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Evidences of human impact on megabenthic assemblages of bathyal sediments in the Alboran Sea (western Mediterranean)

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Abstract

Megabenthic assemblages in deep-sea sedimentary environments receive far less attention than those occurring on rocky environments, despite they have been widely impacted by destructive trawling activities, mainly due to their association with important commercial species. ROV dives conducted on bathyal muds of the Alboran Sea continental slope (western Mediterranean) were used to characterize megabenthic assemblages, as well as assess their response to trawling and benthic litter. We identified a multispecific assemblage, dominated by the isidid *Isidella elongata*, and two monospecific assemblages composed by the sea-pens *Funiculina quadrangularis* and *Kophobelemnon stelliferum*. These assemblages are defined as vulnerable marine ecosystems by international institutions. Trawled areas exhibit significant low densities of habitat-forming species and a striking impoverishment of habitat complexity and diversity. Plastic debris and lost fishing gears were the most abundant components of the marine litter. This study highlights the destructive effects of human activities on bathyal muds, emphasizing the need for urgent conservation measures.

Keywords: Mediterranean Sea, vulnerable marine ecosystems, megabenthos, soft sediments, bottom trawling, marine litter.

1. Introduction

The present-day advances in underwater technologies eased the access to scientific Remotely Operated Vehicles (ROVs), Autonomous Underwater Vehicles (AUVs) and high-frequency imaging systems, able to operate at high depths during increasingly long acquisition periods (Wynn et al., 2014; Aguzzi et al., 2019; Price et al., 2019; Bo et al., 2020). These advances generate a sudden expansion on the deep-sea benthic ecology studies, and related physical processes based on image analysis and high-resolution acoustic mapping (e.g. Benoist et al., 2019; Corbera et al., 2019). In parallel, the technological expansion in marine sciences enhanced the awareness that human-related activities exert a strong and pervasive impact on deep-sea ecosystems, with a detrimental effect on habitat complexity and biodiversity (de Moura-Neves et al., 2015; Clark et al., 2019).

Image-based investigations of deep-sea habitats were mainly focused on benthic communities or key structuring species occurring on hard substrates (Mortensen and Buhl-Mortensen, 2005; Althaus et al., 2009; Price et al., 2019), while similar studies on deep-sea sedimentary settings are scarce (Robert and Juniper, 2012; De Leo et al., 2017). This represents a significant knowledge gap, considering that deep-sea sediments are among the most widespread benthic habitats in the world, covering vast areas of continental shelves, slopes and abyssal plains (Gray, 2002). Deep-sea sedimentary environments have been traditionally considered as barren fields sparsely populated by megabenthic organisms (Jones et al., 2007). However, in specific areas, deep-sea sedimentary floors have been shown to host three-dimensional complex megabenthic assemblages, such as sponge grounds or sessile anthozoan aggregations (de Moura-Neves et al., 2015; Maldonado et al., 2015). These assemblages locally enhance biomass and act as biodiversity hotspots, as they provide habitat for a large variety of associated species, including a wide range of species of economic interest

(Maynou and Cartes, 2012; de Moura-Neves et al., 2015; Maldonado et al., 2017; Mastrototaro et al., 2017; Pierdomenico et al., 2018).

Deep-sea benthic communities are generally composed of fragile organisms having very slow recovering rates (Clark et al., 2019). Consequently, the impact of over-exploiting human activities produce extremely long-lasting degrading effects (Jobstvogt et al., 2014; Vanreusel et al., 2016; Clark et al, 2019).

In 2006, the need to preserve Vulnerable Marine Ecosystems (VMEs) from the negative impacts caused by human activities, led to the development of specific management and conservation measures to prevent their degradation (UNGA, 2007). To achieve this goal, several international strategies, such as the "*International guideline for the management of the deep-sea fisheries in the high seas*" (FAO, 2009) or the EU's Marine Strategy Directive (2008/56/CE), have been developed during the last decade. Deep-sea soft sedimentary environments of the Mediterranean Sea host several VMEs, mainly represented by the isidid *Isidella elongata* and the pennatulaceans *Funiculina quadrangularis* and *Kophobelemnon stelliferum.*

Amongst the many anthropogenic impacts on the marine environment, bottom trawling is the widest spread physical disturbance in the world's seabed (Watling, 2013; Hiddink et al., 2017). Impacts associated to bottom trawling include removal or damage to epibenthic fauna, reduction of habitat complexity and alteration of sedimentary and geochemical processes (e.g. De Leo et al., 2017; Hiddink et al., 2017; Paradis et al., 2019). With bottom trawling progressively expanding to deeper environments, VMEs associated to sedimentary bottoms have undergone a vast decimation (Sardà et al., 2004; Gerovasileiou et al., 2019; Pusceddu et al., 2014), restricting their distribution to isolated regions or areas inaccessible to trawlers, such as sub-vertical walls or regions surrounded by rocky outcrops (Bo et al., 2015; Pierdomenico et al., 2018).

Marine litter represents a further extremely pervasive and fast-growing anthropogenic pollution affecting the world’s oceans (Bergmann et al., 2015). Specifically, benthic marine debris, defined as the macro-litter that settles on the seafloor (Spengler and Costa, 2008), can damage benthic organisms through entanglement or coverage (Pham et al., 2014; Bergman et al., 2015). Litter and especially plastic debris are a considerable source of persistent organic pollutants and chemical additives, which are toxic to marine species (Rochman et al., 2013). The breakdown products of plastic, the so-called microplastics, are proven to be ingested by a variety of organisms, with potential ecotoxicological effects still poorly assessed (Browne et al., 2013; Carreras-Colom et al., 2018; Cau et al., 2019a).

Most of the information regarding VMEs of deep-sea sediments derives from invasive sampling gears (e.g. experimental trawling, epi-benthic sledge) (Gili and Ros, 1987; Maynou and Cartes, 2012; Mastrototaro et al., 2013), which despite providing relevant information on species occurrence, deliver limited knowledge on sessile taxa abundance, assemblage composition, habitat characteristics and their state of conservation (Soltwedel et al., 2009; Chimienti et al., 2018). Recently, several image-based studies carried out in the canyon heads of the Gulf of Lion and Tyrrhenian Sea, and the open slope of the Balearic archipelago, have provided new insights on the VMEs of sedimentary environments, highlighting in most cases their poor conservation status (e.g. Fabri et al., 2014; Mastrototaro et al., 2017; Pierdomenico et al., 2018). Overall, our knowledge of deep-sea assemblages located on soft bottoms is still limited and thus, it is urgent to improve our understanding of these VMEs in order to support sound conservation and management measures (Maldonado et al., 2015; Mastrototaro et al., 2015).

Based on the analyses of ROV footage collected within a depth range of 500-860 m, we aim at (1) characterizing the composition of deep megabenthic assemblages found on sedimentary bottoms of the Alboran Sea (western Mediterranean), and (2) quantify the effects of human activities on these assemblages, with a major focus on changes in habitat complexity and biodiversity caused by bottom trawling.

