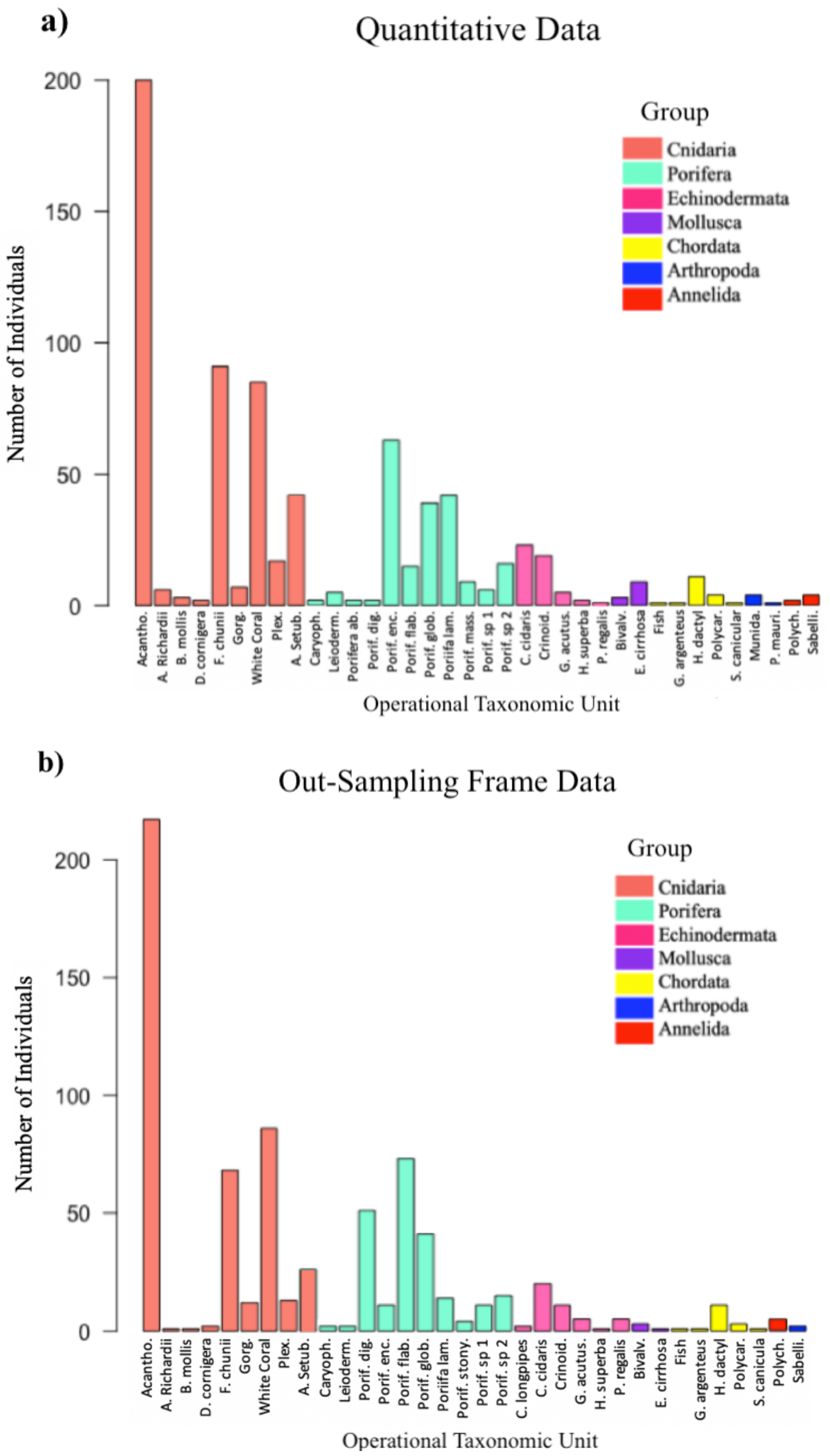

Figure 9. *Number of individuals counted per Operational Taxonomic Unit (OTU), categorized by source of data;* ***a)*** *Quantitative data,* ***b)*** *Out-sampling frame data. Complete names for OTUs can be found in the complete taxonomic list in Annex 1.*

In terms of important deep-sea sponges, *Asconema setubalense* was one of the few identified to species level and also one of the dominant habitat-forming species with 42 individuals counted (belonging to the quantitative data set). Other abundant OTUs were Porifera lamellate with a total of 42 individuals and Porifera encrusting with the highest number of counts (with 67 individuals) overall for deep-sea sponges (all belonging to the quantitative data set).

For the out-sampling frame data set, the same OTUs remained as the most frequent and abundant. Regarding the out-sampling frame data, a total of 1448 individuals were counted, and the same patterns for abundance of OTUs were observed (Fig.9b) as the quantitative data set (Fig. 9a).

4.4 Densities and spatial patterns of megabenthic organisms

The six OTUs that showed to be the most frequent and abundant in the linear transect sequences (Fig. 10), were studied in further detail; a) *Acanthogorgia* spp., b) White Corals, c) *Asconema setubalense*, d) *Flabellum chunii*, e) Porifera globular, and f) Porifera lamellate. Total densities were calculated and averaged in number of individuals per square meter (Table 1, Fig. 11). Results showed that the majority of individuals and OTUs tended to form assemblages in large aggregations or patches, which we will refer to as *clusters* (Fig. 11, Annex 2). The six most frequent and abundant OTUs showed aggregation patterns in association with substrate type. Furthermore, four clusters of variable sizes have been identified and described along the linear transect from GMV (Fig. 11). Cluster 1 was covered with dense aggregations of deep-sea sponges including *A. setubalense*, Porifera globular and Porifera lamellate. Sponge taxa tended to be more frequent and abundant here in cluster 1, yet the colonial coral; *Acanthogorgia* spp. also showed the highest density here for CWCs. Cluster 2 showed other emergent fauna, such as the solitary coral *F. chunii* (Table 1, Fig. 11). While clusters 3 and 4 were composed mainly of the White Corals.

Cluster 1 was the largest in distance navigated and area covered, but also the one with the highest taxonomic diversity in terms of number of OTUs (Table 1). Indeed, Cluster 1 also showed to have the highest densities for most OTUs observed in this study (Table 1). A large variation of density values between the six most abundant OTUs (at least one order of magnitude) was observed, yet showed large differences of density values between the different cluster locations (Table 1). All individuals of *Acanthogorgia* spp. *(a)* were found in Cluster 1 at high densities (Table 1, Fig. 12) settled on soft flagstone and some hard flagstone (Fig. 11). The colonies were mixed among sponge fields, consisting mainly of small and large *A. setubalense (c)* individuals (Table 1, Fig. 12). Some Porifera globular, Porifera lamellate, Porifera flabellate and *Leiodermatium* sp. were observed among the assemblage as well (Fig. 11, 12). White Corals *(b)* and Porifera globular *(e)* showed to monopolize cluster 3, mainly nearing the summit of the volcano on hard rocky substrate (Fig. 11). Porifera globular, Porifera lamellate *(f),* and *A. setubalense* all showed similar distribution patterns with little density variation (Table 1, Fig. 12), aggregated in both clusters 1 and 3. Few of the six most abundant OTUs tended to aggregate in cluster 4 where a mixture of rock and soft flagstone covered the bottoms, yet other important CWC OTUs (*Bebryce mollis*, *Dendrohyllia cornigera* and *Plexauridae* sp.) did happen to colonize the hard substratum instead (Annex 2). Lastly individuals of the actinian species, *A. richardiii* and the sclearctinian *F. chunii* (d) were found dominating in cluster 2 on detritic substrate, with no observations in any other clusters (Fig. 11, 12).

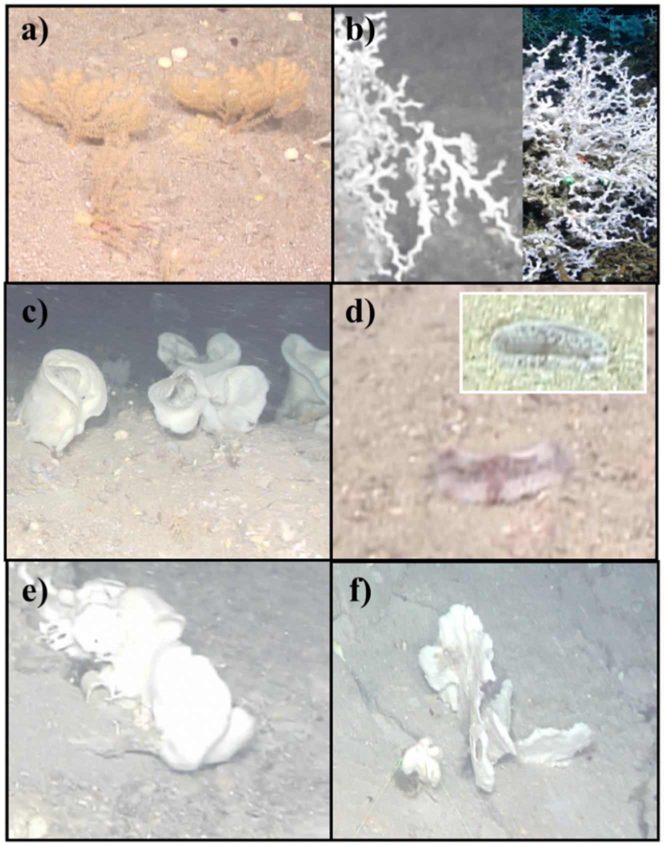

Figure 10. *The six most frequent and abundant operational taxonomic units from the linear transect at the Gazul Mud Volcano.* ***a)*** *gorgonian, cold-water coral; Acanthogorgia* spp., ***b)*** *reef-building 'White corals'; Madrepora oculata on left, Lophelia pertusa on right, (Source: NOAA-Pelagic Research Services),* ***c)*** *deep-sea sponge; Asconema setubalense,* ***d)*** *solitary scleractinian coral; Flabellum chunii****,*** *and examples of the Porifera morphotypes included as the Operational Taxonomic Units;* ***e)*** *Porifera globular****,*** *and* ***f)*** *Porifera lamellate*

Location	***Acanthogorgia* spp.** (ind · m^{-2})	***Asconema setubalense*** (ind · m^{-2})	**White Corals** (ind · m^{-2})	***Flabellum chunii*** (ind · m^{-2})	**Porifera globular** (ind · m^{-2})	**Porifera lamellate** (ind · m^{-2})	**Navigated distance** (m)	**Average depth** (m)	**Total OTUs**	**Area covered (m^{-2})**
Cluster 1	0.314	0.056	0	0.002	0.045	0.068	168 - 741	452 - 457	20	573
Cluster 2	0.029	0	0	0.040	0.002	0	922 - 1375	452	5	453
Cluster 3	0.031	0.038	0.085	0	0.023	0.238	1434 - 1564	423	16	130
Cluster 4	0	0.022	0	0	0	0	2016 - 2057	393	15	46
Linear Transect	0.374	0.116	0.226	0.042	0.07	0.306	1197	393 - 457	56	1202

Table 1. *Characteristics of the linear transect and the four main clusters observed at Gazul Mud Volcano. The biological information includes the total number of Operational Taxonomic Units (OTUs) and average densities (individuals · m^{-2}) for each of the six most frequent and abundant OTUs. The navigated distance (m) within the video transect, depth (m) and approximated area covered (m^{2}) is described for each location. See Figure 11 for location of each cluster within the analyzed video transect.*

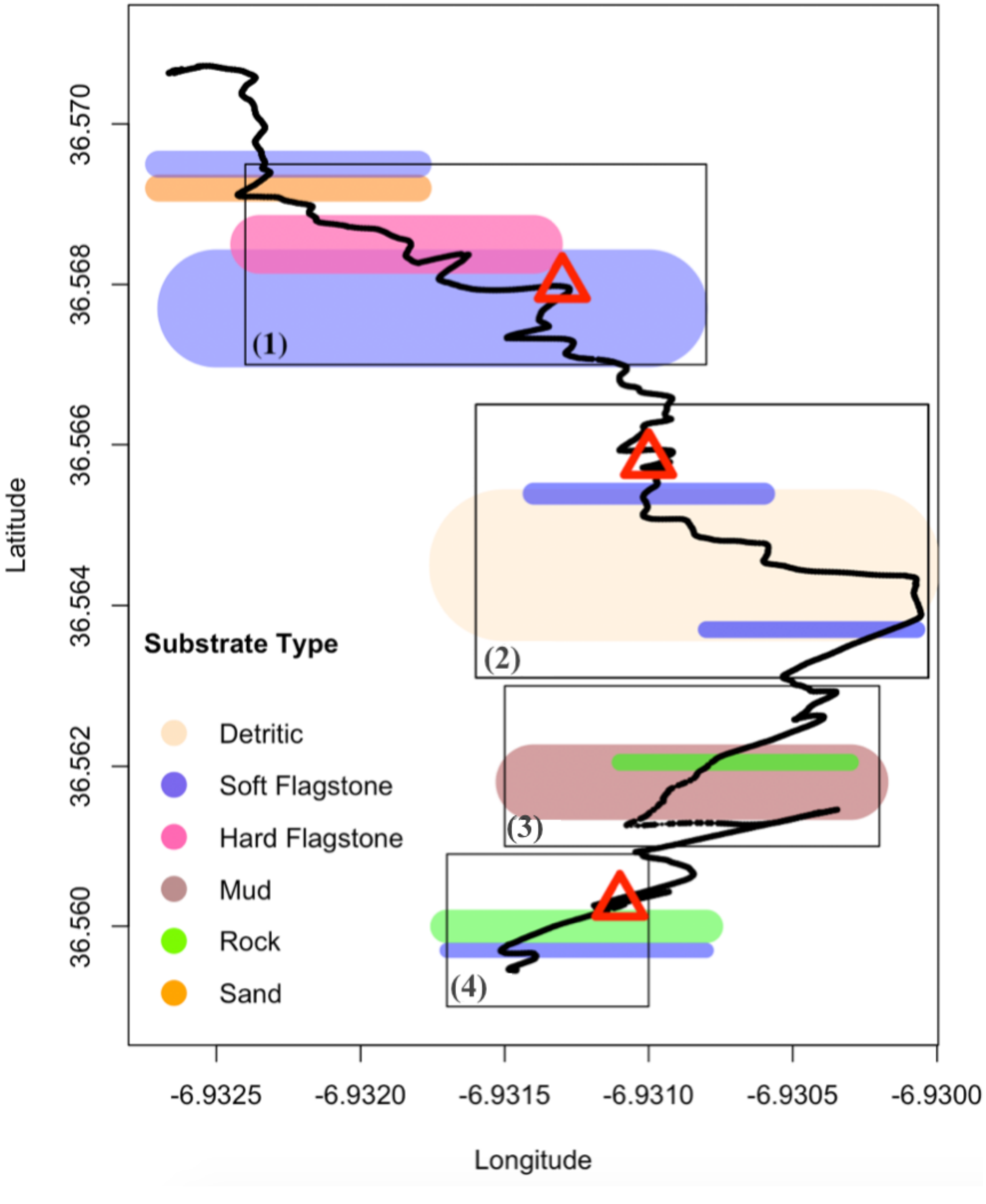

Figure 11. *Spatial patterns of the megabenthic communities along the linear transect of Gazul Mud Volcano. Entire transect track is denoted by the black line (see Figure 2 for location within the study area). Clusters are displayed in rectangular boxes labelled with the corresponding number. Substrate types are represented along the transect track with different colours (see legend). Red triangles indicate fishing impacts observed.*

