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### ► To cite this version:

Jordi Grinyó, Claudio Lo Iacono, Martina Pierdomenico, Suzanne Conlon, Guillem Corbera, et al.. Evidences of human impact on megabenthic assemblages of bathyal sediments in the Alboran Sea (western Mediterranean). *Deep Sea Research Part I: Oceanographic Research Papers*, 2020, 165, pp.103369 -. 10.1016/j.dsr.2020.103369 . hal-03493746

**HAL Id: hal-03493746**

<https://hal.science/hal-03493746v1>

Submitted on 17 Oct 2022

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

3  
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18  
19 Abstract

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

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

## 36 1. Introduction

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

48 Image-based investigations of deep-sea habitats were mainly focused on benthic  
49 communities or key structuring species occurring on hard substrates (Mortensen and  
50 Buhl-Mortensen, 2005; Althaus et al., 2009; Price et al., 2019), while similar studies  
51 on deep-sea sedimentary settings are scarce (Robert and Juniper, 2012; De Leo et al.,  
52 2017). This represents a significant knowledge gap, considering that deep-sea  
53 sediments are among the most widespread benthic habitats in the world, covering vast  
54 areas of continental shelves, slopes and abyssal plains (Gray, 2002). Deep-sea  
55 sedimentary environments have been traditionally considered as barren fields sparsely  
56 populated by megabenthic organisms (Jones et al., 2007). However, in specific areas,  
57 deep-sea sedimentary floors have been shown to host three-dimensional complex  
58 megabenthic assemblages, such as sponge grounds or sessile anthozoan aggregations  
59 (de Moura-Neves et al., 2015; Maldonado et al., 2015). These assemblages locally  
60 enhance biomass and act as biodiversity hotspots, as they provide habitat for a large  
61 variety of associated species, including a wide range of species of economic interest

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

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

68 In 2006, the need to preserve Vulnerable Marine Ecosystems (VMEs) from the  
69 negative impacts caused by human activities, led to the development of specific  
70 management and conservation measures to prevent their degradation (UNGA, 2007).

71 To achieve this goal, several international strategies, such as the “*International*  
72 *guideline for the management of the deep-sea fisheries in the high seas*” (FAO, 2009)  
73 or the EU’s Marine Strategy Directive (2008/56/CE), have been developed during the  
74 last decade. Deep-sea soft sedimentary environments of the Mediterranean Sea host  
75 several VMEs, mainly represented by the isidid *Isidella elongata* and the  
76 pennatulaceans *Funiculina quadrangularis* and *Kophobelemnion stelliferum*.

77 Amongst the many anthropogenic impacts on the marine environment, bottom  
78 trawling is the widest spread physical disturbance in the world’s seabed (Watling,  
79 2013; Hiddink et al., 2017). Impacts associated to bottom trawling include removal or  
80 damage to epibenthic fauna, reduction of habitat complexity and alteration of  
81 sedimentary and geochemical processes (e.g. De Leo et al., 2017; Hiddink et al.,  
82 2017; Paradis et al., 2019). With bottom trawling progressively expanding to deeper  
83 environments, VMEs associated to sedimentary bottoms have undergone a vast  
84 decimation (Sardà et al., 2004; Gerovasileiou et al., 2019; Pusceddu et al., 2014),  
85 restricting their distribution to isolated regions or areas inaccessible to trawlers, such

86 as sub-vertical walls or regions surrounded by rocky outcrops (Bo et al., 2015;  
87 Pierdomenico et al., 2018).

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

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

110 order to support sound conservation and management measures (Maldonado et al.,  
111 2015; Mastrototaro et al., 2015).

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

117

## 118 2. Material and Methods

### 119 2.1. The Alboran Sea

120

121 The Alboran Sea is the westernmost basin of the Mediterranean, which is enclosed  
122 between the Iberian Peninsula and the North Africa, connected to the Atlantic Ocean  
123 through the Straits of Gibraltar (Fig. 1). The Alboran Sea displays a relatively  
124 complex seafloor geomorphology, including three main sub-basins (i.e. West, East  
125 and South Alboran Basins) separated by the Alboran Ridge, a major NE-SW oriented  
126 structural high (Muñoz et al., 2008; Lo Iacono et al., 2014; Gràcia et al., 2006, 2012,  
127 2019; Fig. 1). The shelf of the Alboran Sea displays a variable extension, from 3.5 to  
128 12 km, and is bounded by the shelf edge at a depth of 100-115 m. The slope is 10 to  
129 50 km wide and displays variable gradients, connecting to the base of slope at a depth  
130 between 575 and 1000 m, transitioning to the deep basins. The sediment dynamics of  
131 the margin are strongly controlled by the prevailing along-slope current regimes,  
132 which gave origin to large contourite deposits (Ercilla et al., 2016, 2019, Juan et al.,  
133 2016). They are locally interrupted by turbidite fans, mass-wasting deposits and  
134 volcanic outcrops (Muñoz et al., 2008; Lo Iacono et al., 2008; Ercilla et al., 2019).  
135 The Alboran Sea is mainly characterized by a siliciclastic sedimentation controlled by  
136 the river sources, together with the aeolian inputs as the main source of terrigenous

