

1 **ECOLOGICAL QUALITY ASSESSMENT OF MARINAS: AN INTEGRATIVE**  
2 **APPROACH COMBINING BIOLOGICAL AND ENVIRONMENTAL DATA.**

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20 **Highlights:**

21 -An integrative method for quality assessment is developed and proposed for marinas

22 -Correlations between fauna and abiotic data are detected at family and order level

23 -Soft-bottom benthic communities are key tools for managing programmes

24

25 **Abstract**

26 The importance of marinas as infrastructures for recreational boating is  
27 increasing substantially. However, information on their soft-bottom benthic  
28 communities, a key tool for managing programmes, is still scarce. We combined  
29 environment features with macro- and meiofaunal soft-bottom community information  
30 for assessing the ecological status of marinas with an integrative approach. To address  
31 this issue, we focused on eight marinas of the Southern Iberian Peninsula. Macro- and  
32 meiofauna data revealed high benthic heterogeneity at a spatial scale. The  
33 environmental variables which correlated best with macrofauna were mainly

34 phosphorus, granulometry, and total organic carbon, and secondarily important  
35 variables were faecal coliforms, the biocide Irgarol, and heavy metals; total  
36 hydrocarbon concentration was also significant for meiofauna. Annelida was the  
37 dominant phylum in terms of number of species (37%) and abundance (66%) and were  
38 better descriptors of the environmental conditions than Arthropoda and Mollusca.  
39 Although identification to the species level is desirable and mandatory for assessing  
40 biological pollution, significant differences among marinas and correlations between  
41 fauna and abiotic variables were already detected at the level of family and order. This  
42 implies that biota assessment at higher levels may still be useful in monitoring  
43 programmes limited by time and budget constraints. The major novelty of this study lies  
44 in the development of an integrative assessment method based on the following selected  
45 ecological indicators: Marinas Environmental Pollution Index (MEPI),  
46 Biocontamination Index (BCI), macrofaunal biotic indices (AMBI, M-AMBI, BENTIX,  
47 MEDOCC and BENFES), macrofaunal taxa richness and Shannon-Wiener's diversity,  
48 and nematode:copepod index. This approach was able to discriminate marinas of the  
49 Southern Iberian Peninsula based on their ecological status, which ranged from poor to  
50 good. The method can be useful to design standards for assigning "sustainable quality  
51 seals" to those marinas with better values of ecological indicators.

52

53 Keywords: Marinas, ecological assessment, macrofauna, meiofauna, soft bottoms,  
54 taxonomic resolution

55

## 56 **1. Introduction**

57 Port areas and marinas in particular, are confined environments subjected to a  
58 number of anthropogenic activities that result in significant environmental disturbances  
59 (Tempesti et al., 2020 and references therein). The number of marinas has significantly  
60 increased during the last years, since they are essential infrastructures for the  
61 recreational boating sector, a key activity of global marine tourism in high demand (Di  
62 Franco et al., 2011). However, marinas modify ecological conditions (Rivero et al.,  
63 2013; Valdor et al., 2019) and their increasing number is recognized as an important  
64 environmental stressor in many regions (e.g. Gómez et al., 2017).

65 Despite the importance of marinas as hotspots of environmental and biological  
66 pollution, knowledge about these novel ecosystems is still very scarce. Previous studies

67 have evaluated the pollution pressure (Mali et al., 2017; Gómez et al., 2019; Guerra-  
68 García et al., 2021), but little effort has been put into proper characterization of their  
69 biological communities (Covazzi Harriague et al., 2012; Chatzinikolaou et al., 2018;  
70 Dimitrou et al., 2020). Faunistic information in marinas is mainly restricted to sessile  
71 communities and/or Non-Indigenous Species (NIS) (e.g. Di Franco et al., 2011;  
72 Oricchio et al., 2016; Kenworthy et al., 2018; Ulman et al., 2019; Ferrairo et al., 2020),  
73 and there is a lack of research targeting mobile macro- or meiofauna. Furthermore, the  
74 few studies dealing with these taxa have focused on arborescent substrates attached to  
75 floating pontoons (Ros et al., 2013; Marchini et al., 2015; Fernández-Romero et al.,  
76 2019; Gavira O’Neill et al., 2018; Martínez-Laiz et al., 2018). Meanwhile, studies on  
77 soft-bottom fauna in marinas are scarce (e.g. Chatzinikolaou et al., 2018, Dimitrou et  
78 al., 2020, Table S1) and generally limited when compared to other assemblages, such as  
79 the biofouling organisms which cause noticeable financial and ecological impacts (Ng  
80 et al., 2019 and references therein). The study of the soft bottom environment in  
81 marinas (usually corresponding to muddy sediments) is especially relevant since (i) it is  
82 the most widespread habitat there, (ii) it represents a more stable substrate for fauna  
83 than the floating structures above (which are temporally cleaned and often subjected to  
84 fluctuations in salinity and temperature), and (iii) sediments accumulate pollutants and  
85 provide more reliable information about environmental conditions than seawater, which  
86 only provides punctual information. Indeed, most of the monitoring programmes for  
87 quality assessment guidelines in marine habitats are focused on sediments (Birch, 2017;  
88 2018; Dimitrou et al., 2020) and previous work (e.g. Martínez-Lladó et al., 2007;  
89 Ondiviela et al., 2013) has already demonstrated that sediments constitute the main  
90 testimony of contaminant episodes in harbours.

91 To achieve the goal of “Bridging the Gap Between Policy and Science in  
92 Assessing the Health Status of Marine Ecosystems”, scientists should provide  
93 policymakers with the best available and updated knowledge (Borja et al., 2016, 2017).  
94 Most of the monitoring programmes are mainly concerned with bathing water, while  
95 marinas and harbours are usually excluded. Regulations in marinas are especially  
96 challenging due to the wide range of uses, pressures and singularities of each marina  
97 (different morphology and hydrodynamic regimes, quick and unpredictable changes due  
98 to local actions, etc) (Gupta et al., 2005; Petrosillo et al., 2010). Management of marinas  
99 also involves conflicting uses by residents, visitors, boat owners, shipping, and industry

100 among others, thus requiring the integration of multi-disciplinary authorities and  
101 stakeholders from different sectors (e.g. engineers, biologists, economists,  
102 environmental agencies, governmental bodies) (Chatzinikolaou et al., 2018).

103 Several methods and models, such as the Complexity Tidal Range Index (CTRI)  
104 or the Marina Environmental Risk Assessment (MERA), have been recently applied to  
105 prioritize environmental strategies in marinas (e.g. Gómez et al., 2019; Valdor et al.,  
106 2019). However, the progress in these tools significantly contrasts with the scarce biotic  
107 and abiotic information available. This basic information is mandatory to develop an  
108 integrative assessment, which is necessary to undertake properly designed management  
109 programs in marine ecosystems (Karydis and Kitsiou, 2013; Dauner et al., 2018;  
110 Carriger and Parker, 2020; Stelzenmüller et al., 2021).

111 Thus, the use of appropriate and effective indicators of environmental conditions  
112 is critical to accurately conduct integrative assessments and to ensure that human  
113 activities are carried out in a sustainable manner (Birch, 2017). In this sense, benthic  
114 invertebrates are very sensitive to physical and chemical perturbations and  
115 recommended as good biological indicators of contamination for integrating  
116 environmental disturbances over time (Albano et al., 2013). Biological data have the  
117 advantage of giving an integrated view of the long-term environmental conditions,  
118 whereas chemical and physical analytical methods provide only a ‘snapshot’ of  
119 conditions at the time of sampling (Saiz-Salinas, 1997; de-la-Ossa-Carretero et al.,  
120 2012; Ng et al., 2019). In fact, the use of benthic communities to assess the  
121 environmental state of marine systems is strongly supported by the Water Framework  
122 Directive (WFD) of the European Union (2000/60/EC). For harbour environments,  
123 Ondiviela et al. (2013) stressed the importance of considering simultaneously abiotic  
124 and biological indicators in integrative assessments to evaluate the ecological status.

125 An integrative approach must, therefore, include inventories of the biodiversity,  
126 since taxonomic information is pivotal for establishing a baseline to properly address  
127 sustainable management of marinas. Ideally, the analysis of benthic communities should  
128 be based on identification to the species level but this is usually a complex and time-  
129 consuming procedure (Terlizzi et al., 2003; Sánchez-Moyano et al., 2006). Indeed, in  
130 many taxa, species identification is laborious and requires appropriate taxonomic  
131 expertise that nowadays is often lacking (Paknia et al., 2015; Chatzinikolaou et al.,  
132 2018). Furthermore, policy makers and funding agencies often demand results and

133 recommendations be achieved within very short timescales. These requirements prevent  
134 identification to species level and promote analyses considering higher taxa (e.g. genus,  
135 family, order). Extensive literature, including a variety of methodologies, habitats and  
136 substrate types, has been developed to explore if environmental changes can be detected  
137 above the species level (see Sánchez-Moyano et al., 2006 and references therein; De  
138 Oliveira et al., 2020; Gerwin et al., 2020). Nonetheless, the response of fauna to  
139 environmental data by considering different taxonomic levels has been scarcely studied  
140 in harbours and marinas (Chatzinikolaou et al., 2018).

141 To fill the gaps described above, the main objective of this study was to explore  
142 the importance of soft-bottom environments, including both biotic and abiotic  
143 information, for assessing the ecological status of marinas from an integrative approach.  
144 To address this issue we aim to: (i) characterize the diversity and spatial distribution  
145 patterns of soft-bottom macro- and meiofauna communities in marinas, (ii) identify the  
146 set of abiotic variables which better explain the biological patterns, (iii) explore the  
147 taxonomic sufficiency required to detect differences among marinas and environmental  
148 patterns, and, eventually, (iv) develop an integrative and easy-to-apply method to assess  
149 the quality status of soft-bottom environments of marinas based on selected ecological  
150 indicators. Most previous studies regarding soft bottoms of marinas have focused on  
151 abiotic parameters, and when they include biotic data, information on the macrofaunal  
152 and meiofaunal communities is neither integrated simultaneously nor do they develop  
153 integrative quality assessments based on a multiparametric approach (Table S1). The  
154 novelty of the present study lies in the integration of abiotic and biotic data for  
155 designing a quality assessment method based, concurrently, on the following selected  
156 ecological indicators: Marinas Environmental Pollution Index (MEPI),  
157 Biocontamination Index (BCI), macrofaunal biotic indices (AMBI, M-AMBI, BENTIX,  
158 MEDOCC and BENFES), macrofaunal taxa richness and Shannon-Wiener's diversity,  
159 and nematode:copepod index.

160 To achieve the aforementioned objectives, the Southern Iberian Peninsula was  
161 selected as the study area since it (i) represents an interesting biogeographical area  
162 which includes an horizontal gradient Atlantic-Mediterranean, (ii) encompasses a very  
163 high number of marinas along the coast and (iii) includes the Strait of Gibraltar, a hot  
164 spot for maritime traffic.

165

166 **2. Material and Methods**

167 *2.1. Sampling survey and laboratory processing*

168 Sampling was conducted from June 26<sup>th</sup> to July 2<sup>nd</sup> 2017. Eight marinas were  
169 randomly selected along the Southern Iberian Peninsula: four in the Atlantic coast  
170 (Faro, Chipiona, Puerto América, Barbate) and four in the Mediterranean (La Línea-  
171 Puerto Chico, Fuengirola, Motril, Almería) (Fig. 1). Details of these marinas  
172 (coordinates, depth, size, number of berths and population density of the adjacent  
173 locality) can be consulted in Guerra-García et al. (2021). Within each marina, three  
174 floating pontoons were randomly selected (each pontoon was considered a replicate for  
175 analyses throughout the whole study; consequently, three replicates per marina were  
176 taken) and the sediment below each pontoon (mostly muddy) was sampled.

177 For macrofauna, a small van Veen grab (area: 15 x 15 cm) was used. Because  
178 the grab was relatively small, the replicate (i.e. pontoon) consisted of three additive  
179 sediment grabs (total area: 675 cm<sup>2</sup>). Samples were washed through a sieve with a mesh  
180 size of 0.5 mm and the retained fraction was fixed in 96% ethanol stained with Rose  
181 Bengal.

182 For meiofauna, only one sediment van Veen grab was collected per pontoon.  
183 Once the grab was recovered, a small corer of 10 cm<sup>2</sup> was introduced into the  
184 undisturbed sediment. Therefore, a total of three replicates (one corer per pontoon) were  
185 obtained per marina and directly fixed in 96% ethanol stained with Bengal Rose.