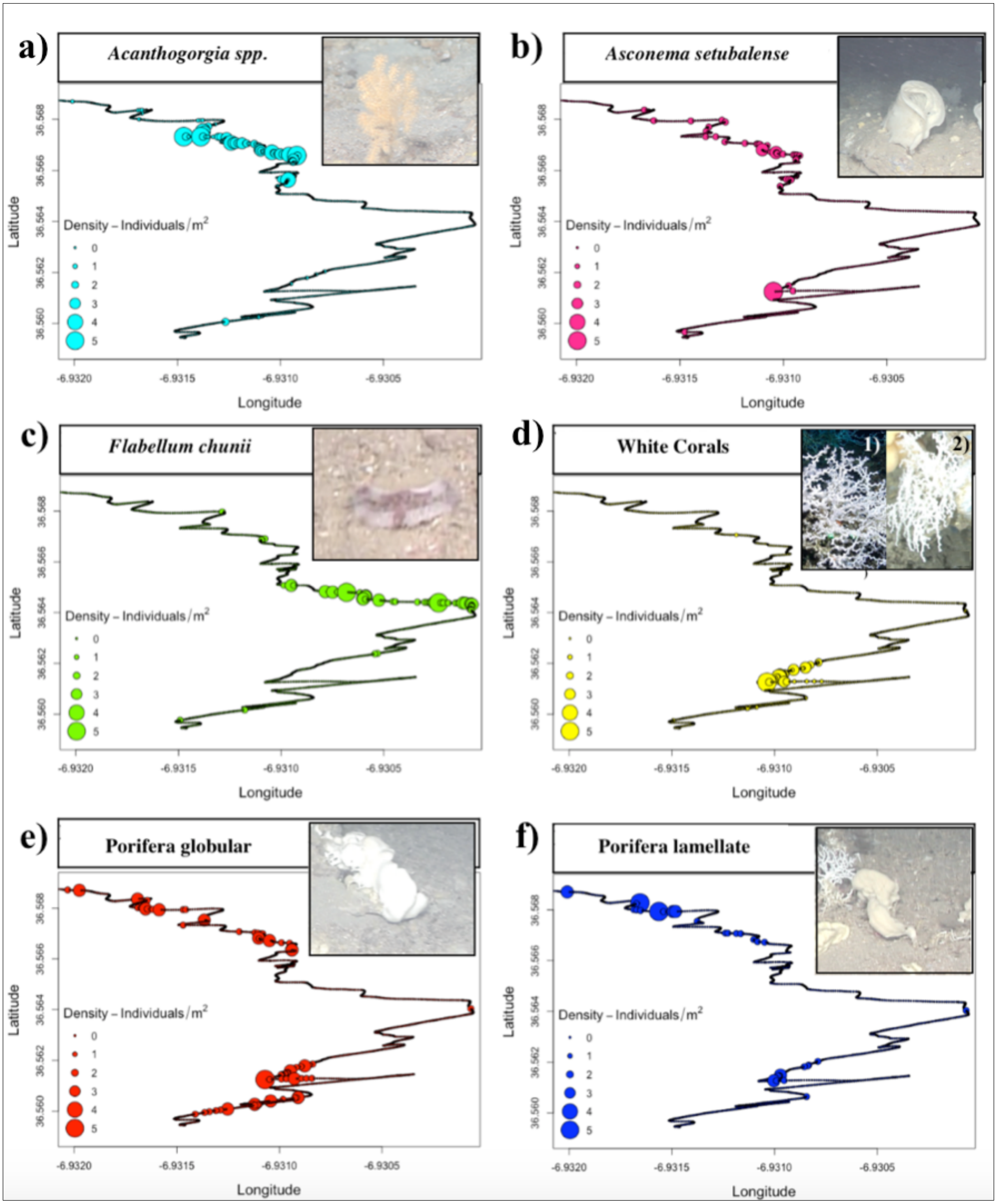

***Figure 12**. Density plots of the six most frequent and abundant Operational Taxonomic Units (OTUs) along the linear transect; **a)** Acanthogorgia spp., **b)** Asconema setubalense, **c)** Flabellum chunii, **d)** White corals; including the combined observations from both **1)** Lophelia pertusa (Source: NOAA-Pelagic Research Services) and **2)** Madrepora oculata, **e)** Porifera globular, and **f)** Porifera lamellate.*

With regards to distribution of the remaining OTUs, results showed that most tended to aggregate among one or more of the four clusters we identified (Annex 2, Fig. 11). Some individuals showed interesting and clearly visible spatial patterns. For Cnidarian taxa; *Bebryce mollis, Dendrophyllia cornigera, Plexauridae* sp1 and *Gorgonacea* sp1, showed a patchy distribution, aggregating together near or at the summit of GMV in cluster 4 (Annex 2, Fig. 11). The cup coral *Caryophyllia* spp. was the only cnidarian OTU found in cluster 2, and nowhere else. For Porifrera taxa; OTUs were found widespread along the entire linear transect (e.g. in all clusters). *Leiodermatium* sp. individuals aggregated in a dense cluster only at the beginning of the video transect, just outside of cluster 1 (Annex 2). Porifera stony morphotype showed unique patterns as it was the only OTU from the Porifera phyla to be found in cluster 4. For Echinodermata taxa; the common urchin *Cidaris cidaris* showed to be the most widespread and most abundant Echinodermata (Annex 2). It should also be noted that Echinoderm *Crinoidea* sp. were difficult to observe, differentiate and therefore properly count the number of individuals. For Chordata taxa; elasmobranch, *Scyliorhinus canicula* was observed in a single location at the beginning of the transect. Most, if not all fish taxa were found widespread along the linear transect as well (Annex 2). The ascidian, *Polycarpa* sp. occurred in 4 distinct and highly dense patches (>100 individuals) amongst cluster 1, 3 and 4. The actinia*, A. richardii* displayed a similar distribution pattern as the solitary scleractinian *F. chunii,* dominating in cluster 2 only, although the actinia showed much lower densities compared to *F. chunii.* For Mollusca taxa; individuals were found in cluster 1 and 3 only, with cephalopod species; *Eledone cirrhosa* found at the bottom of the volcano and at the summit in cluster 4 (Annex 2, Fig. 11).

4.5 Fishing impact in the analyzed video transect

The total number of fishing impacts were counted and objects human debris or leftover fishing gear were annotated along the video transect (Fig. 13). A total number of three separate fishing remains were recorded in three separate locations (Fig. 13) along the video transect. One large fishing net (Fig. 13) found at 450 m depth in an area of hard flagstone mixed with detritic bottoms. The colonization of *Crinoidea* sp*., Acanthogorgia* spp*.,* Porifera globular, Porifera lamellate, Porifera encrusting and White Corals, all exist, some directly attached to the mesh of the net. Three remains of fishing lines (Fig. 13) were found at 447 m depth, surrounded by different habitat-engineering OTUs; several large *A. setubalense* and Porifera lamellate individuals, more than 12 colonies / individuals of *Acanthogorgia* spp. and several *Crinoidea* spp. individuals were attached directly on the line.

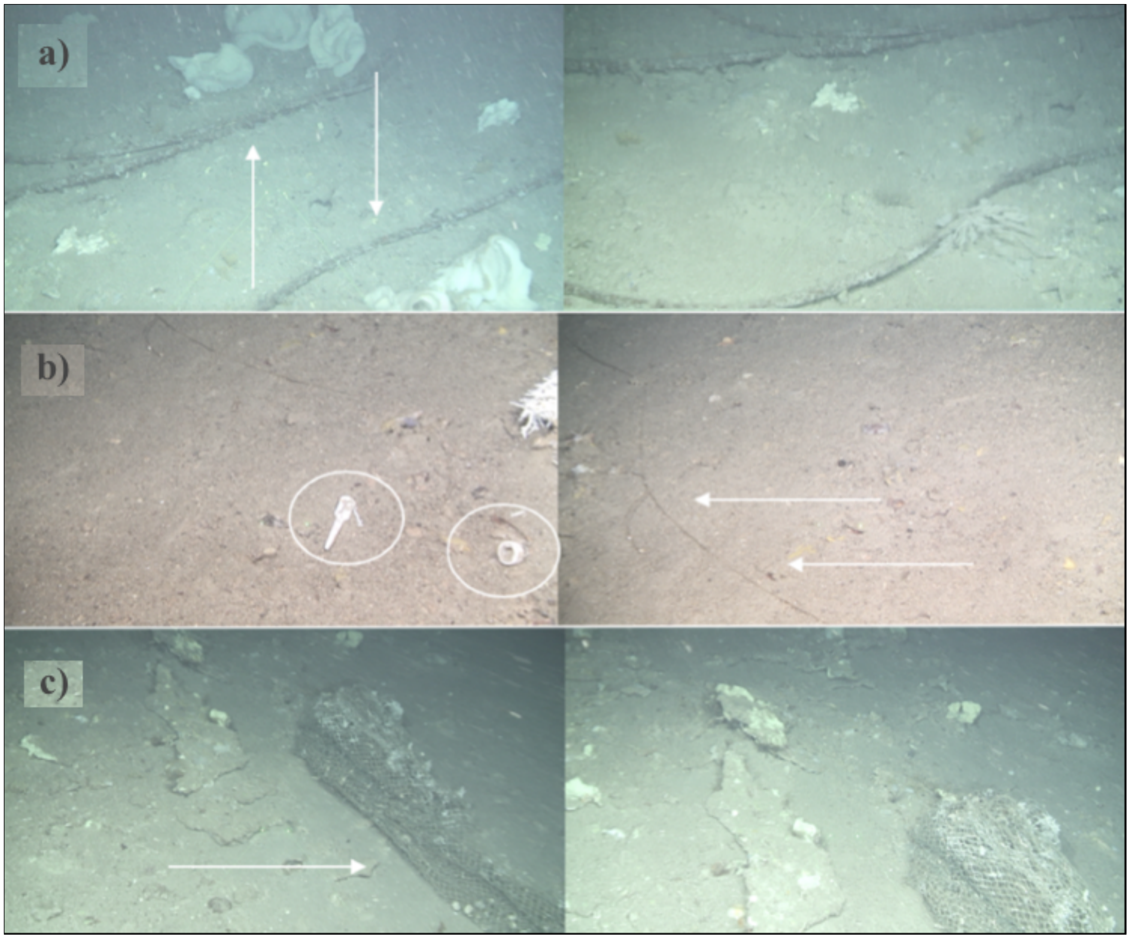

Figure 13. *Three different fishing impacts observed along the video transect at Gazul Mud Volcano;* ***a)*** *thick fishing line,* ***b)*** *thin fishing line and debris, and* ***c)*** *fishing net. Location of fishing impacts within the video transect can be found in Figure 11.*

One fishing line and two pieces of fishing debris (Fig. 13) were found at 405 m depth, in a soft substrate. In the surrounding area of the remains, *F. chunii* dominates, with diverse taxa intermingling; *M. oculata, Munida* sp., *Asteroidea* sp., *Cidaris cidaris,* Porifera globular and Porifera encrusting (Fig. 13).

5. DISCUSSION

5.1 Video Analysis

Our main results from the ROV video analysis at GMV showed that the study area hosts a large number of OTUs found in different deep-sea benthic communities. The sampled area also shows to have a high density of OTUs, compared to nearby soft-bottoms in the GoC (Rueda et al., 2012; Sitjà et al., 2018). Our study area focused on only a short-navigated distance and yet our overall findings still supported our hypothesis in that the GMV acts as a host of many different VMEs and VME indicator taxa. CWCs and sponges are both important players in deep-sea ecosystems, acting as habitat providers (Guinan et al., 2009; Davies et al., 2017; Pham et al., 2015; De la Torriente et al., 2018) to a variety of diverse organisms. Indeed, there is a particular interest for conservation of reef habitats in the Habitat Directive (Reef-1170, (Directive 93/42/EC Habitat). As part of the Spanish and European natural heritage, the GMV and eleven others within the GoC have previously been added to the Habitats Directive (Evans, 2006). and the Marine Strategy Framework Directive for conservation of resources (European Parliament and Coucil of the EU, 2008).These past implementations put us one step ahead and closer to complete protection of these vulnerable fauna, our findings and data collected from GMV could greatly contribute to the overall knowledge on this specific location, in hopes of attaining more and more conservation.

5.2 Biodiversity and densities of megabenthic fauna

Densities of the main scleractinian CWCs at GMV showed to be higher than average in comparison to the same species (*M. oculata, L. pertusa*) in nearest surrounding areas, e.g. the GoC and other northern locations in the NE Atlantic (Cathalot et al., 2015; Wienberg et al., 2009, 2010; Davies et al., 2017; Van den Beld et al., 2017; Cunha et al., 2011). Average density values for White Corals at GMV (0.05 ind · m^{-2}) showed to be most comparable to those from the Bay of Biscay (NE Atlantic) (0.01-0.04 ind · m^{-2}; Arnaud-Haond et al., 2017; Reveillaud et al., 2008). Both regions are biogeographically located within a subtropical / boreal transition zone and both regions display similar ecological richness and diversity (Andonegi et al., 2015). The two areas are shaped by strong hydrodynamics with an increase in food availability, making them both suitable for benthic suspension feeders such as CWCs (Clippele et al., 2017; Thiem et al., 2006). The hydrodynamics at the Bay of Biscay undergoes upwelling, is highly influenced by river runoff, the Gulf Stream and gyres (Andonegi et al., 2015). Whereas the hydrodynamics at the GoC basin involves the connection of the North Atlantic Ocean to the Mediterranean Sea, being highly influenced by two different water masses; the MOW and the NACW. Indeed, the presence of various water conditions may influence both areas biodiversity. The warmer Mediterranean waters, such as the MOW in the GMV might benefit the *M. oculata* growth over *L. pertusa*. *Madrepora oculata* is known to be more tolerant to slightly warmer temperatures, ranging in the Mediterranean Sea between 11-13 °C (Naumann et al., 2014) and up to a maximal upper limit of 20 °C. *Madrepora oculata* is also more tolerant to abrupt environmental changes (Keller and Os'kina, 2008; Wienberg et al., 2009) and acts as the dominant CWC framework species in the Mediterranean Sea (Weinberg et al., 2009). In comparison to *L. pertusa* which is commonly found between temperatures of 4 - 12 °C (Frank et al., 2011) and seems to dominate more cooler locations in the North Atlantic (Arnaud-Haond et al., 2017 Naumann et al., 2014). *Madrepora oculata* seemed to dominate over *L. pertusa* in the analyzed video transect at GMV, as usually occur in this area (Keller and Os'kina, 2008). However, these results should be taken with caution due to the difficulties in the identification of the two species using underwater ROV footage only.

The alcyonacean CWC *Acanthogorgia* spp showed to be the overall most abundant and frequent OTU in the linear transect, it dominated in cluster 1 and was often found living amongst sponge fields at deeper depths of the MV (~450m). This species is also considered an important ecosystem-structuring individual responsible for forming dense aggregations (Braga-Henriques et al., 2013), known as 'coral gardens'. Similarly, as scleractinian coral reefs, the complex structure and 3D-framework (Pham et al., 2015) of the coral gardens also provide stability (Gori et al., 2012) as well as other ecosystem benefits and services for many associated organisms, including habitat (Arnaud-Haond et al., 2017) and nursery provisioning (Pham et al., 2015).

In addition to CWCs, sponge taxa show equal importance in the deep-sea as they play key roles in habitat supply, nutrient cycling (Thurber et al., 2014), and give structural complexity, increasing local biodiversity (Ramiro-Sánchez et al., 2019). Unfortunately, available information regarding distribution and biology of these organisms remain limited, especially in areas below 200 m (Kazanidis et al., 2019). Our findings at GMV, for Porifera density values (e.g. flabellate globular, *etc.*) are comparable to those observed at the Faroe-Shetland Channel (FSC) in the NE Atlantic (Kazanidis et al., 2019). Densities of common sponge OTUs ranged between 0.009 – 0.056 ind · m^{-2} at GMV (mean ~ 0.116 ind ·m^{-2}) whereas densities within the FSC-NCMPA (Faroe-Shetland Channel Nature Conservation Marine Protected Area) ranged from 0.011 to 0.338 ind ·m^{-2} (mean ~ 0.175 ind ·m^{-2} ; Kazanidis et al., 2019). Both areas present very different characteristics in terms of location and oceanography (Kazanidis et al., 2019), yet still show similar mean densities for deep-sea sponge fauna. It could be thought that substrate, salinity and temperature all influence these organisms distributions at both locations and may give reasoning for such similarities in density values. At FSC-NCMPA, Porifera densities were highest on harder substrate (e.g. rock, cobble; Kazanidis et al., 2019), showing comparable findings to GMV where porifera organisms also preferred harder substrate (e.g. detritic, flagstone) opposed to sand. At FSC-

NCMPA, densities were highest in the salinity range of 34.91 to 35.13 psu and temperatures between 6.52 to 8.98 °C. Although temperatures at GMV are slightly warmer (reaching 13.1°C), salinities were very similar (ranging from 35.9 to 36.0 psu). Overall, Porifera fauna at FSC-NCMPA aggregated in a narrow depth zone between 450 – 530 m in warmer and more saline water masses (Kazanidis et al., 2019). These findings show many similarities to our results from GMV, as most Porifera also aggregated around 440 to 460 m depth, also highly influenced by warmer water masses (MOW). Therefore it could be suggested that the salinity and depth range of the Faroe-Shetland area coincides with the MOW in the Faroe-Shetland area producing similar influences on the deep-sea sponge communities for both regions.