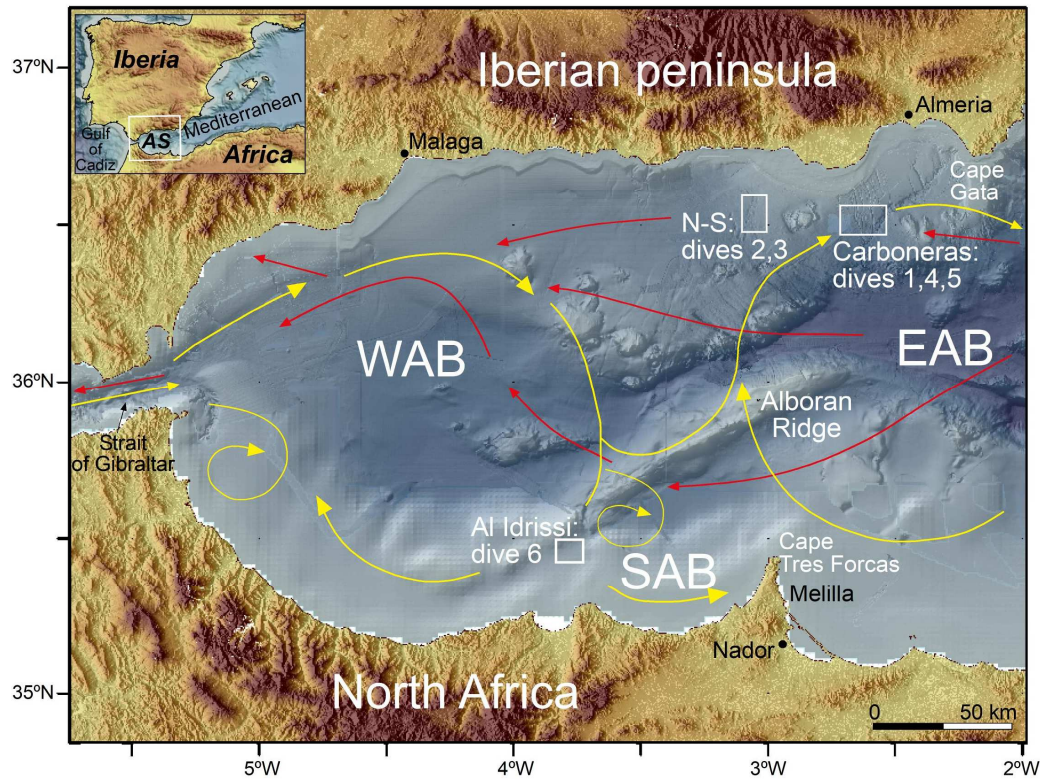
137 sediments (Moreno et al., 2002). Nevertheless, the high input of organic-rich  
138 sediments is associated to the relatively high-productivity of this basin, in contrast  
139 with the general oligotrophic characteristics of the Mediterranean Sea (Oguz et al.,  
140 2014). Phytoplankton primary productivity is mainly related to the local circulation  
141 pattern, controlled by the entrance of Atlantic surface waters into the Mediterranean  
142 and the development of anticyclonic gyres (Sarhan et al., 2000). Oceanographic  
143 conditions in the Alboran Sea are strictly related to the interaction between Atlantic  
144 and Mediterranean water masses, which create a regional thermohaline circulation  
145 (Vargas-Yáñez et al., 2010). The Atlantic waters (AW), warmer and less salty than  
146 Mediterranean ones ( $T \sim 15 \text{ }^\circ\text{C}$ ,  $S \sim 36.2 \text{ psu}$ ,  $T \sim 13.5 \text{ }^\circ\text{C}$ ,  $S \sim 38.4 \text{ psu}$ , respectively),  
147 enter the Alboran Sea through the Straits of Gibraltar and flow within the first 150–  
148 200 m of the water column at a velocity of  $\sim 50$  to up to  $150 \text{ cm}\cdot\text{s}^{-1}$  (Millot and  
149 Taupier-Letage, 2005). The AW circulation is driven by two semi-permanent anti-  
150 cyclonic gyres: The Western Alboran Gyre and the Eastern Alboran Gyre (Renault et  
151 al., 2012; Fig. 1). Deeper than the AW, the colder and salty Mediterranean Water  
152 ( $T \sim 13.5 \text{ }^\circ\text{C}$ ,  $S \sim 38.4 \text{ psu}$ ), named Levantine Intermediate Water (LIW), flows towards  
153 the Straits of Gibraltar at  $\sim 10 \text{ cm}\cdot\text{s}^{-1}$  (García Lafuente et al., 1998) (Fig. 1). The LIW  
154 extends down to 600 - 700 m and flows above the underlying high density western  
155 Mediterranean deep water ( $< 12.7\text{-}12^\circ\text{C}$ ,  $S \sim 38.40\text{-}38.52 \text{ psu}$ ), whose circulation is  
156 largely conditioned by the local physiography of the basin (Millot, 2009). Despite the  
157 lack of exhaustive studies on the deep-sea benthic assemblages of sedimentary  
158 substrates in the Alboran Sea, muddy substrates in interspersed patches within hard  
159 frameworks of cold-water coral communities have been reported to host colonies of *I.*  
160 *elongata* and the pennatulacean *K. stelliferum* (Hebbeln et al. 2009).

161

## 162 2.2. The ROV video acquisition

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





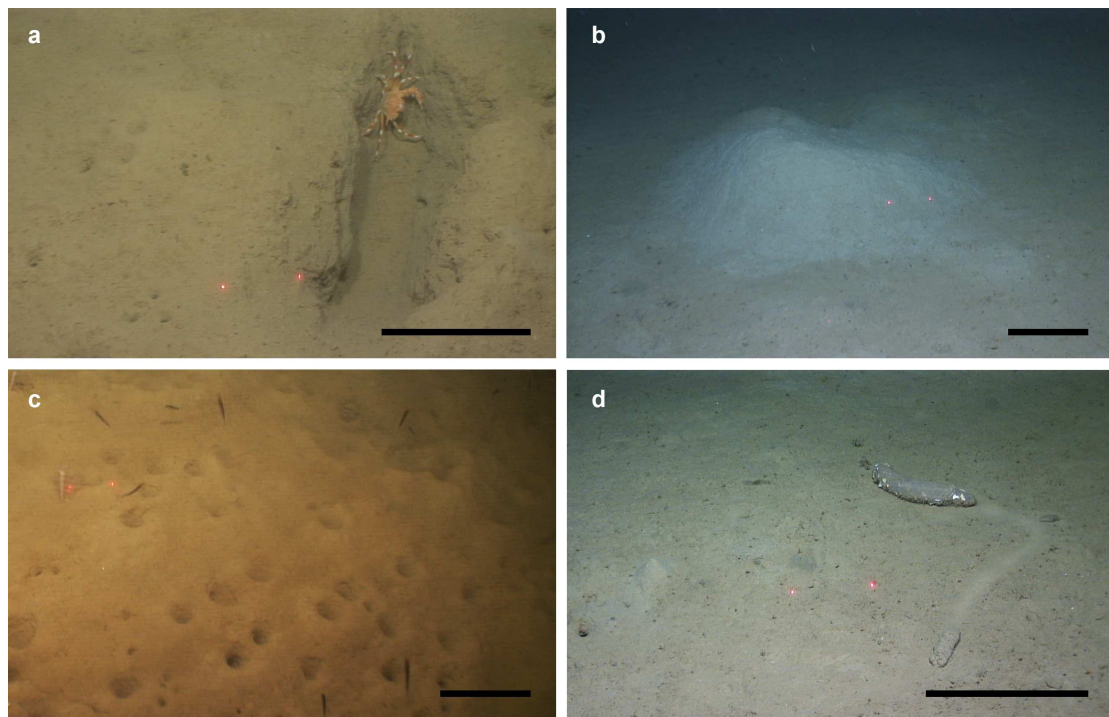
181  
 182 Fig. 1. Topographic and bathymetric map of the Alboran Sea, white rectangles indicate the  
 183 location of dives, whose location correspond to different fault systems: North-South fault  
 184 system (N-S), Carboneras fault system and Al-Idrissi Fault System. WAB: West Alboran  
 185 Basin, EAB: East Alboran basin, SAB: South Alboran Basin. Yellow and red lines indicate  
 186 the simplified patterns of the Atlantic Water (AW) gyres and the Mediterranean Levantine  
 187 Intermediate Waters (LIW), respectively. Upper left inset: Western Mediterranean region  
 188 indicate the Alboran Sea (AS).

189

### 190 2.3. Video analysis

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

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



204

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

208

209 Litter items were categorized according to their composition into five classes: plastics  
210 debris (i.e. plastic bags, bottles, small plastic fragments), lost fishing gears (long-lines  
211 and nets), metal debris, glass bottles and clothes. Additionally, two categories of  
212 substratum were defined: untrawled and trawled bottoms. Sediment texture and  
213 seafloor gradients were not considered in the video analysis due to the homogeneity  
214 of these variables. Each observation (organism, trawl mark, lebensspuren, marine

215 litter) was assigned a position within the dive, following the methodology described  
216 by Grinyó et al. (2016).

