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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

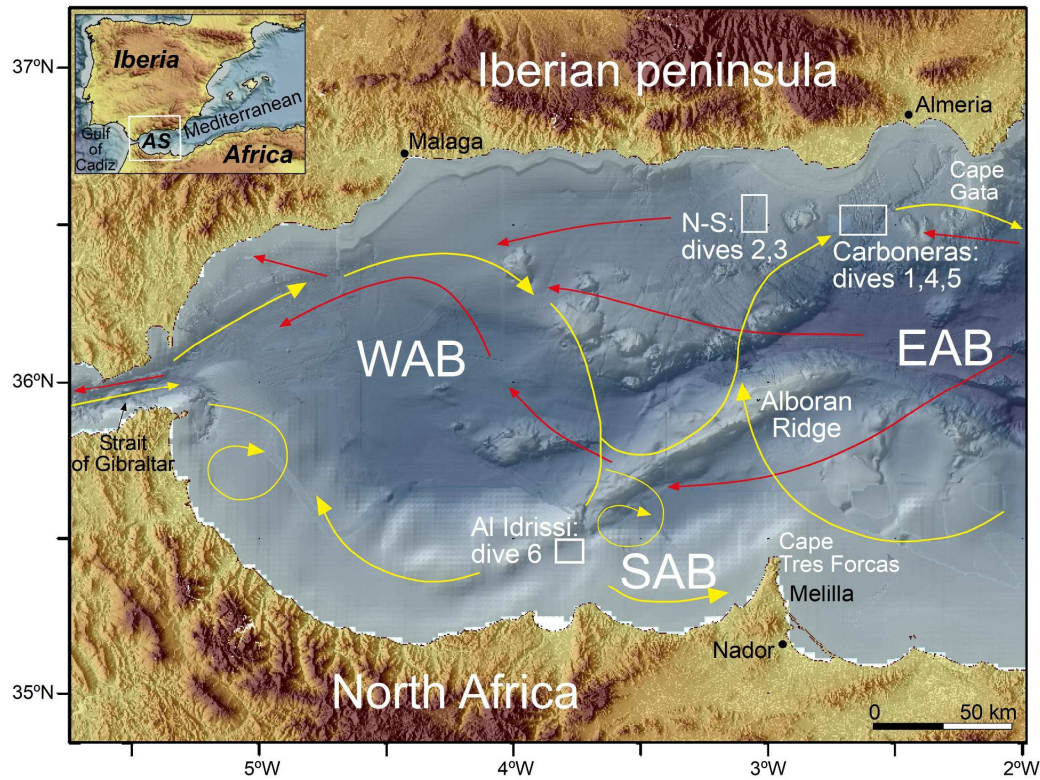
121 The Alboran Sea is the westernmost basin of the Mediterranean, which is enclosed
122 between the Iberian Peninsula and the North Africa, connected to the Atlantic Ocean
123 through the Straits of Gibraltar (Fig. 1). The Alboran Sea displays a relatively
124 complex seafloor geomorphology, including 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 ($T \sim 15$ °C, $S \sim 36.2$ psu, $T \sim 13.5$ °C $S \sim 38.4$ 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 ~ 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 ($T \sim 13.5$ °C, $S \sim 38.4$ 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 - 12 °C, $S \sim 38.40$ - 38.52 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).