186 In the laboratory, macrofaunal samples were rinsed with fresh water over a 0.5-  
187 mm sieve, sorted, identified to species level whenever possible, and counted. For  
188 macrofauna, the status of each species (N: Native, I: Introduced, C: Cryptogenic, U:  
189 Undetermined) was also considered. Species names and systematic arrangement of taxa  
190 follows the World Register of Marine Species (WoRMS, 2020).

191 Meiofaunal samples were sieved in the laboratory through a 0.5-mm mesh to  
192 exclude macrofauna, with a 30-µm mesh below to retain meiofauna. Specimens were  
193 sorted under a stereomicroscope and identified to high taxonomic levels (e.g. phylum,  
194 class or order; Sedano et al., 2014, 2020)

195 Additionally, to quantify abiotic variables in sediment for each marina, three  
196 sediment samples (one per pontoon) were taken from the bottom using the van Veen  
197 grab. All samples were immediately frozen (-20°C) until laboratory analyses. The  
198 following environmental variables were measured: sand, silt, clay, total organic carbon

199 (TOC), hydrocarbons, faecal coliforms, biocide Irgarol, and major, minor and trace  
200 elements As, Cd, Co, Cr, Cu, N, Ni, P, Pb, S and Zn. Analytical procedures are detailed  
201 in Guerra-García et al. (2021).

202

## 203 2.2. Statistical analyses

204 The experimental design included two factors: ‘Location’ (Lo), a fixed factor  
205 with two levels (Mediterranean vs Atlantic) and ‘Marina’ [Ma(Lo)], a random factor  
206 with four levels, nested with Lo. The number of replicates was  $n=3$ , corresponding to  
207 the three pontoons randomly selected for each marina.

208 Regarding macrofauna, number of taxa (considering the species, genus, family,  
209 order, class and phylum levels separately), total abundance and Shannon-Wiener’s  
210 Diversity at species level ( $H'$ ) were calculated for each replicate ( $675\text{ cm}^2$ ). Data were  
211 also calculated separately for the three main phyla, i.e. Annelida, Arthropoda and  
212 Mollusca. Total taxa richness (i.e. total number of different species) and  $H'$  were also  
213 determined for each marina. For meiofauna, number of taxa (for high taxonomic levels),  
214 abundance (individuals/ $10\text{ cm}^2$ ) and the nematode:copepod ratio (Rubal et al., 2009)  
215 were also calculated.

216 Two-way ANOVAs with the above design were conducted to explore  
217 differences in the biological descriptive parameters of macro- and meiofauna among  
218 marinas (factor ‘Marina’) along the Mediterranean-Atlantic axis (factor ‘Location’).  
219 Homogeneity of variances was checked using Cochran’s test. When ANOVA detected  
220 significant differences for a given factor, the source of the variance was identified by  
221 applying the Student-Newman-Keuls (SNK) test (Underwood, 1997).

222 Non-parametric multidimensional scaling (MDS) was used to show the  
223 relationship among locations (Atlantic vs Mediterranean) and marinas based on  
224 macrofaunal abundances; this was done considering the data matrix for each of the six  
225 selected taxonomic levels (species, genus, family, order, class and phylum) separately  
226 and also for the three main phyla (i.e. Annelida, Arthropoda and Mollusca) considering  
227 only the species level. The Bray-Curtis similarity index was applied after transformation  
228 of abundance values by the square-root. An additional MDS was conducted using  
229 abundances of meiofaunal higher taxa.

230 The macrobenthic community patterns obtained from the six different taxonomic  
231 levels were compared using a second-stage MDS, by computing a weighted Spearman

232 rank correlation coefficient between the corresponding elements of each pair of marinas  
233 from the respective similarity matrices (Sommerfield and Clarke, 1995; Chatzinikolaou et  
234 al., 2018). Similarly, a second stage MDS was conducted to compare the patterns  
235 obtained separately for Annelida, Arthropoda and Mollusca with that of the total  
236 community.

237 Differences in the macro- and meiofaunal assemblage among locations and  
238 marinas were tested by permutational multivariate analyses of variance  
239 (PERMANOVA), using the same experimental design explained above, and considering  
240 the aforementioned six taxonomic levels. Additional PERMANOVAs were conducted  
241 for Annelida, Arthropoda and Mollusca independently. Analyses were based on Bray-  
242 Curtis similarities and Monte Carlo tests were included. Significant P-values were  
243 obtained by computing 9999 permutations of residuals under a reduced model, as this  
244 method gives the most accurate Type I error for complex design (Anderson, 2005).  
245 Pairwise comparisons were then used. Additionally, to test the dispersion among  
246 samples for the factors 'Location' and 'Marina', a permutational analysis of multivariate  
247 dispersions (PERMDISP) was used.

248 Distance-based linear modelling (DistLM) was used to assess the relative  
249 contribution of abiotic parameters to the variability observed in the macro- and  
250 meiofaunal community structure (Anderson et al., 2008). The analysis was performed  
251 based on the matrix of square-root transformed abundance data. Abiotic data were  
252 transformed by  $\log(x+1)$  and normalized to reduce the effects of differences in unit  
253 scales. The model was constructed using the best combination of predictors (*Best*  
254 *procedure*) using AIC (Akaike's Information Criteria) (Burham and Anderson, 2004;  
255 Leonardi et al., 2020; Mulik et al., 2020; Wei et al., 2020). Prior to the DistLM analysis,  
256 multicollinearity between abiotic factors and skewness of data were evaluated using  
257 Draftsman plot based on Spearman correlations. Sets of variables with Spearman's test  
258 values higher than 0.8 ( $p < 0.01$ ) were omitted (Chatzinikolaou et al., 2018). Distance-  
259 based Redundancy Analysis (dbRDA) was performed to provide a visual representation  
260 of the macrobenthic community fitted to the significant predictor variables in the multi-  
261 dimensional space (Mulik et al., 2020; Wei et al., 2020). To increase robustness of  
262 results, the BEST routine (BIO-ENV) was also employed to explore correlations  
263 between the matrix of faunal abundances and environmental predictors from a different  
264 analytical perspective (Anderson et al., 2008). The Bray-Curtis similarities matrix was

265 based on faunal abundances while the Euclidean distances-based matrices derived from  
266 all the possible sequential combinations of the abiotic variables. Spearman rank  
267 coefficient was used to identify the best environmental variables that explained the  
268 observed patterns on 999 permutations (Marchini et al., 2020). To explore the response  
269 to abiotic parameters of each considered taxonomic level (species to phylum) and the  
270 three main phyla, DistLM and BIO-ENV were conducted for each category.

271 PERMANOVA, PERMDISP, MDS, DistLM, dbRDA and BIO-ENV analyses  
272 were carried out using the PRIMER v.6+PERMANOVA package (Clarke and Gorley,  
273 2006; Anderson et al., 2008) and ANOVA analyses were conducted on the GMAV5  
274 software (Underwood et al., 2002).

275

### 276 *2.3. Integrative assessment*

277 For an integrative assessment of the ecological quality status of marinas  
278 considering biotic and abiotic information of soft-bottom environments, the following  
279 six ecological indicators were selected and calculated for each marina (Fig. 2):

280

281 A) Marinas Environmental Pollution Index (MEPI). This index was recently  
282 developed by Guerra-García et al. (2021) to characterize the environmental  
283 pollution levels in marinas based on 15 selected stressors measured in the  
284 sediments.

285 B) Biocontamination Index (BCI). It was developed by Arbačiauskas et al. (2008)  
286 to assess the biopollution level (in terms of exotic species) in inland waters. We  
287 have used this index here as a proxy to evaluate the contribution of NIS to the  
288 soft-bottom benthic community in each marina.

289 C) Macrofaunal taxa richness. The total number of species found in each marina  
290 was calculated. The number of genera, families and orders were also included  
291 for comparison.

292 D) Macrofaunal diversity (at species level) was measured by the Shannon-Wiener's  
293 index ( $H'$ ).

294 E) Macrofaunal biotic indices. Many biotic indices have been developed during the  
295 last years. In the present study we have used AMBI (Borja et al. 2000), M-  
296 AMBI (Muxika et al., 2007), BENTIX (Simboura and Zenetos, 2002),  
297 MEDOCC (Pinedo et al., 2015) and BENFES (Sánchez-Moyano et al., 2017).

298 Compiled information about the boundaries and intervals for these indices can  
299 be found in Keeley et al. (2012). Biotic indices have been considered suitable  
300 tools for assessing the ecological status of Mediterranean ports (Dimitriou et al.,  
301 2020)

302 F) Meiofaunal nematode:copepod index proposed by Raffaelli and Mason (1981).  
303 This ratio was proposed as a fast, easy, and reliable method to study the effects  
304 of organic matter enrichment in coastal sediments (see Rubal et al., 2009).

305

306 The intervals and boundaries assigned in the literature for MEPI, BCI, AMBI,  
307 M-AMBI, BENTIX, MEDOCC and BENFES (high, good, moderate, poor, bad) were  
308 adopted in the present study. Intervals for taxa richness,  $H'$  and nematode:copepod ratio  
309 were established in the present study according to the results obtained in the marinas  
310 from the Southern Iberian Peninsula.

311 The following methodological protocol was developed to assess a quality status  
312 based on the selected ecological indicators (Fig. 2):

313 (i) Once the six indicators were calculated, a score for each indicator was  
314 assigned according to the colour categories for each ecological status: blue (high) was  
315 scored with 4 points, green (good) with 3 points, yellow (moderate) with 2 points,  
316 orange (poor) with 1 point and red (bad) with 0 points. All parameters have the same  
317 five intervals (blue, green, yellow, orange and red), except for MEPI which includes  
318 only green (good), yellow (moderate) and red (bad) (see Guerra-García et al., 2021). In  
319 this case green was scored with 4 points, yellow with 2 points and red with 0 points.

320 (ii) The global score of each marina (ranging from 0 to 24) was obtained by  
321 summing the scores assigned to each of the six selected indicators (MEPI, BCI, Taxa  
322 richness,  $H'$ , biotic indices and nematode:copepod ratio) based on its ecological status  
323 (i). If several biotic indices are available, as in the present study, the value used in the  
324 assessment would result from the mean of the scores obtained for each index. Although  
325 the number of species is advisable as an indicator of taxa richness, the number of  
326 genera, families or orders could also be used in the assessment.

327 (iii) Establishment of global quality status of each marina according to the total  
328 score. We proposed the following intervals: (0-5: bad, 6-10: poor, 11-15: moderate, 16-  
329 20: good, 21-24: high)

330 Additionally, the relationships between all the indicators used were explored by  
331 Spearman correlation for all datasets.

332

### 333 **3. Results**

#### 334 *3.1. Macrofaunal community*

335 8339 individuals belonging to at least 166 macrofaunal species were found during the  
336 present study in sediments from eight marinas on the Southern Iberian Peninsula (Table  
337 1 and Tables S2, S3). This included 61 annelid species (5514 specimens, mainly  
338 represented by polychaetes), 58 arthropods (1837 specimens, mainly peracarid  
339 crustaceans), 41 molluscs (789 specimens, mainly bivalves), 3 echinoderms (9  
340 specimens) and 190 additional specimens belonging to the phyla Nematoda, Nemertea  
341 and Phoronida. In all marinas, Annelida was the dominant phylum (Fig. 3A) both in  
342 species richness and abundance, except in Almería where Arthropoda was the most  
343 abundant phylum (due to the high density of the tanaid *Apseudopsis latreilli*). The most  
344 common and abundant taxa were the annelids *Aphelochaeta marioni*, *Capitella*  
345 *capitata*, *Chaetozone gibber*, *Cirriformia tentaculata*, *Cirrophorus furcatus* and  
346 *Fabricia stellaris*, the crustaceans *Phtisica marina*, *Iphinoe tenella* and *A. latreillii*, and  
347 the molluscs *Abra tenuis*, *Corbula gibba* and *Parvicardium exiguum* (Table 1). Detailed  
348 information on the taxonomical categories of each species and their status (native,  
349 cryptogenic or introduced) is included in Tables S2 and S3. Most of the species  
350 inhabiting sediments from marinas can be considered native or cryptogenic, while only  
351 four were exotic species, all of them crustaceans, namely the amphipods *Caprella*  
352 *scaura* and *Jassa slateryi*, the isopod *Paranthura japonica* and the decapod *Alpheus* sp.  
353 (Table S2).