## 217 2.4. Data treatment

### 218 2.4.1. Megabenthic fauna occupancy and abundance

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

226

### 227 2.4.2. Sessile megabenthic assemblage composition

228 Sessile megabenthic assemblages, defined here as assemblages constituted by sessile  
229 and low motile invertebrates such as echinoderms (Grinyó et al., 2018), were  
230 identified based on species composition by means of a non-metric multidimensional  
231 scaling ordination (nMDS) of the sampling units, with species abundances being  
232 square root transformed, and ordination performed via a Bray-Curtis dissimilarity  
233 matrix. Adonis permutation multivariate analysis of variance and subsequent pairwise  
234 tests were used to test for significant differences amongst assemblages. The nMDS  
235 and adonis test were performed using the R-language function “metaMDS” and  
236 “Adonis”, available in the vegan library of the R software platform (Oksanen et al.,  
237 2016). Species occurring less than 3 times were excluded from the nMDS analysis.

238

### 239 2.4.3. Influence of anthropogenic impacts

240 Anthropogenic impact was assessed by estimating the occurrence of trawl marks and  
241 the density and typology of litter in each sampling unit. The effect of human  
242 disturbance on megafauna was analyzed within dives by comparing the frequency of  
243 occurrence of each megabenthic species in trawled (sampling units where trawl-marks  
244 were present) and un-trawled (sampling units where trawl marks were absent)  
245 sampling units, with similar environmental conditions in terms of substrate and  
246 inclination. Furthermore, we quantified the variation in habitat complexity and  
247 diversity between trawled (dives where trawl marks were present) and untrawled (dives  
248 were trawl marks were absent) areas, by comparing lebensspuren abundance (Gage,  
249 1996) and the variation in species turnover (beta-diversity) by means of a randomized  
250 species accumulation curve (De Leo et al., 2014). This statistical approach allows us  
251 to find the average species accumulation curve and its standard deviation from  
252 random permutations of data (n=100), eluding misrepresentation in the curves due to  
253 differences in species abundance and sampling effort (Gotelli and Colwell, 2001).  
254 Megabenthic species, also including decapods and ichthyofauna, were considered to  
255 calculate the randomized species accumulation curve, although species that present  
256 positive phototactism were not considered (e.g. myctophids or squids). Randomized  
257 species accumulation curves were calculated with R-language function “specaccum”,  
258 using the vegan library of the R software platform (Oksanen et al., 2016).

259

### 260 3. Results

#### 261 3.1. Description of physical characteristics along ROV dives

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

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

269

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

273

Zone	Dv	Start		End		Length (m)	N° 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

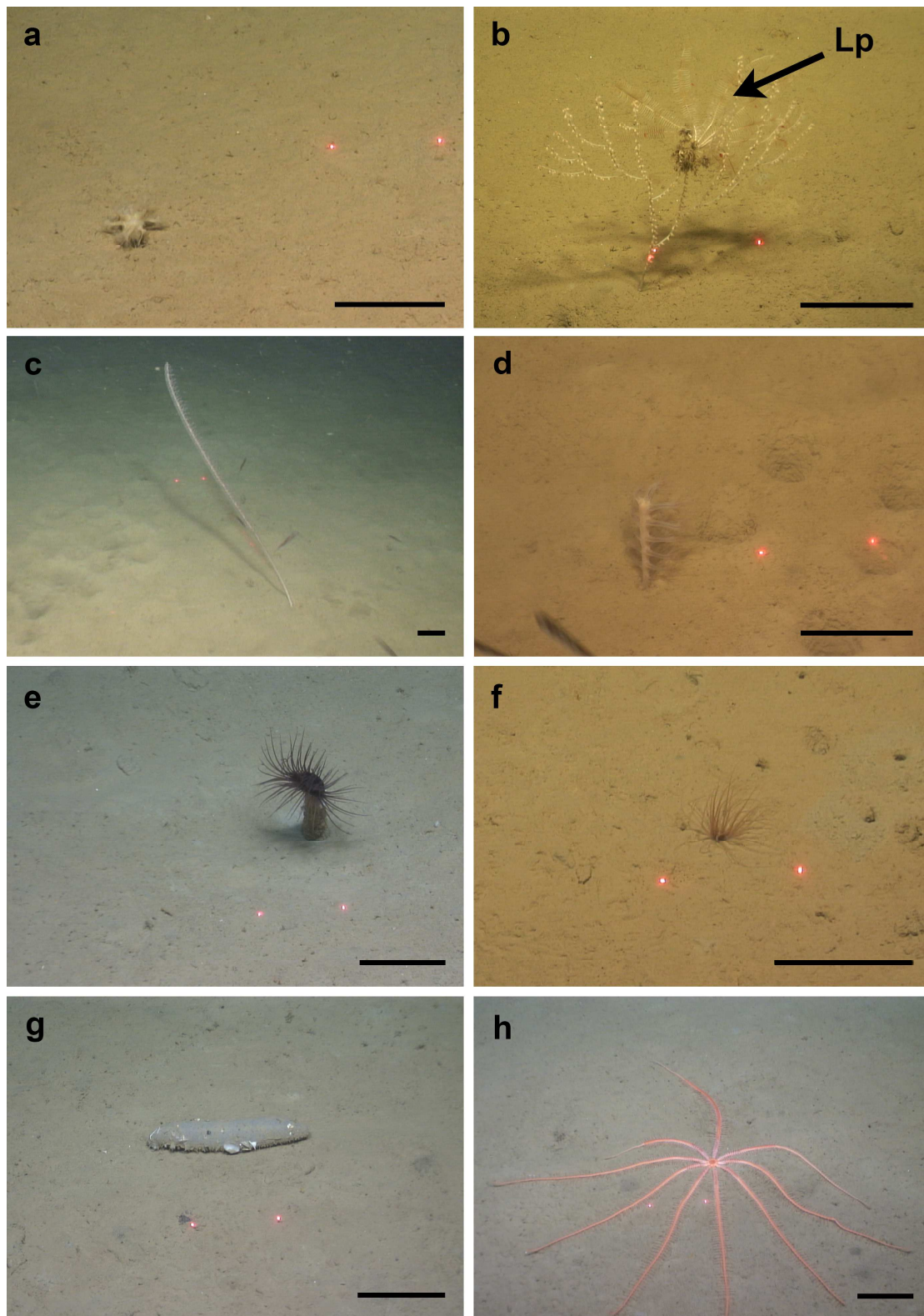
274

### 275 3.2. Megabenthic organisms: Occupancy and abundance

276 A total of 447 organisms from 9 different species belonging to 5 different phyla were  
 277 observed in the study area (Fig. 3 and Table 2), occurring in 17.3% of the 1904  
 278 sampling units. The bamboo coral *Isidella elongata*, the cerianthid *Arachnanthus*  
 279 *oligopodus* and the sponge *Thenea muricata* (Figs. 3a, 3b and 3f) were the most  
 280 abundant species, representing 26.4%, 23.7% and 16.6% of all observed organisms,  
 281 respectively. *A. oligopodus* was the most frequent species, occurring in 30.7% of the  
 282 occupied sampling units, followed by both *I. elongata* and *T. muricata*, which both  
 283 occurred in 21.6% of the occupied sampling units (Table 2). Abundance and  
 284 frequency progressively decreased from the asteroid *Hymenodiscus coronata* to the  
 285 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).