2. Material and Methods

2.1. The Alboran Sea

The Alboran Sea is the westernmost basin of the Mediterranean, which is enclosed between the Iberian Peninsula and the North Africa, connected to the Atlantic Ocean through the Straits of Gibraltar (Fig. 1). The Alboran Sea displays a relatively complex seafloor geomorphology, including three main sub-basins (i.e. West, East and South Alboran Basins) separated by the Alboran Ridge, a major NE-SW oriented structural high (Muñoz et al., 2008; Lo Iacono et al., 2014; Gràcia et al., 2006, 2012, 2019; Fig. 1). The shelf of the Alboran Sea displays a variable extension, from 3.5 to 12 km, and is bounded by the shelf edge at a depth of 100-115 m. The slope is 10 to 50 km wide and displays variable gradients, connecting to the base of slope at a depth between 575 and 1000 m, transitioning to the deep basins. The sediment dynamics of the margin are strongly controlled by the prevailing along-slope current regimes, which gave origin to large contourite deposits (Ercilla et al., 2016, 2019, Juan et al., 2016). They are locally interrupted by turbidite fans, mass-wasting deposits and volcanic outcrops (Muñoz et al., 2008; Lo Iacono et al., 2008; Ercilla et al., 2019). The Alboran Sea is mainly characterized by a siliciclastic sedimentation controlled by the river sources, together with the aeolian inputs as the main source of terrigenous sediments (Moreno et al., 2002). Nevertheless, the high input of organic-rich sediments is associated to the relatively high-productivity of this basin, in contrast with the general oligotrophic characteristics of the Mediterranean Sea (Oguz et al., 2014). Phytoplankton primary productivity is mainly related to the local circulation pattern, controlled by the entrance of Atlantic surface waters into the Mediterranean and the development of anticyclonic gyres (Sarhan et al., 2000). Oceanographic conditions in the Alboran Sea are strictly related to the interaction between Atlantic and Mediterranean water masses, which create a regional thermohaline circulation (Vargas-Yáñez et al., 2010). The Atlantic waters (AW), warmer and less salty than Mediterranean ones (T∼15 °C, S∼36.2 psu, T~13.5 ºC S~38.4 psu, respectively), enter the Alboran Sea through the Straits of Gibraltar and flow within the first 150–200 m of the water column at a velocity of ∼50 to up to 150 $cm \cdot s^{-1}$ (Millot and Taupier-Letage, 2005). The AW circulation is driven by two semi-permanent anti-cyclonic gyres: The Western Alboran Gyre and the Eastern Alboran Gyre (Renault et al., 2012; Fig. 1). Deeper than the AW, the colder and salty Mediterranean Water (T∼13.5 °C, S∼38.4 psu), named Levantine Intermediate Water (LIW), flows towards the Straits of Gibraltar at ∼10 $cm \cdot s^{-1}$ (García Lafuente et al., 1998) (Fig. 1). The LIW extends down to 600 - 700 m and flows above the underlying high density western Mediterranean deep water (<12.7-l2°C, S∼38.40-38.52 psu), whose circulation is largely conditioned by the local physiography of the basin (Millot, 2009). Despite the lack of exhaustive studies on the deep-sea benthic assemblages of sedimentary substrates in the Alboran Sea, muddy substrates in interspersed patches within hard frameworks of cold-water coral communities have been reported to host colonies of *I. elongata* and the pennatulacean *K. stelliferum* (Hebbeln et al. 2009).

2.2. The ROV video acquisition

A total of six dives were recorded during the oceanographic expedition "SHAKE", onboard the R/V “Sarmiento de Gamboa” (May-June, 2015) (SHAKE Cruise: CGL2011-3005-C02-02) (Fig. 1, Table 1). Dives were carried out with the ROV “Max-Rover”, owned by the Hellenic Centre for Marine Research. It was equipped with a HD video camera (1920x1080 pixels), a manipulator arm for sampling tasks and two parallel laser beams (10 cm apart) that provided a reference scale to measure targets and define a fixed width of 1 m for the subsequent video analysis. ROV positioning was achieved by means of an ultra-short baseline (USBL) transponder mounted on the vehicle, which tracked their positioning every 3 seconds, approximately. Dives were conducted on both, northern and southern sectors of the eastern Alboran Sea between 500 and 860 m depth (Table 1), along three areas characterized by the occurrence of active faults: Carboneras Fault, North-South Faults, Al-Idrissi Fault (Gràcia et al., 2006, 2012, 2019; Fig. 1). The ROV moved at a constant speed of ~0.3 knots. Along each dive, voucher organisms were collected to confirm the taxonomic identification determined in the image analyses. Sampled organisms were fixed and preserved in 10% formalin for further analyses, were identified with the support of taxonomic experts and a manual on deep megabenthic fauna of the Mediterranean Sea (Fourt et al., 2017).

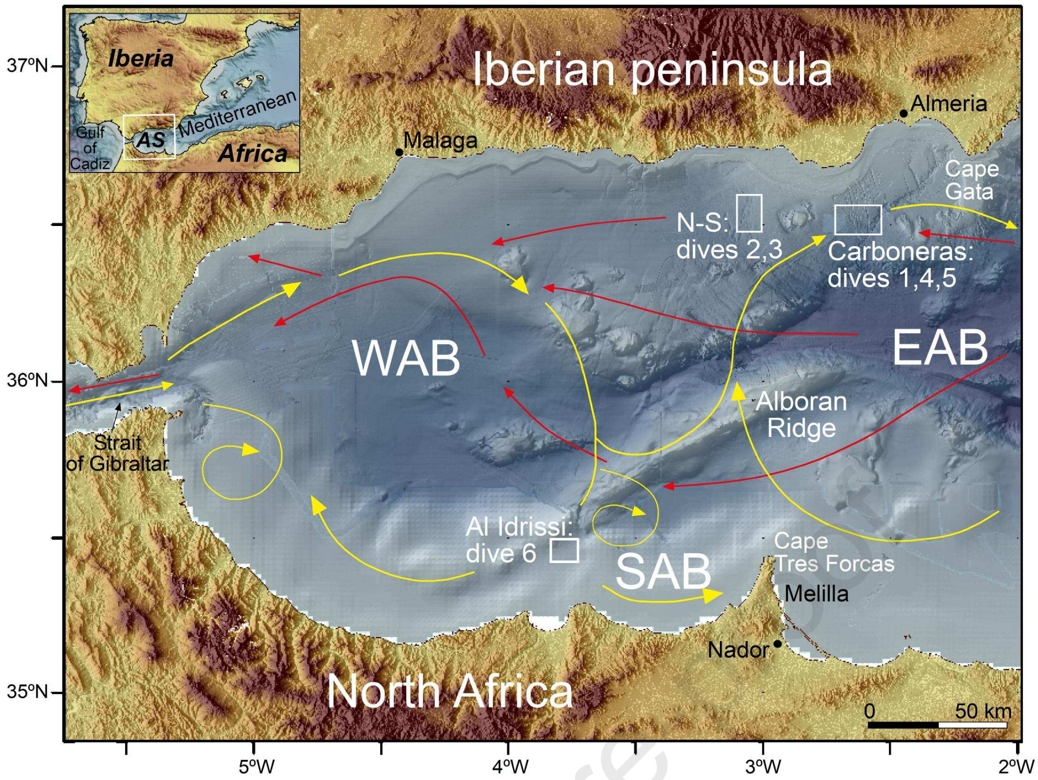

Fig. 1. Topographic and bathymetric map of the Alboran Sea, white rectangles indicate the location of dives, whose location correspond to different fault systems: North-South fault system (N-S), Carboneras fault system and Al-Idrissi Fault System. WAB: West Alboran Basin, EAB: East Alboran basin, SAB: South Alboran Basin. Yellow and red lines indicate the simplified patterns of the Atlantic Water (AW) gyres and the Mediterranean Levantine Intermediate Waters (LIW), respectively. Upper left inset: Western Mediterranean region indicate the Alboran Sea (AS).

2.3. Video analysis

Quantitative video analysis was performed according to the methodology described in Grinyó et al. (2016), using the software Final Cut Pro 7 (Apple Inc.). When the ROV was stopped or moving in loops, sequences were removed to avoid over-estimation of megabenthic organisms' abundance and dive length. Where the ROV was too detached from the seafloor or suspended sediments prevented a clear view of the seafloor, sequences were considered unusable and discarded from analyses. ROV dives covered a total distance of 5842 m, of which 5521 m was considered suitable for post-processing video analysis, corresponding to 94.5% of the total footage.

Video analysis was aimed at quantifying the encountered megabenthic organisms, the lebensspuren items (i.e. sedimentary forms produced by metazoans biological activity) (Fig. 2), trawl marks and marine litter. All these items were quantified within a width of 1 m (estimated from the laser beam distance). Lebensspuren was classified into four categories: furrows, mounds, holes and trail marks (Fig. 2).