354 Regarding the total number of species per marina, Puerto América showed the  
355 highest species richness (76) followed by Almería (53), Motril (52) and La Línea (47).  
356 The marinas with the lowest number of species were Chipiona (25), Barbate (30),  
357 Fuengirola (33) and Faro (37). This pattern was also obtained for the number of species  
358 per replicate (Fig. 3B). The highest values of the Shannon-Wiener's diversity ( $H'$ )  
359 (based on the global data per marina) was also measured in Puerto América (3.23)  
360 followed by La Línea (2.82), Motril (2.41) and Faro (2.03). Barbate (1.56), Chipiona  
361 (1.64), Almería (1.86) and Fuengirola (1.92) showed the lowest values.

362 The number of species, total abundance and H' differed significantly among  
363 marinas, although no differences were obtained for the factor 'location' (Atlantic vs  
364 Mediterranean) (Table 2). The highest heterogeneity among marinas was obtained for  
365 the Mediterranean (see SNK tests in ANOVAs of Table 2). The two-dimensional MDS  
366 plot based on species abundances showed segregation of marinas (Fig. 4A).  
367 PERMANOVA confirmed significant differences for all marinas (see pair-wise tests in  
368 Table 2) with no significant differences for the factor location (Table 2).

369 When each of the three main phyla (Annelida, Arthropoda and Mollusca) was  
370 analysed separately, the species richness also differed significantly among marinas  
371 (Table S4A, Fig. 3C). The pattern was more evident for annelids and molluscs than for  
372 arthropods (Table S4A). In fact, SNK tests indicated no differences among  
373 Mediterranean marinas for arthropods. At the community level, the factor 'Marina' was  
374 significant for the three phyla according to PERMANOVA results (Table S5A) and  
375 MDS plots graphically showed segregation among marinas again (Fig. 5A). Molluscs  
376 also revealed some segregation between Mediterranean and Atlantic marinas, although  
377 'Location' was not significant according to PERMANOVA (Pseudo-F=1.9178,  
378 p=0.0961, Table S5A). The second stage MDS (Fig. 5B) demonstrated that the pattern  
379 derived from the annelid assemblage was very similar to that considering all taxa,  
380 showing that this taxon was most responsible for driving the observed pattern for the  
381 whole community.

382

### 383 3.2. Meiofaunal community

384 A total of 16 higher taxa were identified (Tables 3, Table S6), 4 of which (Copepoda,  
385 nauplius larvae of crustaceans, Nematoda and Polychaeta) were always present at all  
386 marinas. Nematoda was the numerically dominant taxon in Faro, Chipiona, P. América,  
387 Barbate and Fuengirola, while Copepoda dominated in La Línea, Motril and Almería  
388 (Fig. 6A).

389 The number of taxa did not differ among marinas or among locations (Atlantic  
390 vs Mediterranean), while total abundance values were higher in Mediterranean marinas  
391 (Fig. 6A, Table 4). The nematode:copepod ratio differed significantly among marinas,  
392 although these differences were mainly due to the high values measured in Fuengirola.  
393 The MDS plot showed segregation of marinas and, differently from macrofauna,  
394 reflected a clear pattern for the factor location, i.e. Atlantic vs Mediterranean marinas

395 (Fig. 6B). PERMANOVA confirmed significant differences for the factors ‘Location’  
396 and ‘Marina’ (Table 4).

397

### 398 *3.3. Influence of environmental data on faunal assemblages*

399 A full dataset of environmental data measured in sediments of the eight studied  
400 marinas can be found in Table S7 (see also Guerra-García et al., 2021, for details on the  
401 environmental assessment).

402 The variables Co, N, As, S, Clay and N were correlated with other variables  
403 ( $r > 0.8$ ,  $p < 0.01$ ) and were not included in the DistLM, so the model was run with 12  
404 variables (Sand, Silt, hydrocarbons, faecal coliforms, biocide Irgarol, Cd, Cr, Cu, Ni, P,  
405 Pb and Zn). According to DistLM, the environmental variables which significantly  
406 correlated with the whole macrofaunal community (identified at species level) were  
407 mainly P (18.2% of variation explained), sand (16.7%) and TOC (16.0%) (Table 5).  
408 Faecal coliforms and the heavy metals Cr, Cu, Pb and Zn were also significant (Table  
409 5). The best model (AIC=180.46,  $R^2=0.073$ ) was obtained for the combination of Sand,  
410 Silt, TOC, P, faecal coliforms, Irgarol, Cr, Cu and Pb (Table 5). The first two axes of  
411 the dbRDA explained 50.9 % of the total variation (Fig. 7). BIO-ENV results showed  
412 that the best model (Rho=0.820,  $p < 0.01$ ) was obtained with the combination of Sand,  
413 TOC, P, faecal coliforms, Cu, Pb and Zn, coinciding with the significant variables  
414 provided by DistLM (Table 6).

415 When the three main phyla (Annelida, Arthropoda and Mollusca) were analysed  
416 separately, DistLM showed similar results to those obtained with the whole community  
417 (Sand, TOC, P, faecal coliforms and heavy metals) (Table 6, Table S8). Variables  
418 explained a higher proportion of variability in annelids, followed by molluscs and  
419 finally arthropods (Table S8). In fact, BIO-ENV only provided a significant model  
420 (Rho=0.797,  $p < 0.05$ ) for Annelida (Table 6), which, therefore, turned out to be the  
421 phylum best reflecting the environmental conditions in marinas.

422 Regarding to meiofauna (based on higher taxa abundances), DistLM selected  
423 Sand (17.1%), Hydrocarbons (14%), Cd (13.7%) and Cu (10%) as the variables that  
424 correlated significantly with the meiofaunal assemblage (Table S9, Table 6). The best  
425 model (AIC=157.62,  $R^2=0.62$ ) was obtained for the combination of Sand, Silt, TOC,  
426 faecal coliforms, Cd, Cr, Cu and Zn. BIO-ENV also showed Hydrocarbons, Cd and Cu

427 as the best combination model. However, the model was not significant ( $Rho=0.630$ ,  
428  $p=0.130$ ) (Table 6).

429

### 430 *3.4. Macrofaunal taxonomic sufficiency*

431 This study revealed the presence of 166 species, 155 genera, 105 families, 44  
432 orders, 16 classes and 8 phyla inhabiting marinas of the Southern Iberian Peninsula. The  
433 number of species was very similar to the number of genera in all marinas (Fig. 3B),  
434 since most of the genera were represented by a single species (Table S2). Number of  
435 families was also close to the number of genera and species (Fig. 3B). Regarding taxa  
436 richness, ANOVAs revealed that the factor ‘Marina’ was significant for all taxonomic  
437 levels considered, although for class and phylum these differences were only due to the  
438 high number of taxa collected in P. América in comparison with other Atlantic marinas  
439 (Table S4B, Fig. 3B). PERMANOVA confirmed that there were significant differences  
440 in community structure among all marinas when separately considering each matrix for  
441 species, genera, families or orders (Table S5B). Although significant differences in the  
442 factor ‘Marina’ were also obtained for classes and phyla, these differences were due to  
443 the influence of certain marinas, such as Faro (Atlantic) or Almería (Mediterranean)  
444 (Table S5B). This pattern was supported by the corresponding MDS ordinations (Fig.  
445 4A). Separation among marinas was evident at the level of species, genus, family and  
446 order, while MDS based on classes and phyla showed a less defined grouping (Fig. 4A).  
447 The second-stage MDS performed on the six considered taxonomic levels (Fig. 4B)  
448 demonstrated that patterns showed by the species/genus levels were similar, and close to  
449 that obtained for family/order levels. Conversely, they differed considerably from those  
450 derived from the higher levels (i.e. phylum and class).

451 When the influence of taxonomic resolution was considered to compare the  
452 environment-fauna relations, DistLM revealed a similar pattern for species, genus,  
453 family and order (Table 6, Table S8). The environmental variables that were significant  
454 at the species level were the same as those found at the level of genus, family and order.  
455 The set of significant variables were quite different when considering classes and phyla  
456 data. Indeed, BIO-ENV provided significant results with similar variables for species,  
457 genus, family and order, but they were not significant for class and phylum (Table 6).

458

### 459 *3.5. Global assessment. Biotic indices and biocontamination*

460 According to the Marinas Environmental Pollution Index (MEPI), Barbate and  
461 Fuengirola were the most polluted marinas (Fig. 8). The Biocontamination Index (BCI)  
462 values were very low in all marinas due to the small number of introduced species  
463 found on sediments. Although quality intervals for MEPI, BCI and Biotic Indices were  
464 defined in the literature, those for taxa richness, diversity ( $H'$ ) and nematode:copepod  
465 ratio were not available so they were established during the present study, based on the  
466 data obtained for marinas of Southern Iberian Peninsula (see Table S10). The global  
467 score, obtained by summing the scores assigned to each selected indicator (MEPI, BCI,  
468 Taxa richness,  $H'$ , Biotic indices and nematode:copepod ratio) ranged from 9 to 17.  
469 Fuengirola, Barbate and Chipiona got the lowest score (9 and 10 points) while La Línea,  
470 Motril and P. América showed better ecological status (16 and 17 points) (Fig. 8).  
471 Therefore, marinas of the Southern Iberian Peninsula ranged from poor (Fuengirola,  
472 Barbate and Chipiona) to moderate (Faro and Almería) and good (P. América, La Línea  
473 and Motril).

474 In general terms, the ecological indicators used to conduct the global assessment  
475 were in good agreement, except for punctual differences (i.e. BENTIX classified all the  
476 marinas as bad or poor, while BENFES classified them as moderate, good or high).  
477 Apart from BCI which showed low values in all marinas and was not able to  
478 discriminate among them, environmental data (MEPI) and biological information (taxa  
479 richness,  $H'$ , biotic indices and nematode:copepod ratio) were more discriminant and  
480 provided similar information (Fig. 8). MEPI was correlated with richness of species and  
481 genera, the biotic index MEDOCC and the nematode:copepod ratio. Taxa richness was  
482 correlated with  $H'$  and with biotic indices (Table S11). Indeed, the marinas classified as  
483 good (P. América, La Línea and Motril) were those with the highest values of  
484 macrofauna taxa richness and Shannon-Wiener's diversity, and the lowest degree of  
485 environmental pollution. Conversely, the most polluted marinas, such as Chipiona,  
486 Barbate and Fuengirola, showed the lowest values for taxa richness, diversity, and the  
487 highest nematode:copepod ratios.

488

## 489 **4. Discussion**

### 490 *4.1. Benthic communities*

491 The present study revealed that the soft bottom of marinas from the Southern  
492 Iberian Peninsula supported a wide variety of macrobenthic taxa (166 species

493 distributed in 155 genera, 105 families, 44 orders, 16 classes and 8 phyla). The number  
494 of soft-bottom macrofauna species per marina (ranging from 25 to 76) was similar to  
495 that reported from marinas worldwide (e.g. McGee et al., 1995; Estacio et al., 1997;  
496 Guerra-García and García-Gómez, 2004 a,b,c; Martínez-Lladó et al., 2007; Covazzi  
497 Harriague et al., 2012; Chatzinikolaou et al., 2018; Ng et al., 2019).

498 Annelida (mostly polychaetes) was the dominant phylum in number of species  
499 (37%) and, especially, in number of individuals (66%). In fact, the patterns of the total  
500 community were mainly driven by annelids (see Fig. 3), that are usually reported as the  
501 dominant macrobenthic taxon in the sediment of marinas worldwide (Moreira et al.,  
502 2010; Ng et al., 2019; Chatzinikolaou et al. 2018) and, in general, in most soft-bottom  
503 habitats (Hutchings, 1998). On the contrary, Arthropoda (mainly Amphipoda inhabiting  
504 arborescent substrates) dominated floating pontoons in terms of abundance and species  
505 richness (Gavira O'Neill et al., 2015, 2018).

506 Although all studied marinas were located across the same geographical area,  
507 univariate and multivariate analyses consistently showed significant differences among  
508 them based on biological data. This heterogeneity among marinas has also been  
509 revealed by environmental stressors (see Guerra-García et al., 2021). Spatial variations  
510 in biotic assemblages among and within marinas have been attributed to differences in  
511 the local environment as well as to the marina design (Toh et al., 2017). In fact, the  
512 singularity of any given marina, largely determined by a variety of local factors (e.g.  
513 environmental conditions, availability of suitable substrate, level of marine traffic) has  
514 proven to be a key factor affecting, for example, several stages of the NIS invasion  
515 process (Martínez-Laiz et al., 2019). Hence, our results reinforce the importance of  
516 considering a local scale perspective for conservation and management purposes.