288

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)

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

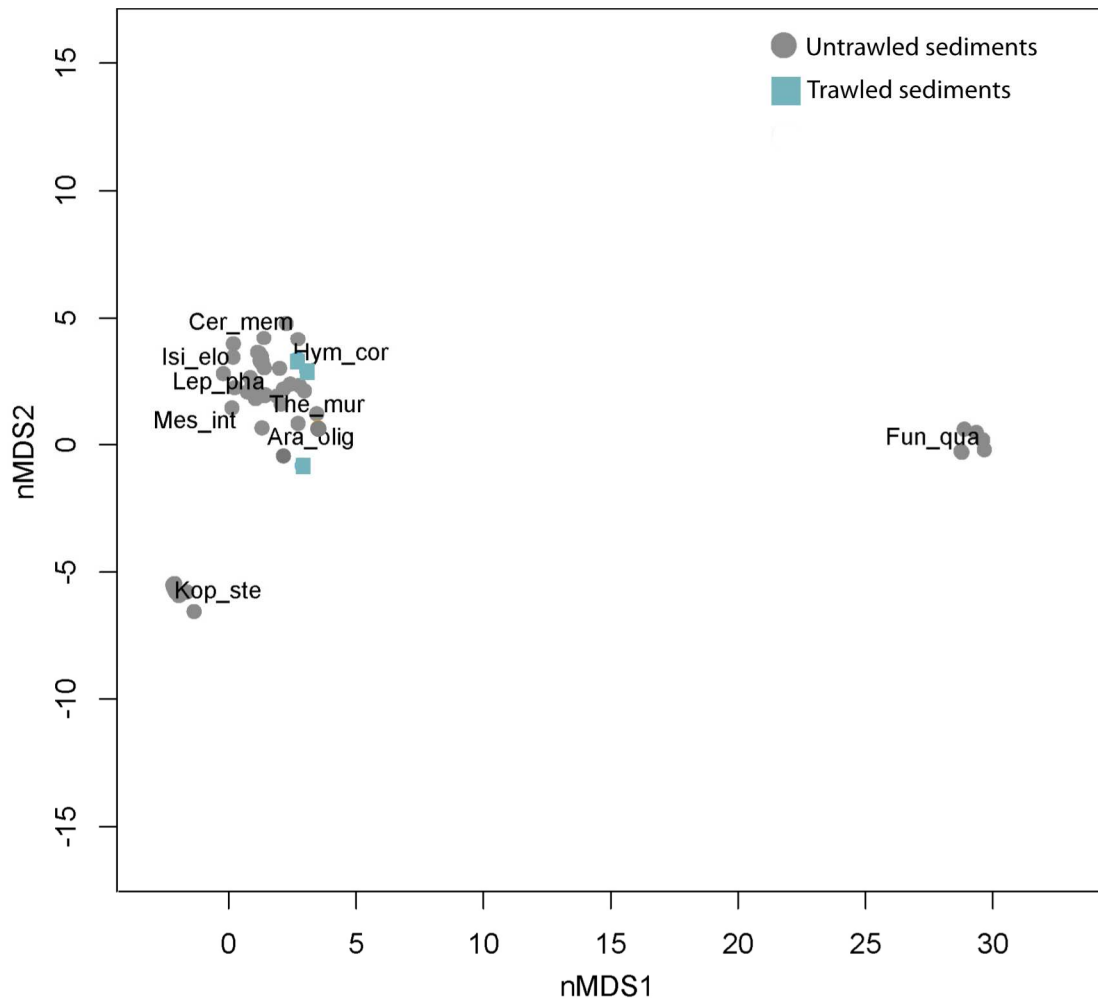
294 Table 2 Total and relative abundance of sessile megabenthic species together with their  
 295 occupancy (n° of sampling units occupied by a species) and frequency (% of occurrence in  
 296 the whole set of occupied sampling units) in the study area. Average and maximum density of  
 297 each sessile species within the occupied sampling units (n=329) are also shown. ind:  
 298 individuals.  
 299

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

300

### 301 3.3. Sessile megabenthic assemblage composition

302 Three megabenthic assemblages, distributed along the six ROV dives, could be  
 303 identified in the nMDS: two monospecific assemblages composed by few colonies of  
 304 the pennatulaceans *K. stelliferum* and *F. quadrangularis* respectively, and a  
 305 multispecific group composed of *I. elongata* (28%), *A. oligopodus* (25%), *T. muricata*  
 306 (18%), *H. coronata* (9%), *L. phalangium* (8%), *M. intestinalis* (8%) and *Cerianthus*  
 307 *membranaceus* (4%) (Fig. 4). The Adonis test and subsequent pairwise test revealed  
 308 that all three assemblages were significantly different (p<0.001) from one another.



309

310 Fig. 4. Non-metric multi-dimensional scaling (nMDS) ordination plot, data were square root  
 311 transformed, and ordination performed via a Bray-Curtis dissimilarity matrix): Sampling  
 312 units containing sessile organisms and echinoderms are represented considering trawling  
 313 presence (9 sampling units) and trawling absence (320 sampling units). Ara\_oli =  
 314 *Arachnanthus oligopodus*, Cer\_mem = *Cerianthus membranaceus*, Fun\_qua = *Funiculina*  
 315 *quadrangularis*, Hym\_cor = *Hymenodiscus coronata*, Isi\_elo = *Isidella elongata*, Kop\_ste =  
 316 *Kophobelemnon stelliferum*, Lep\_pha = *Leptometra phalangium*, Mes\_int = *Mesothuria*  
 317 *intestinalis*, The\_mur = *Thenea muricata*.