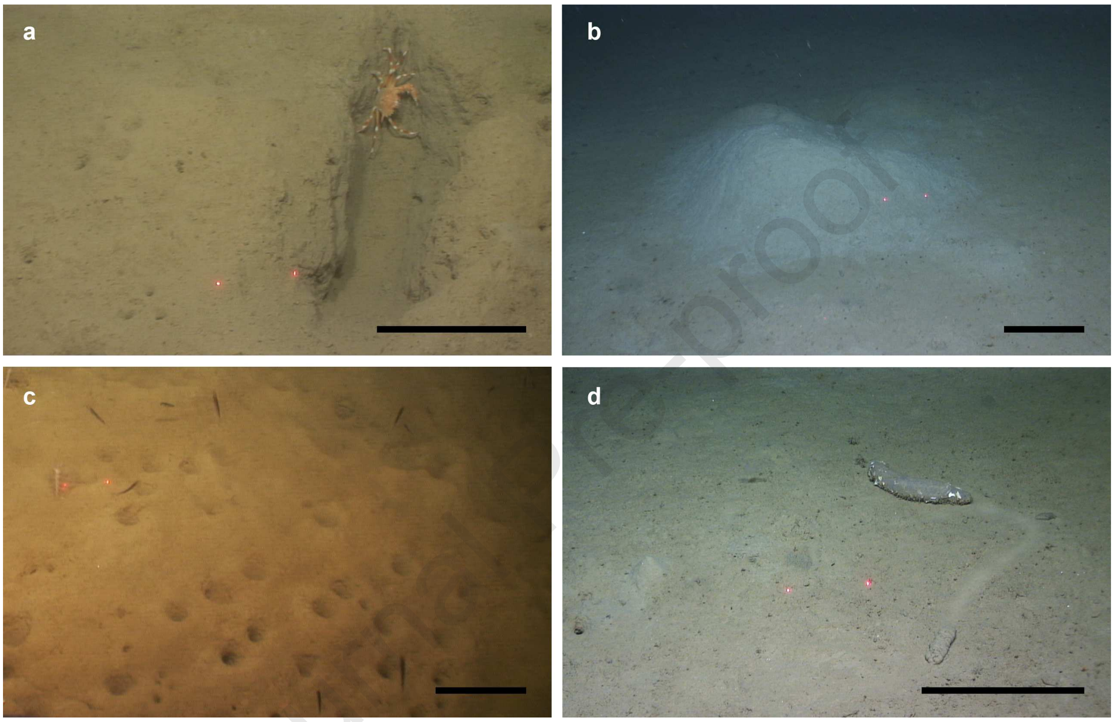

Fig. 2. Lebensspuren categories: a) furrow with a *Batynectes maravigna* crab inside, b) mound, c) hole aggregations, and d) trail-mark generated by *Mesothuria intestinalis*. Scale bar: 20 cm.

Litter items were categorized according to their composition into five classes: plastics debris (i.e. plastic bags, bottles, small plastic fragments), lost fishing gears (long-lines and nets), metal debris, glass bottles and clothes. Additionally, two categories of substratum were defined: untrawled and trawled bottoms. Sediment texture and seafloor gradients were not considered in the video analysis due to the homogeneity of these variables. Each observation (organism, trawl mark, lebensspuren, marine litter) was assigned a position within the dive, following the methodology described by Grinyó et al. (2016).

2.4. Data treatment

2.4.1. Megabenthic fauna occupancy and abundance

To measure megabenthic fauna occupancy (i.e. the frequency of occurrence in the set of sampling units) and abundance (i.e. the number of individuals per sampling unit) and to assess megabenthic assemblage composition, each dive was split into 2 m^2 sampling units (0.7 m width and 2.9 m long). A total of 1904 sampling units were obtained. Each sampling unit was characterized by the abundance of organisms, number of sessile megabenthic species, lebensspuren and litter items. On the basis of trawl mark presence, sampling units were classified as trawled or untrawled.

2.4.2. Sessile megabenthic assemblage composition

Sessile megabenthic assemblages, defined here as assemblages constituted by sessile and low motile invertebrates such as echinoderms (Grinyó et al., 2018), were identified based on species composition by means of a non-metric multidimensional scaling ordination (nMDS) of the sampling units, with species abundances being square root transformed, and ordination performed via a Bray-Curtis dissimilarity matrix. Adonis permutation multivariate analysis of variance and subsequent pairwise tests were used to test for significant differences amongst assemblages. The nMDS and adonis test were performed using the R-language function “metaMDS” and “Adonis”, available in the vegan library of the R software platform (Oksanen et al., 2016). Species occurring less than 3 times were excluded from the nMDS analysis.

2.4.3. Influence of anthropogenic impacts

Anthropogenic impact was assessed by estimating the occurrence of trawl marks and the density and typology of litter in each sampling unit. The effect of human disturbance on megafauna was analyzed within dives by comparing the frequency of occurrence of each megabenthic species in trawled (sampling units where trawl-marks were present) and un-trawled (sampling units where trawl marks were absent) sampling units, with similar environmental conditions in terms of substrate and inclination. Furthermore, we quantified the variation in habitat complexity and diversity between trawled (dives were trawl marks were present) and untrawled (dives were trawl marks were absent) areas, by comparing lebensspuren abundance (Gage, 1996) and the variation in species turnover (beta-diversity) by means of a randomized species accumulation curve (De Leo et al., 2014). This statistical approach allows us to find the average species accumulation curve and its standard deviation from random permutations of data (n=100), eluding misrepresentation in the curves due to differences in species abundance and sampling effort (Gotelli and Colwell, 2001). Megabenthic species, also including decapods and ichthyofauna, were considered to calculate the randomized species accumulation curve, although species that present positive phototactism were not considered (e.g. myctophids or squids). Randomized species accumulation curves were calculated with R-language function “specaccum”, using the vegan library of the R software platform (Oksanen et al., 2016).

3. Results

3.1. Description of physical characteristics along ROV dives

Dive lengths ranged between 168 m to 1700 m depth (Table 1). In all the dives, the seafloor was considered sub-horizontal and consisted of muddy sediments. A total of nine pockmarks were observed, one in dive 4 and eight in dive 5 (Table 1).

Pockmarks displayed a circular shape, with diameters ranging between 1.5 and 3 m and a maximum depth of ~40-50 cm. No carbonate concretions nor fluid or gas release were observed within the pockmarks, suggesting that these features are currently inactive.

Table 1 Location (geographic coordinates in WGS84), length, pockmark abundance and depth (minimum and maximum depth) of the SHAKE ROV dives. CFS: Carboneras fault system, NSFS: North-South fault system, AIFS: Al-Idrissi fault system, and Dv: Dive.

Zone	Dv	Start		End		Length (m)	Nº of Pockmarks	Depth range (m)
		Lat° N	Lon° W	Lat° N	Lon° W			
CFS	1	36.524	3.088	36.519	3.077	1700	0	704 - 722
NSFS	2	36.481	2.695	36.481	2.694	1109	0	639 - 685
NSFS	3	36.515	3.051	36.520	3.052	1368	1	638 - 688
CFS	4	36.532	2.619	36.541	2.618	168	0	836 - 860
CFS	5	35.498	3.737	35.504	3.739	725	8	821 - 842
AIFS	6	36.501	2.660	36.503	2.662	773	0	500 - 526

3.2. Megabenthic organisms: Occupancy and abundance

A total of 447 organisms from 9 different species belonging to 5 different phyla were observed in the study area (Fig. 3 and Table 2), occurring in 17.3% of the 1904 sampling units. The bamboo coral *Isidella elongata*, the cerianthid *Arachnanthus oligopodus* and the sponge *Thenea muricata* (Figs. 3a, 3b and 3f) were the most abundant species, representing 26.4%, 23.7% and 16.6% of all observed organisms, respectively. *A. oligopodus* was the most frequent species, occurring in 30.7% of the occupied sampling units, followed by both *I. elongata* and *T. muricata*, which both occurred in 21.6% of the occupied sampling units (Table 2). Abundance and frequency progressively decreased from the asteroid *Hymenodiscus coronata* to the crinoid *Leptometra phalangium* and the holothurian *Mesothuria intestinalis* (Table 2).

286 The remaining species accounted for less than 5% of all observed organisms and 287 occurred in less than 6% of sampling units (Table 2).