517 Although significant differences in the factor 'marina' were relevant throughout  
518 the whole study, the factor 'Location' (Atlantic vs Mediterranean) was not relevant for  
519 macrofauna, only showing certain degree of segregation when considering molluscs  
520 separately. However, this factor was significant for meiofauna and should also be  
521 considered in management programmes.

522 The present study only considered a spatial scale, and therefore further research  
523 should explore if the observed patterns remain constant through time. Although  
524 epifaunal communities associated with floating pontoons suffer significant seasonal  
525 fluctuations (e.g. Ros et al., 2013), Chatzinikolaou et al. (2018) did not find important

526 seasonal differences in soft-bottom communities of port and marinas. On the contrary,  
527 Moreira et al. (2010) detected strong fluctuations among and within seasons mostly  
528 related to recruitment of opportunistic species and some perturbations (i.e. proliferation  
529 of ephemeral algae on the sediment in summer). This again emphasizes that the  
530 environmental singularity of any given marina must be considered in management  
531 programmes. In this sense, sediment monitoring should ideally be based on previous  
532 baseline data in order to design the appropriate sampling for each marina and reduce the  
533 monetary, time and labor costs of such activities as much as possible.

534

#### 535 *4.2. Influence of environmental data on faunal assemblages*

536 The multivariate pattern of the macrofaunal community in the present study was  
537 mainly explained by P, Sand, TOC, faecal coliforms, and heavy metals. Although  
538 hydrocarbon concentration clearly discriminated among marinas (Table S7, Guerra-  
539 García et al., 2021) and has been traditionally considered a significant factor affecting  
540 biota (Blanchard et al., 2002), it did not contribute to explain the observed macrofaunal  
541 patterns but was, in turn, relevant for meiofauna. In this sense, Covazzi Harriague et al.  
542 (2012) pointed out that the macrofaunal and meiofaunal assemblage can respond  
543 differently to human pressure.

544 Most explanatory variables selected by our analyses agree with those reported in  
545 previous studies in harbours and ports. Granulometry is considered one of the main  
546 factors modulating soft-bottom communities, including those inhabiting harbour areas  
547 (e.g. Moreira et al., 2005). Guerra-García et al. (2004 a,b,c) also reported granulometry,  
548 TOC, P and heavy metals as important variables to explain the distribution of molluscs,  
549 crustaceans and polychaetes in the harbour of Ceuta, while Chatzinikolaou et al. (2018)  
550 found heavy metals to be relevant explanatory variables of biotic patterns. Among the  
551 many potential sources, metal-based antifouling systems, mainly Cu and Zn, clearly  
552 play a major role in contamination in marinas (Briant et al., 2013).

553 When each of the three main phyla were analysed separately, Annelida was  
554 more related to the considered environmental conditions in marinas than others.  
555 Arthropods in general, and crustaceans in particular, are traditionally considered better  
556 bioindicators due to their higher sensitivity to marine pollution (Navarro-Barranco et al.,  
557 2020) and better reflect differences at the spatial scale (i.e. among localities) than  
558 molluscs and annelids (Sánchez-Moyano and García-Gómez, 1998). However, in

559 harbours and marinas, annelids (mainly polychaetes) become the best taxon to  
560 discriminate between sampling sites, especially when oxygen is not a limiting factor  
561 (Guerra-García and García-Gómez, 2005a).

562

### 563 *4.3. Taxonomic sufficiency*

564 The use of higher taxonomic levels instead of species as a proxy for  
565 identification has been extensively tested in both terrestrial and marine habitats, and on  
566 such taxa as insects, fish, fauna associated with algae and seagrasses, meiofauna and  
567 benthic macrofauna in general (see Sánchez-Moyano et al., 2006 and references  
568 therein). However, it is important to properly analyse the cost-benefits of such  
569 approaches to select the appropriate level of taxonomic resolution in benthic monitoring  
570 programmes (Bouchard et al., 2005).

571 Although Ferraro and Cole (1990) indicated that the species level would be the  
572 most sensitive for the evaluation of pollution impacts, Sánchez-Moyano et al. (2006),  
573 based on several datasets within a wide range of habitats and substrate types along the  
574 southern Iberian coastline, concluded that studies at the different levels of taxonomic  
575 resolution (species, family, order) led to similar results both as regards community  
576 distribution and composition, and their relationship to environmental variables.

577 The present study found similar patterns to those provided by Sánchez-Moyano  
578 et al. (2006) and Chatzinikolaou et al. (2018). On the one hand, differences among  
579 marinas were also detected using high taxonomic levels, and on the other hand, the set  
580 of environmental variables which better explained macrofaunal assemblages were the  
581 same at the species, genus, family and order level, but differed at class and phylum  
582 levels. The second stage MDS revealed that identification to genus provided almost  
583 identical results to those obtained when considering species due to many genera being  
584 represented by only one species in each marina. Family and order levels yielded similar  
585 results between them but were less informative with respect to species and genus levels  
586 (Fig. 4). For all these reasons, family and order levels could be an alternative in large-  
587 scale monitoring programmes with significant time and budget constraints.

588 Conversely, taxonomical resolution to the species level is mandatory to  
589 accurately identify NIS in marinas, and to correctly calculate biocontamination indices  
590 such as BCI (Arbačiauskas et al., 2008). Indeed, early detection of NIS is often delayed  
591 when taxa are taxonomically challenging, such as small-sized marine organisms which

592 require great taxonomic expertise (Marchini and Cardeccia, 2017; Martínez-Laiz et al.,  
593 2020). Therefore, the potential efficacy of identification to high taxonomic levels in  
594 some monitoring programmes does not replace by any means the information obtained  
595 at the species level, which is paramount for basic and applied ecological studies (de  
596 Oliveira et al., 2020).

597

#### 598 *4.4. Global assessment*

599 The quality assessment applied in the present study and developed specifically  
600 for marinas was based on six selected ecological indicators (MEPI, BCI, Taxa Richness,  
601 H', biotic indices and nematode:copepod ratio), encompassing five of the eleven  
602 descriptors of MSFD: D1 (Biological diversity), D2 (NIS), D5 (Human-induced  
603 eutrophication), D6 (Sea floor integrity) and D8 (Concentrations of contaminants) (Berg  
604 et al., 2015; Piroddi et al., 2015). The assessment was useful for discriminating among  
605 marinas, even when they were closely located. The evaluation simultaneously combined  
606 abiotic and biotic information, integrating chemical pollution, NIS risk and attributes of  
607 the macro- and meiofaunal assemblages.

608 Regarding taxa richness in the assessment method, identification to the species  
609 level is advisable, but this study showed that other categories such as genus, family or  
610 even order could provide similar ecological intervals (see Fig. 8 and Table S10). Indeed,  
611 values of these four taxonomic categories were significantly correlated ( $r>0.9$ ,  $p<0.01$ )  
612 (Table S11). Therefore, this global assessment for marinas can be conducted whenever  
613 the macrofauna is, at least identified to the order level.

614 In recent years, a variety of biotic indices dealing with the structure of  
615 macrobenthic communities have been proposed to address the objectives of European  
616 Directives (e.g., Marine Strategy Framework Directive -MSFD- 2008/56/EC, EU  
617 Biodiversity Strategy to 2020 and Water Framework Directive -WFD- 2000/60/EC).  
618 Indeed, biotic indices have been recently demonstrated to be suitable for assessing the  
619 ecological status of Mediterranean ports and marinas (Dimitrou et al., 2020).

620 Although a unified protocol within the EU has not yet been adopted (Dimitriou  
621 et al., 2020) and there is no agreement on what index or indices should be used by  
622 environmental managers to establish benthic quality (Borja et al., 2015), the most used  
623 ones are AMBI (AZTI's Marine Biotic Index; Borja et al., 2000), M-AMBI  
624 (multivariate AMBI; Muxika et al., 2007) and BENTIX (Simboura and Zenetos, 2002).

625 The MEDOCC index was an adaptation of the AMBI for the Mediterranean area  
626 (Pinedo et al., 2015). All these indices rely on the taxonomic identification of species.  
627 Recently, a new index based on identification to the family level, i.e. BENFES, was  
628 developed (Sánchez-Moyano et al., 2017). The application of these indices in marinas  
629 revealed important differences in the ecological status assigned (Fig. 8). BENFES was  
630 the most favourable (most marinas classified as good). Conversely, BENTIX was  
631 clearly more restrictive (most marinas qualified as bad or poor), coinciding with the  
632 results obtained by Dimitrou et al. (2020). BENTIX did not correlate with any other  
633 index, nor with other selected ecological indicators, so its use in marinas, at least in the  
634 Southern Iberian Peninsula, is discouraged. MEDOCC was the only index correlating  
635 with environmental pollution (MEPI), so its calculation in marinas can be useful.  
636 Therefore, we recommend using the biotic indices in marinas with caution and,  
637 whenever possible, calculating several to obtain mean scores and reduce potential biases  
638 in the quality assessment.

639 The selected meiofaunal indicator for assessment was the nematode:copepod  
640 ratio, which is relatively easy to undertake in a marina. Undoubtedly, considering the  
641 difficulty and time-consuming process of taxonomic identification of nematodes and  
642 copepods, the use of taxonomic sufficiency is one of the most attractive features of this  
643 ratio (Rubal et al., 2019). Hence, this index has become a common method for  
644 evaluating organic enrichment and chemical pollution (Rohal et al., 2020). A higher  
645 ratio is usually associated to chemical and organic pollution (Raffaelli and Mason,  
646 1981), because copepods, specifically harpacticoids, are more sensitive to pollutants  
647 than nematodes (Elarbaoui et al., 2015). In fact, Moreno et al. (2008) found a significant  
648 correlation between nematodes and concentrations of environmental contaminants in  
649 tourist marinas of the Mediterranean Sea. The present study revealed that the index  
650 discriminates well among marinas. It correlated with other biotic indices and, most  
651 importantly, with the environmental pollution levels as defined by the MEPI.  
652 Accordingly, we consider that the measure of nematode:copepod ratio in marinas can be  
653 an adequate proxy for environmental pollution.

654 Concerning exotic species, recreational boating is currently considered a  
655 significant vector for the introduction and secondary spread of NIS, especially at local  
656 scales (e.g. Ros et al., 2013; Ashton et al., 2014; Foster et al., 2016; Ferrario et al.,  
657 2017; Martínez-Laiz et al., 2018, 2019; Ulman et al., 2019). In the present study, we

658 used the Biocontamination Index (BCI) for marinas. Alternatively, a more recent index,  
659 ALEX, has also been proposed to assess the impacts of alien species on benthic  
660 communities (Çinar and Bakir, 2014). While BCI is based on presence and abundance  
661 of alien species *sensu lato*, ALEX does not take into account abundances and its  
662 calculation requires classification of alien species as ‘causal’, ‘established’ or  
663 ‘invasive’. However, these categories are hard to assign when considering small  
664 invertebrates in marinas. For this reason, we have not calculated ALEX in the present  
665 study; instead, we consider BCI to be more appropriate for marinas. Despite marinas  
666 being deemed ‘hot spots’ for exotics, the number of alien species inhabiting soft  
667 bottoms was remarkably low, with only 4 NIS (all crustaceans, 3 peracarids and 1  
668 decapod) out of 166 species. It should be noted though, that the native range of more  
669 than a third of the reported taxa is unknown (their status remains undetermined or  
670 cryptogenic), which highlights the lack of baseline information and taxonomic  
671 uncertainty for many of these small mobile taxa. In any case, the low number of alien  
672 species reported here contrasts considerably with the high number of NIS (both mobile  
673 and sessile taxa) inhabiting floating pontoons (e.g. Martínez-Laiz et al., 2018; Tempesti  
674 et al., 2020). In fact, higher numbers of NIS in hard substrates in comparison with soft  
675 bottoms have been reported by other authors (Ruiz et al., 2009; Jimenez et al., 2018).  
676 Our results confirm this pattern in marinas and suggest that exploring NIS only in  
677 sediments is not enough to properly characterize biopollution levels in recreational  
678 harbours. The contrasting invasion pattern observed between hard and soft-bottom  
679 habitats also illustrates the importance of sampling across a variety of habitats (Jiménez  
680 et al., 2018).