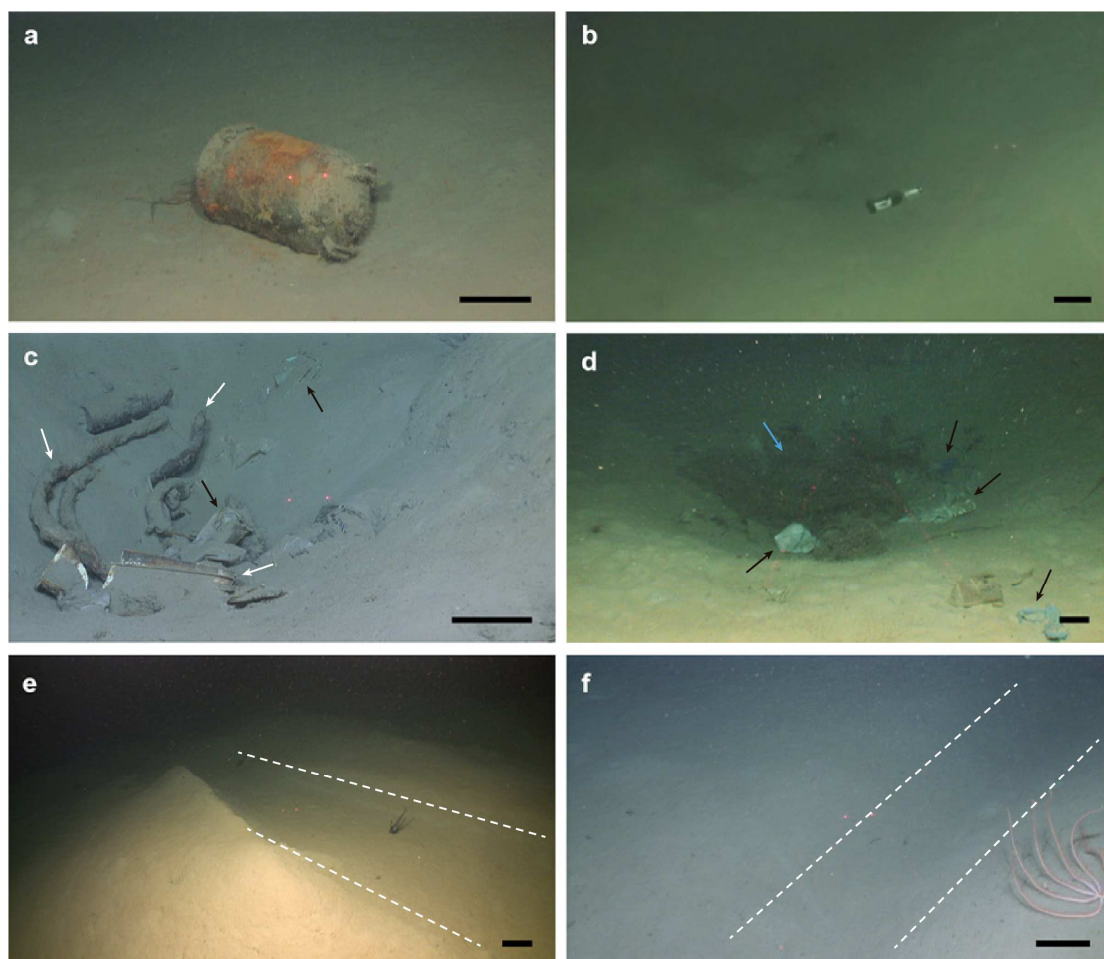
318

#### 319 3.4. Distribution of anthropogenic impacts

320 Marine litter was observed in all the ROV dives, except dive 6 (Table 3). Overall, 44  
 321 items were found, with plastics debris being the most abundant component (46%),



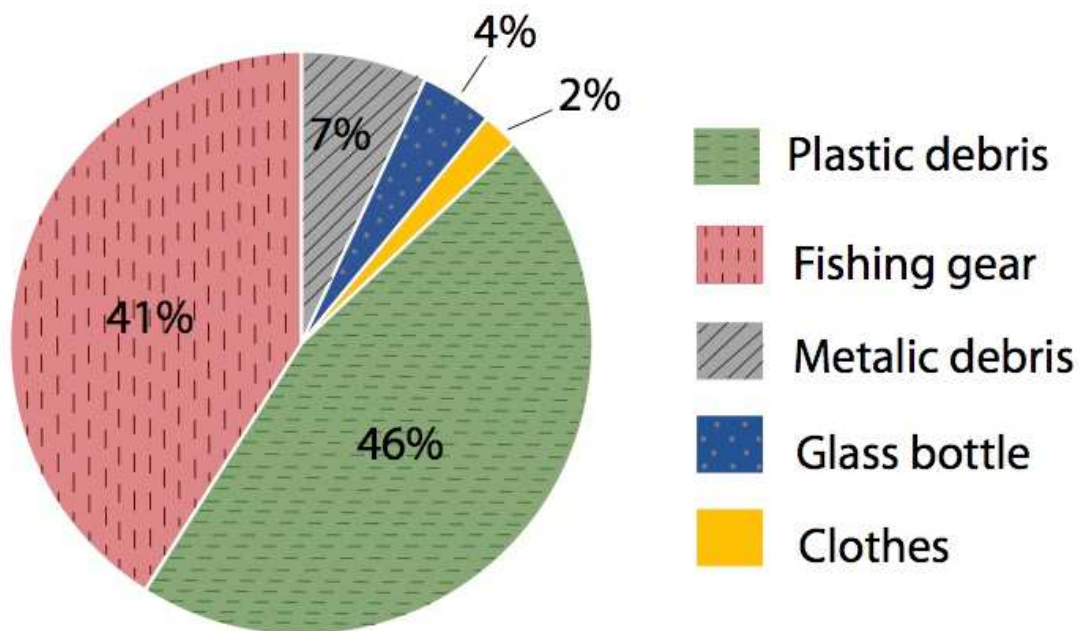
322 followed by lost fishing gears (41%), metallic debris (7%), such as a cot and an empty  
323 butane cylinder (Fig. 5a), glass bottles (4%) (Fig. 5b) and clothes (2%) (Fig. 6). In  
324 dives 4 and 5, 33% and 100% of marine litter items were found within pockmarks  
325 (Figs. 5c, 5d and 7c). Marine litter average densities ranged between  $0.001\pm 0.09$  to  
326  $0.01\pm 0.002$  items  $m^{-2}$ , with maximum densities of 2 items  $m^{-2}$  occurring within  
327 pockmarks. Trawl marks were observed in three of the six dives (Fig. 5e, 5f), with  
328 almost the totality of them concentrated in dive 3 (Table 3). A total of 93 trawl marks  
329 were observed, affecting 93 sampling units (4.88% of all sampling units).



330

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

335 arrows: metallic debris, black arrows: plastic debris, blue arrow: lost net, discontinuous line:  
 336 trawl mark delimitation.  
 337



338  
 339 Fig. 6. Marine litter composition.