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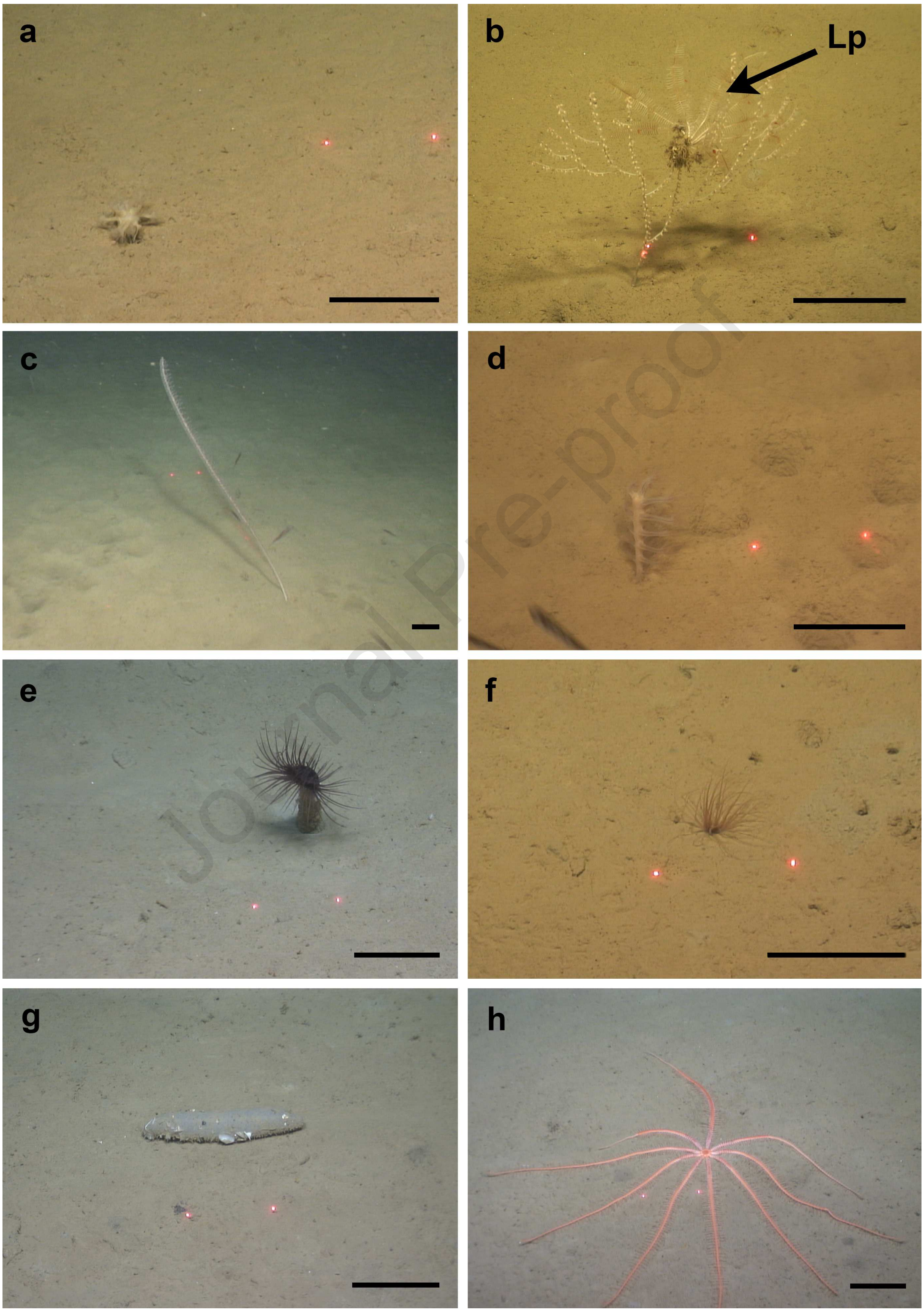

289 Fig. 3. Most common sessile and low motile megabenthic species occurring in the Alboran 290 sedimentary bottom. a) *Thenea muricata*, b) *Isidella elongata* and *Leptometra phalangium* (Lp), c)

Funiculina quadrangularis, d) *Kophobelemnon stelliferum*, e) *Cerianthus membranaceus*, f) *Arachnanthus oligopodus*, g) *Mesothuria intestinalis*, and h) *Hymenodiscus coronata*. Scale bar: 10 cm.

Table 2 Total and relative abundance of sessile megabenthic species together with their occupancy (n° of sampling units occupied by a species) and frequency (% of occurrence in the whole set of occupied sampling units) in the study area. Average and maximum density of each sessile species within the occupied sampling units (n=329) are also shown. ind: individuals.

Class	Species	Abundance		Occupancy		Average density ± SD	Max density
		Number	(%)	Number	(%)	(ind. m^{-2})	(ind. m^{-2})
Desmospongia	*Thenea muricata*	74	16.6	71	21.6	1.04±0.2	2
Anthozoa	*Isidella elongata*	118	26.4	71	21.6	1.5±0.9	7
	Arachnanthus oligopodus	106	23.7	101	30.7	1.03±0.2	2
	Kophobelemnon stelliferum	21	4.7	20	6.1	1.04±0.2	2
	Cerianthus membranaceus	17	3.8	17	5.2	1	1
	Funiculina quadrangularis	7	1.6	7	2.1	1	1
Crinoidea	*Leptometra phalangium*	35	7.8	26	7.9	1.2±0.6	3
Astroidea	*Hymenodiscus coronata*	36	8	36	10.9	1	1
Holothuria	*Mesothuria intestinalis*	33	7.4	33	10	1	1

3.3. Sessile megabenthic assemblage composition

Three megabenthic assemblages, distributed along the six ROV dives, could be identified in the nMDS: two monospecific assemblages composed by few colonies of the pennatulaceans *K. stelliferum* and *F. quadrangularis* respectively, and a multispecific group composed of *I. elongata* (28%), *A. oligopodus* (25%), *T. muricata* (18%), *H. coronata* (9%), *L. phalangium* (8%), *M. intestinalis* (8%) and *Cerianthus membranaceus* (4%) (Fig. 4). The Adonis test and subsequent pairwise test revealed that all three assemblages were significantly different (p<0.001) from one another.

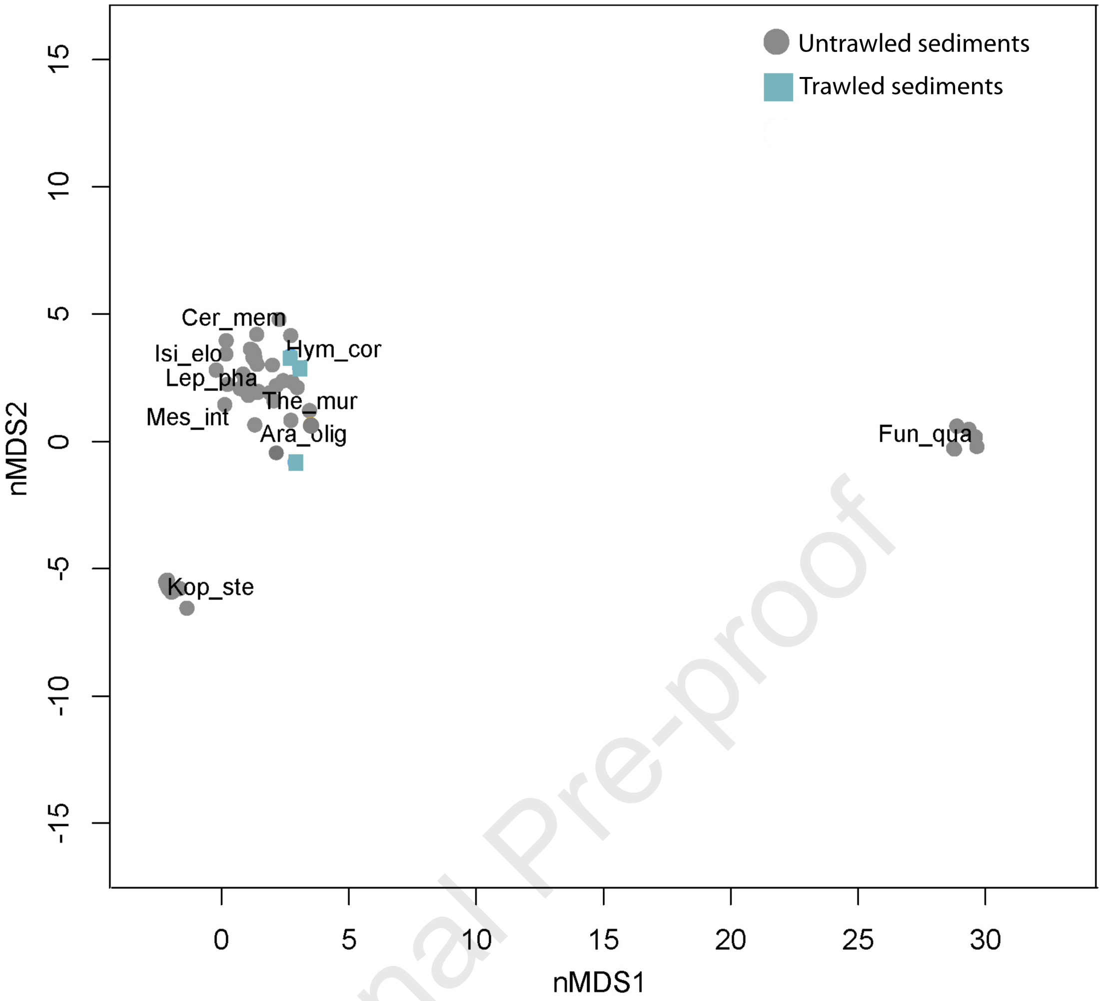

Fig. 4. Non-metric multi-dimensional scaling (nMDS) ordination plot, data were square root transformed, and ordination performed via a Bray-Curtis dissimilarity matrix): Sampling units containing sessile organisms and echinoderms are represented considering trawling presence (9 sampling units) and trawling absence (320 sampling units). Ara_oli = *Arachnanthus oligopodus*, Cer_mem = *Cerianthus membranaceus*, Fun_qua = *Funiculina quadrangularis*, Hym_cor = *Hymenodiscus coronata*, Isi_elo = *Isidella elongata*, Kop_ste = *Kophobelemnon stelliferum*, Lep_pha = *Leptometra phalangium*, Mes_int = *Mesothuria intestinalis*, The_mur = *Thenea muricata*.