681

#### 682 4.5. Towards Blue-flag marinas

683 A suitable management of marinas and ports should be a priority in the context  
684 of implementation of MWFDD and United Nations (UN) sustainability goals 2030. One  
685 of these goals includes marine life and aims to reduce pollution in coastal waters by  
686 increasing scientific knowledge and developing research capacities and transference of  
687 marine technology. Therefore, monitoring anthropogenic pressure and ecological  
688 quality of marinas should be an important issue for local authorities (D’Alessandro et  
689 al., 2020). The assessment of quality status can allow for design of standards when  
690 assigning “sustainable quality seals” to those marinas with better values of ecological

691 indicators. Environmental quality is, indeed, one of the main criteria to assign a Blue  
692 Flag to a marina (<http://www.banderaazul.org/puertos-y.embarcaciones-2020>, accessed  
693 8 August 2020). In our study, the marinas at P. América, La Línea and Motril reached  
694 the ‘good’ status according to the global environmental assessment based on soft-  
695 bottom habitats. This assessment based on sediment indicators could provide a tool for  
696 marinas to be awarded a Blue Bag.

697 To reduce environmental impact and increase biological diversity, the design of  
698 new marinas should allow water exchange by building more openings or having a  
699 through-flow design to facilitate exchange with the adjacent open waters (Guerra-García  
700 and García-Gómez 2004a,b,c, 2005a,b; Ng et al., 2019). In fact, marinas that are well  
701 designed, with adequate structural complexity and good water quality, can even  
702 function as nurseries for juvenile fish (Bouchoucha et al., 2016) and small invertebrates  
703 (pers. observ.).

704 Marinas may support a rich and diverse marine biodiversity and are easily  
705 visible to the public (Toh et al., 2017). Pontoons are full of sessile and mobile colourful  
706 fauna representing different phyla, and can be visited at any time by walking (no need  
707 for snorkeling or SCUBA diving). Beside hard substrates, the bottom of marinas usually  
708 corresponds to muddy sediments that can easily be sampled with small grabs for macro-  
709 and meiofauna. These manageable grabs can be effortlessly hand-carried and deployed  
710 from the pontoon in the marina without the need of a boat or any motorized lift system.  
711 For all these reasons, marinas become suitable enclaves to develop teaching activities  
712 on sampling methods in hard and soft bottoms, citizenship education and biodiversity  
713 conservation programmes as well as training courses (e.g. NIS identification), among  
714 others. Furthermore, by using social perception surveys and media programmes, boat  
715 owners can become aware of the marine life inhabiting pontoons and sediments, and get  
716 involved in reaching more ecologically sustainable marinas.

717

## 718 **5. Conclusions**

719

720 The present study reveals that the proposed method based on selected abiotic and biotic  
721 indicators [Marinas Environmental Pollution Index (MEPI), Biocontamination Index  
722 (BCI), macrofaunal biotic indices (AMBI, M-AMBI, BENTIX, MEDOCC and  
723 BENFES), macrofaunal taxa richness, Shannon-Wiener’s diversity, and

724 nematode:copepod index], can be useful for integrative assignment of the ecological  
725 status of marinas. Although the consideration of several indicators is advisable in terms  
726 of scientific integrity, it could be questioned that this would be user-friendly and easy to  
727 apply for management stakeholders. As scientists, we must encourage policy makers,  
728 governments and funding agencies to invest in rigorous scientific studies to properly  
729 address specific monitoring guidelines before making any decision. Alternatively, we  
730 must provide easy tools to facilitate this work, so a reasonable commitment must be  
731 reached with a proper balance between scientific integrity and a manageable approach.  
732 Although our proposed method involves several parameters and indicators, it can be  
733 flexible according to the available data. For example, taxa can be identified to family  
734 and order instead of species, and MEPI can be adjusted based on the environmental data  
735 available (see Guerra-García et al., 2021). Similarly, species richness can be  
736 alternatively substituted by the number of genera, family or orders. Values of the  
737 nematode:copepod index are not difficult to calculate and, regarding biotic indices, the  
738 score can be calculated on the basis of the indices available, and some of them do not  
739 require species identification. The only index that requires identification at the species  
740 level is the BCI, since it needs the correct identification of exotic species. But, if  
741 necessary, under time and budget constraints, the global score can be done by  
742 eliminating some of the markers, such as BCI, and extrapolating the score to the new  
743 intervals. Future studies must be conducted to test the method proposed across different  
744 biogeographical and seasonal scales.

745

#### 746 **Declaration of competing interest.**

747 We declare no conflict of interest.

748

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756

757 **Appendix A. Supplementary data**

758 Supplementary data related to this article can be found online at  
759 <https://doi.org/xxxxxxxxx>.

760

761

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1148 <http://www.marinespecies.org> at VLIZ.

1149

### 1150 **Figure legends**

1151 Fig. 1. Map of the study area showing the location of the eight studied marinas.

1152

1153 Fig. 2. Flowchart showing the methodology of the assessment method proposed in the  
1154 present study.

1155

1156 Fig. 3. A) Abundance of the three main macrofaunal phyla (Annelida, Arthropoda and  
1157 Mollusca) in each marina; “others” include remaining phyla. B) Taxa richness for each  
1158 considered taxonomic level (species, genus, family, order, class and phylum). C)  
1159 Species richness of the three main phyla. Values represent the mean (A–C) and standard  
1160 error (B–C) (n=3) per marina.

1161

1162 Fig. 4. A) Non-parametric multidimensional scaling (MDS) showing the relationship  
1163 among locations (Atlantic vs Mediterranean) and marinas according to macrofauna  
1164 separately for the six considered taxonomic levels (species, genus, family, order, class  
1165 and phyla). B) Second stage MDS considering all macrofaunal taxonomic levels.

1166

1167 Fig. 5. A) Non-parametric multidimensional scaling (MDS) showing the relationship  
1168 among locations (Atlantic vs Mediterranean) and marinas according to macrofauna  
1169 (separately for Annelida, Arthropoda and Mollusca). B) Second stage MDS for the three  
1170 main phyla and the total macrofauna community.

1171

1172 Fig. 6. A) Nematode:copepod ratio and abundance of the main meiofaunal taxa  
1173 measured in each marina. B) Non-parametric multidimensional scaling (MDS) showing  
1174 the relationship among locations (Atlantic vs Mediterranean) and marinas according to  
1175 meiofauna (higher taxa abundances).

1176

1177 Fig. 7. Graphic representation of the dbRDA showing ordination of marinas and  
1178 variables with respect to the first two axes. Only significant variables according to the  
1179 DistLM (see Table 5) are included.

1180

1181 Fig. 8. Global assessment of ecological quality status of the marinas from southern  
1182 Iberian Peninsula based on six selected indicators: Marinas Environmental Pollution  
1183 Index -MEPI-, Biocontamination Index -BCI-, macrofaunal taxa richness, Shannon-  
1184 Wiener's diversity ( $H'$ ), macrofaunal biotic indices and meiofaunal nematode:copepod  
1185 ratio. Total score for each marina (0-24) is based on the sum of the scores for each  
1186 indicator.

1 **ECOLOGICAL QUALITY ASSESSMENT OF MARINAS: AN INTEGRATIVE**  
2 **APPROACH COMBINING BIOLOGICAL AND ENVIRONMENTAL DATA.**

3  
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19  
20 **Highlights:**

21 -An integrative method for quality assessment is developed and proposed for marinas

22 -Correlations between fauna and abiotic data are detected at family and order level

23 -Soft-bottom benthic communities are key tools for managing programmes

24  
25 **Abstract**

26 The importance of marinas as infrastructures for recreational boating is  
27 increasing substantially. However, information on their soft-bottom benthic  
28 communities, a key tool for managing programmes, is still scarce. We combined  
29 environment features with macro- and meiofaunal soft-bottom community information  
30 for assessing the ecological status of marinas with an integrative approach. To address  
31 this issue, we focused on eight marinas of the Southern Iberian Peninsula. Macro- and  
32 meiofauna data revealed high benthic heterogeneity at a spatial scale. The  
33 environmental variables which correlated best with macrofauna were mainly

34 phosphorus, granulometry, and total organic carbon, and secondarily important  
35 variables were faecal coliforms, the biocide Irgarol, and heavy metals; total  
36 hydrocarbon concentration was also significant for meiofauna. Annelida was the  
37 dominant phylum in terms of number of species (37%) and abundance (66%) and were  
38 better descriptors of the environmental conditions than Arthropoda and Mollusca.  
39 Although identification to the species level is desirable and mandatory for assessing  
40 biological pollution, significant differences among marinas and correlations between  
41 fauna and abiotic variables were already detected at the level of family and order. This  
42 implies that biota assessment at higher levels may still be useful in monitoring  
43 programmes limited by time and budget constraints. The major novelty of this study lies  
44 in the development of an integrative assessment method based on the following selected  
45 ecological indicators: Marinas Environmental Pollution Index (MEPI),  
46 Biocontamination Index (BCI), macrofaunal biotic indices (AMBI, M-AMBI, BENTIX,  
47 MEDOCC and BENFES), macrofaunal taxa richness and Shannon-Wiener's diversity,  
48 and nematode:copepod index. This approach was able to discriminate marinas of the  
49 Southern Iberian Peninsula based on their ecological status, which ranged from poor to  
50 good. The method can be useful to design standards for assigning “sustainable quality  
51 seals” to those marinas with better values of ecological indicators.

52

53 Keywords: Marinas, ecological assessment, macrofauna, meiofauna, soft bottoms,  
54 taxonomic resolution

55

## 56 **1. Introduction**

57 Port areas and marinas in particular, are confined environments subjected to a  
58 number of anthropogenic activities that result in significant environmental disturbances  
59 (Tempesti et al., 2020 and references therein). The number of marinas has significantly  
60 increased during the last years, since they are essential infrastructures for the  
61 recreational boating sector, a key activity of global marine tourism in high demand (Di  
62 Franco et al., 2011). However, marinas modify ecological conditions (Rivero et al.,  
63 2013; Valdor et al., 2019) and their increasing number is recognized as an important  
64 environmental stressor in many regions (e.g. Gómez et al., 2017).

65 Despite the importance of marinas as hotspots of environmental and biological  
66 pollution, knowledge about these novel ecosystems is still very scarce. Previous studies

67 have evaluated the pollution pressure (Mali et al., 2017; Gómez et al., 2019; Guerra-  
68 García et al., 2021), but little effort has been put into proper characterization of their  
69 biological communities (Covazzi Harriague et al., 2012; Chatzinikolaou et al., 2018;  
70 Dimitrou et al., 2020). Faunistic information in marinas is mainly restricted to sessile  
71 communities and/or Non-Indigenous Species (NIS) (e.g. Di Franco et al., 2011;  
72 Oricchio et al., 2016; Kenworthy et al., 2018; Ulman et al., 2019; Ferrairo et al., 2020),  
73 and there is a lack of research targeting mobile macro- or meiofauna. Furthermore, the  
74 few studies dealing with these taxa have focused on arborescent substrates attached to  
75 floating pontoons (Ros et al., 2013; Marchini et al., 2015; Fernández-Romero et al.,  
76 2019; Gavira O’Neill et al., 2018; Martínez-Laiz et al., 2018). Meanwhile, studies on  
77 soft-bottom fauna in marinas are scarce (e.g. Chatzinikolaou et al., 2018, Dimitrou et  
78 al., 2020, Table S1) and generally limited when compared to other assemblages, such as  
79 the biofouling organisms which cause noticeable financial and ecological impacts (Ng  
80 et al., 2019 and references therein). The study of the soft bottom environment in  
81 marinas (usually corresponding to muddy sediments) is especially relevant since (i) it is  
82 the most widespread habitat there, (ii) it represents a more stable substrate for fauna  
83 than the floating structures above (which are temporally cleaned and often subjected to  
84 fluctuations in salinity and temperature), and (iii) sediments accumulate pollutants and  
85 provide more reliable information about environmental conditions than seawater, which  
86 only provides punctual information. Indeed, most of the monitoring programmes for  
87 quality assessment guidelines in marine habitats are focused on sediments (Birch, 2017;  
88 2018; Dimitrou et al., 2020) and previous work (e.g. Martínez-Lladó et al., 2007;  
89 Ondiviela et al., 2013) has already demonstrated that sediments constitute the main  
90 testimony of contaminant episodes in harbours.