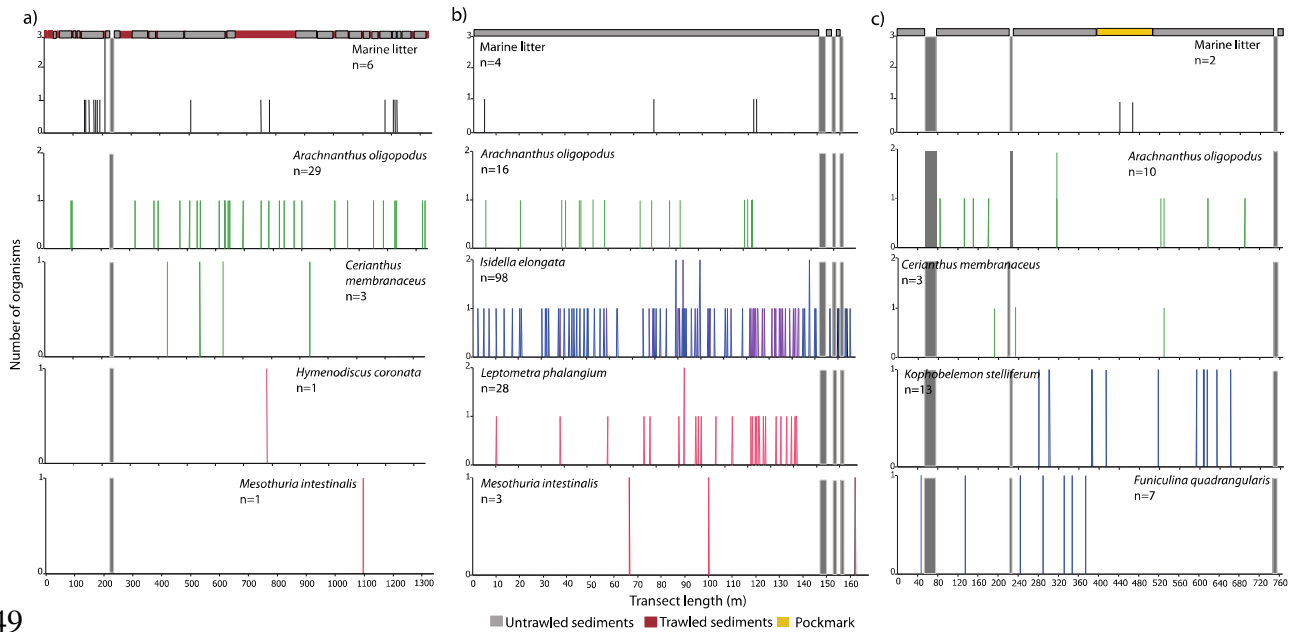
340

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

346  
 347

Zone	Dv	Marine litter				Trawl marks			
		Abundance (N°)	Occupancy (N°)	Av. density (Mean±SD)	SO (N°)	Abundance (N°)	Occupancy (N°)	Av. density (Mean±SD)	SO (N°)
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

348



349

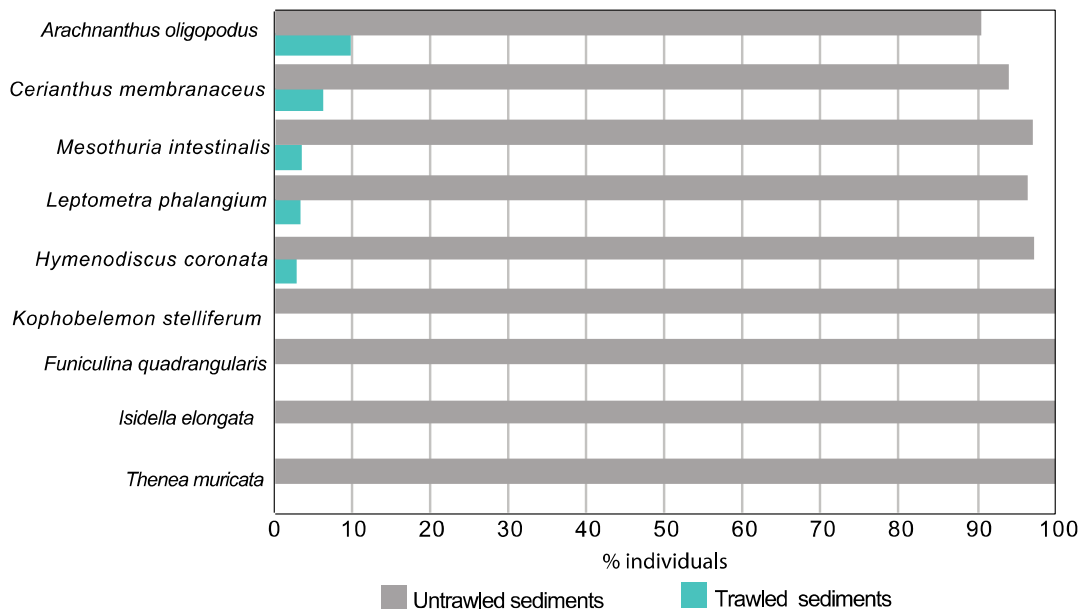
350 Fig. 7. Density plots showing variation of the abundance of different species and  
 351 anthropogenic impacts along representative dives. (a) Density plots of an area impacted by  
 352 bottom trawling (dive 3). (b) Density plots of an untrawled area covered by an *I. elongata*  
 353 meadow (dive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

359

### 360 3.5. Relationship between anthropogenic impacts and sessile megabenthic fauna

361 With the exception of a lost gear line, which occurred next to an *I. elongata* colony  
 362 within dive 2 (Table 3), marine litter and megabenthic species did not co-occur. Only  
 363 9 out of the 93 sampling units containing trawl-marks were colonized by megabenthic  
 364 species (Table 3), mainly represented by *A. oligopodus*, *C. membranaceus* and, to a  
 365 lesser extent, by *M. intestinalis*, *L. phalangium* and *H. coronata* (Figs. 7a ,8).  
 366 Overall, megabenthic densities in dives where trawl-marks were present ( $0.5 \pm 0.2$  ind.

367  $\text{m}^{-2}$  mean $\pm$ SD) were significantly lower (Adonis, PSEUDO-F = 26.9,  $p < 0.001$ ) than  
 368 those where trawl-marks were absent ( $1.5 \pm 0.7$  ind.  $\text{m}^{-2}$  mean $\pm$ SD).  
 369 Uprising non-retractile sessile species were exclusively found on untrawled sediment  
 370 (Figs. 7b, 7c and 8).

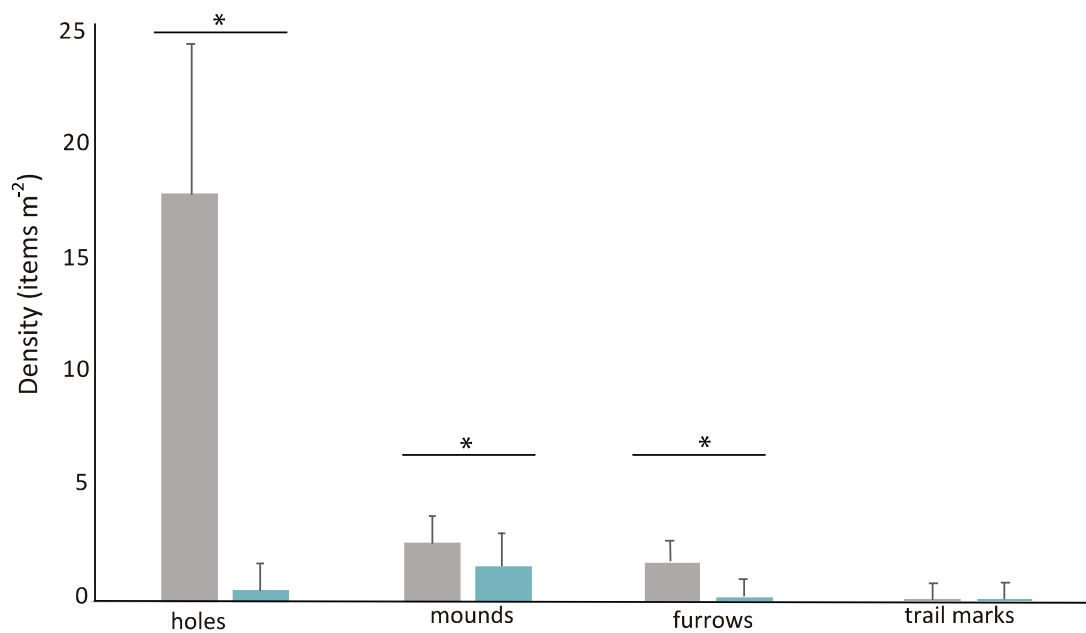


371  
 372 Fig. 8. Percentage of occurrence of sessile and low motile species in untrawled (grey) and  
 373 trawled (green) sampling units. untrawled sampling units: 320, trawled sampling units: 9.