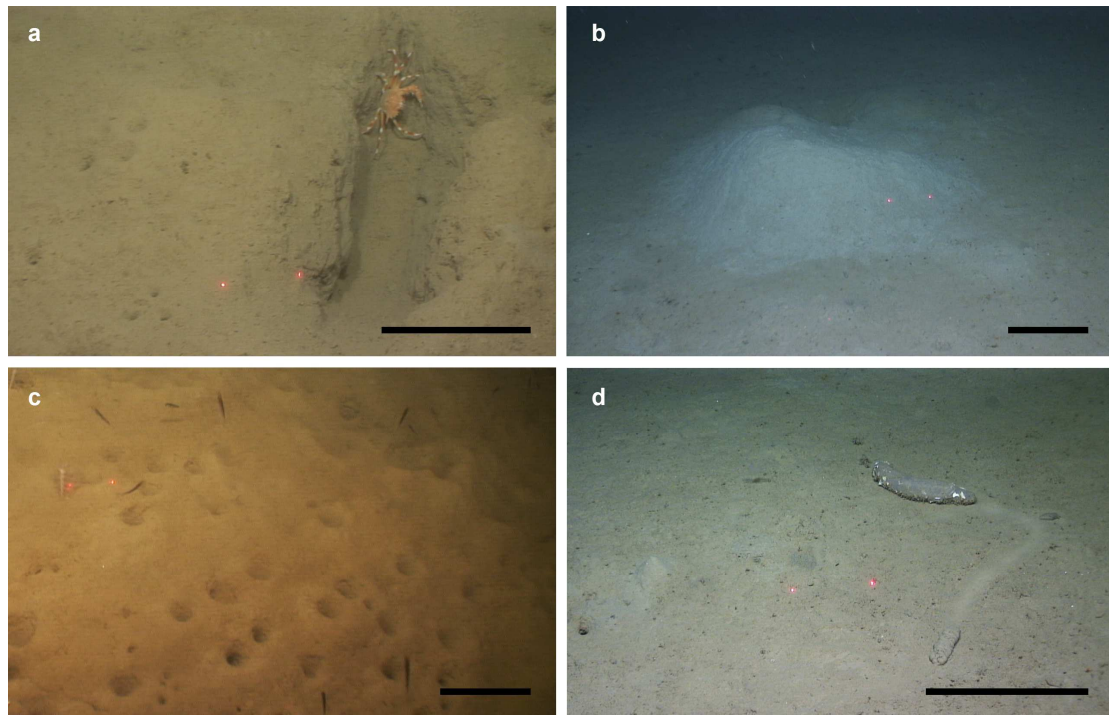
181
 182 Fig. 1. Topographic and bathymetric map of the Alboran Sea, white rectangles indicate the
 183 location of dives, whose location correspond to different fault systems: North-South fault
 184 system (N-S), Carboneras fault system and Al-Idrissi Fault System. WAB: West Alboran
 185 Basin, EAB: 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 m²
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