3.4. Distribution of anthropogenic impacts

Marine litter was observed in all the ROV dives, except dive 6 (Table 3). Overall, 44 items were found, with plastics debris being the most abundant component (46%), followed by lost fishing gears (41%), metallic debris (7%), such as a cot and an empty butane cylinder (Fig. 5a), glass bottles (4%) (Fig. 5b) and clothes (2%) (Fig. 6). In dives 4 and 5, 33% and 100% of marine litter items were found within pockmarks (Figs. 5c, 5d and 7c). Marine litter average densities ranged between 0.001±0.09 to 0.01±0.002 items m^{-2}, with maximum densities of 2 items m^{-2} occurring within pockmarks. Trawl marks were observed in three of the six dives (Fig. 5e, 5f), with almost the totality of them concentrated in dive 3 (Table 3). A total of 93 trawl marks were observed, affecting 93 sampling units (4.88% of all sampling units).

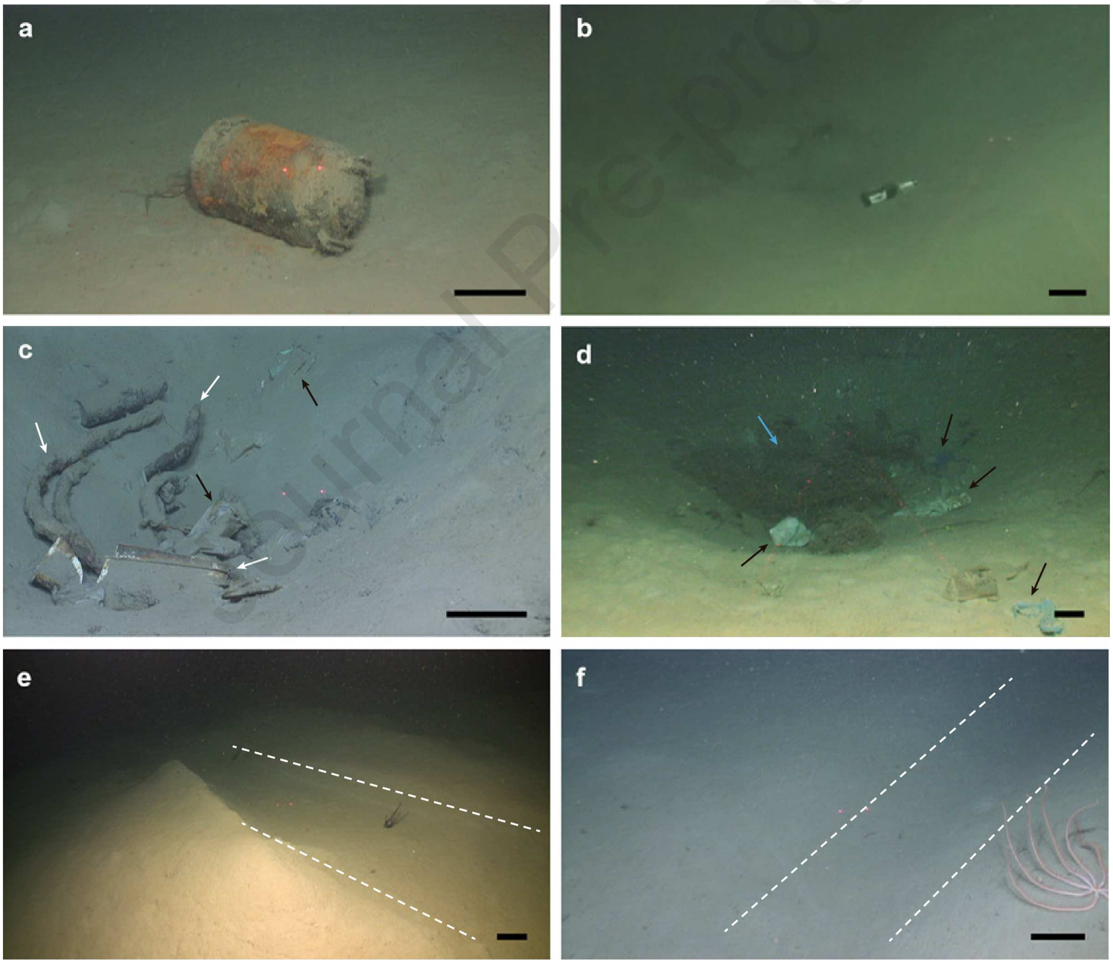

Fig. 5. Evidences of anthropogenic impact on the deep-sea sedimentary floor of the Alboran Sea: a) butane cylinder, b) glass bottle, debris accumulation inside pockmarks, c) reinforced rope, metal plates and plastic debris, d) lost net and light plastic debris, e) trawl-mark with macrourid fish, f) old trawl-mark with *Hymenodiscus coronata*. Scale bar: 20 cm. white

arrows: metallic debris, black arrows: plastic debris, blue arrow: lost net, discontinuous line: trawl mark delimitation.

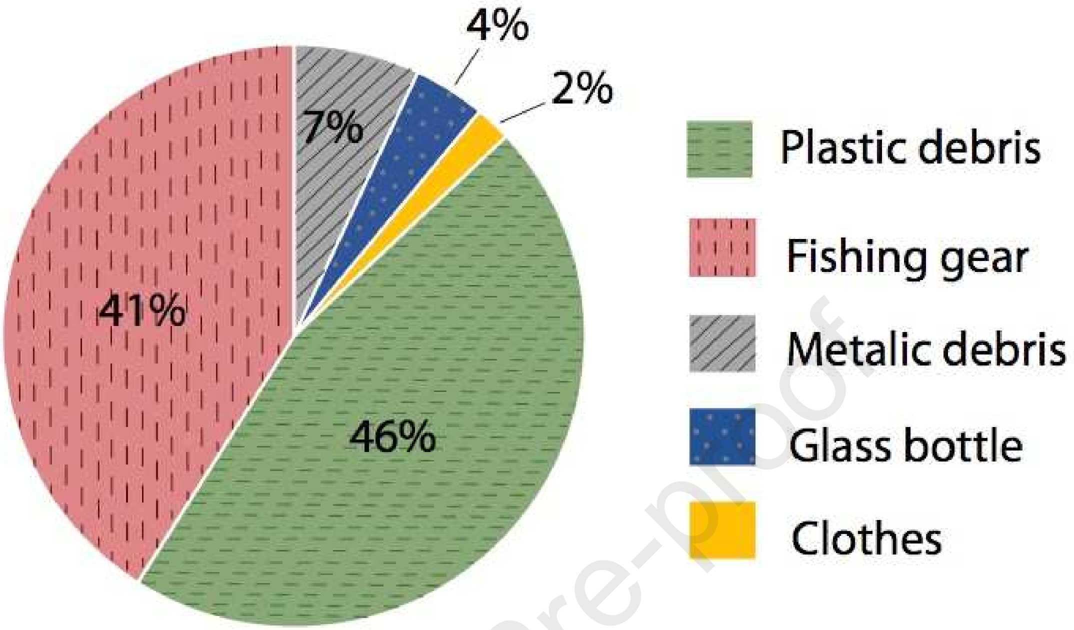

Fig. 6. Marine litter composition.

Table 3 Abundance and frequency of the occurrence of marine litter and trawl-marks in the study area. Sampling units with organisms (SO): number of sampling units simultaneously occupied by sessile megabenthic species and marine litter items or trawl-marks. CFS: Carboneras fault system, NSFS: North South fault system, AIFS: Al-Idrissi fault system; Dv: dive, Av. Density: Average density.