374

### 375 3.6. Variations in habitat complexity and biodiversity in trawled vs un-trawled 376 areas

377 A total of 15730 lebensspuren items were observed, occurring on 67.5% of the  
 378 sampling units. Holes were the most abundant category (90%) followed by mounds  
 379 (8%), furrows (1.3%) and trail-marks (0.7%) (Fig. 9). Overall, lebensspuren densities  
 380 in dives where trawled marks were absent ( $26 \pm 16$  mean $\pm$ SD items  $\text{m}^{-2}$ ) were  
 381 significantly higher (Adonis, PSEUDO-F = 1778,  $p < 0.001$ ) than those where trawling  
 382 was present ( $1.8 \pm 3.2$  mean $\pm$ SD items  $\text{m}^{-2}$ ). Such pattern was observed for all  
 383 lebensspuren categories with the exception of trail-marks, for which no significant  
 384 differences were found (Fig. 9).



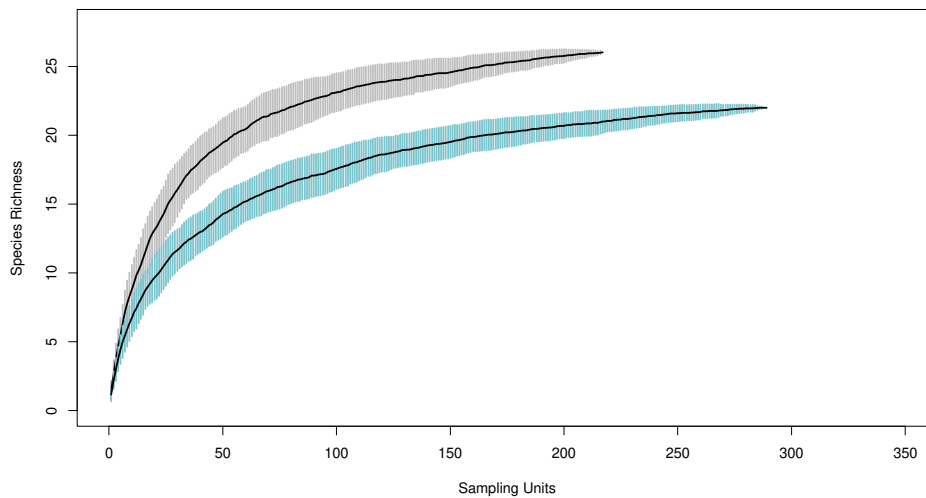
386  
387

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

392

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

397



398

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

403

#### 404 4. Discussion

##### 405 4.1. Sessile megabenthic assemblages

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

414 The two monospecific assemblages observed in this study were respectively  
 415 dominated by the sea-pens *Funiculina quadrangularis* and *Kophobelemnon*  
 416 *stelliferum* (Figs. 3c, 3d and 4), both being common inhabitants of bathyal muds of  
 417 the western Mediterranean Sea (Gili and Ros, 1987; Fabri et al., 2014; De La Torriente

418 et al., 2018), where they have already been reported to form monospecific  
419 assemblages (Pérès, 1967; Mastrototaro et al., 2013; Pierdomenico et al., 2018).  
420 Although *F. quadrangularis* were described to form dense aggregations on  
421 Mediterranean bathyal muds (Pérès and Picard, 1964), the low densities observed in  
422 this study resemble previous observations from shelf-edge and submarine canyon  
423 environments of the western Mediterranean (Fabri et al., 2014; Grinyó et al., 2018;  
424 Pierdomenico et al., 2018). Conversely, *K. stelliferum* assemblages presented high  
425 densities than those reported by Mastrototaro et al. (2013) in Santa Maria di Leuca  
426 (Ionian Sea - Central Mediterranean). De Clippele et al. (2015) observed that both  
427 species increase diversity at a local scale. Certainly, 22% of *K. stelliferum* colonies,  
428 found in the present study, were associated with decapods and macrourid fishes,  
429 reinforcing the general consensus that this species may provide shelter and act as a  
430 feeding grounds for associated species (De Clippele et al., 2015). Conversely, *F.*  
431 *quadrangularis* colonies were not associated to any other megabenthic species.  
432 However, this observation might have been affected by the low number of colonies  
433 reported on this study.

434 The multispecific assemblage was widely represented, occurring in 99% of the  
435 occupied sampling units (Fig. 4). In this assemblage, the bamboo coral *Isidella*  
436 *elongata* (Fig. 3b) was the most abundant species (Table 1), forming meadows that  
437 extended over wide areas (Fig. 7b), which punctually reach densities up to 7 colonies  
438 m<sup>-2</sup>. These density values are substantially higher than those recorded in other regions  
439 of the Mediterranean Sea (Mastrototaro et al., 2017; Pierdomenico et al., 2018;  
440 Ingrassia et al., 2019), where *I. elongata* reached maximum densities of 2.5 colonies  
441 m<sup>-2</sup> (Bo et al., 2015). *I. elongata* populations appeared to be in good conservation  
442 status, as no dead colonies were observed and partial necrosis was present in less

443 than 6% of the observed colonies. This low necrosis ratio contrasted with what has  
444 been observed in impacted *I. elongata* populations located on trawling grounds of the  
445 western Mediterranean (Pierdomenico et al., 2018). *I. elongata* has a major ecological  
446 role in bathyal environments of the Mediterranean Sea, providing essential habitat for  
447 several ichthyic and decapod species (Cartes et al., 2013; Mastrototaro et al., 2017),  
448 representing a secondary biological hard substratum, elevated from the surrounding  
449 sediments (Mastrototaro et al., 2017). On this regard, 47% of *I. elongata* colonies  
450 where epiphyted by the crinoid *Leptometra phalangium* (Fig. 3b), and to a lesser  
451 extent, by the decapod *Anamathia rissoana*. More than 90% of *L. phalangium*  
452 individuals occurred on top of *I. elongata* colonies, generally hosting 1 crinoid  
453 ( $1.7 \pm 1.0$  ind./colony mean  $\pm$  SD), although largest colonies (>20 cm height) could host  
454 up to 5 crinoids. Since *L. phalangium* is a suspension feeder, it is likely that these  
455 crinoids are using *I. elongata* colonies as a physical support allowing to be detached  
456 from the seafloor and gaining exposure to more intense and food-rich currents,  
457 favoring their feeding rates. A similar functional relationship has been observed in the  
458 bathyal muds of the Balearic archipelago between *I. elongata* and the crinoid *Antedon*  
459 *mediterraneum* (Mastrototaro et al., 2017).