A noteworthy pattern was observed at GMV regarding other existing fauna. The diversity in terms of OTUs tended to show an increase when CWCs and sponges were present (Table 1, Fig. 11). Cluster 1 showed the highest diversity (20 OTUs), which positively associates with the presence of CWCs and sponges which dominates this cluster. Whereas cluster 2 has the lowest diversity (5 OTUs) likely due to the fact that only small solitary corals and actinians were present, but none with complex structure were present in this cluster. The presence of CWCs and sponges provide services and functions to many other organisms, such as refuge (Guinan et al., 2009), feeding, nursery and reproductive grounds (Pham et al., 2015), cycling (Van den Beld et al., 2017) carbon sequestration (Sweetman et al., 2017), *etc*. It could also be suggested that with a higher biodiversity in the area, comes more colonization and growth of CWC individuals (Komyakova et al., 2018; Clements et al., 2019), which gives reasoning to the biologically diversified ecosystems at GMV. Indeed, the structures and ecosystems formed by underwater mud volcanoes themselves may also favour diversity of organisms, simply due to the presence of marked slopes, enhanced currents and in general due to the specific oceanographic conditions surrounding the area (Cunha et al., 2013; Morato et al., 2013; Rueda et al., 2015).

5.3 Distribution patterns

In terms of distribution patterns, results showed that almost all OTUs tended to aggregate in four main clusters (Fig. 12, 13), rather than randomly along the analyzed linear transect. Clustering patterns has previously been suggested in the GoC for both CWCs and sponges by Cunha et al (2013). These authors stated that few locations in the GoC are known where CWCs and sponges occur, but generally aggregating at small carbonate mounds and on flanks of the volcanoes. Although no presence of carbonate mounds were observed at GMV, flanks of underwater MVs in the GoC probably show similar sediment characteristics, topography and slope, which make our findings comparable to the study of Cunha et al (2013). Indeed, similar spatial patterns were found, where diverse CWCs and sponge OTUs tended to aggregate on the flanks of GMV, as clearly observed in cluster 1.

The main aggregations along the linear transect may have been obvious and apparent simply due to the true occurrence and distribution of these organisms as a patchy distribution is very common in benthic communities (Bett and Rice, 1992; Cosson et al., 1997; Orejas et al., 2009b). However, the processing technique used involved the selection of only certain sequences to be analyzes from video transect and in consequence, the observed patchy distribution of OTUs could have been determined artificially. However, the non-useful sequences of the video transect generally showed two contrasting settings; 1) bare bottoms with sand and detritic or 2) harder bottoms with organisms aggregated together. Thus, despite the fact that those sequences were not included in the quantitative data analysis, they are useful to confirm the patchy distribution patterns found. Overall, it seems as though organisms at GMV tend to show a more patchy distribution rather than a continuous change.

A main influential factor to determine spatial patterns was substrate type, as fauna aggregations and clusters were commonly associated with different substratum types observed. The main hard substrates observed in GMV included carbonates crusts and slabs, rock and in a further extent coral rubble (classified as detritic sediment) . Same substrate types has been observed covering the surfaces of other MVs within the GoC (e.g. Hespérides, Faro; Léon et al., 2007, 1999). Harder substrates usually offer better settling grounds, for most CWC species (Léon et al., 1999; Henry and Roberts, 2007; Weinberg et al., 2009. These surfaces provide a hard substratum for white corals, *Acanthogorgia* spp., Porifera OTUs and many other benthic taxa of GMV (Díaz-del-Rio et al., 2003; Léon et al., 2007; Somoza et al., 2014. Overall, our findings are supported by those of Purser et al. (2013) in that different taxa favours different substrate in terms of settlement, growth and distribution. As observed in our study area, CWCs and sponges mainly existed on hard substrate such as soft flagstone, hard flagstone and rock (e.g. clusters 1, 3 and 4). Deep-sea sponges (*A. setubalense*, *Leiodermatium* sp., and most Porifera morphotypes) OTUs were mainly found on hard substrates in cluster 1. In agreement with the study of Sitjà et al. (2018), in the Galicia Bank (NE Atlantic), sponge OTUs at GMV tended to aggregate on the less steep areas (Sitjà et al., 2018) and deepest depths (450 m). No large habitat forming CWCs or sponge OTUs were found on soft substrate (cluster 2; Fig. 11). However, there are other coral species (e.g. *F. chunii, Caryophyllia* spp.) that have adapted to settle on soft bottoms (Corbera et al., 2019). Sedimentary habitats are also noteworthy sea floor components within the MV of the Spanish margin (Rueda et al., 2012). Weinberg et al. (2009) documented that soft sediment dwellers showed to be more common on the lower flanks of MVs in the GoC compared to the summit region. In GMV, when soft substrate was present, individuals of *F. chunii* and *A. richardii* cohabited and were found in flatter, deeper areas (450 m) of the linear transect.

Beside substrate type and geomorphology other environmental and biological factors influence the distribution patterns of benthic fauna at GMV, as observed previously in other studies (e.g. Clippele et al., 2017; Guinan et al., 2009; Sánchez et al., 2014). Among these factors; hydrodynamic conditions play a role in spatial patterns and species growth (Clippele et al., 2017; Thiem et al., 2006). For instance, CWCs; *M. oculata* and *L. pertusa* were observed intermingling on the summit of the volcano in cluster 3, probably due to the ideal conditions seen at the summit of the volcano. Compared to other parts of the volcano, temperatures reached 13.1 °C and salinities were lower between 35.9 – 36.0 psu, with strong current speeds of $> 0.3\ \mathrm{m} \cdot \mathrm{s}^{-1}$ [MEDWAVES; ATLAS project unpublished data]. In contrast, colonial *Acanthogorgia* spp. were found among all four clusters in high densities, distributed all along the linear transect. It could be added that these diverse communities may favour the GMV for its unique conditions, specifically regarding hydrodynamics and water masses. Throughout the video transect, large amounts of detritus and suspended particles were continuously being transported which probably acts as a primary source of food for benthic suspension feeder organisms (e.g. CWCs, sponges), especially because these organisms display an opportunistic behaviour of passive suspension feeding (Van Rooij et al, 2010; Sánchez et al, 2014; Corbera et al., 2019). Unfortunately, due to time constraints, our study was unable to analyze all of these driving factors, nevertheless hydrodynamics and other factors, such anthropogenic impacts (see details below), cannot be disregarded as partially responsible for the observed spatial patterns at GMV.

5.4 Vulnerable Marine Ecosystems (VMEs), fishing impact and conservation

Vulnerable Marine Ecosystems are areas which, among others, may include unique features such as seamounts, hydrothermal vents, *etc*., that may be vulnerable to fishing impacts (FAO, 2015). According to the ICES (2018), FAO (2019) and other studies (e.g. Ardron et al. 2013), VMEs are formed by organisms that possess uniqueness or rarity, functional significance of the habitat, fragility, life-history traits that make recovery difficult (*e.g.* slow growth rates, long life span) and structural complexity. It is generally difficult to define exactly when an assemblage of organisms or a habitat should be considered a VME or simply a VME indicator organism. According to the FAO (2016), VME indicator organisms are defined as benthic taxa which indicate the occurrence or likely occurrence of a VME. These indicator organisms in the deep-sea include: black corals, gorgonians, sea-pens, soft corals, sponges, stony corals, anemones, stalked crinoids, sponges, among others (ICES, 2018; FAO, 2019). However, some VME or VME indicator organisms are more vulnerable (fragile vs. resilient) than others (FAO, 2015). According to ICES (2018) guidelines for VMEs; numerous assemblages, habitat types and organisms may be considered VMEs or indicators of VMEs in GMV (Annex I). At least 4 different VMEs were clearly identified as: a) Mud and sand emergent fauna (*A. richardii, F. chunii)*, b) Cold-water coral reefs (*M. oculata, L. pertusa*), c) coral gardens (*Acanthogorgia* spp.), and d) deep-sea sponge aggregations (*A. setubalense*, Porifera OTUs).

Unfortunately, VMEs all around the world and their associated organisms are currently subject to many human threats, including fishing and pollution and will be subject to future threats such as climate change, oil and gas exploitation, mining, shipping, *etc*.; which also play a key role in shaping benthic communities (Fisheries and Oceans Canada, 2012; FAO, 2016 Lo Iacono et al., 2018; Orejas et al., 2009a; Hall-Spencer et al., 2002; Pham et al., 2015; Sweetman et al., 2017). Fishing activities at GMV could negatively influence benthic fauna (Sitjà et al., 2018). A recent study by Sitjà et al. (2018), suggested that GMV deals with low (0.5) fishing activity (0 = none, 1= 1 vessel, 2= 2 - 5 vessels, 3= >5 vessels) within the GoC area. Although fishing activity may be labelled as low to moderate, remains of fishing gear can still be observed at GMV from our video transect (Fig. 15). Perhaps this could be due to higher intensity of fishing activities in past time (Coll et al., 2014). The three impacted locations observed at GMV were probably old since signs of recolonization by various taxa were shown. Indeed, the man-made materials may actually provide benthic organisms a hard substrate for attachment and settlement needs (Sampaio et al., 2012).

The conservation of VMEs, such as the reef-building CWCs, from these impacts is of substantial importance and require protection measurements. The International Union for Conservation of Nature (IUCN) assigned global status' for taxa that shows threats of endangerment (Annex 1).

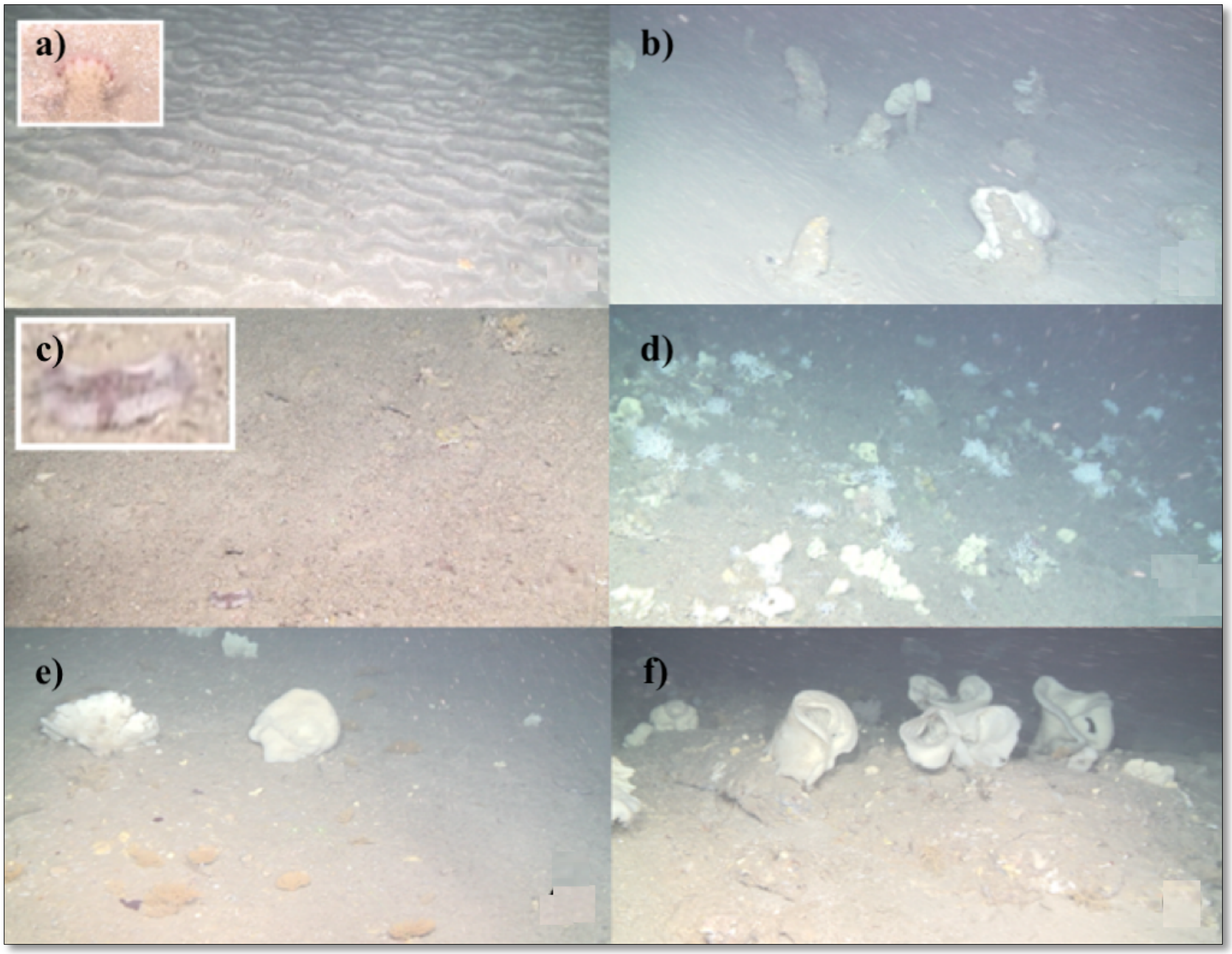

Figure 14. *Vulnerable Marine Ecosystems found in the video transect analyzed at Gazul Mud Volcano:* ***a)*** *Mud and sand emergent fauna, dominated by anemones, colonized on sandy bottoms by Actinauge richardii,* ***b)*** *Aggregation of mud chimneys on muddy slopes,* ***c)*** *Mud and sand emergent fauna, dominated by Flabellum chunii on sandy bottoms,* ***d)*** *Cold-water coral aggregations composed of Madrepora oculata, Lophelia pertusa mixed with sponges on detritic bottoms,* ***e)*** *Coral garden created by Acanthogorgia spp. mixed with sponges on soft bottoms,* ***f)*** *Deep-sea sponge aggregations made up of Asconema setubalense on soft flagstone.*

Certain CWCs observed at GMV, such as *M. oculata* and *L. pertusa* are listed as ‘critically endangered’ (CR) as well as the solitary coral *D. cornigera* (IUCN, 2019). Other alcyonacean CWCs observed at GMV such as *Callogorgia verticillatta* are listed as ‘near threatened’ (NT) and some species of the family *Plexauridae* are labelled as ‘vulnerable’ (VU) (IUCN, 2019). Conservation of VMEs help to improve ecosystem stability, which in turn improves the status and recruitment of commercially important species (Komyakova et al., 2018; Clements et al., 2019). For instance, the ‘curled octopus’ *E. cirrhosa*, which is exploited particularly in the Western Mediterranean (Belcari et al, 2002), was observed frequently in the video transect at GMV (Fig. 15). These species have been previously suggested to migrate to shallower, coastal areas for reproduction with only adults living in deep waters

(Sánchez et al., 1998; Sartor et al., 1998; Belcari et al., 2002; Regueira et al., 2013). However, all throughout the video transect at GMV, every observation was of small, juvenile individuals. This may require further investigation regarding the previous knowledge on *E. cirrhosa* lifecycles (Relini et al., 2006). Also, the small-spotted catshark *Scyliorhinus canicula*, another species of commercial interest, was recorded in the GMV (Fig. 15). Sharks are protected in numerous areas and are of critical importance as they are great indicators of a good environmental status (Kousteni et al., 2014). In addition, both the catshark and octopus are thought to be potential competitors (Kousteni et al., 2014; Puerta et al., 2016). Furthermore, such trophic interaction may show evidence for hunting and feeding grounds, suggesting the presence of enough food and resources for both species to co-exist at GMV (Puerta et al., 2016).

Protection is required for all VME associated fauna as the entire ecosystem deserves conservation (Douvere et al., 2008; Hughes et al., 2005). An ecosystem-based approach may be the best strategy moving forward because not just one species or individual should be given special consideration, rather, all taxa and habitats should be paid attention to (Crowder et al., 2008; Personnic et al., 2014). Our study could contribute to progressing the knowledge on deep-sea VMEs at the GoC and help to protect them and their associated fauna.

Summarising, this study provides insight in the spatial distribution and biological diversity of VMEs at GMV which may be used in contribution for future planning and management of the area. Using image methodologies (a non-invasive approach) can help to develop a baseline of VMEs in the region which can be used to evaluate other impacts (e.g. anthropogenic influences, environmental changes, *etc*.; Chimienti et al., 2018). This non-invasive approach, using an ROV to document and characterize benthic communities can help to build our scientific findings in deep-sea research and contribute to the conservation of vulnerable organisms like ecosystem engineers (reef-building CWCs and deep-sea sponges).