91 To achieve the goal of “Bridging the Gap Between Policy and Science in  
92 Assessing the Health Status of Marine Ecosystems”, scientists should provide  
93 policymakers with the best available and updated knowledge (Borja et al., 2016, 2017).  
94 Most of the monitoring programmes are mainly concerned with bathing water, while  
95 marinas and harbours are usually excluded. Regulations in marinas are especially  
96 challenging due to the wide range of uses, pressures and singularities of each marina  
97 (different morphology and hydrodynamic regimes, quick and unpredictable changes due  
98 to local actions, etc) (Gupta et al., 2005; Petrosillo et al., 2010). Management of marinas  
99 also involves conflicting uses by residents, visitors, boat owners, shipping, and industry

100 among others, thus requiring the integration of multi-disciplinary authorities and  
101 stakeholders from different sectors (e.g. engineers, biologists, economists,  
102 environmental agencies, governmental bodies) (Chatzinikolaou et al., 2018).

103 Several methods and models, such as the Complexity Tidal Range Index (CTRI)  
104 or the Marina Environmental Risk Assessment (MERA), have been recently applied to  
105 prioritize environmental strategies in marinas (e.g. Gómez et al., 2019; Valdor et al.,  
106 2019). However, the progress in these tools significantly contrasts with the scarce biotic  
107 and abiotic information available. This basic information is mandatory to develop an  
108 integrative assessment, which is necessary to undertake properly designed management  
109 programs in marine ecosystems (Karydis and Kitsiou, 2013; Dauner et al., 2018;  
110 Carriger and Parker, 2020; Stelzenmüller et al., 2021).

111 Thus, the use of appropriate and effective indicators of environmental conditions  
112 is critical to accurately conduct integrative assessments and to ensure that human  
113 activities are carried out in a sustainable manner (Birch, 2017). In this sense, benthic  
114 invertebrates are very sensitive to physical and chemical perturbations and  
115 recommended as good biological indicators of contamination for integrating  
116 environmental disturbances over time (Albano et al., 2013). Biological data have the  
117 advantage of giving an integrated view of the long-term environmental conditions,  
118 whereas chemical and physical analytical methods provide only a ‘snapshot’ of  
119 conditions at the time of sampling (Saiz-Salinas, 1997; de-la-Ossa-Carretero et al.,  
120 2012; Ng et al., 2019). In fact, the use of benthic communities to assess the  
121 environmental state of marine systems is strongly supported by the Water Framework  
122 Directive (WFD) of the European Union (2000/60/EC). For harbour environments,  
123 Ondiviela et al. (2013) stressed the importance of considering simultaneously abiotic  
124 and biological indicators in integrative assessments to evaluate the ecological status.

125 An integrative approach must, therefore, include inventories of the biodiversity,  
126 since taxonomic information is pivotal for establishing a baseline to properly address  
127 sustainable management of marinas. Ideally, the analysis of benthic communities should  
128 be based on identification to the species level but this is usually a complex and time-  
129 consuming procedure (Terlizzi et al., 2003; Sánchez-Moyano et al., 2006). Indeed, in  
130 many taxa, species identification is laborious and requires appropriate taxonomic  
131 expertise that nowadays is often lacking (Paknia et al., 2015; Chatzinikolaou et al.,  
132 2018). Furthermore, policy makers and funding agencies often demand results and

133 recommendations be achieved within very short timescales. These requirements prevent  
134 identification to species level and promote analyses considering higher taxa (e.g. genus,  
135 family, order). Extensive literature, including a variety of methodologies, habitats and  
136 substrate types, has been developed to explore if environmental changes can be detected  
137 above the species level (see Sánchez-Moyano et al., 2006 and references therein; De  
138 Oliveira et al., 2020; Gerwin et al., 2020). **Nonetheless, the response of fauna to  
139 environmental data by considering different taxonomic levels has been scarcely studied  
140 in harbours and marinas (Chatzinikolaou et al., 2018).**

141 To fill the gaps described above, the main objective of this study was to explore  
142 the importance of soft-bottom environments, including both biotic and abiotic  
143 information, for assessing the ecological status of marinas from an integrative approach.  
144 To address this issue we aim to: (i) characterize the diversity and spatial distribution  
145 patterns of soft-bottom macro- and meiofauna communities in marinas, (ii) identify the  
146 set of abiotic variables which better explain the biological patterns, (iii) explore the  
147 taxonomic sufficiency required to detect differences among marinas and environmental  
148 patterns, and, eventually, (iv) develop an integrative and easy-to-apply method to assess  
149 the quality status of soft-bottom environments of marinas based on selected ecological  
150 indicators. **Most previous studies regarding soft bottoms of marinas have focused on  
151 abiotic parameters, and when they include biotic data, information on the macrofaunal  
152 and meiofaunal communities is neither integrated simultaneously nor do they develop  
153 integrative quality assessments based on a multiparametric approach (Table S1). The  
154 novelty of the present study lies in the integration of abiotic and biotic data for  
155 designing a quality assessment method based, concurrently, on the following selected  
156 ecological indicators: Marinas Environmental Pollution Index (MEPI),  
157 Biocontamination Index (BCI), macrofaunal biotic indices (AMBI, M-AMBI, BENTIX,  
158 MEDOCC and BENFES), macrofaunal taxa richness and Shannon-Wiener's diversity,  
159 and nematode:copepod index.**

160 To achieve the aforementioned objectives, the Southern Iberian Peninsula was  
161 selected as the study area since it (i) represents an interesting biogeographical area  
162 which includes an horizontal gradient Atlantic-Mediterranean, (ii) encompasses a very  
163 high number of marinas along the coast and (iii) includes the Strait of Gibraltar, a hot  
164 spot for maritime traffic.

165

166 **2. Material and Methods**

167 *2.1. Sampling survey and laboratory processing*

168 Sampling was conducted from June 26<sup>th</sup> to July 2<sup>nd</sup> 2017. Eight marinas were  
169 randomly selected along the Southern Iberian Peninsula: four in the Atlantic coast  
170 (Faro, Chipiona, Puerto América, Barbate) and four in the Mediterranean (La Línea-  
171 Puerto Chico, Fuengirola, Motril, Almería) (Fig. 1). Details of these marinas  
172 (coordinates, depth, size, number of berths and population density of the adjacent  
173 locality) can be consulted in Guerra-García et al. (2021). Within each marina, three  
174 floating pontoons were randomly selected (each pontoon was considered a replicate for  
175 analyses throughout the whole study; consequently, three replicates per marina were  
176 taken) and the sediment below each pontoon (mostly muddy) was sampled.

177 For macrofauna, a small van Veen grab (area: 15 x 15 cm) was used. Because  
178 the grab was relatively small, the replicate (i.e. pontoon) consisted of three additive  
179 sediment grabs (total area: 675 cm<sup>2</sup>). Samples were washed through a sieve with a mesh  
180 size of 0.5 mm and the retained fraction was fixed in 96% ethanol stained with Rose  
181 Bengal.

182 For meiofauna, only one sediment van Veen grab was collected per pontoon.  
183 Once the grab was recovered, a small corer of 10 cm<sup>2</sup> was introduced into the  
184 undisturbed sediment. Therefore, a total of three replicates (one corer per pontoon) were  
185 obtained per marina and directly fixed in 96% ethanol stained with Bengal Rose.

186 In the laboratory, macrofaunal samples were rinsed with fresh water over a 0.5-  
187 mm sieve, sorted, identified to species level whenever possible, and counted. For  
188 macrofauna, the status of each species (N: Native, I: Introduced, C: Cryptogenic, U:  
189 Undetermined) was also considered. Species names and systematic arrangement of taxa  
190 follows the World Register of Marine Species (WoRMS, 2020).

191 Meiofaunal samples were sieved in the laboratory through a 0.5-mm mesh to  
192 exclude macrofauna, with a 30-µm mesh below to retain meiofauna. Specimens were  
193 sorted under a stereomicroscope and identified to high taxonomic levels (e.g. phylum,  
194 class or order; Sedano et al., 2014, 2020)

195 Additionally, to quantify abiotic variables in sediment for each marina, three  
196 sediment samples (one per pontoon) were taken from the bottom using the van Veen  
197 grab. All samples were immediately frozen (-20°C) until laboratory analyses. The  
198 following environmental variables were measured: sand, silt, clay, total organic carbon

199 (TOC), hydrocarbons, faecal coliforms, biocide Irgarol, and major, minor and trace  
200 elements As, Cd, Co, Cr, Cu, N, Ni, P, Pb, S and Zn. Analytical procedures are detailed  
201 in Guerra-García et al. (2021).

202

## 203 *2.2. Statistical analyses*

204 The experimental design included two factors: ‘Location’ (Lo), a fixed factor  
205 with two levels (Mediterranean vs Atlantic) and ‘Marina’ [Ma(Lo)], a random factor  
206 with four levels, nested with Lo. The number of replicates was n=3, corresponding to  
207 the three pontoons randomly selected for each marina.

208 Regarding macrofauna, number of taxa (considering the species, genus, family,  
209 order, class and phylum levels separately), total abundance and Shannon-Wiener’s  
210 Diversity at species level ( $H'$ ) were calculated for each replicate (675 cm<sup>2</sup>). Data were  
211 also calculated separately for the three main phyla, i.e. Annelida, Arthropoda and  
212 Mollusca. Total taxa richness (i.e. total number of different species) and  $H'$  were also  
213 determined for each marina. For meiofauna, number of taxa (for high taxonomic levels),  
214 abundance (individuals/10 cm<sup>2</sup>) and the nematode:copepod ratio (Rubal et al., 2009)  
215 were also calculated.

216 Two-way ANOVAs with the above design were conducted to explore  
217 differences in the biological descriptive parameters of macro- and meiofauna among  
218 marinas (factor ‘Marina’) along the Mediterranean-Atlantic axis (factor ‘Location’).  
219 Homogeneity of variances was checked using Cochran’s test. When ANOVA detected  
220 significant differences for a given factor, the source of the variance was identified by  
221 applying the Student-Newman-Keuls (SNK) test (Underwood, 1997).

222 Non-parametric multidimensional scaling (MDS) was used to show the  
223 relationship among locations (Atlantic vs Mediterranean) and marinas based on  
224 macrofaunal abundances; this was done considering the data matrix for each of the six  
225 selected taxonomic levels (species, genus, family, order, class and phylum) separately  
226 and also for the three main phyla (i.e. Annelida, Arthropoda and Mollusca) considering  
227 only the species level. The Bray-Curtis similarity index was applied after transformation  
228 of abundance values by the square-root. An additional MDS was conducted using  
229 abundances of meiofaunal higher taxa.

230 The macrobenthic community patterns obtained from the six different taxonomic  
231 levels were compared using a second-stage MDS, by computing a weighted Spearman

232 rank correlation coefficient between the corresponding elements of each pair of marinas  
233 from the respective similarity matrices (Sommerfield and Clarke, 1995; Chatzinikolaou et  
234 al., 2018). Similarly, a second stage MDS was conducted to compare the patterns  
235 obtained separately for Annelida, Arthropoda and Mollusca with that of the total  
236 community.

237 Differences in the macro- and meiofaunal assemblage among locations and  
238 marinas were tested by permutational multivariate analyses of variance  
239 (PERMANOVA), using the same experimental design explained above, and considering  
240 the aforementioned six taxonomic levels. Additional PERMANOVAs were conducted  
241 for Annelida, Arthropoda and Mollusca independently. Analyses were based on Bray-  
242 Curtis similarities and Monte Carlo tests were included. Significant P-values were  
243 obtained by computing 9999 permutations of residuals under a reduced model, as this  
244 method gives the most accurate Type I error for complex design (Anderson, 2005).  
245 Pairwise comparisons were then used. Additionally, to test the dispersion among  
246 samples for the factors 'Location' and 'Marina', a permutational analysis of multivariate  
247 dispersions (PERMDISP) was used.