		Marine litter				**Trawl marks**			
Zone	Dv	Abundance (Nº)	Occupancy (Nº)	Av. density (Mean±SD)	SO (Nº)	Abundance (Nº)	Occupancy (Nº)	Av. density (Mean±SD)	SO (Nº)
CFS	1	15	1	0.01±0.002	0	6	6	0.002±0.04	1
NSFS	2	7	4	0.060±0.200	1	0	0	0	0
NSFS	3	14	16	0.015±0.102	0	84	84	0.081±0.215	7
CFS	4	1	2	0.001±0.092	0	3	3	0.004±0.043	1
CFS	5	7	4	0.014±0.131	0	0	0	0	0
AIFS	6	0	0	0	0	0	0	0	0

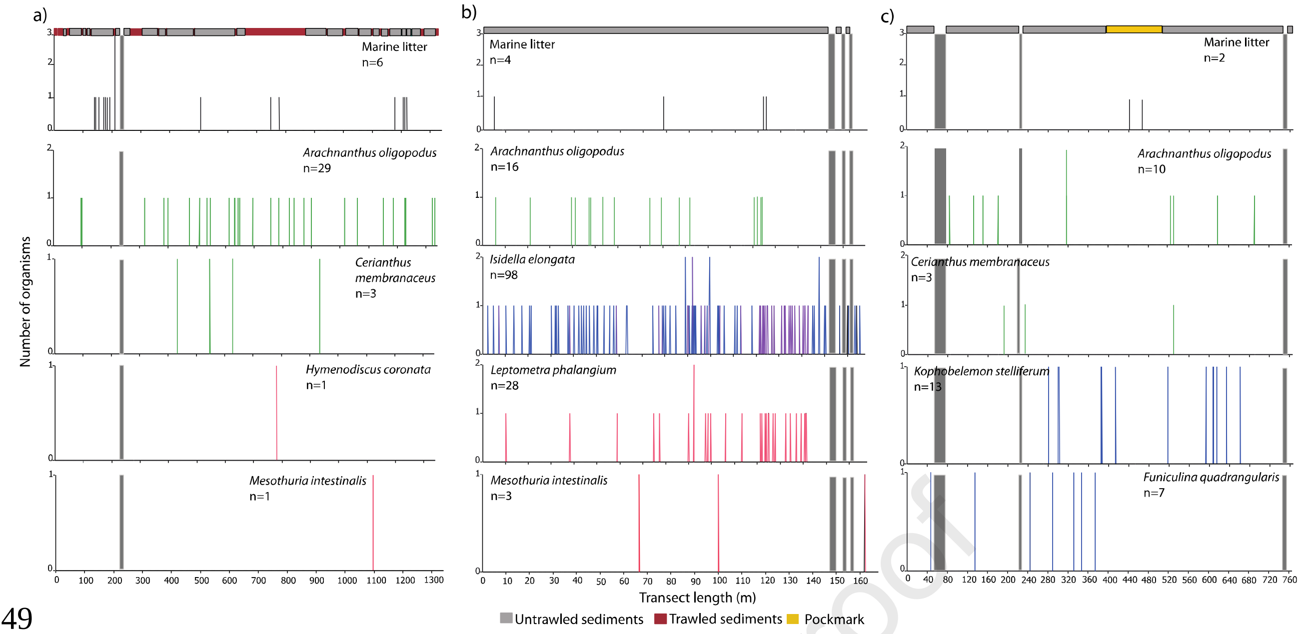

Fig. 7. Density plots showing variation of the abundance of different species and anthropogenic impacts along representative dives. (a) Density plots of an area impacted by bottom trawling (dive 3). b) Density plots of an untrawled area covered by an *I. elongata* meadow (dive 4). (c) Density plots of an untrawled area with pockmarks. This area was dominated by the sea pens *F. quadrangularis* and *K. stelliferum* (dive 6). Vertical gray lines indicate dive sequences that could not be analyzed due to a poor image quality, n=number of litter items/individuals/colonies. Sessile taxa with retractile and non-retractile capacity have been marked in green and blue, respectively. *Isidella elongata* colonies epiphyted by *L. phalangium* are marked in purple. Taxa with motile capacity have been marked in red.

3.5. Relationship between anthropogenic impacts and sessile megabenthic fauna

With the exception of a lost gear line, which occurred next to an *I. elongata* colony within dive 2 (Table 3), marine litter and megabenthic species did not co-occur. Only 9 out of the 93 sampling units containing trawl-marks were colonized by megabenthic species (Table 3), mainly represented by *A. oligopodus*, *C. membranaceus* and, to a lesser extent, by *M. intestinalis*, *L. phalangium* and *H. coronata* (Figs. 7a ,8). Overall, megabenthic densities in dives where trawl-marks were present (0.5±0.2 ind.

m^{-2} mean±SD) were significantly lower (Adonis, PSEUDO-F = 26.9, $p<0.001$) than those where trawl-marks were absent (1.5 ± 0.7 ind. m^{-2} mean±SD).

Uprising non-retractile sessile species were exclusively found on untrawled sediment (Figs. 7b, 7c and 8).

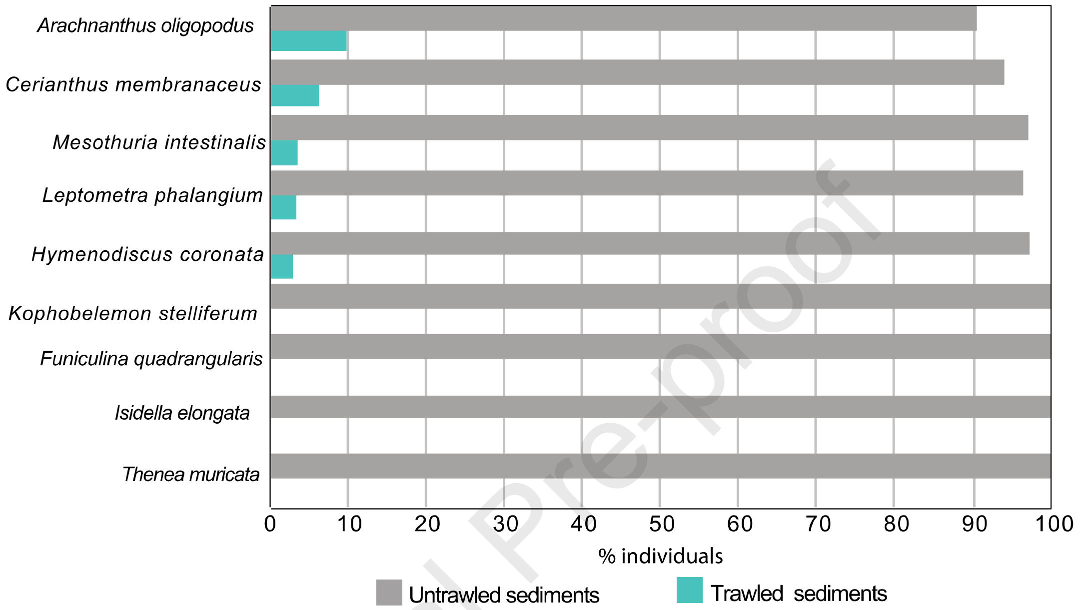

Fig. 8. Percentage of occurrence of sessile and low motile species in untrawled (grey) and trawled (green) sampling units. untrawled sampling units: 320, trawled sampling units: 9.

3.6. Variations in habitat complexity and biodiversity in trawled vs un-trawled areas

A total of 15730 lebensspuren items were observed, occurring on 67.5% of the sampling units. Holes were the most abundant category (90%) followed by mounds (8%), furrows (1.3%) and trail-marks (0.7%) (Fig. 9). Overall, lebensspuren densities in dives where trawled marks were absent (26±16 mean±SD items m^{-2}) were significantly higher (Adonis, PSEUDO-F = 1778, $p<0.001$) than those where trawling was present (1.8±3.2 mean±SD items m^{-2}). Such pattern was observed for all lebensspuren categories with the exception of trail-marks, for which no significant differences were found (Fig. 9).