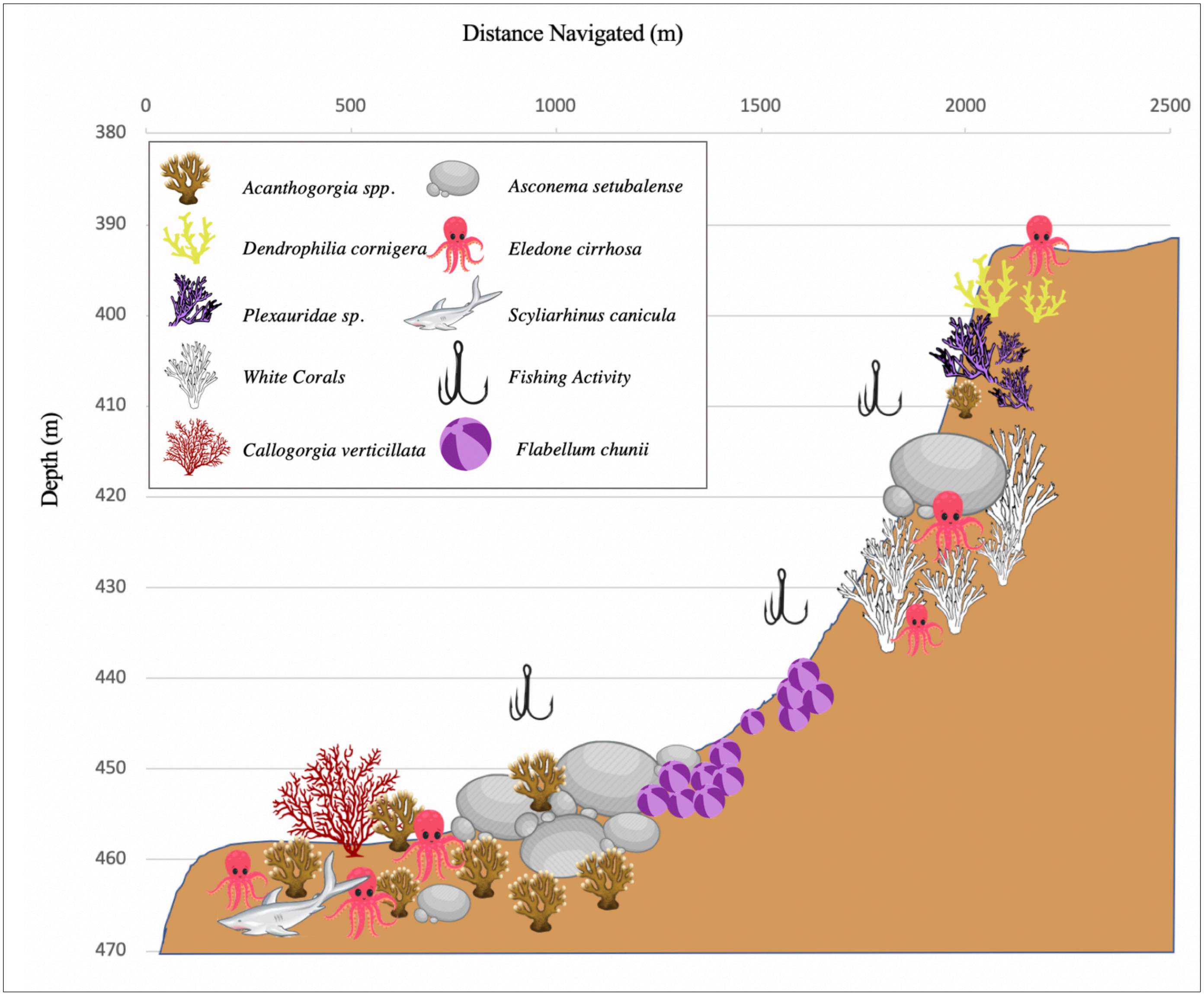

Figure 15. Diagram of the Vulnerable Marine Ecosystems and key indicator organisms found in the bathymetric gradient at the Gazul Mud Volcano.

5.5 Difficulties and limitations of the methodological approach

Numerous difficulties arose from this non-invasive method of sampling, that we tried to overcome during the analysis. Problems regarding ROV video sampling may have reduced our sample size and the majority of the video footage was unusable for the kind of quantitative analysis we planned to conduct. For example, a long video sequence of a dense sponge-field of *A. setubalense* was recorded at GMV for a couple of minutes, but unfortunately the lasers were off, making it a non-useful sequence. In addition, the footage that was good and useful was sometimes difficult to analyze due to bad visibility and image quality, overall adding inaccurate taxonomic identifications and/or a potential bias in counting. Another example of misidentification could have been between the morphotypes (e.g. Porifera lamellate and Porifera flabellate).

For future perspectives, it is most beneficial to analyze the footage multiple times in order to capture any observations which may not have been noted in the initial viewing, as we did in our study. Although it is quite out of our hands, good environmental conditions offer more successful functioning of the ROV which provides us with more useful and quantitative data. For example; with unfavourable environmental conditions in the study area, such as a strong current, comes high flows of sedimentation and in our case, low visibility. To conclude, it would have also been very beneficial and advantageous to analyse our data in relation to the different water masses, to better understand how the different hydrodynamic conditions affect different VMEs. It would be interesting to compare taxonomic diversity between the NACW and MOW masses, although unfortunately time was a constraint.

6. CONCLUSIONS

- Gazul Mud Volcano hosts diverse megabenthic communities with 745 total organisms/colonies counted in one video transect (~1200 m^2); belonging to 46 different Operational Taxonomic Units identified within 7 phyla, including organisms like; cold-water corals, deep-sea sponges, sharks, octopus, sea urchins, fish *etc*.
- The six most frequent and abundant Operational Taxonomic Units observed in the deep-sea benthic communities at Gazul Mud Volcano consisted of all cnidaria and porifera phyla (in order of decreasing abundance): 1) *Acanthogorgia* spp., 2) Porifera lamellate, 3) White Corals (*Madrepora oculata* and *Lophelia pertusa*), 4) *Flabellum chunii*, 5) *Asconema setubalense*, and 6) Porifera globular.
- Density values showed a large variation, of at least one order of magnitude, between the six most frequent and abundant Operational Taxonomic Units; the highest values arose from *Acanthogorgia* spp. with an average total density value of 0.096 ind · m^{-2}.
- Most of the observed benthic fauna and particularly the key habitat-forming Operational Taxonomic Units (cold-water corals and sponges) from Gazul Mud Volcano tended to aggregate into four main clusters, which associated with substrate type. Cluster 1 covered the largest area (573 m^{-2}) and showed the highest biodiversity with a total of 20 different Operational Taxonomic Units observed.

- Density values of the six most frequent and abundant Operational Taxonomic Units also presented large differences among the different cluster locations. For example; cluster 1 shows the highest total densities (0.52 ind·m^{-2}) mainly composed of cold-water corals and sponges, whereas cluster 2 shows the lowest total densities (0.02 ind·m^{-2}), mainly composed of *Flabellum chunii* individuals.
- Harder substrate types (such as soft flagstone, hard flagstone, rock) tended to host biodiversity in terms of Operational Taxonomic Unit diversity and density in comparison to bottoms dominated by soft substrate. In addition, the presence of habitat-forming cold-water corals, which mainly colonized on hard substrate, increased the biodiversity.
- At least four different Vulnerable Marine Ecosystem types were observed at Gazul Mud Volcano (following ICES, 2018 criteria) including: (1) Mud and sand emergent fauna, (2) Cold-water coral reefs, (3) Coral gardens, and (4) Deep-sea sponge aggregations. In addition, numerous indicator organisms were also observed such as *Madrepora oculata, Lophelia pertusa, Asconema setubalense, Dendrophyllia cornigera, etc.,* and organisms of commercial interest or importance such as *Eledone cirrhosa* and *Scyliorhinus canicula.*

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8. REFERENCE LIST

1. Andonegi, Eider and Llope M. "Comparison of the trophic control dynamics between two areas of the Western European shelf seas, the Bay of Biscay and Gulf of Cadiz". *Final Report of the ICES Science Fund Project*, 2015, pp. 2–25. http://www.ices.dk/community/icessciencefund/Pages/Comparison-of-trophic-control-dynamics-between-two-areas-of-the-western-European-Shelf-Seas,-the-Bay-of-Biscay-and-Gulf-of-.aspx

2. Ardron, Jeff A., Clark M.R., Penney A.J., Hourigan T.F., Rowden A.A., Dunstan P.K., Watling L., Shank T.M., Tracey D.M., Dunn M.R., and Parker S.J. "A Systematic Approach towards the Identification and Protection of Vulnerable Marine Ecosystems." *Marine Policy*, vol. 49, 2013, pp. 146, https://doi.org/10.1016/j.marpol.2013.11.017

3. Arnaud-Haond, S., Beld V.I.M.J., Ronan B., and Orejas C. "Two 'Pillars of Cold-Water Coral Reefs along Atlantic European Margins: Prevalent Association of Madrepora Oculata with Lophelia Pertusa, from Reef to Colony Scale." *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 145, 2017, pp. 110–119, doi:10.1016/j.dsr2.2015.07.013.

4. Belcari, Paola, Tserpes G., Gonzalez M., and Lefkaditou E.G. "Distribution and Abundance of *Eledone Cirrhosa* (Lamarck, 1798) and *Eledone Moschata* (Lamarck, 1798) (Cephalopoda: Octopoda) in the Mediterranean Sea." *Scientia Marina*, vol. 66, no. S2, 2002, p. 143, doi:10.3989/scimar.2002.66s2143.

5. Bett, B. J., and Rice, A.L. "The Influence of Hexactinellid Sponge (Pheronema Carpenteri) Spicules on the Patchy Distribution of Macrobenthos in the Porcupine Seabight (Bathyal Ne Atlantic)." *Ophelia*, vol. 36, no. 3, 1992, pp. 217–226, doi:10.1080/00785326.1992.10430372.

6. Braga-Henriques, Andreia, Porteiro F.M., Ribeiro P.A., de Matos V., Sampaio Í., Ocaña O., and Santos R.S. "Diversity, distribution and spatial structure of the cold-water coral fauna of the Azores (NE Atlantic)." *Biogeosciences*, vol. 10, no. 6, 2013, pp. 4009–4036, https://doi.org/10.5194/bg-10-4009-2013

7. Buhl-Mortensen, Lene, Vanreusel A., Gooday A.J., Levin L.A., Priede I.M., Buhl-Mortensen P., Gheerardyn H., King N.J., and Raes M. "Biological Structures as a Source of Habitat Heterogeneity and

Biodiversity on the Deep Ocean Margins.” *Marine Ecology*, vol. 31, no. 1, 2010, pp. 21–50., doi:10.1111/j.1439-0485.2010.00359.x.

8. Chimienti, Giovanni., Bo, M., and Mastrototaro, F. “Know the distribution to assess the changes: Mediterranean cold-water coral bioconstructions.” *Rendiconti Lincei*, vol. 29, no. 3, 2018, pp. 583–588, https://doi.org/10.1007/s12210-018-0718-3

9. Chimienti, Giovanni, Bo M., Taviani M., and Mastrototaro F. “19 Occurrence and Biogeography of Mediterranean Cold-Water Corals.” In: *Mediterranean Cold-Water Corals: Past, Present and Future Coral Reefs of the World*, 2019, pp. 213–243., doi:10.1007/978-3-319-91608-8_19.

10. Clements, Cody S., and Mark E. Hay. “Biodiversity Enhances Coral Growth, Tissue Survivorship and Suppression of Macroalgae.” *Nature Ecology & Evolution*, vol. 3, no. 2, 2019, pp. 178–182, doi:10.1038/s41559-018-0752-7.

11. Clippele, L. H. De, Huvenne V.A.I., Orejas C., Lundälv T., Fox A., Hennige S.J., and Roberts J.M. “The Effect of Local Hydrodynamics on the Spatial Extent and Morphology of Cold-Water Coral Habitats at Tisler Reef, Norway.” *Coral Reefs*, vol. 37, no. 1, 2017, pp. 253–266, doi:10.1007/s00338-017-1653-y.

12. Coll, Marta, Carreras M., Ciércoles C., Cornax M.J., Gorelli G., Morote E., and Sáez R. “Assessing Fishing and Marine Biodiversity Changes Using Fishers Perceptions: The Spanish Mediterranean and Gulf of Cadiz Case Study.” *PLoS ONE*, vol. 9, no. 1, 2014, pp. 1-15, doi:10.1371/journal.pone.0085670.

13. Corbera, Guillem, Lo Iacono C., Gràcia E., Grinyó J., Pierdomenico M., Huvenne V.A.I., Aguilar R., and Gili J.M. “Ecological Characterisation of a Mediterranean Cold-Water Coral Reef: Cabliers Coral Mound Province (Alboran Sea, Western Mediterranean).” *Progress in Oceanography*, vol. 175, 2019, pp. 245–62, doi:10.1016/j.pocean.2019.04.010.

14. Cosson, N., Sibuet M., and Galeron J. “Community Structure and Spatial Heterogeneity of the Deep-Sea Macrofauna at Three Contrasting Stations in the Tropical Northeast Atlantic.” *Deep Sea Research Part I: Oceanographic Research Papers*, vol. 44, no. 2, 1997, pp. 247–269, doi:10.1016/s0967-0637(96)00110-0.

15. Crowder, Larry B., Hazen E.L., Avissar N., Bjorkland R., Latanich C., Ogburn M.B. “The Impacts of Fisheries on Marine Ecosystems and the Transition to Ecosystem-Based Management.” *Annual Review of Ecology, Evolution, and Systematics*, vol. 39, no. 1, 2008, pp. 259–278, doi:10.1146/annurev.ecolsys.39.110707.173406.

16. Cunha, Marina, Rodrigues C.F., Génio L., Hilário A., Ravara A., and Pfannkuche O. “Macrofaunal assemblages from mud volcanoes in the Gulf of Cadiz: Abundance, biodiversity and diversity partitioning across spatial scales.” *Biogeosciences*, vol. 10, no. 4, 2013, pp. 2553–68, doi.org/10.5194/bg-10-2553-2013.

17. Cunha, Marina, G.L.J. P., Amaro T., and Blackbird S.J. “Biodiversity of Macrofaunal Assemblages from Three Portuguese Submarine Canyons (NE Atlantic).” *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 58, no. 23-24, 2011, pp. 2433–47, doi:10.1016/j.dsr2.2011.04.007.

18. Davies, Andrew J., Duineveld G.C.A., Lavaleye M.S.S., Bergman M.J.N., van Haren H., and Roberts J.M. “Downwelling and Deep-Water Bottom Currents as Food Supply Mechanisms to the Cold-Water Coral Lophelia Pertusa (Scleractinia) at the Mingulay Reef Complex.” *Limnology and Oceanography*, vol. 54, no. 2, 2009, pp. 620–629., doi:10.4319/lo.2009.54.2.0620.

19. Davies, Jaime., Guillaumont B., Tempera F., and Vertino A. “A New Classification Scheme of European Cold-Water Coral Habitats: Implications for Ecosystem-Based Management of the Deep Sea.” *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 145, 2017, pp. 102–09, doi:10.1016/j.dsr2.2017.04.014.

20. De la Torriente, A., Serrano A., Fernández-Salas L.M., García M., Aguilar R. “Identifying epibenthic habitats on the Seco de los Olivos Seamount: Species assemblages and environmental characteristics.” *Deep-Sea Research Part I: Oceanographic Research Papers*, vol. 135*,* 2018, pp. 9–22, https://doi.org/10.1016/j.dsr.2018.03.015

21. De Mol, Ben, Henriet J., and Canals M.. “Development of Coral Banks in Porcupine Seabight: Do They Have Mediterranean Ancestors?” *Cold-Water Corals and Ecosystems*, 2005, pp. 515–533, doi:10.1007/3-540-27673-4_26.

22. Díaz-del-Río, V., Somoza L., Martínez-Frías J., Mata M.P., Delgado A., Hernandez F.J., Lunar R., Martínez-Rubí J.A., Maestro A., Fernández M.C., León R., Llave E., Medialdea T., Vázquez J.T. “Vast fields of hydrocarbon-derived carbonate chimneys related to the accretionary wedge/olistostrome of the Gulf of Cádiz.” *Marine Geology*, vol. 195, no. 1–4, 2003, pp. 177–200, doi.org/10.1016/S0025-3227(02)00687-4

23. D’Onghia, Gianfranco., Maiorano P., Sion L., Giove A., Capezzuto F., Carlucci R., and Tursi A. “Effects of Deep-Water Coral Banks on the Abundance and Size Structure of the Megafauna in the Mediterranean Sea.” *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 57, no. 5-6, 2010, pp. 397–411, doi:10.1016/j.dsr2.2009.08.022.

24. D’Onghia, Gianfranco. “30 Cold-Water Corals as Shelter, Feeding and Life-History Critical Habitats for Fish Species: Ecological Interactions and Fishing Impact.” *Mediterranean Cold-Water Corals: Past, Present and Future Coral Reefs of the World*, 2019, pp. 335–356, doi:10.1007/978-3-319-91608-8_30.

25. Douvere, Fanny. “The Importance of Marine Spatial Planning in Advancing Ecosystem-Based Sea Use Management.” *Marine Policy*, vol. 32, no. 5, 2008, pp. 762–771, doi:10.1016/j.marpol.2008.03.021.