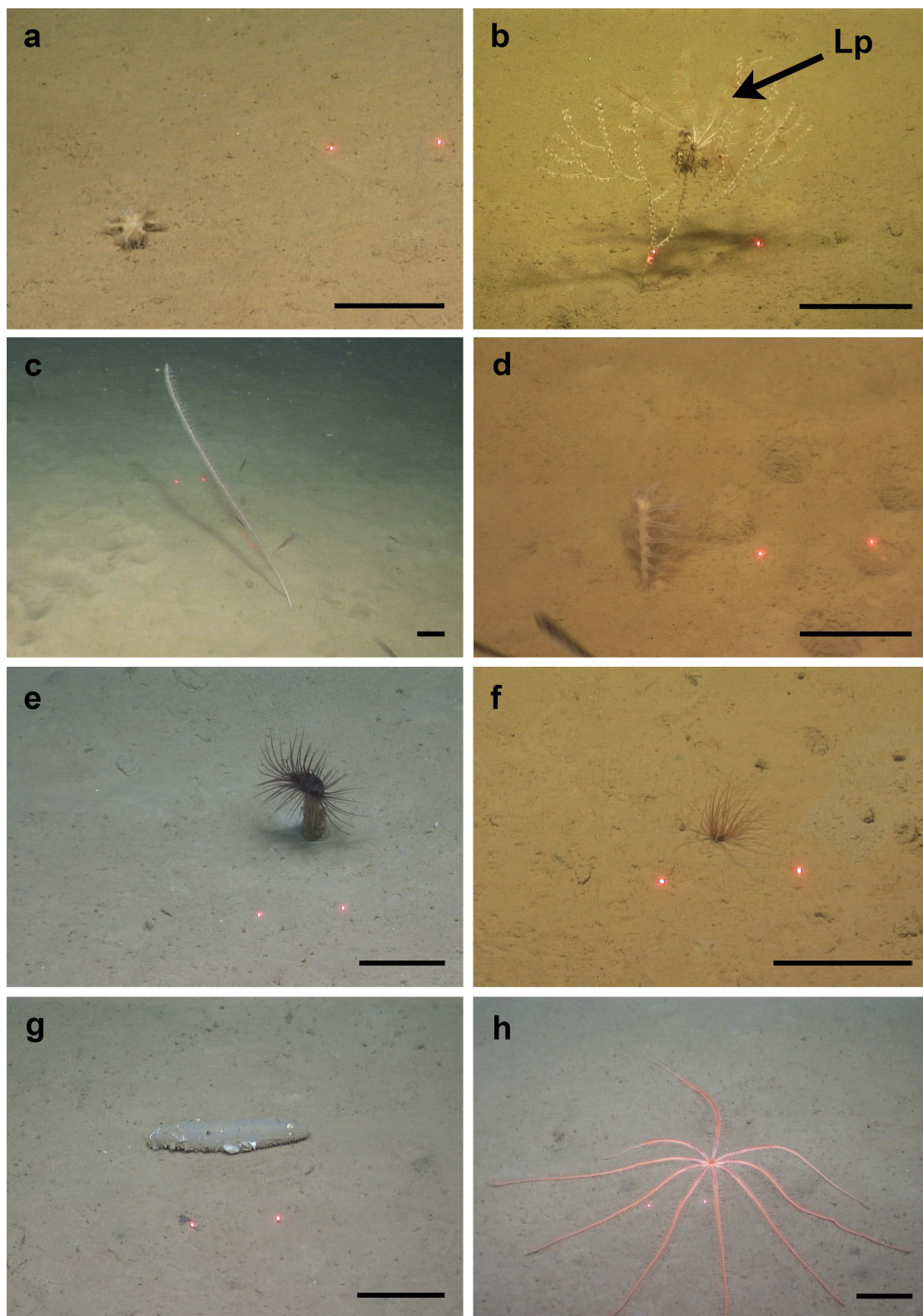
Zone	Dv	Start		End		Length (m)	N° of Pockmarks	Depth range (m)
		Lat° N	Lon° W	Lat° N	Lon° W			
CFS	1	36.524	3.088	36.519	3.077	1700	0	704 - 722
NSFS	2	36.481	2.695	36.481	2.694	1109	0	639 - 685
NSFS	3	36.515	3.051	36.520	3.052	1368	1	638 - 688
CFS	4	36.532	2.619	36.541	2.618	168	0	836 - 860
CFS	5	35.498	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) *Kophobelemnon stelliferum*, e) *Cerianthus membranaceus*, f)
 292 *Arachnanthus oligopodus*, g) *Mesothuria intestinalis*, and h) *Hymenodiscus coronata*. Scale bar: 10
 293 cm.