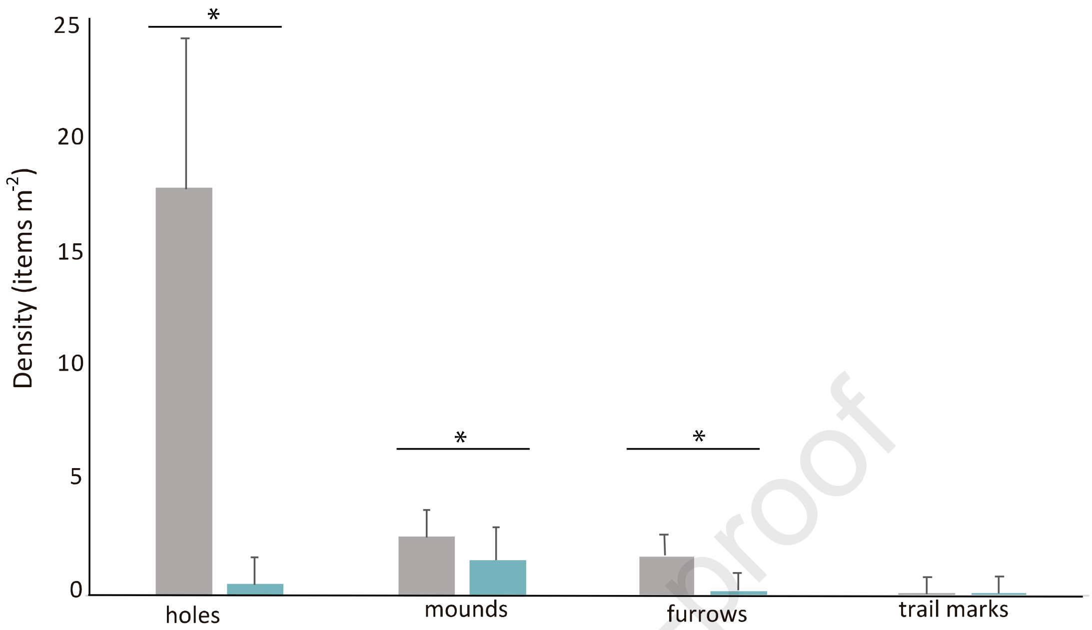

Fig. 9. Histograms comparing mean densities of different lebensspuren items in untrawled (grey) and trawled (green) dives. * Represents significant differences (p<0.001, Adonis). Error bars represent the standard deviation. A total 527 sampling units from dives were trawling was present and 622 sampling units were trawling was absent were used.

Species richness was higher on untrawled dives, where 26 megabenthic species were observed, than in trawled dives, where 22 species were found. A similar pattern was observed for species turnover, which resulted higher in untrawled dives than in trawled ones (Fig. 10).

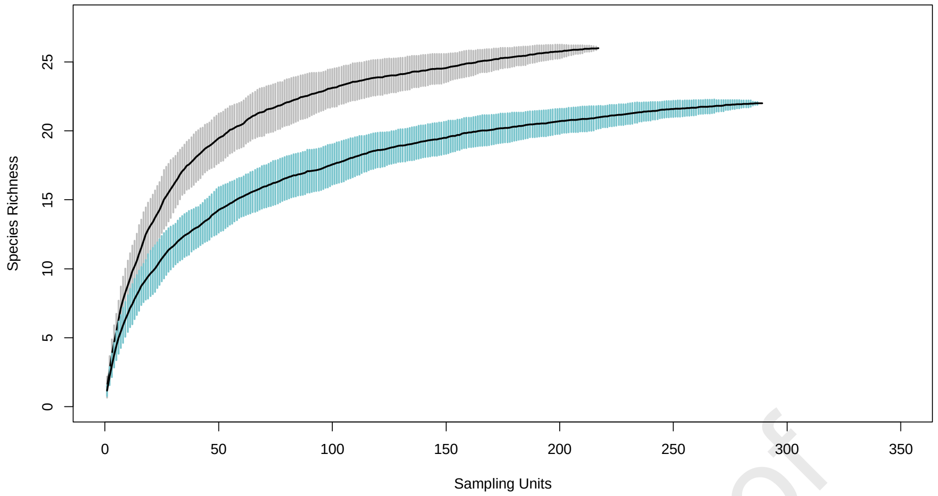

Fig. 10. Randomized species accumulation curve calculated for trawled (green) and untrawled dives (grey). Colored areas around the curve represent the confidence intervals from standard deviation. A total of 290 sampling units from dives were trawling was present and 218 sampling units from dives were trawling was absent were used for this analysis.

4. Discussion

4.1. Sessile megabenthic assemblages

The three megabenthic assemblages identified in this study (Fig. 4) were sparsely populated and dominated by passive suspension feeders (Table 1), agreeing with previous observations on the Mediterranean bathyal mud environments (Pérès, 1967; Emig, 1997; Mastrototaro et al., 2013, 2017). This contrasts with the bathyal soft sediment assemblages in more productive oceanic basins, such as those found in New Zealand or California, which received high amounts of phytodetrital material and are dominated by dense aggregations of deposit feeders (Smith and Hamilton, 1983; De Leo et al., 2010).

The two monospecific assemblages observed in this study were respectively dominated by the sea-pens *Funiculina quadrangularis* and *Kophobelemnon stelliferum* (Figs. 3c, 3d and 4), both being common inhabitants of bathyal muds of the western Mediterranean Sea (Gili and Ros,1987; Fabri et al., 2014; De La Torriente et al., 2018), where they have already been reported to form monospecific assemblages (Pérès, 1967; Mastrototaro et al., 2013; Pierdomenico et al., 2018). Although *F. quadrangularis* were described to form dense aggregations on Mediterranean bathyal muds (Pérès and Picard, 1964), the low densities observed in this study resemble previous observations from shelf-edge and submarine canyon environments of the western Mediterranean (Fabri et al., 2014; Grinyó et al., 2018; Pierdomenico et al., 2018). Conversely, *K. stelliferum* assemblages presented high densities than those reported by Mastrototaro et al. (2013) in Santa Maria di Leuca (Ionian Sea - Central Mediterranean). De Clippele et al. (2015) observed that both species increase diversity at a local scale. Certainly, 22% of *K. stelliferum* colonies, found in the present study, were associated with decapods and macrourid fishes, reinforcing the general consensus that this species may provide shelter and act as a feeding grounds for associated species (De Clippele et al., 2015). Conversely, *F. quadrangularis* colonies were not associated to any other megabenthic species. However, this observation might have been affected by the low number of colonies reported on this study.

The multispecific assemblage was widely represented, occurring in 99% of the occupied sampling units (Fig. 4). In this assemblage, the bamboo coral *Isidella elongata* (Fig. 3b) was the most abundant species (Table 1), forming meadows that extended over wide areas (Fig. 7b), which punctually reach densities up to 7 colonies m^{-2}. These density values are substantially higher than those recorded in other regions of the Mediterranean Sea (Mastrototaro et al., 2017; Pierdomenico et al., 2018; Ingrassia et al., 2019), where *I. elongata* reached maximum densities of 2.5 colonies m^{-2} (Bo et al., 2015). *I. elongata* populations appeared to be in good conservation status, as no dead colonies where observed and partial necrosis was present in less than 6% of the observed colonies. This low necrosis ratio contrasted with what has been observed in impacted *I. elongata* populations located on trawling grounds of the western Mediterranean (Pierdomenico et al., 2018). *I. elongata* has a major ecological role in bathyal environments of the Mediterranean Sea, providing essential habitat for several ichthyic and decapod species (Cartes et al., 2013; Mastrototaro et al., 2017), representing a secondary biological hard substratum, elevated from the surrounding sediments (Mastrototaro et al., 2017). On this regard, 47% of *I. elongata* colonies where epiphyted by the crinoid *Leptometra phalangium* (Fig. 3b), and to a lesser extent, by the decapod *Anamathia rissoana*. More than 90% of *L. phalangium* individuals occurred on top of *I. elongata* colonies, generally hosting 1 crinoid (1.7±1.0 ind./colony mean±SD), although largest colonies (>20 cm height) could host up to 5 crinoids. Since *L. phalangium* is a suspension feeder, it is likely that these crinoids are using *I. elongata* colonies as a physical support allowing to be detached from the seafloor and gaining exposure to more intense and food-rich currents, favoring their feeding rates. A similar functional relationship has been observed in the bathyal muds of the Balearic archipelago between *I. elongata* and the crinoid *Antedon mediterraneum* (Mastrototaro et al., 2017).

Among the other uprising sessile fauna that integrated the multispecific assemblage, we encountered the Demosponge *Thenea muricata* and the Cerianthids *Arachnanthus oligopodus* and *Cerianthus membranaceus* (Figs. 3a, e and f). *Thenea muricata* has been described as a typical inhabitant of Mediterranean bathyal muds (Vacelet, 1969; Maldonado et al., 2015). In the study area, this species presented a sparse distribution and occurred in low densities, in accordance with other studies from the western Mediterranean (Santín et al., 2018). Conversely, cerianthids were amongst the most abundant species (38% of all sessile fauna), contrasting with previous observations depicting them as a rare group in Mediterranean bathyal muds (Cartes et al., 2009; Mastrototaro et al., 2013). Up to date, information regarding megabenthic assemblages in deep sedimentary seafloor, mostly derives from experimental bottom trawls or benthic sledges (e.g. Gili and Ros, 1987; Pansini and Musso, 1991; Maynou and Cartes, 2012; Mastrototaro et al., 2013). Although these gears have provided a great amount of occurrence data, they cannot be considered trustworthy from a quantitative point of view, often providing weak information about the abundance and distribution of sessile species (Chimienti et al., 2018). This lack of reliability is magnified for those species that have soft bodies and burrowing or withdrawing behaviors (Durden et al., 2015; Chimienti et al., 2018). Therefore, since cerianthids rapidly and fully retract into sediments, it is likely that the abundance of this groups in Mediterranean bathyal muds has been widely underestimated.