26. Dullo, Wolf-Christian, Flögel, Sascha and Rüggeberg, Andres. “Cold-water coral growth in relation to the hydrography of the Celtic and Nordic European continental margin.” *Marine Ecology Progress Series*, vol. 371, 2008, pp. 165–76, doi.org/10.3354/meps07623

27. Escobar, V. and Johnson D. “Annual Report 2010/2011: Protecting and conserving the North-East Atlantic and its resources.” London, *OSPAR Commission,* 2011*,* pp. 1-16.

28. EU. 2019. Chapter 4: The Protection of European Habitats and Species, P.G.G. Davies (Ed.). European Union Environmental Law – An Introduction to Key Selected Issues. Brussels. 37 pp.

29. Evans, Doug. "The Habitats of the European Union Habitats Directive." *Biology & Environment: Proceedings of the Royal Irish Academy*, vol. 106, no. 3, 2006, pp. 167–173, doi:10.3318/bioe.2006.106.3.167.

30. FAO. 2009. The State of Food and Agriculture: Livestock in the balance. Food and Agriculture Organization of the United Nation. Rome. 180 pp. (http://www.fao.org/3/a-i0680e.pdf)

31. FAO. 2015. The State of Food and Agriculture (SOFA): Social Protection and Agriculture: Breaking the Cycle of Rural Poverty. Food and Agriculture Organization of the United Nations. Rome. 151 pp. (http://www.fao.org/publications/ card/en/c/ab825d80-c277-4f12-be11-fb4b384cee35/)

32. FAO. 2016. The State of the World Fisheries and Aquaculture, Food and Agriculture Organization of the United Nations. Contributing to Food Security and Nutrition For All. Rome. 106 pp. (https://doi.org/10.18356/e68e16bb-en)

33. FAO. 2019. The State of the World's Biodiversity for Food and Agriculture, J. Bélanger and D. Pilling (eds.). FAO Commission on Genetic Resources for Food and Agriculture Assessments. Rome. 572 pp. (http://www.fao.org/3/CA3129EN/CA3129EN.pdf)
Licence: CC BY-NC-SA 3.0 IGO.

34. Fisheries and Oceans Canada. "Canada's State of the Oceans Report, 2012". *Ecosystems and Oceans Science*. Ottawa: Communications Branch, Fisheries and Oceans Canada, 2012, pp. 1-38, http://www.dfo-mpo.gc.ca/oceans/publications/soto-rceo/2012/page07-eng.html.

35. Frank, N., Freiwald A., Correa M.L., Wienberg C., Eisele M., Hebbeln D., Van Rooij D., Henriet J.P., Colin C., van Weering T., de Haas H., Buhl-Mortensen P., Roberts J.M., De Mol B., Doubille E., Blamart D., and Hatté C. "Northeastern Atlantic Cold-Water Coral Reefs and Climate." *Geology*, vol. 39, no. 8, 2011, pp. 743–746, doi:10.1130/g31825.1.

36. Gage, J. D., and B. J. Bett. "Deep-Sea Benthic Sampling." *Methods for the Study of Marine Benthos*, Edition 3, 2007, pp. 273-325, doi:10.1002/9780470995129.ch7.

37. Gori, Andrea, Orejas C., Madurell T., Bramanti L., Martins M., Quintanilla E., Marti-Puig P., Lo Iacono C., Puig P., Requena S., Greenacre M., and Gili J.M. "Bathymetrical distribution and size structure of cold-water coral populations in the Cap de Creus and Lacaze-Duthiers canyons (northwestern Mediterranean)." *Biogeosciences*, vol. 10, no. 3, 2012, pp. 2049–60, doi.org/10.5194/bg-10-2049-2013.

38. Gori, Andrea, Rossi S., Linares C., Berganzo E., Orejas C., and Dale M.R.T. "Size and Spatial Structure in Deep versus Shallow Populations of the Mediterranean Gorgonian Eunicella Singularis (Cap de Creus, Northwestern Mediterranean Sea)." *Marine Biology*, vol. 158, no. 8, 2011, pp. 1721–32, doi:10.1007/s00227-011-1686-7.

39. Guinan, Janine, Grehan A.J., Dolan M.F.J., and Brown C. "Quantifying Relationships between Video Observations of Cold-Water Coral Cover and Seafloor Features in Rockall Trough, West of Ireland." *Marine Ecology Progress Series*, vol. 375, 2009, pp. 125–38, doi:10.3354/meps07739.

40. Hall–Spencer, Jason, Allain V., and Fosså J.H. "Trawling Damage to Northeast Atlantic Ancient Coral Reefs." *Proceedings of the Royal Society of London. Series B: Biological Sciences*, vol. 269, no. 1490, 2002, pp. 507–511, doi:10.1098/rspb.2001.1910.

41. Henry, Lea-Anne, and Roberts, Murray. "Biodiversity and Ecological Composition of Macrobenthos on Cold-Water Coral Mounds and Adjacent off-Mound Habitat in the Bathyal Porcupine Seabight, NE Atlantic." *Deep Sea Research Part I: Oceanographic Research Papers*, vol. 54, no. 4, 2007, pp. 654–672, doi:10.1016/j.dsr.2007.01.005.

42. Hernández-Molina, F.j., Sierro F.J., Llave E., Roque C., Stow D.A.V., Williams T., Lofi J., Van der Schee M., Arnáiz A., Ledesma S., Rosales C., Rodríquez-Tovar F.J., Pardo-Igúzquiza E., and Brackenridge R.E. "Evolution of the Gulf of Cadiz Margin and Southwest Portugal Contourite Depositional System: Tectonic, Sedimentary and Paleoceanographic Implications from IODP Expedition 339." *Marine Geology*, vol. 377, 2016, pp. 7–39, doi:10.1016/j.margeo.2015.09.013.

43. Hughes, T, Bellwood D.R., Folke C., Steneck R.S., and Wilson J. "New Paradigms for Supporting the Resilience of Marine Ecosystems." *Trends in Ecology & Evolution*, vol. 20, no. 7, 2005, pp. 380–386, doi:10.1016/j.tree.2005.03.022.

44. ICES. 2018. Report of the ICES/NAFO Joint Working Group on Deep-water Ecology (WGDEC), 5-9 March 2018, Dartmouth, Nova Scotia, Canada. ICES CM 2018/ACOM:26. 126 pp.

45. IUCN. International Union for the Conservation of Nature. "The IUCN Red List of Threatened Species." Version 2019-2. http://www.iucnredlist.org. Downloaded on 18 July 2019.

46. Kazanidis, Georgios, Vad J., Henry L., Neat F., Berx B., Georgoulas K., and Roberts J.M."Distribution of Deep-Sea Sponge Aggregations in an Area of Multisectoral Activities and Changing Oceanic Conditions." *Frontiers in Marine Science*, vol. 6, 2019, doi:10.3389/fmars.2019.00163.

47. Keller, N. B., and N. S. Os'Kina. "Habitat Temperature Ranges of Azooxantellate Scleractinian Corals in the World Ocean." *Oceanology*, vol. 48, no. 1, 2008, pp. 77–84, doi:10.1134/s0001437008010098.

48. Komyakova, Valeriya, Jones G.J., and Munday P.L."Strong Effects of Coral Species on the Diversity and Structure of Reef Fish Communities: A Multi-Scale Analysis." *Plos ONE*, vol. 13, no. 8, 2018, doi:10.1371/journal.pone.0202206.

49. Kousteni, Vasiliki, Kasapidis P., Kotoulas G., and Megalofonou P. "Strong Population Genetic Structure and Contrasting Demographic Histories for the Small-Spotted Catshark (*Scyliorhinus canicula*) in the Mediterranean Sea." *Heredity*, vol. 114, no. 3, 2014, pp. 333–343, doi:10.1038/hdy.2014.107.

50. León, Federico R., Somoza L., Medialdea T., González F.J., Díaz-del-Rio V., Fernández-Puga M.C., and Maestro A. "Sea-Floor Features Related to Hydrocarbon Seeps in Deepwater Carbonate-Mud.

Mounds of the Gulf of Cádiz: From Mud Flows to Carbonate Precipitates." *Geo-Marine Letters*, vol. 27, no. 2–4, 2007, pp. 237–47, doi:10.1007/s00367-007-0074-2.

51. León, Ricardo, Somoza L., Medialdea T., and Vazquez J.T. "New Discoveries of Mud Volcanoes on the Moroccan Atlantic Continental Margin (Gulf of Cádiz): Morpho-Structural Characterization." *Geo-Marine Letters*, vol. 32, no. 5–6, 2012, pp. 473–88, doi:10.1007/s00367-012-0275-1.

52. Levin, Lisa A., Etter R.J., Rex M.A., Gooday A.J., Smith C.R., Pineda J., Stuart C.T., Hessler R.R., and Pawsonn D. "Environmental Influences on Regional Deep-Sea Species Diversity." *Annual Review of Ecology and Systematics*, vol. 32, no. 1, 2001, pp. 51–93, doi:10.1146/annurev.ecolsys.32.081501.114002.

53. Lo Iacono, Claudio, Robert K., Gonzalez-Villanueva R., Gori A., Gili J.M., and Orejas C. "Predicting Cold-Water Coral Distribution in the Cap de Creus Canyon (NW Mediterranean): Implications for Marine Conservation Planning." *Progress in Oceanography*, vol. 169, 2018, pp. 169–80, doi:10.1016/j.pocean.2018.02.012.

54. Medialdea, Teresa, Somoza L., Pinheiro L., and Fernández-Puga. "Tectonics and Mud Volcano Development in the Gulf of Cádiz." *Marine Geology*, vol. 261, no. 1-4, 2009, pp. 48–63, doi:10.1016/j.margeo.2008.10.007.

55. Morato, Telmo, Kvile K.Ø., Taranto G.H., Tempera F., Narayanaswamy B.E., Hebbeln D., Menezes G.,M., Wienberg C., Santos R.S., and Pitcher T.J. "Seamount Physiography and Biology in the North-East Atlantic and Mediterranean Sea." *Biogeosciences*, vol. 10, no. 5, 2013, pp. 3039–54, doi:10.5194/bg-10-3039-2013.

56. Naumann, Malik S. Orejas C., and Ferrier-Pagès C. "Species-Specific Physiological Response by the Cold-Water Corals Lophelia Pertusa and Madrepora Oculata to Variations within Their Natural Temperature Range." *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 99, 2014, pp. 36–41, doi:10.1016/j.dsr2.2013.05.025.

57. Orejas, Covadonga, Addamo A.,; Alvarez M., Aparicio A., Alcoverro D., Arnaud-Haond S., Bilan M., Boavida J., Cainzos V., Calderon R., Cambeiro P., Castano M., Fox, A., Gallardo, M., Gori A., Guitierrez C., Henry L.A., Hermida M., Jimenez J.A.A., Lopez-Jurado J.L., Lozano P., Mateo-Ramirez A., Mateu G., Matoso J.L., Mendez C., Morillas A., Movilla J., Olariaga A., Paredes M., Pelayo V., Pineiro S., Rakka M., Ramirez T., Ramos M.R.J., Rivera J., Romero A., Rueda J.L.L., Salvador T., Sampaio I., Sanchez H., Santiago R., Serrano A., Taranto G., Urra J., Velez-Belchi P., Viladrich N., and Zein M (2017). Cruise Summary Report – MEDWAVES Survey. (MEDiterranean out flow WAter and Vulnerable EcosystemS) ATLAS Project H2020. 211 pp and Appendixes.

58. Orejas, Covadonga, Gori A., Lo Iacono C., and Puig P. "Cold-Water Corals in the Cap de Creus Canyon, Northwestern Mediterranean: Spatial Distribution, Density and Anthropogenic Impact." *Marine Ecology Progress Series*, vol. 397, 2009a, pp. 37–51, doi:10.3354/meps08314.

59. Orejas, Covadonga, and Andrea Gori. "Special Distribution of Madrepora Oculata, Lophelia Pertusa and Dendrophyllia Cornigera in the Submarine Canon of Cap de Creus (NorthWest Mediterannean) from Video Transect Analysis." *Marine Ecology in Progress Series*, vol. 397, no. 37-51, 2009b, doi: 10.3354/meps08314.

60. Palanques, Albert, Durrieu de Madron X., Puig P., Fabres J., Guillén J., Calafat A., Canals M., Heussner S., and Bonnin J. "Suspended Sediment Fluxes and Transport Processes in the Gulf of Lions Submarine Canyons. The Role of Storms and Dense Water Cascading." *Marine Geology*, vol. 234, no. 1-4, 2006, pp. 43–61, doi:10.1016/j.margeo.2006.09.002.

61. Palomino, Desirée, Lopez-Gonzalez N., Vazquez J.T., Fernández Salas L.M. "Multidisciplinary Study of Mud Volcanoes and Diapirs and Their Relationship to Seepages and Bottom Currents in the Gulf of Cádiz Continental Slope (Northeastern Sector)." *Marine Geology*, vol. 378, 2016, pp. 196–212, doi:10.1016/j.margeo.2015.10.001.

62. Personnic, Sébastien, Boudouresque C.F., Astruch P., Ballesteros E., Blouet S., Bellan-Santini D., Bonhomme P., Thibault-Botha D., Feunteun E., Harmelin-Vivien M., Pergent G., Pergent-Martini C., Pastor J., Poggiale J.C., Renaud F., Thibaut T., and Ruitton S. "An Ecosystem-Based Approach to Assess the Status of a Mediterranean Ecosystem, the Posidonia Oceanica Seagrass Meadow." *PLoS ONE*, vol. 9, no. 6, 2014, doi:10.1371/journal.pone.0098994.

63. Pham, Christopher K., Frederic V., Gui M., Filipe P., Eduardo I., and Telmo M. "The Importance of Deep-Sea Vulnerable Marine Ecosystems for Demersal Fish in the Azores." *Deep-Sea Research Part I: Oceanographic Research Papers*, vol. 96, 2015, pp. 80–88, doi:10.1016/j.dsr.2014.11.004.

64. Pinheiro, L.M., Ivanov M.K., Sautkin A., Akhmanov G., Volonskaya A., Monteiro J.H., Somoza L., Gardner J., Hamoumi N., and Cunha M.R., "Mud Volcanism in the Gulf of Cadiz: Results from the TTR-10 Cruise." *Marine Geology*, vol. 195, no. 1-4, 2003, pp. 131–151, doi:10.1016/s0025-3227(02)00685-0.

65. Puerta, Patricia, Hunsicker M.E., Hidalgo M., Reglero P., Ciannelli L., Esteban A., González M., Quetglas A. "Community–Environment Interactions Explain Octopus-Catshark Spatial Overlap." *ICES Journal of Marine Science: Journal Du Conseil*, vol. 73, no. 7, 2016, pp. 1901–1911, doi:10.1093/icesjms/fsw053.

66. Purser, Autun, Orejas C., Gori A., and Tong., R. "Local Variation in the Distribution of Benthic Megafauna Species Associated with Cold-Water Coral Reefs on the Norwegian Margin." *Continental Shelf Research*, vol. 54, 2013, pp. 37–51, doi:10.1016/j.csr.2012.12.013.

67. Ramiro-Sánchez, Berta, González-Irusta J.M., Henry L.A., Cleland J., Yeo I., Xavier J.R., Carreiro-Silva M., Sampaio I., Spearman J., Victorero L., Messing C.G., Kazanidis G., Roberts J.M., and Muron B. "Characterization and Mapping of a Deep-Sea Sponge Ground on the Tropic Seamount (Northeast Tropical Atlantic): Implications for Spatial Management in the High Seas." *Frontiers in Marine Science*, vol. 6, 2019, doi:10.3389/fmars.2019.00278.

68. Relini, L. Orsi, Mannini A., Fiorentino F., and Palandri G. “Biology and Fishery of Eledone Cirrhosa in the Ligurian Sea.” *Fisheries Research*, vol. 78, no. 1, 2006, pp. 72–88, doi:10.1016/j.fishres.2005.12.008.

69. Regueira, Marcos, González A.F. and Guerra A., Soares A. “Reproductive Traits of Horned Octopus Eledone Cirrhosa in Atlantic Iberian Waters.” *Journal of the Marine Biological Association of the United Kingdom*, vol. 93, no. 6, 2013, pp. 1641–1652, doi:10.1017/s0025315413000118.

70. Reveillaud, Julie, Freiwald A., Van Rooij D., Le Guillou E., Altuna A., Foubert A., Olu-Le Roy K., and Henriet J.P. “The Distribution of Scleractinian Corals in the Bay of Biscay, NE Atlantic.” *Facies*, vol. 54, no. 3, 2008, pp. 317–331, doi:10.1007/s10347-008-0138-4.