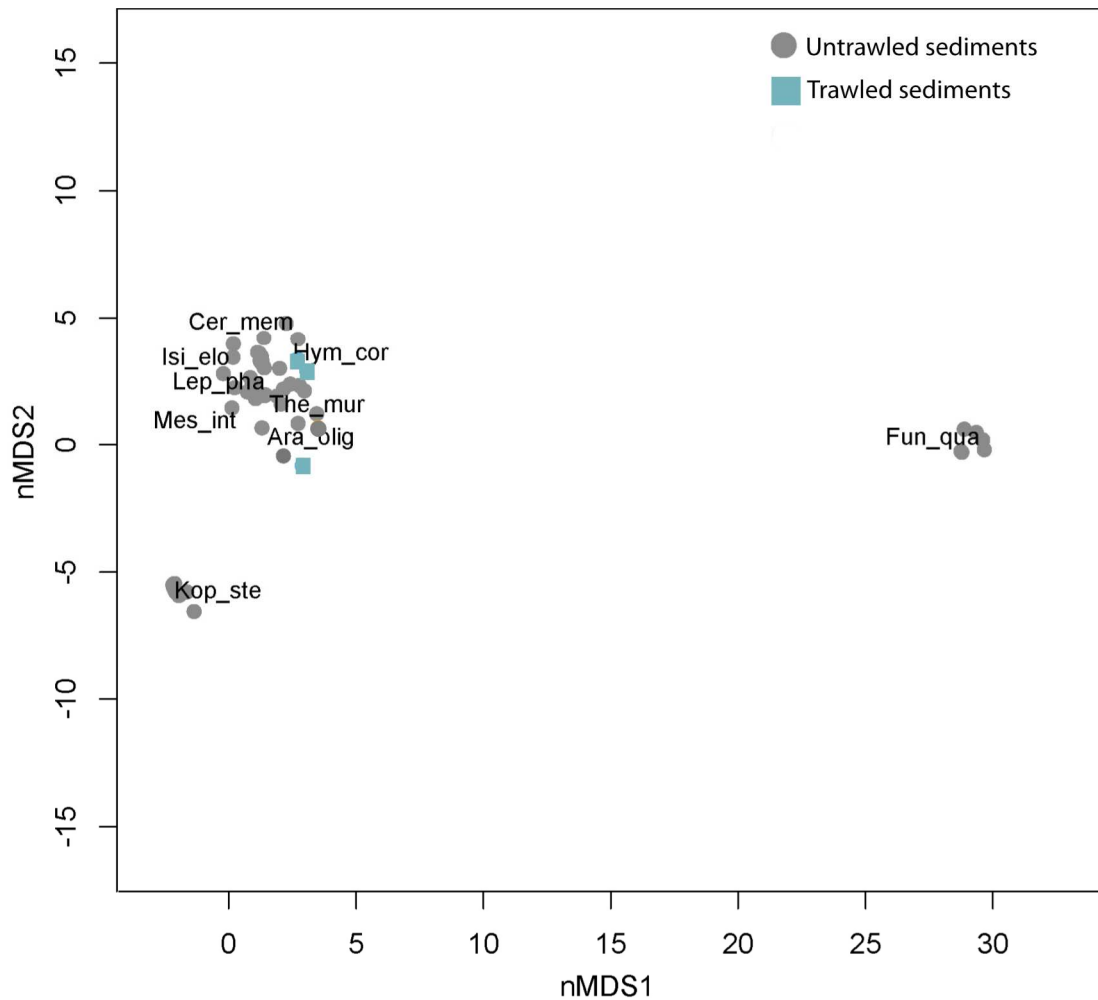
294 Table 2 Total and relative abundance of sessile megabenthic species together with their
 295 occupancy (n° of sampling units occupied by a species) and frequency (% of occurrence in
 296 the whole set of occupied sampling units) in the study area. Average and maximum density of
 297 each sessile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

Class	Species	Abundance		Occupancy		Average density \pm SD	Max density
		Number	(%)	Number	(%)	(ind. m ⁻²)	(ind. m ⁻²)
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>Kophobelemnon 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