248 Distance-based linear modelling (DistLM) was used to assess the relative  
249 contribution of abiotic parameters to the variability observed in the macro- and  
250 meiofaunal community structure (Anderson et al., 2008). The analysis was performed  
251 based on the matrix of square-root transformed abundance data. Abiotic data were  
252 transformed by  $\log(x+1)$  and normalized to reduce the effects of differences in unit  
253 scales. The model was constructed using the best combination of predictors (*Best*  
254 procedure) using AIC (Akaike's Information Criteria) (Burnham and Anderson, 2004;  
255 Leonardi et al., 2020; Mulik et al., 2020; Wei et al., 2020). Prior to the DistLM analysis,  
256 multicollinearity between abiotic factors and skewness of data were evaluated using  
257 Draftsman plot based on Spearman correlations. Sets of variables with Spearman's test  
258 values higher than 0.8 ( $p < 0.01$ ) were omitted (Chatzinikolaou et al., 2018). Distance-  
259 based Redundancy Analysis (dbRDA) was performed to provide a visual representation  
260 of the macrobenthic community fitted to the significant predictor variables in the multi-  
261 dimensional space (Mulik et al., 2020; Wei et al., 2020). To increase robustness of  
262 results, the BEST routine (BIO-ENV) was also employed to explore correlations  
263 between the matrix of faunal abundances and environmental predictors from a different  
264 analytical perspective (Anderson et al., 2008). The Bray-Curtis similarities matrix was

265 based on faunal abundances while the Euclidean distances-based matrices derived from  
266 all the possible sequential combinations of the abiotic variables. Spearman rank  
267 coefficient was used to identify the best environmental variables that explained the  
268 observed patterns on 999 permutations (Marchini et al., 2020). To explore the response  
269 to abiotic parameters of each considered taxonomic level (species to phylum) and the  
270 three main phyla, DistLM and BIO-ENV were conducted for each category.

271 PERMANOVA, PERMDISP, MDS, DistLM, dbRDA and BIO-ENV analyses  
272 were carried out using the PRIMER v.6+PERMANOVA package (Clarke and Gorley,  
273 2006; Anderson et al., 2008) and ANOVA analyses were conducted on the GMAV5  
274 software (Underwood et al., 2002).

275

### 276 *2.3. Integrative assessment*

277 For an integrative assessment of the ecological quality status of marinas  
278 considering biotic and abiotic information of soft-bottom environments, the following  
279 six ecological indicators were selected and calculated for each marina (Fig. 2):

280

281 A) Marinas Environmental Pollution Index (MEPI). This index was recently  
282 developed by Guerra-García et al. (2021) to characterize the environmental  
283 pollution levels in marinas based on 15 selected stressors measured in the  
284 sediments.

285 B) Biocontamination Index (BCI). It was developed by Arbačiauskas et al. (2008)  
286 to assess the biopollution level (in terms of exotic species) in inland waters. We  
287 have used this index here as a proxy to evaluate the contribution of NIS to the  
288 soft-bottom benthic community in each marina.

289 C) Macrofaunal taxa richness. The total number of species found in each marina  
290 was calculated. The number of genera, families and orders were also included  
291 for comparison.

292 D) Macrofaunal diversity (at species level) was measured by the Shannon-Wiener's  
293 index ( $H'$ ).

294 E) Macrofaunal biotic indices. Many biotic indices have been developed during the  
295 last years. In the present study we have used AMBI (Borja et al. 2000), M-  
296 AMBI (Muxika et al., 2007), BENTIX (Simboura and Zenetos, 2002),  
297 MEDOCC (Pinedo et al., 2015) and BENFES (Sánchez-Moyano et al., 2017).

298 Compiled information about the boundaries and intervals for these indices can  
299 be found in Keeley et al. (2012). Biotic indices have been considered suitable  
300 tools for assessing the ecological status of Mediterranean ports (Dimitriou et al.,  
301 2020)

302 F) Meiofaunal nematode:copepod index proposed by Raffaelli and Mason (1981).  
303 This ratio was proposed as a fast, easy, and reliable method to study the effects  
304 of organic matter enrichment in coastal sediments (see Rubal et al., 2009).

305

306 The intervals and boundaries assigned in the literature for MEPI, BCI, AMBI,  
307 M-AMBI, BENTIX, MEDOCC and BENFES (high, good, moderate, poor, bad) were  
308 adopted in the present study. Intervals for taxa richness,  $H'$  and nematode:copepod ratio  
309 were established in the present study according to the results obtained in the marinas  
310 from the Southern Iberian Peninsula.

311 The following methodological protocol was developed to assess a quality status  
312 based on the selected ecological indicators (Fig. 2):

313 (i) Once the six indicators were calculated, a score for each indicator was  
314 assigned according to the colour categories for each ecological status: blue (high) was  
315 scored with 4 points, green (good) with 3 points, yellow (moderate) with 2 points,  
316 orange (poor) with 1 point and red (bad) with 0 points. All parameters have the same  
317 five intervals (blue, green, yellow, orange and red), except for MEPI which includes  
318 only green (good), yellow (moderate) and red (bad) (see Guerra-García et al., 2021). In  
319 this case green was scored with 4 points, yellow with 2 points and red with 0 points.

320 (ii) The global score of each marina (ranging from 0 to 24) was obtained by  
321 summing the scores assigned to each of the six selected indicators (MEPI, BCI, Taxa  
322 richness,  $H'$ , biotic indices and nematode:copepod ratio) based on its ecological status  
323 (i). If several biotic indices are available, as in the present study, the value used in the  
324 assessment would result from the mean of the scores obtained for each index. Although  
325 the number of species is advisable as an indicator of taxa richness, the number of  
326 genera, families or orders could also be used in the assessment.

327 (iii) Establishment of global quality status of each marina according to the total  
328 score. We proposed the following intervals: (0-5: bad, 6-10: poor, 11-15: moderate, 16-  
329 20: good, 21-24: high)

330 Additionally, the relationships between all the indicators used were explored by  
331 Spearman correlation for all datasets.

332

### 333 **3. Results**

#### 334 *3.1. Macrofaunal community*

335 8339 individuals belonging to at least 166 macrofaunal species were found during the  
336 present study in sediments from eight marinas on the Southern Iberian Peninsula (Table  
337 1 and Tables S2, S3). This included 61 annelid species (5514 specimens, mainly  
338 represented by polychaetes), 58 arthropods (1837 specimens, mainly peracarid  
339 crustaceans), 41 molluscs (789 specimens, mainly bivalves), 3 echinoderms (9  
340 specimens) and 190 additional specimens belonging to the phyla Nematoda, Nemertea  
341 and Phoronida. In all marinas, Annelida was the dominant phylum (Fig. 3A) both in  
342 species richness and abundance, except in Almería where Arthropoda was the most  
343 abundant phylum (due to the high density of the tanaid *Apseudopsis latreilli*). The most  
344 common and abundant taxa were the annelids *Aphelochaeta marioni*, *Capitella*  
345 *capitata*, *Chaetozone gibber*, *Cirriformia tentaculata*, *Cirrophorus furcatus* and  
346 *Fabricia stellaris*, the crustaceans *Phtisica marina*, *Iphinoe tenella* and *A. latreilli*, and  
347 the molluscs *Abra tenuis*, *Corbula gibba* and *Parvicardium exiguum* (Table 1). Detailed  
348 information on the taxonomical categories of each species and their status (native,  
349 cryptogenic or introduced) is included in Tables S2 and S3. Most of the species  
350 inhabiting sediments from marinas can be considered native or cryptogenic, while only  
351 four were exotic species, all of them crustaceans, namely the amphipods *Caprella*  
352 *scaura* and *Jassa slateryi*, the isopod *Paranthura japonica* and the decapod *Alpheus* sp.  
353 (Table S2).

354 Regarding the total number of species per marina, Puerto América showed the  
355 highest species richness (76) followed by Almería (53), Motril (52) and La Línea (47).  
356 The marinas with the lowest number of species were Chipiona (25), Barbate (30),  
357 Fuengirola (33) and Faro (37). This pattern was also obtained for the number of species  
358 per replicate (Fig. 3B). The highest values of the Shannon-Wiener's diversity ( $H'$ )  
359 (based on the global data per marina) was also measured in Puerto América (3.23)  
360 followed by La Línea (2.82), Motril (2.41) and Faro (2.03). Barbate (1.56), Chipiona  
361 (1.64), Almería (1.86) and Fuengirola (1.92) showed the lowest values.

362 The number of species, total abundance and H' differed significantly among  
363 marinas, although no differences were obtained for the factor 'location' (Atlantic vs  
364 Mediterranean) (Table 2). The highest heterogeneity among marinas was obtained for  
365 the Mediterranean (see SNK tests in ANOVAs of Table 2). The two-dimensional MDS  
366 plot based on species abundances showed segregation of marinas (Fig. 4A).  
367 PERMANOVA confirmed significant differences for all marinas (see pair-wise tests in  
368 Table 2) with no significant differences for the factor location (Table 2).

369 When each of the three main phyla (Annelida, Arthropoda and Mollusca) was  
370 analysed separately, the species richness also differed significantly among marinas  
371 (Table S4A, Fig. 3C). The pattern was more evident for annelids and molluscs than for  
372 arthropods (Table S4A). In fact, SNK tests indicated no differences among  
373 Mediterranean marinas for arthropods. At the community level, the factor 'Marina' was  
374 significant for the three phyla according to PERMANOVA results (Table S5A) and  
375 MDS plots graphically showed segregation among marinas again (Fig. 5A). Molluscs  
376 also revealed some segregation between Mediterranean and Atlantic marinas, although  
377 'Location' was not significant according to PERMANOVA (Pseudo-F=1.9178,  
378 p=0.0961, Table S5A). The second stage MDS (Fig. 5B) demonstrated that the pattern  
379 derived from the annelid assemblage was very similar to that considering all taxa,  
380 showing that this taxon was most responsible for driving the observed pattern for the  
381 whole community.

382

### 383 3.2. Meiofaunal community

384 A total of 16 higher taxa were identified (Tables 3, Table S6), 4 of which (Copepoda,  
385 nauplius larvae of crustaceans, Nematoda and Polychaeta) were always present at all  
386 marinas. Nematoda was the numerically dominant taxon in Faro, Chipiona, P. América,  
387 Barbate and Fuengirola, while Copepoda dominated in La Línea, Motril and Almería  
388 (Fig. 6A).

389 The number of taxa did not differ among marinas or among locations (Atlantic  
390 vs Mediterranean), while total abundance values were higher in Mediterranean marinas  
391 (Fig. 6A, Table 4). The nematode:copepod ratio differed significantly among marinas,  
392 although these differences were mainly due to the high values measured in Fuengirola.  
393 The MDS plot showed segregation of marinas and, differently from macrofauna,  
394 reflected a clear pattern for the factor location, i.e. Atlantic vs Mediterranean marinas

395 (Fig. 6B). PERMANOVA confirmed significant differences for the factors ‘Location’  
396 and ‘Marina’ (Table 4).

397

### 398 3.3. Influence of environmental data on faunal assemblages

399 A full dataset of environmental data measured in sediments of the eight studied  
400 marinas can be found in Table S7 (see also Guerra-García et al., 2021, for details on the  
401 environmental assessment).

402 The variables Co, N, As, S, Clay and N were correlated with other variables  
403 ( $r > 0.8$ ,  $p < 0.01$ ) and were not included in the DistLM, so the model was run with 12  
404 variables (Sand, Silt, hydrocarbons, faecal coliforms, biocide Irgarol, Cd, Cr, Cu, Ni, P,  
405 Pb and Zn). According to DistLM, the environmental variables which significantly  
406 correlated with the whole macrofaunal community (identified at species level) were  
407 mainly P (18.2% of variation explained), sand (16.7%) and TOC (16.0%) (Table 5).  
408 Faecal coliforms and the heavy metals Cr, Cu, Pb and Zn were also significant (Table  
409 5). The best model (AIC=180.46,  $R^2=0.073$ ) was obtained for the combination of Sand,  
410 Silt, TOC, P, faecal coliforms, Irgarol, Cr, Cu and Pb (Table 5). The first two axes of  
411 the dbRDA explained 50.9 % of the total variation (Fig. 7). BIO-ENV results showed  
412 that the best model ( $Rho=0.820$ ,  $p < 0.01$ ) was obtained with the combination of Sand,  
413 TOC, P, faecal coliforms, Cu, Pb and Zn, coinciding with the significant variables  
414 provided by DistLM (Table 6).

415 When the three main phyla (Annelida, Arthropoda and Mollusca) were analysed  
416 separately, DistLM showed similar results to those obtained with the whole community  
417 (Sand, TOC, P, faecal coliforms and heavy metals) (Table 6, Table S8). Variables  
418 explained a higher proportion of variability in annelids, followed by molluscs and  
419 finally arthropods (Table S8). In fact, BIO-ENV only provided a significant model  
420 ( $Rho=0.797$ ,  $p < 0.05$ ) for Annelida (Table 6), which, therefore, turned out to be the  
421 phylum best reflecting the environmental conditions in marinas.