71. Rossi, Sergio, Bramanti L., Gori A., and Orejas C. *Marine Animal Forests*, New York City, Springer, 2017, pp. 1-1369, doi:10.1007/978-3-319-17001-5_1-1.

72. Rueda, José L., González-García E., Marina P., and Oporto T. “Biodiversity and Geodiversity in the Mud Volcano Field of the Spanish Margin (Gulf of Cádiz).” *7 Simpósio sobre a Margem Ibérica Atlântica,* 2015, pp. 137–41, doi:10.13140/2.1.3244.3849.

73. Rueda, José L., and Nieves Lopez-gonzalez. “De Especies Bentónicas En El Talud Continental Del Golfo de Cádiz Interacción Entre Las Masas de Agua , Los Relieves Submarinos y La Distribución de Especies Bentónicas En El Talud Continental Del Golfo de Cádiz.” *Ministerio de Agricultura, Alimentación y Medio Ambiente*, 2012.

74. Rueda, José L., González-García E., Krutzky C., and López-Rodriguez F.J. “From Chemosynthesis-Based Communities to Cold-Water Corals: Vulnerable Deep-Sea Habitats of the Gulf of Cádiz.” *Marine Biodiversity*, vol. 46, no. 2, 2016, pp. 473–82, doi:10.1007/s12526-015-0366-0.

75. Sampaio, Íris., Braga-Henriques A., Pham C., Ocaña O., de Matos V., Morato T., and Porteiro F.M. “Cold-Water Corals Landed by Bottom Longline Fisheries in the Azores (North-Eastern Atlantic).” *Journal of the Marine Biological Association of the United Kingdom*, vol. 92, no. 7, 2012, pp. 1547–1555, doi:10.1017/s0025315412000045.

76. Sánchez, Francisco, González-Pola C., Druet M., García-Alegre A., Acosta J., Cristobo J., Parra S., Ríos P., Altuna A., Gómez-Ballesteros M., Muñoz-Recio A., Rivera J., and Diaz del Rio G.“Habitat Characterization of Deep-Water Coral Reefs in La Gaviera Canyon (Avilés Canyon System, Cantabrian Sea).” *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 106, 2014, pp. 118–140, doi:10.1016/j.dsr2.2013.12.014.

77. Sánchez-Leal, Ricardo F., Bellanco M.J., Fernández-Sala L.M., Lafuente J.G., Gasser-Rubinat M., González-Pola C., Hernández-Molina F.J., Pelegri J.L., Peliz A., Relvas P., Roque D., Ruiz-Villarreal M., Sammartino S., and Sánchez-Garrido J.C. “The Mediterranean Overflow in the Gulf of Cadiz: A Rugged Journey.” *Science Advances*, vol. 3, no. 11, 2017, doi:10.1126/sciadv.aao0609.

78. Sánchez, Pedro., Beclari P., and Sartor P. "Composition and Spatial Distribution of Cephalopods in Two North-Western Mediterranean Areas." *South African Journal of Marine Science*, vol. 20, no. 1, 1998, pp. 17–24, doi:10.2989/025776198784126584.

79. Sartor, P., Beclari P., Carboell A., and González M. "The Importance of Cephalopods to Trawl Fisheries in the Western Mediterranean." *South African Journal of Marine Science*, vol. 20, no. 1, 1998, pp. 67–72, doi:10.2989/025776198784126313.

80. Sitjà, Cèlia, and Manuel Maldonado. "New and Rare Sponges from the Deep Shelf of the Alboran Island (Alboran Sea, Western Mediterranean)." *Zootaxa*, vol. 3760, no. 2, 2014, p. 141, doi:10.11646/zootaxa.3760.2.2.

81. Sitjà, Cèlia., Maldonado M., Farias C., and Rueda J.L. "Deep-Water Sponge Fauna from the Mud Volcanoes of the Gulf of Cadiz (North Atlantic, Spain)." *Journal of the Marine Biological Association of the United Kingdom*, vol. 99, no. 4, 2018, pp. 1–25, doi:10.1017/s0025315418000589.

82. Somoza, Luis, Ercilla G., Urgorri V., León R., Medialdea T., Paredes M., Gonzalez F.J., and Nombela M.A. "Detection and Mapping of Cold-Water Coral Mounds and Living Lophelia Reefs in the Galicia Bank, Atlantic NW Iberia Margin." *Marine Geology*, vol. 349, 2014, pp. 73–90, doi:10.1016/j.margeo.2013.12.017.

83. Sweetman, Andrew K., Thurber A.R., Smith C.R., Levin L.A., Mora C., Wei C.L., Gooday A.J., Jones D.O.B., Rex M., Yasuhara M., Ingels J., Ruhl H.A., Frieder C.A., Danovaro R., Würzberg L., Baco A., Grupe B.M., Pasulka A., Meyer K.S., Dunlop K.M., Henry L.A., Roberts J.M. "Major Impacts of Climate Change on Deep-Sea Benthic Ecosystems." *Elementa: Science of the Anthropocene*, vol. 5, no. 0, 2017, pp. 4, doi:10.1525/elementa.203.

84. Thiem, Øyvind, Ravagnan E., Fosså J.H., Berntsen J. "Food Supply Mechanisms for Cold-Water Corals along a Continental Shelf Edge." *Journal of Marine Systems*, vol. 60, no. 3-4, 2006, pp. 207–219, doi:10.1016/j.jmarsys.2005.12.004.

85. Thurber, Andrew. R., Sweetman A.K., Narayanaswamy B.E., Jones D.O.B., Ingels J., and Hansman R.L. "Ecosystem Function and Services Provided by the Deep Sea." *Biogeosciences*, vol. 11, no. 14, 2014, pp. 3941–3963, doi:10.5194/bg-11-3941-2014.

86. Van den Beld, Inge M. J., Bourillet J.F., Arnaud-Haond S., Chambure L., Davies J.S., Guillaumont B., Olu K., and Menot L. "Cold-Water Coral Habitats in Submarine Canyons of the Bay of Biscay." *Frontiers in Marine Science*, vol. 4, pp. 1-30, 2017, doi:10.3389/fmars.2017.00118.

87. Vanreusel, Ann, et al. "Biodiversity of Cold Seep Ecosystems Along the European Margins." *Oceanography*, vol. 22, no. 1, 2009, pp. 110–127, doi:10.5670/oceanog.2009.12.

88. Van Rooij, D., Iglesias J., Hernández-Molina F.J., Ercilla G., Gomez-Ballesteros M., Casas D., Llave E., De Hauwere A., García-Gil S., Acosta J., and Henriet J.P. "The Le Danois Contourite Depositional System: Interactions between the Mediterranean Outflow Water and the Upper Cantabrian Slope (North

Iberian Margin).” *Marine Geology*, vol. 274, no. 1-4, 2010, pp. 1–20, doi:10.1016/j.margeo.2010.03.001.

89. Wienberg, Claudia, Hebbeln D., Fink H.G., Mienis F., Dorchel B., Vertino A., López Correa M., and Freiwald A. “Scleractinian Cold-Water Corals in the Gulf of Cádiz-First Clues about Their Spatial and Temporal Distribution.” *Deep-Sea Research Part I: Oceanographic Research Papers*, vol. 56, no. 10, 2009, pp. 1873–93, doi:10.1016/j.dsr.2009.05.016.

90. Wienberg, Claudia, Frank N., Mertens K.N., Stuut J.B., Merchant M., Fietzke J., Mienis F. and Hebbeln D. “Glacial Cold-Water Coral Growth in the Gulf of Cádiz: Implications of Increased Palaeo-Productivity.” *Earth and Planetary Science Letters*, vol. 298, no. 3-4, 2010, pp. 405–416, doi:10.1016/j.epsl.2010.08.017.

91. Willig, Michael R., Kaufman D.M. and Stevens R.D. “Latitudinal Gradients of Biodiversity: Pattern, Process, Scale, and Synthesis.” *Annual Review of Ecology, Evolution, and Systematics*, vol. 34, no. 1, 2003, pp. 273–309, doi:10.1146/annurev.ecolsys.34.012103.144032

LIST OF ANNEXES

Annex 1.

Complete list of all Operational Taxonomic Units (OTUs) observed in the linear transect at Gazul Mud Volcano (GMV).

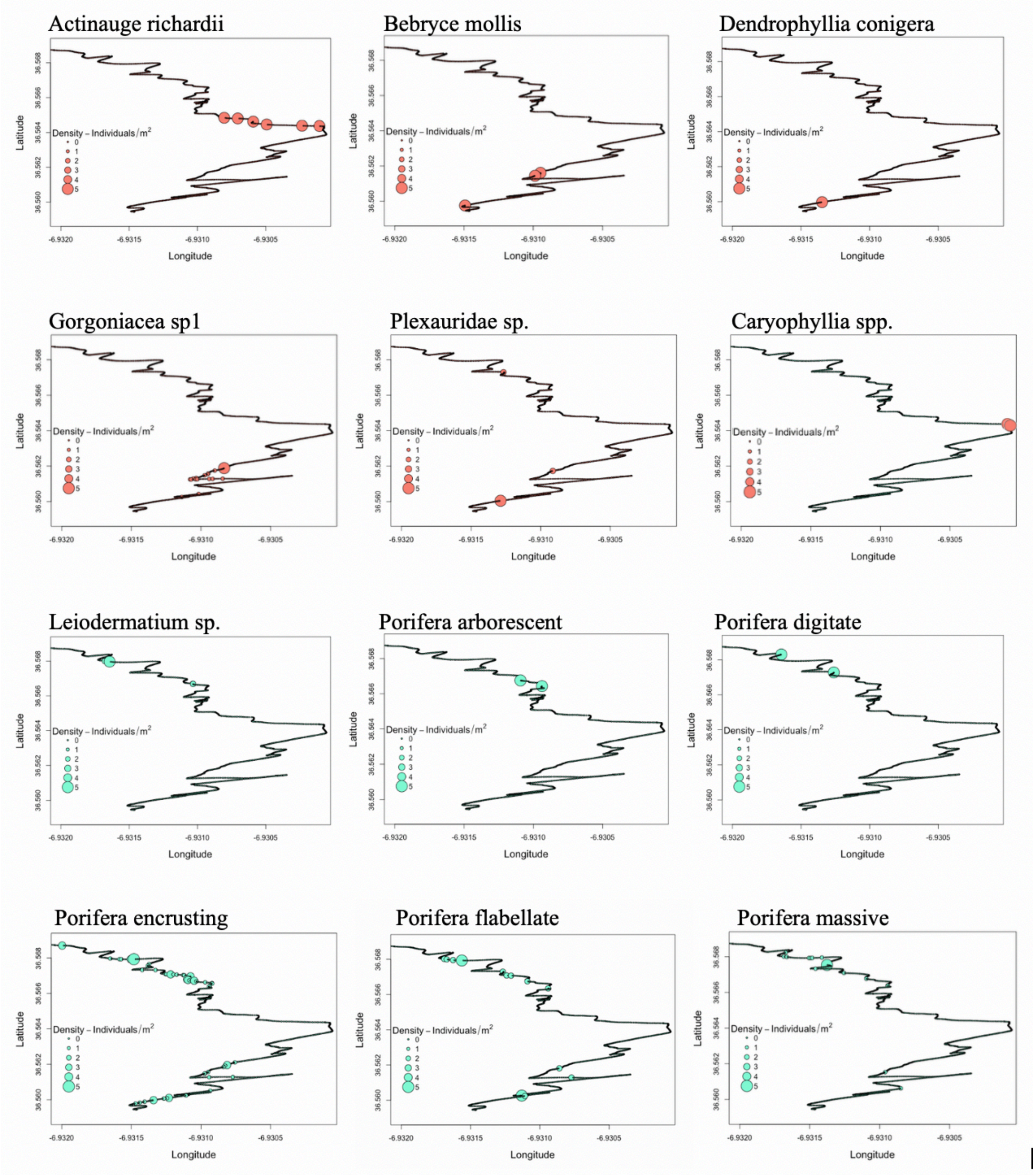
Phylum	Class	Order	Family	OTU	Threatened Status	ID Number
Arthropoda	Malaconstraca	Decapoda	Munididae	*Munida* sp.		1
			Palinuridae	*Palnurus mauritanicus*		2
			Unidentified	Malaconstraca sp1		3
Annelida	Polychaeta	Sabellida	Sabellidae	Sabellidae sp1		4
		Unidentified	Unidentified	Polychaeta sp1		5
Chordata	Actinopterygii	Gadiformes	Macrouidae	*Ceolorhynchus coelorhynchus*		6
			Gadidae	*Gadiculus argenteus*	LC	7
		Scorpaeniformes	Scorpaenidae	*Helicolenus dactylopterus*	LC	8
		Unidentified	Unidentified	Fish		37
	Ascidiacea	Enterogona	Ascidiidae	*Polycarpa* sp1	LC	9
	Chondrichthyes	Carcharhiniforme	Scyliorhinidae	*Scyliorhinus canicula*	LC	10
Cnidaria	Anthozoa	Actinaria	Hormathiidae	*Actinauge richardii*	DD	11
		Alcyonacea	Acanthogorgiida	*Acanthogorgia* spp.	DD / LC	12
			Chrysogorgiidae	*Radicipes cf. gracilis*		13
			Primnoidae	*Callogorgia verticillatta*	NT	14
			Plexauridae	*Bebryce mollis*	DD	15
			Plexauridae	Plexauridae sp.	VU	16
		Scleratinia	Caryophyliidae	*Caryophyllia* spp.	LC	17
				Lophelia pertusa	EN	18
			Dendrophyliidae	*Dendrophyllia cornigera*	EN	19
			Flabellidae	*Flabellum chunii*	DD	20
			Gorgoniidae	Gorgoniacea sp1		41

			Oculinidae	*Madrepora oculata*	CR	21
Echinodermata	Asteroidea	Unidentified	Unidentified	Asteroidea sp1		22
		Diadematoida	Diadematidae	*Centrostephanus longispinus*		23
		Valvatida	Chaetasteridae	*Chaetaster longpipes*		24
			Ophidiasteridae	*Hacelia superba*		25
	Crinoidea	Unidentified	Unidentified	*Crinoidea* sp1		26
	Echinoidea	Camarodonta	Echinidae	*Graciilechinus acutus*		27
		Cidaroida	Cidaridae	*Cidaris cidaris*		28
	Holothuroidea	Synallactida	Stichopodidae	*Parastichopus regalis*		29
Mollusca	Bivalvia	Unidentified	Unidentified	Bivalvia sp1		30
	Cephlapoda	Octopoda	Eledonidae	*Eledone cirrhosa*	LC	31
Porifera	Desmospongaie	Tetractinellida	Azoricidae	*Leiodermatium* sp.	LC	33
		Axinellida	Axinellidae	*Phakelia ventilabrum*		32
		Lyssacinosida	Rossellidae	*Asconema setubalense*	LC	34
	Hexactinellida	Unidentified	Unidentified	Porifera encrusting		35, j), k), l)
	Unidentified			Porifera digitate		38
				Porifera flabellate		39, a), b)
				Porifera globular		40, c), d)
				Porifera lamellate		42, e), f), g)
				Porifera massive		43, h), j)
				Porifera stony		44
				Porifera sp1		36
				Porifera sp2		45

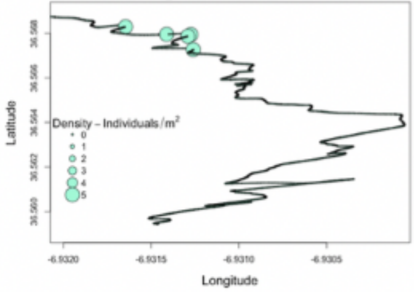
Operational Taxonomic Units (OTUs) are grouped by phylum, class, order, family then species level. When identification was not possible, a general morphotype name was given. The global threatened status' are listed for some important OTUs (IUCN, 2019): LC = least concern, DD = data deficient, NT = not threatened, VU = vulnerable, EN = endangered, CR = critically endangered. Photo identification of all OTUs can be found below in Annex 3.

Annex 2.

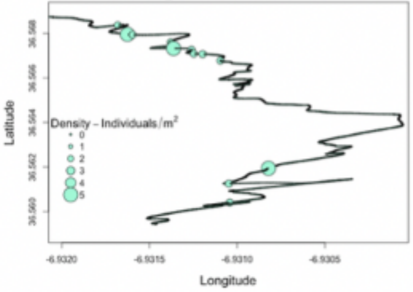
Density plots for each Operational Taxonomic Unity (OTU) observed along the linear transect at Gazul Mud Volcano.