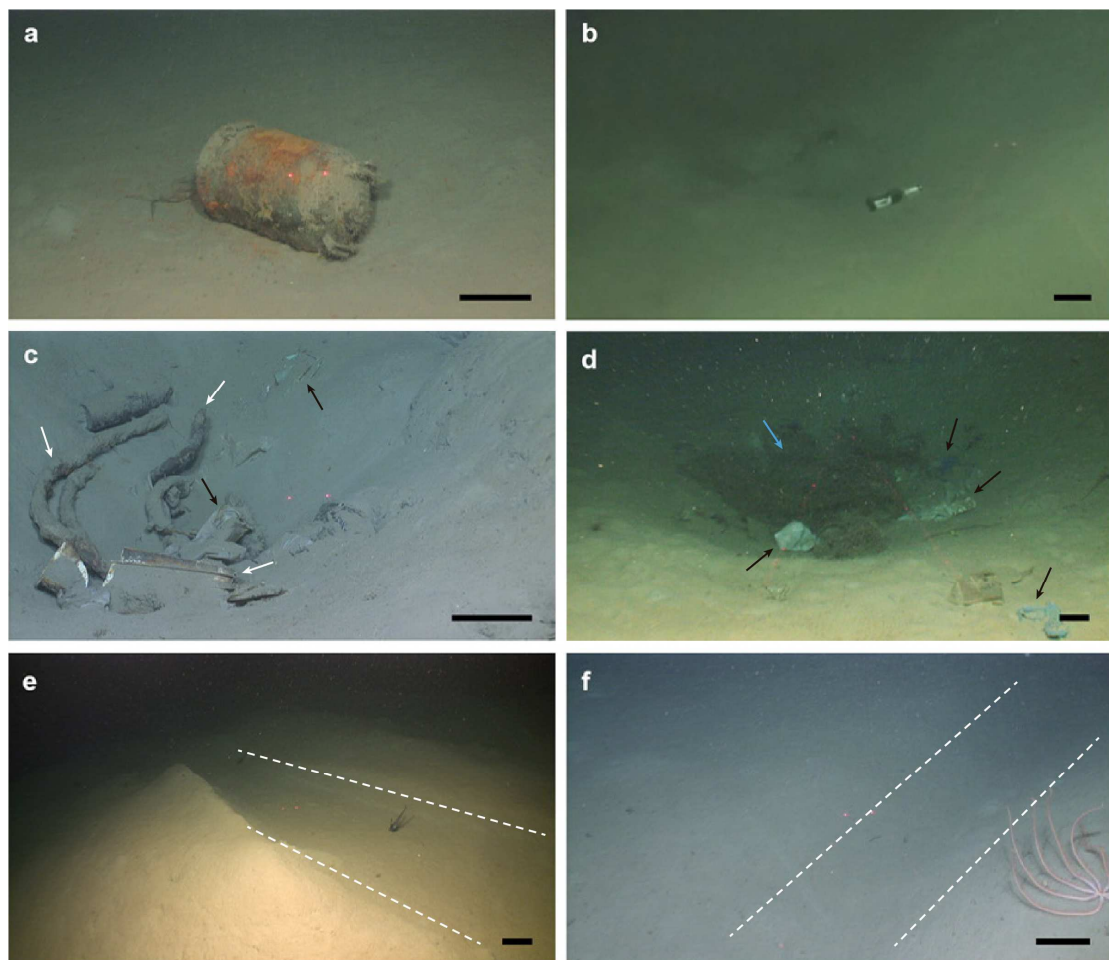
310 Fig. 4. Non-metric multi-dimensional scaling (nMDS) ordination plot, data were square root
 311 transformed, and ordination performed via a Bray-Curtis dissimilarity matrix): Sampling
 312 units containing sessile organisms and echinoderms are represented considering trawling
 313 presence (9 sampling 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 *Kophobelemnion 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%),