The asteroid *Hymenodiscus coronata* and the holothurian *Mesothuria intestinalis* (Figs. 3g and h), typical inhabitants of Mediterranean abyssal muds (Mecho et al., 2018), occurred as isolated individuals, being the most sparsely distributed component of the multispecific assemblage (Figs. 7a and b). In open slope environments, both species have generally presented low abundances (Mecho et al., 2018), although *M. intestinalis* has been observed to form massive aggregations coinciding with increments of labile organic matter (Cartes et al., 2009).

The multispecific assemblage occurred on untrawled sediments (95% sampling units), trawled sediments (3.5% sampling units) and on pockmark fields (0.5% sampling units) (Figs. 4, 7b and 7c). Although active pockmarks can potentially influence species distribution and abundance due to the influence of fluid seepage on water chemistry (Zeppilli et al., 2012), in the study area no chemical nor physical changes were observed through the water column (gas seepage) or surrounding sediments (carbonate crusts) in correspondence to these features. This suggests that inactive pockmarks in this area do not exert any direct influence on megabenthic fauna distribution.

No environmental parameters are able to explain the segregation among assemblages, which could be identified from the video analyses. However, according to Peres and Picard (1964), this segregation may derive from substrate fluidity. While, *F. quadrangularis* and *K. stelliferum* are associated to viscous muds *I. elongata* is associated to compact muds (Peres and Picard, 1964). Therefore, further studies are warranted to assess potential differences in the physical, geological and geochemical characteristics of the sediments (e.g. grain-size, porosity, water content, composition, and organic content) to further understand which natural processes contribute to drive the distribution of these assemblages.

4.2. Marine litter

Litter observations in this study resemble what has already been observed in other deep Mediterranean environments, where plastic represents the most abundant component (Cau et al., 2019b; Pierdomenico et al., 2019a; Gerigny et al., 2019). Most marine litter items occurred in low densities, despite higher concentrations within pockmarks, which hosted plastic bags and fishing gears (Figs. 5c and f). In line with previous observations, pockmarks may act as a sink (Taviani et al., 2013; Mecho et al., 2018), constraining bottom-currents around them and inducing the accumulation of lighter litter, such as plastic bags or certain fishing-related debris (i.e. long-line fragments). Although the mechanisms behind the accumulation of large and heavier debris, such as steel reinforced rope (Fig. 5c), remain largely unknown, it is reasonable to hypothesize that heavy items mostly derive from ships' discard. A maritime-based origin for heavy litter in open-slope environments has been suggested for other areas of the Mediterranean and North-Atlantic Ocean (Ramírez-Llodra et al., 2013; Pham et al., 2014).

It is worth mentioning, that over 25% of global traffic of trading ships use the north Alboran Sea (around 60000 transits per year), making it the world’s second busiest sea route (IUCN, 2007). For navigation safety reasons, the International Maritime Organization (IMO) stablished two mandatory routes named "traffic separation schemes" that divide the northern Alboran Sea. One of this traffic separation schemes, is coincident with the Carboneras and the North-South fault systems (Fig. 1). This could explain the contrasting situation observed between dives performed in the northern Alboran Sea and the Al Idrissi dive were no marine litter items where observed (Table. 3). However, this pattern should be carefully considered based on the small sampling effort made on the south Alboran Sea.

Finally, no interactions between marine litter and motile or sessile fauna were observed in the ROV dives, in contrast with what has been observed in regions with higher litter densities (Pierdomenico et al., 2019a; 2019b).

4.3. The impact of bottom trawling

Despite the low sampling effort of our study (six ROV dives covering 5521 m) our results clearly show that bottom trawling affected sessile megabenthic species distribution and abundance, and caused a reduction in habitat complexity and beta diversity (Figs. 9 and 10). *I. elongata*, *T. muricata*, *F. quadrangularis* and *K. stelliferum* were restricted to untrawled sampling units (Fig. 8). These species are extremely sensitive to bottom trawling (Massutí and Reñones 2005; Mastrototaro et al., 2013; Pierdomenico et al., 2018) and used to be common components of the

Mediterranean trawling by-catch (Relini et al., 1986; Gili and Ros, 1987; Pansini and Musso, 1991). In fact, in ROV dives where trawl marks were present they presented significantly lower densities (0.5±0.1 ind. m^{-2} mean±SD; Adonis, PSEUDO-F=15.28; $p<0.0001$) than those where trawl marks were absent (0.87±0.73 ind. m^{-2} mean±SD). These species populations have been widely decimated in the last decades, and are now mostly restricted to areas inaccessible to bottom trawling (Mastrototaro et al., 2013; 2017; Bo et al., 2015; Santín et al., 2018).

In trawled sampling units, occupancy was rather low and was restricted to cerianthids and echinoderms (Fig. 8). Cerianthids might be able to withstand bottom trawling through their ability to rapidly retract within tubes that are rooted tens of centimeters below the sediment surface (Picton and Manuel, 1985). Echinoderms in deep Mediterranean environments are extremely susceptible to the destructive effects of bottom trawling, being unable to escape or withstand them (Smith et al., 2000; Cartes et al., 2009). Thus, the presence of *H. coronata* in trawled sampling units (Fig. 8) might be explained by its motile capacity, which may allow re-occupancy of sampling units after the passage of trawl gears. However, several *H. coronata* individuals displayed a peculiar behavior in trawled sampling units. Individuals were observed on the edge of trawl marks extending their arms into the water column (Fig. 5f). In this slightly uplifted position, suspension feeders may be able to reach water layers exposed to more intense hydrodynamics, improving their feeding rates (Carlier et al., 2009).

Lebensspuren density and megabenthic diversity were higher in untrawled dives than in trawled ones (Figs. 9 and 10), resembling similar patterns observed in other bathyal Mediterranean environments (Mastrototaro et al., 2017). As nets, trawl-doors and chains are dragged on the seafloor, sedimentary structures are flattened and bioturbator and habitat-forming species are removed or damaged (Auster et al., 1996; Olsgard et al., 2008; Pierdomenico et al., 2018), resulting in a reduction of habitat complexity and, therefore, local biodiversity (Probert et al., 1997). Indeed, bottom trawling can greatly alter benthic ecosystem functioning and productivity (Jennings et al., 2001), potentially having crucial long-term consequences for local fisheries (Victorero et al., 2018). Considering the low recuperation rates of most species characterizing the observed assemblages (Maynou and Cartes 2012), our study's findings arise grave concern on the conservation status of these vulnerable benthic assemblages in the deep muddy bottoms of the Alboran Sea.

5. Conclusions

- Three megabenthic assemblages dominated by *Funiculina quadrangularis*, *Kophobelemnon stelliferum* and *Isidella elongata* respectively, were found in bathyal sedimentary environments of the Alboran Sea, within a depth range of 500-860 m.
- *I. elongata* and *K. stelliferum* were associated to motile taxa suggesting that these species may provide shelter and feeding grounds for associated species.
- Marine litter occurred in low densities. Plastic and fishing gears were the most abundant component of marine litter. Local circulation of bottom currents may force the accumulation of marine litter within pockmark depressions.
- Our results yield clear evidences that bottom trawling decreased the abundance of habitat forming species and lebensspuren items, reducing habitat complexity and negatively affecting biodiversity, however more extensive studies would be advisable to confirm this trend.

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Author contribution

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Martina Pierdomenico: Conceptualization, methodology, writing - review & editing.

Suzanne Conlon: Methodology, data curation.

Guillem Corbera: Visualization, writing - review & editing.

Eulàlia Gràcia: Conceptualization, Writing - review & editing, Resources, Funding acquisition.

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Highlights

- Three benthic assemblages could be identified in the study area.
- *I. elongata* and *K. stelliferum* provide shelter and feeding grounds for other taxa.
- Plastic and lost fishing gear were the most abundant fraction of marine litter.
- Trawling decreased the abundances of bioengineering species.
- Trawling reduced habitat complexity, negatively affecting biodiversity.

Declaration of interests

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