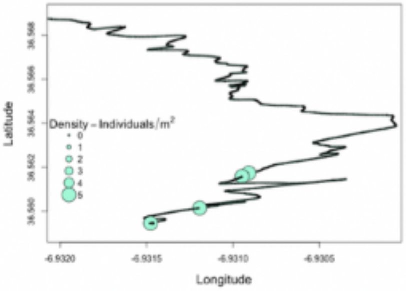
Porifera sp1



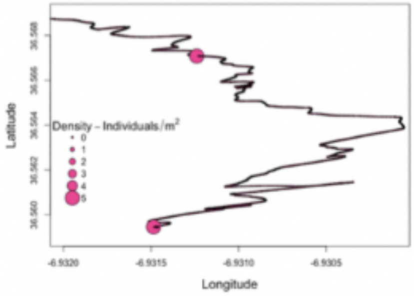
Porifera sp2



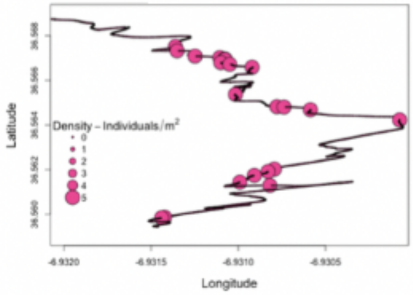
Porifera stony



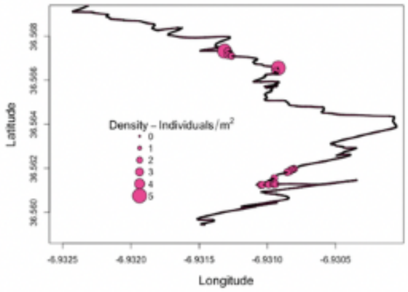
Chaetaster longpipes



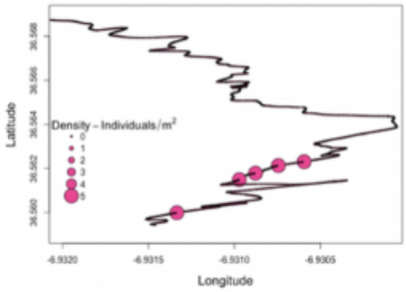
Cidaris cidaris



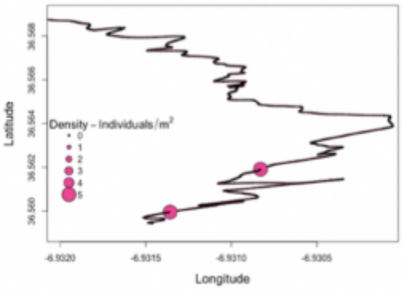
Crinoidea sp1



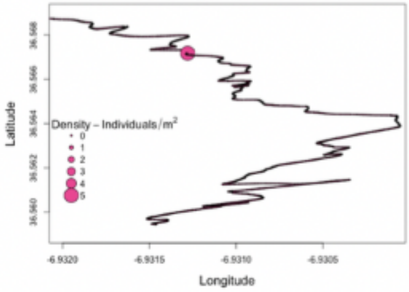
Graciliechinus acutus



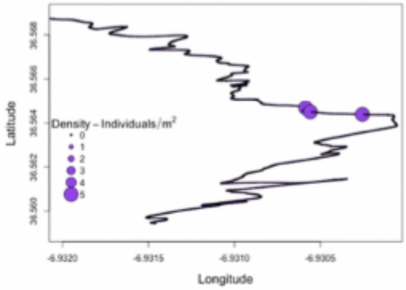
Hacelia superba



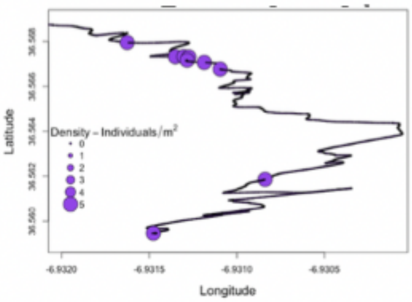
Parastichopus regalis



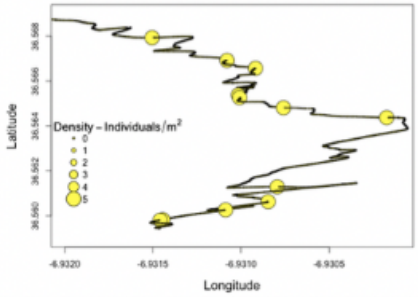
Bivalvia Sp1

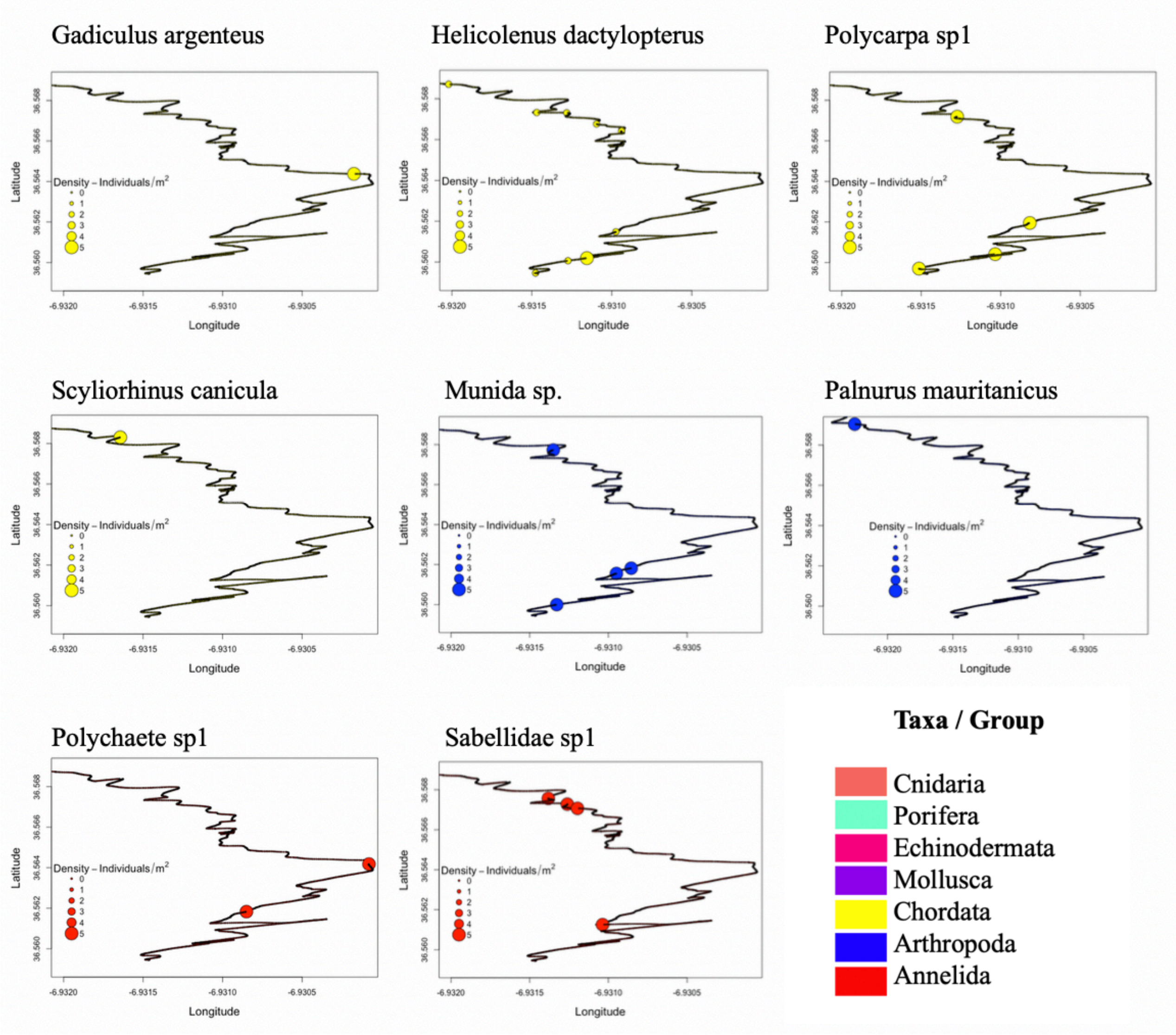


Eledone cirrhosa



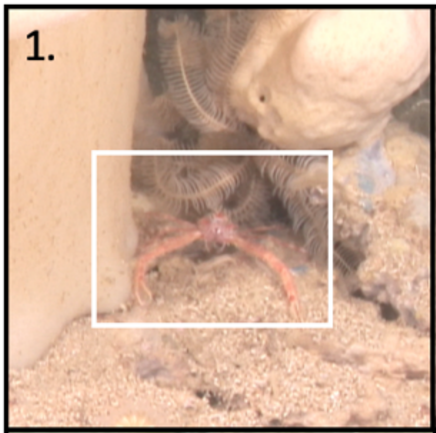
Fish

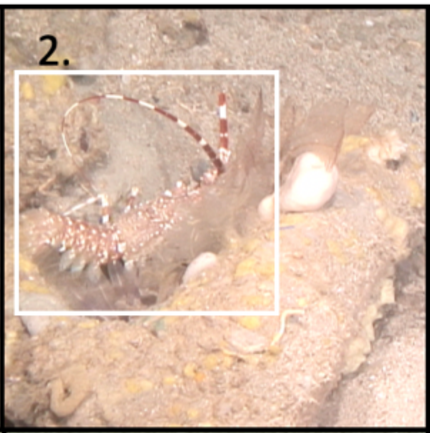


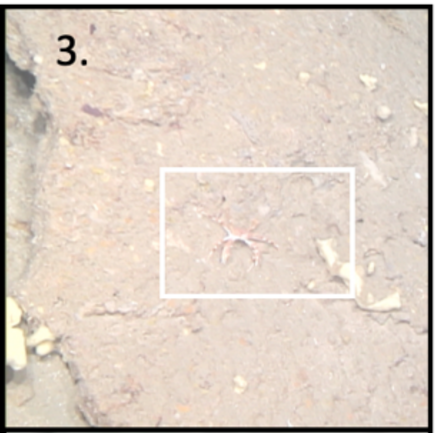

Gadiculus argenteus
Helicolenus dactylopterus
Polycarpa sp1
Scyliorhinus canicula
Munida sp.
Palnurus mauritanicus
Polychaete sp1
Sabellidae sp1
Latitude
Longitude
Density – Individuals/m²
0
1
2
3
4
5
36.560
36.562
36.564
36.566
36.568
-6.9320
-6.9315
-6.9310
-6.9305
Taxa / Group
Cnidaria
Porifera
Echinodermata
Mollusca
Chordata
Arthropoda
Annelida

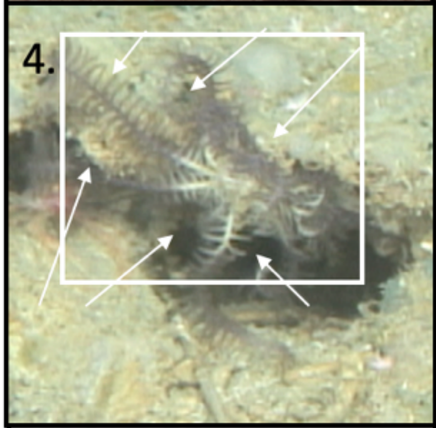
Annex 3.

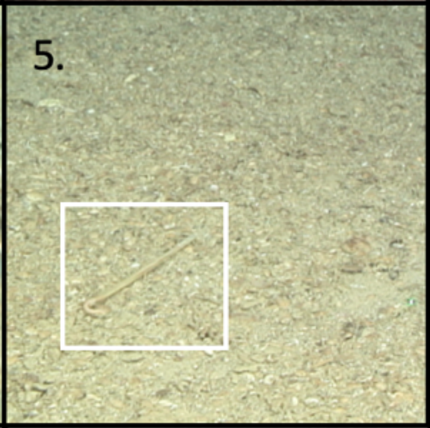
Operational Taxonomic Unit (OTU) photo catalogue for visual identification. All photos were obtained from the Remote Operated Vehicle video footage except photo #18, source: NOAA-Pelagic Research Services. Identification numbers in the photos refer to ID number in Annex 1, whereas identification letters in the photos refer to the exact morphotype of the various OTUs.

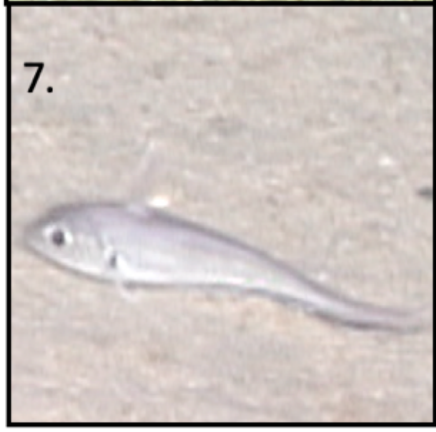


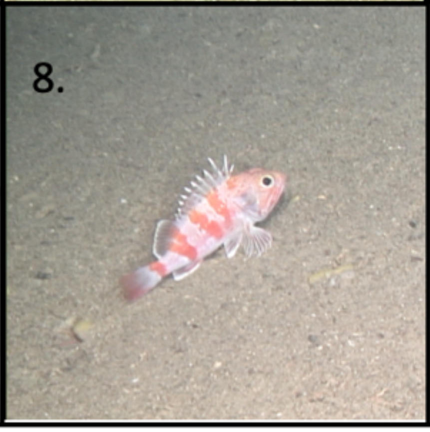


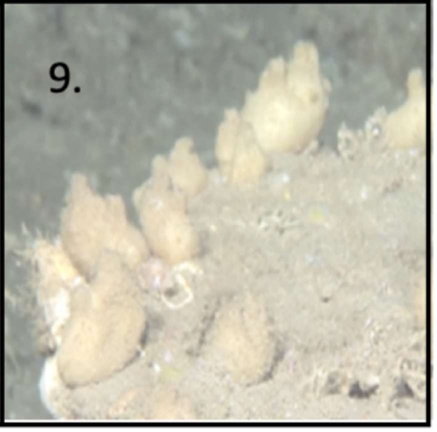












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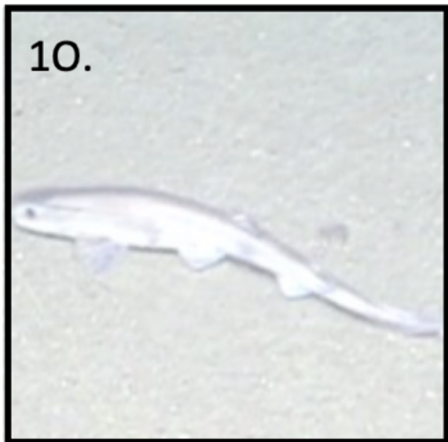

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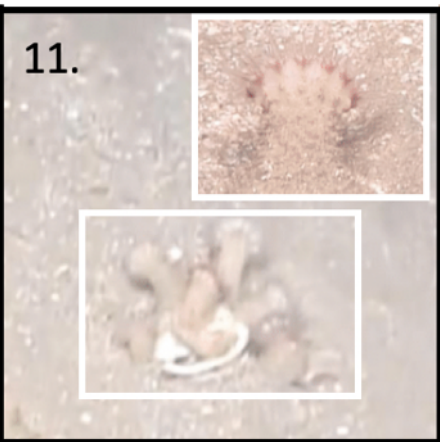

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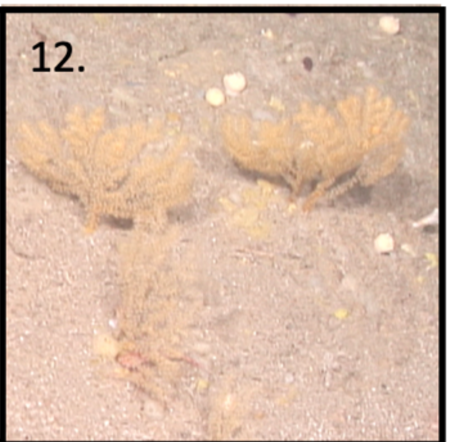

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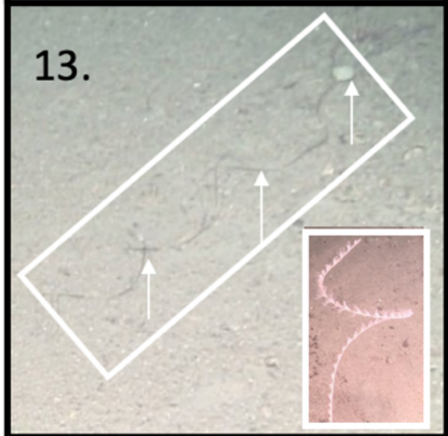
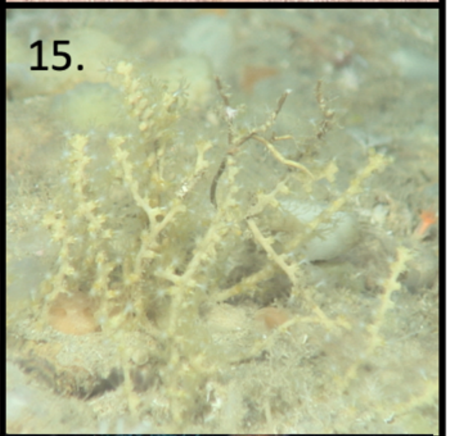


14.