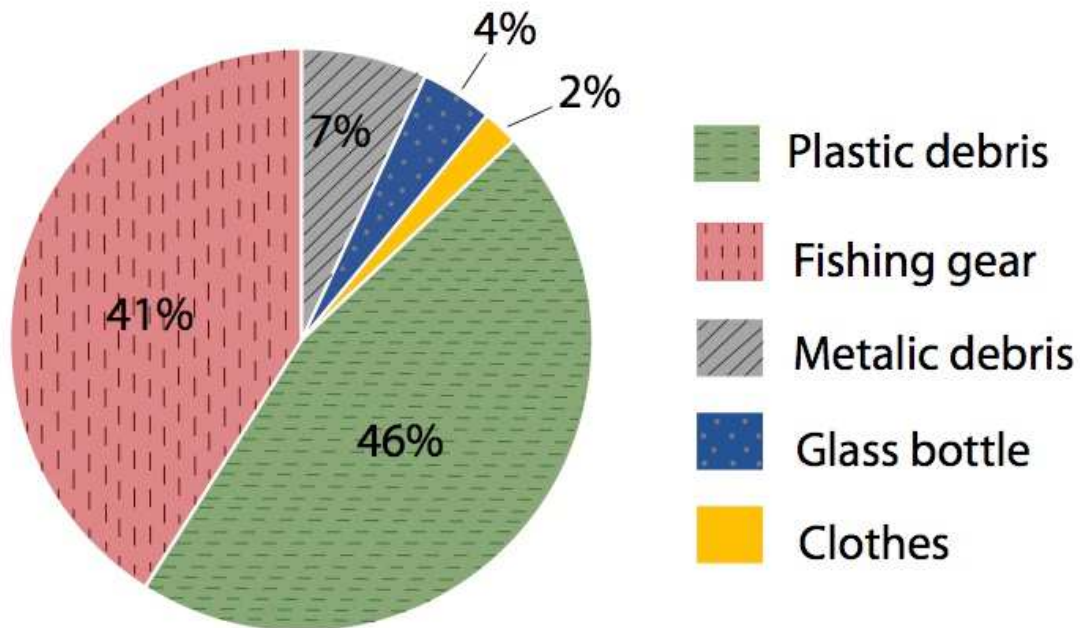
322 followed by lost fishing gears (41%), metallic debris (7%), such as a cot and an empty
323 butane cylinder (Fig. 5a), glass bottles (4%) (Fig. 5b) and clothes (2%) (Fig. 6). In
324 dives 4 and 5, 33% and 100% of marine litter items were found within pockmarks
325 (Figs. 5c, 5d and 7c). Marine litter average densities ranged between 0.001 ± 0.09 to
326 0.01 ± 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.