460 Among the other uprising sessile fauna that integrated the multispecific assemblage,  
461 we encountered the Demosponge *Thenia muricata* and the Cerianthids *Arachnanthus*  
462 *oligopodus* and *Cerianthus membranaceus* (Figs. 3a, e and f). *Thenia muricata* has  
463 been described as a typical inhabitant of Mediterranean bathyal muds (Vacelet, 1969;  
464 Maldonado et al., 2015). In the study area, this species presented a sparse distribution  
465 and occurred in low densities, in accordance with other studies from the western  
466 Mediterranean (Santín et al., 2018). Conversely, cerianthids were amongst the most  
467 abundant species (38% of all sessile fauna), contrasting with previous observations



468 depicting them as a rare group in Mediterranean bathyal muds (Cartes et al., 2009;  
469 Mastrototaro et al., 2013). Up to date, information regarding megabenthic  
470 assemblages in deep sedimentary seafloor, mostly derives from experimental bottom  
471 trawls or benthic sledges (e.g. Gili and Ros, 1987; Pansini and Musso, 1991; Maynou  
472 and Cartes, 2012; Mastrototaro et al., 2013). Although these gears have provided a  
473 great amount of occurrence data, they cannot be considered trustworthy from a  
474 quantitative point of view, often providing weak information about the abundance and  
475 distribution of sessile species (Chimienti et al., 2018). This lack of reliability is  
476 magnified for those species that have soft bodies and burrowing or withdrawing  
477 behaviors (Durden et al., 2015; Chimienti et al., 2018). Therefore, since cerianthids  
478 rapidly and fully retract into sediments, it is likely that the abundance of this groups in  
479 Mediterranean bathyal muds has been widely underestimated.

480 The asteroid *Hymenodiscus coronata* and the holothurian *Mesothuria intestinalis*  
481 (Figs. 3g and h), typical inhabitants of Mediterranean abyssal muds (Mecho et al.,  
482 2018), occurred as isolated individuals, being the most sparsely distributed  
483 component of the multispecific assemblage (Figs. 7a and b). In open slope  
484 environments, both species have generally presented low abundances (Mecho et al.,  
485 2018), although *M. intestinalis* has been observed to form massive aggregations  
486 coinciding with increments of labile organic matter (Cartes et al., 2009).

487 The multispecific assemblage occurred on untrawled sediments (95% sampling units),  
488 trawled sediments (3.5% sampling units) and on pockmark fields (0.5% sampling  
489 units) (Figs. 4, 7b and 7c). Although active pockmarks can potentially influence  
490 species distribution and abundance due to the influence of fluid seepage on water  
491 chemistry (Zeppilli et al., 2012), in the study area no chemical nor physical changes  
492 were observed through the water column (gas seepage) or surrounding sediments

493 (carbonate crusts) in correspondence to these features. This suggests that inactive  
494 pockmarks in this area do not exert any direct influence on megabenthic fauna  
495 distribution.

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

505

#### 506 4.2. Marine litter

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

518 maritime-based origin for heavy litter in open-slope environments has been suggested  
519 for other areas of the Mediterranean and North-Atlantic Ocean (Ramírez-Llodra et al.,  
520 2013; Pham et al., 2014).

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

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

534

#### 535 4.3. The impact of bottom trawling

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

543 Mediterranean trawling by-catch (Relini et al., 1986; Gili and Ros, 1987; Pansini and  
544 Musso, 1991). In fact, in ROV dives where trawl marks were present they presented  
545 significantly lower densities ( $0.5 \pm 0.1$  ind.  $m^{-2}$  mean  $\pm$  SD; Adonis, PSEUDO-F=15.28;  
546  $p < 0.0001$ ) than those where trawl marks were absent ( $0.87 \pm 0.73$  ind.  $m^{-2}$  mean  $\pm$  SD).  
547 These species populations have been widely decimated in the last decades, and are  
548 now mostly restricted to areas inaccessible to bottom trawling (Mastrototaro et al.,  
549 2013; 2017; Bo et al., 2015; Santín et al., 2018).

550 In trawled sampling units, occupancy was rather low and was restricted to cerianthids  
551 and echinoderms (Fig. 8). Cerianthids might be able to withstand bottom trawling  
552 through their ability to rapidly retract within tubes that are rooted tens of centimeters  
553 below the sediment surface (Picton and Manuel, 1985). Echinoderms in deep  
554 Mediterranean environments are extremely susceptible to the destructive effects of  
555 bottom trawling, being unable to escape or withstand them (Smith et al., 2000; Cartes  
556 et al., 2009). Thus, the presence of *H. coronata* in trawled sampling units (Fig. 8)  
557 might be explained by its motile capacity, which may allow re-occupancy of sampling  
558 units after the passage of trawl gears. However, several *H. coronata* individuals  
559 displayed a peculiar behavior in trawled sampling units. Individuals were observed on  
560 the edge of trawl marks extending their arms into the water column (Fig. 5f). In this  
561 slightly uplifted position, suspension feeders may be able to reach water layers  
562 exposed to more intense hydrodynamics, improving their feeding rates (Carlier et al.,  
563 2009).

564 Lebensspuren density and megabenthic diversity were higher in untrawled dives than  
565 in trawled ones (Figs. 9 and 10), resembling similar patterns observed in other bathyal  
566 Mediterranean environments (Mastrototaro et al., 2017). As nets, trawl-doors and  
567 chains are dragged on the seafloor, sedimentary structures are flattened and

568 bioturbator and habitat-forming species are removed or damaged (Auster et al., 1996;  
569 Olsgard et al., 2008; Pierdomenico et al., 2018), resulting in a reduction of habitat  
570 complexity and, therefore, local biodiversity (Probert et al., 1997). Indeed, bottom  
571 trawling can greatly alter benthic ecosystem functioning and productivity (Jennings et  
572 al., 2001), potentially having crucial long-term consequences for local fisheries  
573 (Victorero et al., 2018). Considering the low recuperation rates of most species  
574 characterizing the observed assemblages (Maynou and Cartes 2012), our study's  
575 findings arise grave concern on the conservation status of these vulnerable benthic  
576 assemblages in the deep muddy bottoms of the Alboran Sea.

577

## 578 5. Conclusions

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

591

## 592 Acknowledgements

593 The data for this study were collected during the Spanish national project SHAKE  
594 (CGL2011-3005-C02-02). We acknowledge the EU Eurofleets-2 Project, which  
595 provided the ROV Max Rover from the Hellenic Centre for Marine Research  
596 (Greece) during the SHAKE cruise (IRIS project - Embarked Equipment 2013 Call  
597 EF2-EE-003. GA 312762), Finally, we gratefully acknowledge all the participants on  
598 the SHAKE cruise and the crew on board of the R/V “Sarmiento de Gamboa” and  
599 Professor Pablo J. Lopez from the University of Seville for his help to identify several  
600 megabenthic species.

601

602 Author contribution

603 Jordi Grinyó: Conceptualization, methodology, data curation, formal analysis, writing  
604 - original draft; Writing - review & editing.

605

606 Claudio Lo Iacono: Conceptualization, methodology, writing - original draft; writing -  
607 review & editing.

608

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

610

611 Suzanne Conlon: Methodology, data curation.

612

613 Guillem Corbera: Visualization, writing - review & editing.

614

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

617

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