15.



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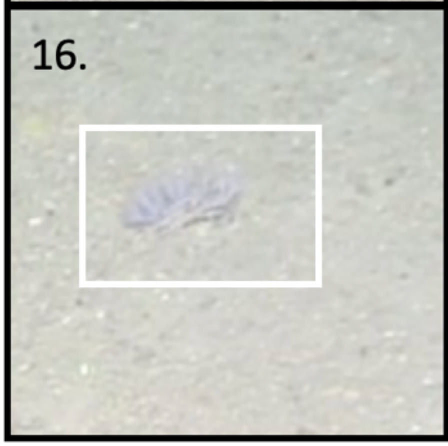

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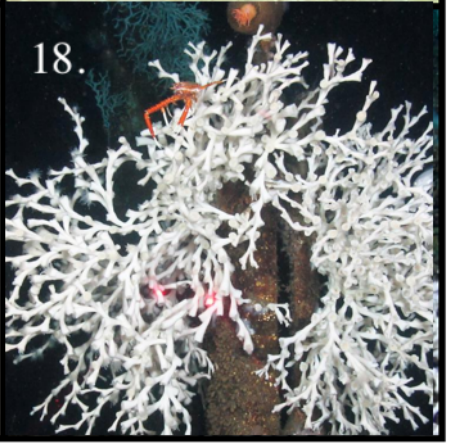

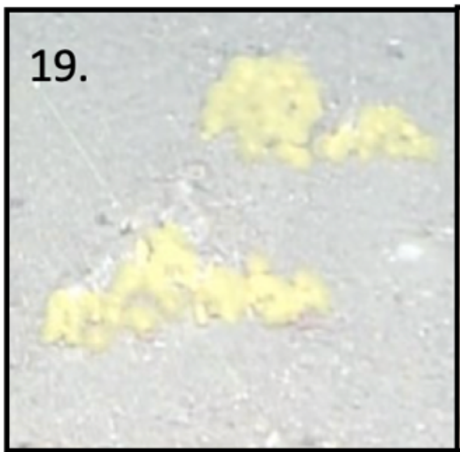
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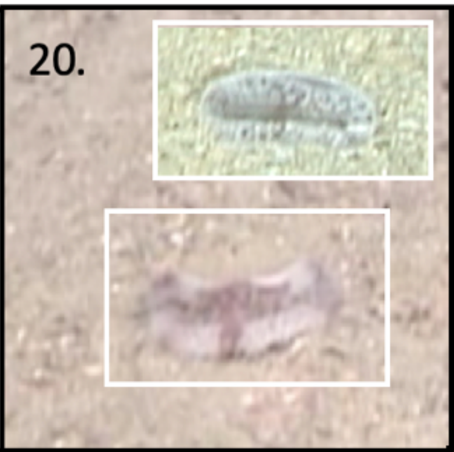
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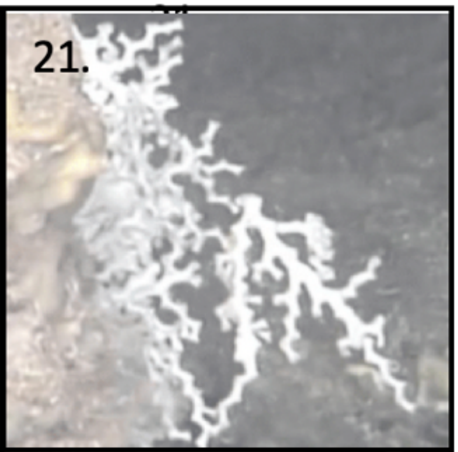
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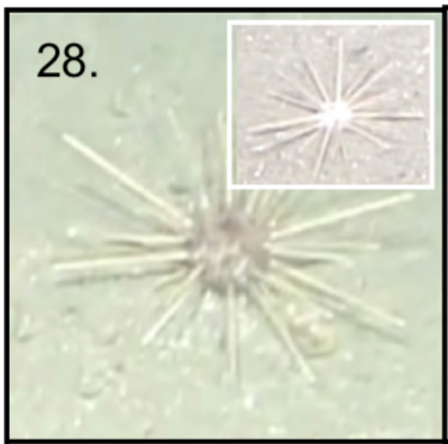
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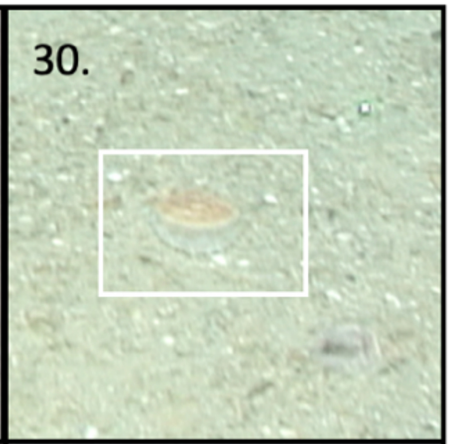
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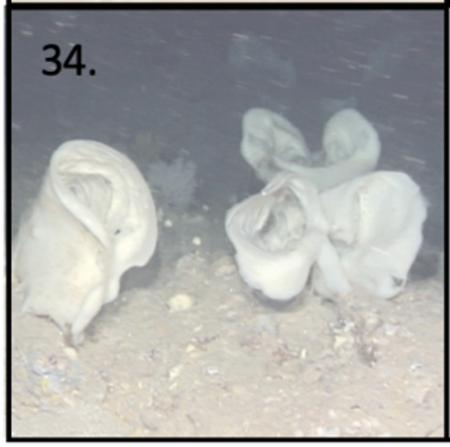
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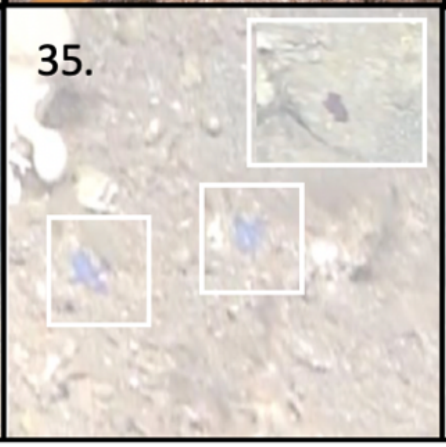
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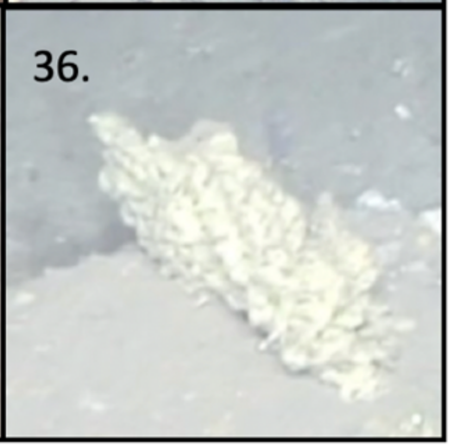
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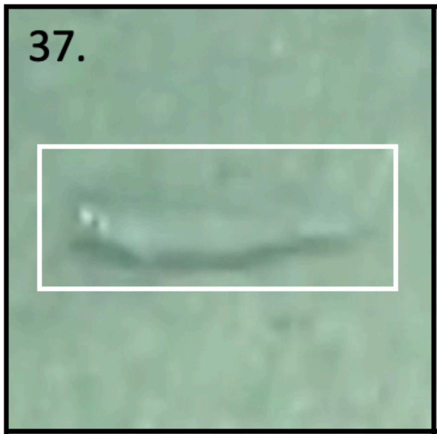

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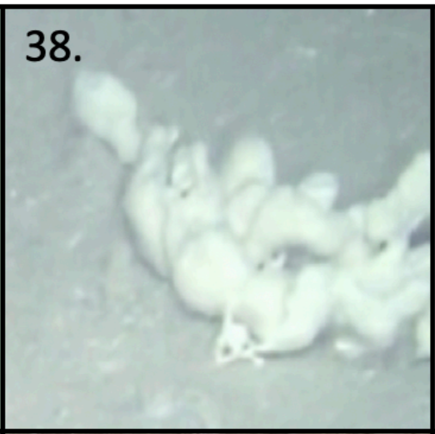

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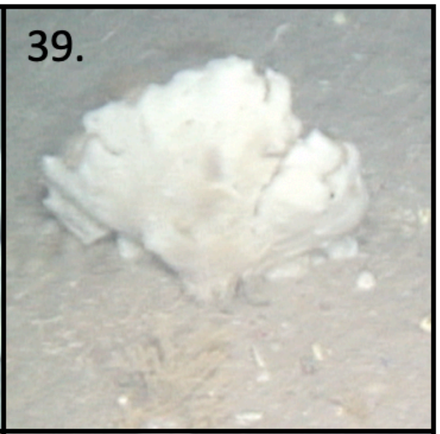

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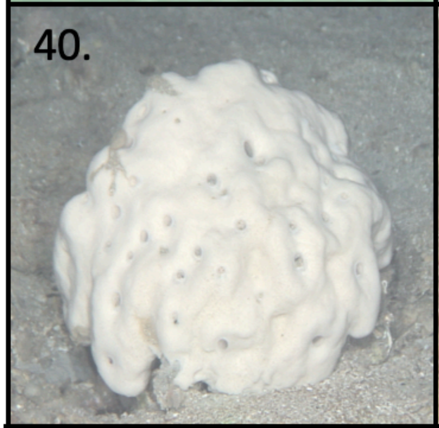

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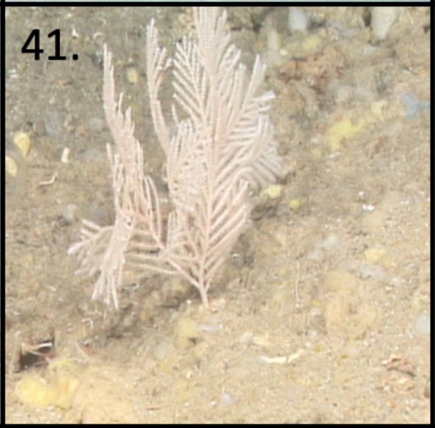

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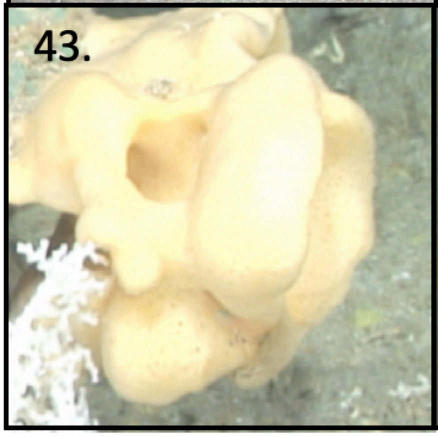

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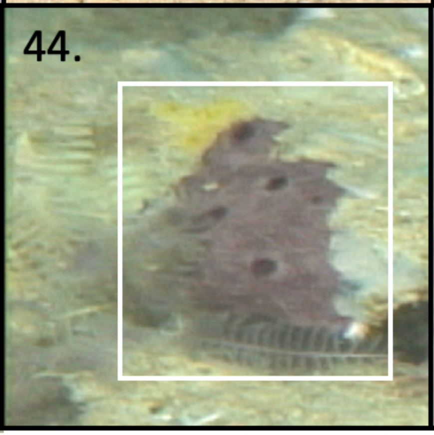

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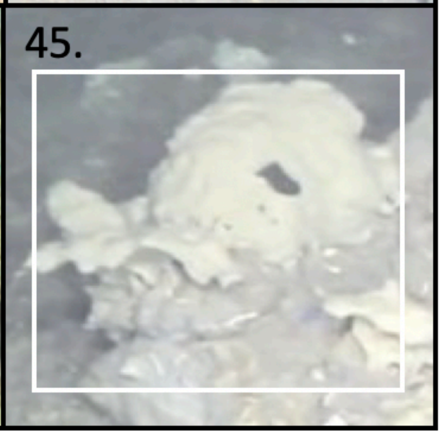

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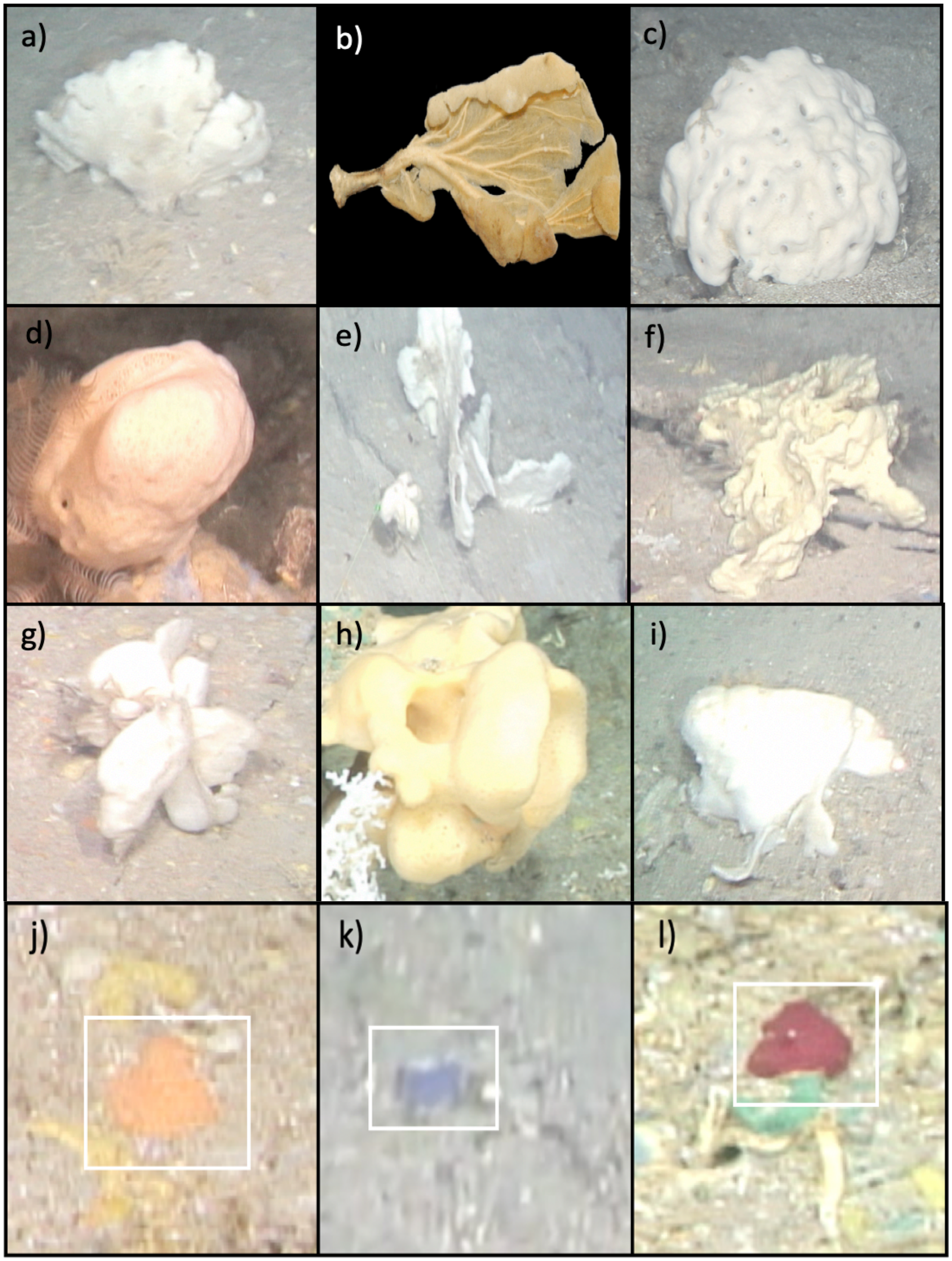

Some Operational Taxonomic Units (OTUs) encompass several taxa with the same morphotype: the OTU Porifera flabellate includes diverse sponge species such as those represented in a) and b). Porifera globular includes c) and d); Porifera lamellate comprises e), f) and g); Porifera massive includes h) and i) and Porifera encrusting includes j), k) and l). The two OTUs of White Corals can be found in photo #18 and #21.