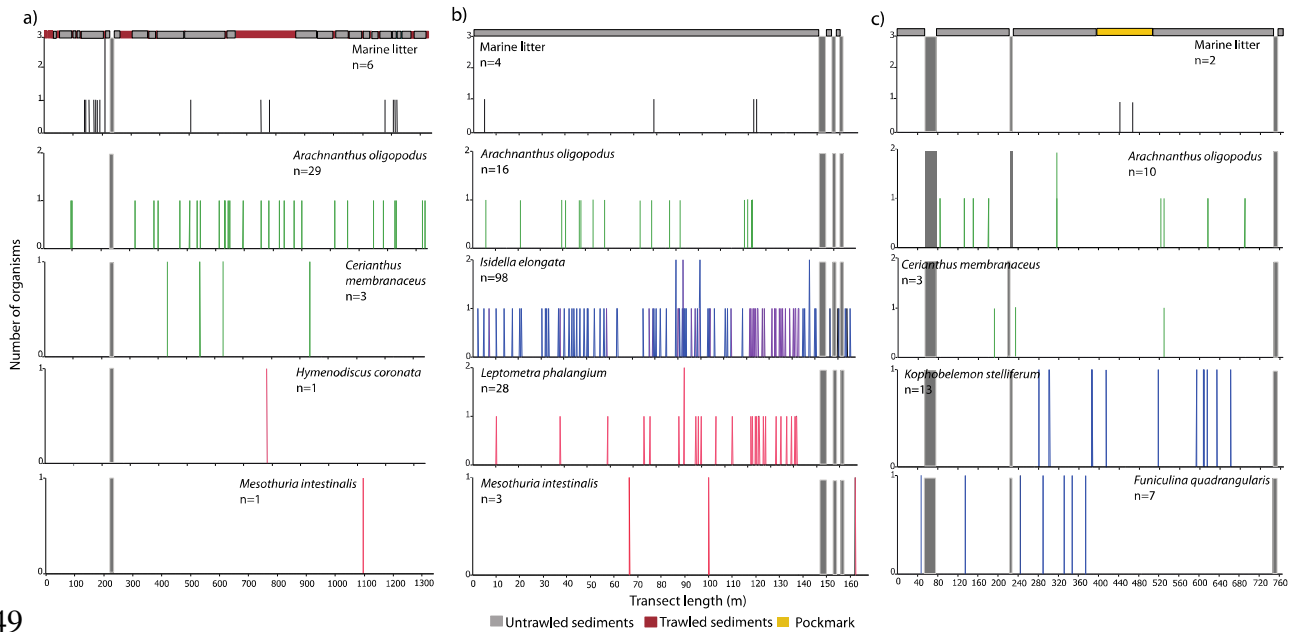
340

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

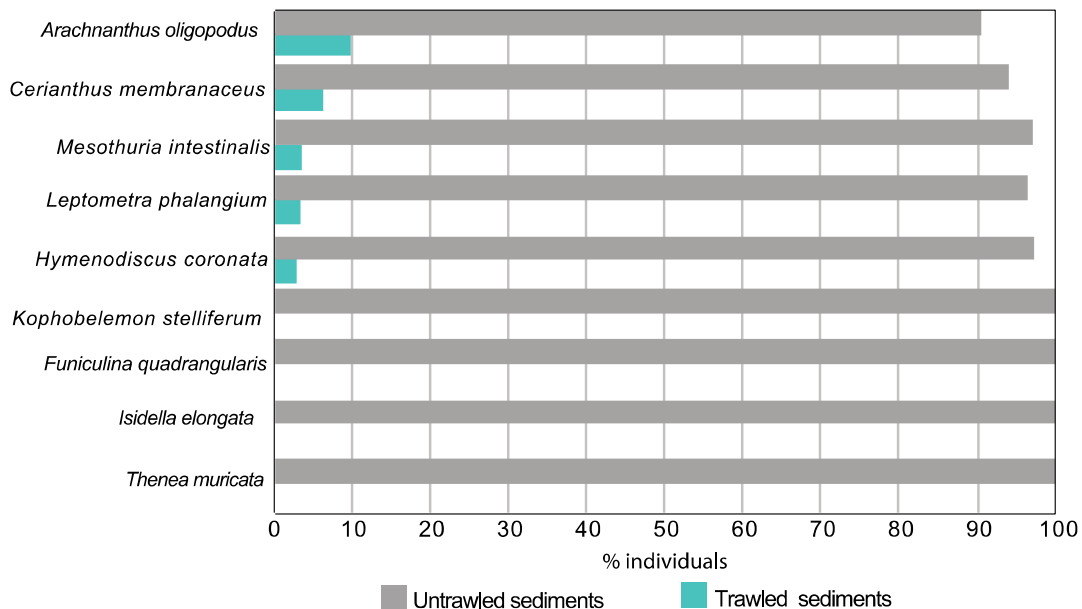
350 Fig. 7. Density plots showing variation of the abundance of different species and
 351 anthropogenic impacts along representative dives. (a) Density plots of an area impacted by
 352 bottom trawling (dive 3). (b) Density plots of an untrawled area covered by an *I. elongata*
 353 meadow (dive 4). (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 ± 0.2 ind.

367 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 ± 0.7 ind. m^{-2} mean \pm SD).
 369 Uprising non-retractile sessile species were exclusively found on untrawled sediment
 370 (Figs. 7b, 7c and 8).

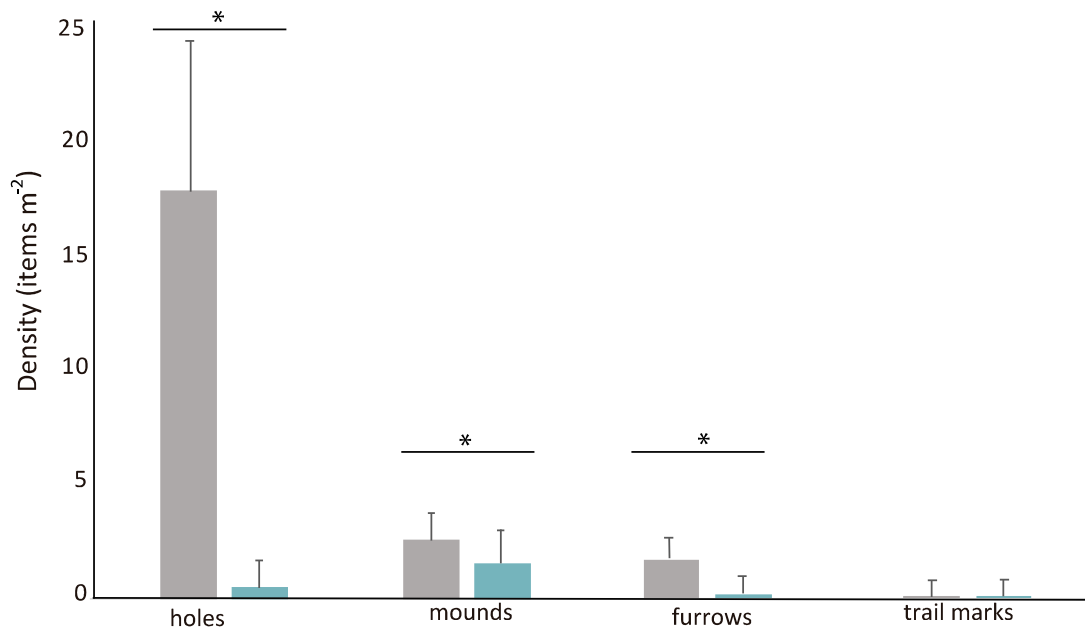


371
 372 Fig. 8. Percentage of occurrence of sessile and low motile species in untrawled (grey) and
 373 trawled (green) sampling units. untrawled sampling units: 320, trawled sampling units: 9.

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 ± 16 mean \pm SD items m^{-2}) were
 381 significantly higher (Adonis, PSEUDO-F = 1778, $p < 0.001$) than those where trawling
 382 was present (1.8 ± 3.2 mean \pm SD items 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).



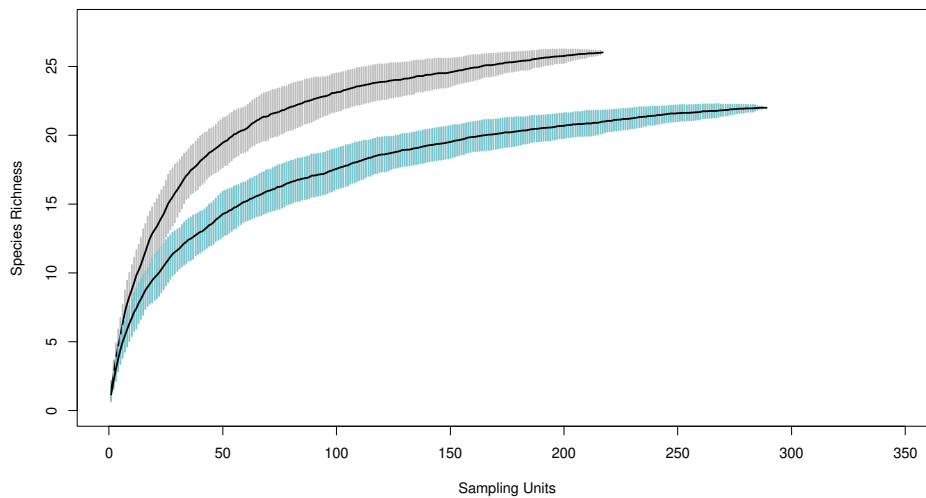
386
387

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⁻². 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⁻² (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 ± 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 ± 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 ± 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

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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

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612

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

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

617

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