422 Regarding to meiofauna (based on higher taxa abundances), DistLM selected  
423 Sand (17.1%), Hydrocarbons (14%), Cd (13.7%) and Cu (10%) as the variables that  
424 correlated significantly with the meiofaunal assemblage (Table S9, Table 6). The best  
425 model (AIC=157.62,  $R^2=0.62$ ) was obtained for the combination of Sand, Silt, TOC,  
426 faecal coliforms, Cd, Cr, Cu and Zn. BIO-ENV also showed Hydrocarbons, Cd and Cu

427 as the best combination model. However, the model was not significant ( $Rho=0.630$ ,  
428  $p=0.130$ ) (Table 6).

429

### 430 3.4. Macrofaunal taxonomic sufficiency

431 This study revealed the presence of 166 species, 155 genera, 105 families, 44  
432 orders, 16 classes and 8 phyla inhabiting marinas of the Southern Iberian Peninsula. The  
433 number of species was very similar to the number of genera in all marinas (Fig. 3B),  
434 since most of the genera were represented by a single species (Table S2). Number of  
435 families was also close to the number of genera and species (Fig. 3B). Regarding taxa  
436 richness, ANOVAs revealed that the factor ‘Marina’ was significant for all taxonomic  
437 levels considered, although for class and phylum these differences were only due to the  
438 high number of taxa collected in P. América in comparison with other Atlantic marinas  
439 (Table S4B, Fig. 3B). PERMANOVA confirmed that there were significant differences  
440 in community structure among all marinas when separately considering each matrix for  
441 species, genera, families or orders (Table S5B). Although significant differences in the  
442 factor ‘Marina’ were also obtained for classes and phyla, these differences were due to  
443 the influence of certain marinas, such as Faro (Atlantic) or Almería (Mediterranean)  
444 (Table S5B). This pattern was supported by the corresponding MDS ordinations (Fig.  
445 4A). Separation among marinas was evident at the level of species, genus, family and  
446 order, while MDS based on classes and phyla showed a less defined grouping (Fig. 4A).  
447 The second-stage MDS performed on the six considered taxonomic levels (Fig. 4B)  
448 demonstrated that patterns showed by the species/genus levels were similar, and close to  
449 that obtained for family/order levels. Conversely, they differed considerably from those  
450 derived from the higher levels (i.e. phylum and class).

451 When the influence of taxonomic resolution was considered to compare the  
452 environment-fauna relations, DistLM revealed a similar pattern for species, genus,  
453 family and order (Table 6, Table S8). The environmental variables that were significant  
454 at the species level were the same as those found at the level of genus, family and order.  
455 The set of significant variables were quite different when considering classes and phyla  
456 data. Indeed, BIO-ENV provided significant results with similar variables for species,  
457 genus, family and order, but they were not significant for class and phylum (Table 6).

458

### 459 3.5. Global assessment. Biotic indices and biocontamination

460 According to the Marinas Environmental Pollution Index (MEPI), Barbate and  
461 Fuengirola were the most polluted marinas (Fig. 8). The Biocontamination Index (BCI)  
462 values were very low in all marinas due to the small number of introduced species  
463 found on sediments. Although quality intervals for MEPI, BCI and Biotic Indices were  
464 defined in the literature, those for taxa richness, diversity ( $H'$ ) and nematode:copepod  
465 ratio were not available so they were established during the present study, based on the  
466 data obtained for marinas of Southern Iberian Peninsula (see Table S10). The global  
467 score, obtained by summing the scores assigned to each selected indicator (MEPI, BCI,  
468 Taxa richness,  $H'$ , Biotic indices and nematode:copepod ratio) ranged from 9 to 17.  
469 Fuengirola, Barbate and Chipiona got the lowest score (9 and 10 points) while La Línea,  
470 Motril and P. América showed better ecological status (16 and 17 points) (Fig. 8).  
471 Therefore, marinas of the Southern Iberian Peninsula ranged from poor (Fuengirola,  
472 Barbate and Chipiona) to moderate (Faro and Almería) and good (P. América, La Línea  
473 and Motril).

474 In general terms, the ecological indicators used to conduct the global assessment  
475 were in good agreement, except for punctual differences (i.e. BENTIX classified all the  
476 marinas as bad or poor, while BENFES classified them as moderate, good or high).  
477 Apart from BCI which showed low values in all marinas and was not able to  
478 discriminate among them, environmental data (MEPI) and biological information (taxa  
479 richness,  $H'$ , biotic indices and nematode:copepod ratio) were more discriminant and  
480 provided similar information (Fig. 8). MEPI was correlated with richness of species and  
481 genera, the biotic index MEDOCC and the nematode:copepod ratio. Taxa richness was  
482 correlated with  $H'$  and with biotic indices (Table S11). Indeed, the marinas classified as  
483 good (P. América, La Línea and Motril) were those with the highest values of  
484 macrofauna taxa richness and Shannon-Wiener's diversity, and the lowest degree of  
485 environmental pollution. Conversely, the most polluted marinas, such as Chipiona,  
486 Barbate and Fuengirola, showed the lowest values for taxa richness, diversity, and the  
487 highest nematode:copepod ratios.

488

## 489 **4. Discussion**

### 490 *4.1. Benthic communities*

491 The present study revealed that the soft bottom of marinas from the Southern  
492 Iberian Peninsula supported a wide variety of macrobenthic taxa (166 species

493 distributed in 155 genera, 105 families, 44 orders, 16 classes and 8 phyla). The number  
494 of soft-bottom macrofauna species per marina (ranging from 25 to 76) was similar to  
495 that reported from marinas worldwide (e.g. McGee et al., 1995; Estacio et al., 1997;  
496 Guerra-García and García-Gómez, 2004 a,b,c; Martínez-Lladó et al., 2007; Covazzi  
497 Harriague et al., 2012; Chatzinikolaou et al., 2018; Ng et al., 2019).

498 Annelida (mostly polychaetes) was the dominant phylum in number of species  
499 (37%) and, especially, in number of individuals (66%). In fact, the patterns of the total  
500 community were mainly driven by annelids (see Fig. 3), that are usually reported as the  
501 dominant macrobenthic taxon in the sediment of marinas worldwide (Moreira et al.,  
502 2010; Ng et al., 2019; Chatzinikolaou et al. 2018) and, in general, in most soft-bottom  
503 habitats (Hutchings, 1998). On the contrary, Arthropoda (mainly Amphipoda inhabiting  
504 arborescent substrates) dominated floating pontoons in terms of abundance and species  
505 richness (Gavira O'Neill et al., 2015, 2018).

506 Although all studied marinas were located across the same geographical area,  
507 univariate and multivariate analyses consistently showed significant differences among  
508 them based on biological data. This heterogeneity among marinas has also been  
509 revealed by environmental stressors (see Guerra-García et al., 2021). Spatial variations  
510 in biotic assemblages among and within marinas have been attributed to differences in  
511 the local environment as well as to the marina design (Toh et al., 2017). In fact, the  
512 singularity of any given marina, largely determined by a variety of local factors (e.g.  
513 environmental conditions, availability of suitable substrate, level of marine traffic) has  
514 proven to be a key factor affecting, for example, several stages of the NIS invasion  
515 process (Martínez-Laiz et al., 2019). Hence, our results reinforce the importance of  
516 considering a local scale perspective for conservation and management purposes.

517 Although significant differences in the factor 'marina' were relevant throughout  
518 the whole study, the factor 'Location' (Atlantic vs Mediterranean) was not relevant for  
519 macrofauna, only showing certain degree of segregation when considering molluscs  
520 separately. However, this factor was significant for meiofauna and should also be  
521 considered in management programmes.

522 The present study only considered a spatial scale, and therefore further research  
523 should explore if the observed patterns remain constant through time. Although  
524 epifaunal communities associated with floating pontoons suffer significant seasonal  
525 fluctuations (e.g. Ros et al., 2013), Chatzinikolaou et al. (2018) did not find important

526 seasonal differences in soft-bottom communities of port and marinas. On the contrary,  
527 Moreira et al. (2010) detected strong fluctuations among and within seasons mostly  
528 related to recruitment of opportunistic species and some perturbations (i.e. proliferation  
529 of ephemeral algae on the sediment in summer). This again emphasizes that the  
530 environmental singularity of any given marina must be considered in management  
531 programmes. In this sense, sediment monitoring should ideally be based on previous  
532 baseline data in order to design the appropriate sampling for each marina and reduce the  
533 monetary, time and labor costs of such activities as much as possible.

534

#### 535 *4.2. Influence of environmental data on faunal assemblages*

536 The multivariate pattern of the macrofaunal community in the present study was  
537 mainly explained by P, Sand, TOC, faecal coliforms, and heavy metals. Although  
538 hydrocarbon concentration clearly discriminated among marinas (Table S7, Guerra-  
539 García et al., 2021) and has been traditionally considered a significant factor affecting  
540 biota (Blanchard et al., 2002), it did not contribute to explain the observed macrofaunal  
541 patterns but was, in turn, relevant for meiofauna. In this sense, Covazzi Harriague et al.  
542 (2012) pointed out that the macrofaunal and meiofaunal assemblage can respond  
543 differently to human pressure.

544 Most explanatory variables selected by our analyses agree with those reported in  
545 previous studies in harbours and ports. Granulometry is considered one of the main  
546 factors modulating soft-bottom communities, including those inhabiting harbour areas  
547 (e.g. Moreira et al., 2005). Guerra-García et al. (2004 a,b,c) also reported granulometry,  
548 TOC, P and heavy metals as important variables to explain the distribution of molluscs,  
549 crustaceans and polychaetes in the harbour of Ceuta, while Chatzinikolaou et al. (2018)  
550 found heavy metals to be relevant explanatory variables of biotic patterns. Among the  
551 many potential sources, metal-based antifouling systems, mainly Cu and Zn, clearly  
552 play a major role in contamination in marinas (Briant et al., 2013).

553 When each of the three main phyla were analysed separately, Annelida was  
554 more related to the considered environmental conditions in marinas than others.  
555 Arthropods in general, and crustaceans in particular, are traditionally considered better  
556 bioindicators due to their higher sensitivity to marine pollution (Navarro-Barranco et al.,  
557 2020) and better reflect differences at the spatial scale (i.e. among localities) than  
558 molluscs and annelids (Sánchez-Moyano and García-Gómez, 1998). However, in

559 harbours and marinas, annelids (mainly polychaetes) become the best taxon to  
560 discriminate between sampling sites, especially when oxygen is not a limiting factor  
561 (Guerra-García and García-Gómez, 2005a).

562

#### 563 *4.3. Taxonomic sufficiency*

564 The use of higher taxonomic levels instead of species as a proxy for  
565 identification has been extensively tested in both terrestrial and marine habitats, and on  
566 such taxa as insects, fish, fauna associated with algae and seagrasses, meiofauna and  
567 benthic macrofauna in general (see Sánchez-Moyano et al., 2006 and references  
568 therein). However, it is important to properly analyse the cost-benefits of such  
569 approaches to select the appropriate level of taxonomic resolution in benthic monitoring  
570 programmes (Bouchard et al., 2005).

571 Although Ferraro and Cole (1990) indicated that the species level would be the  
572 most sensitive for the evaluation of pollution impacts, Sánchez-Moyano et al. (2006),  
573 based on several datasets within a wide range of habitats and substrate types along the  
574 southern Iberian coastline, concluded that studies at the different levels of taxonomic  
575 resolution (species, family, order) led to similar results both as regards community  
576 distribution and composition, and their relationship to environmental variables.

577 The present study found similar patterns to those provided by Sánchez-Moyano  
578 et al. (2006) and Chatzinikolaou et al. (2018). On the one hand, differences among  
579 marinas were also detected using high taxonomic levels, and on the other hand, the set  
580 of environmental variables which better explained macrofaunal assemblages were the  
581 same at the species, genus, family and order level, but differed at class and phylum  
582 levels. The second stage MDS revealed that identification to genus provided almost  
583 identical results to those obtained when considering species due to many genera being  
584 represented by only one species in each marina. Family and order levels yielded similar  
585 results between them but were less informative with respect to species and genus levels  
586 (Fig. 4). For all these reasons, family and order levels could be an alternative in large-  
587 scale monitoring programmes with significant time and budget constraints.

588 Conversely, taxonomical resolution to the species level is mandatory to  
589 accurately identify NIS in marinas, and to correctly calculate biocontamination indices  
590 such as BCI (Arbačiauskas et al., 2008). Indeed, early detection of NIS is often delayed  
591 when taxa are taxonomically challenging, such as small-sized marine organisms which

592 require great taxonomic expertise (Marchini and Cardeccia, 2017; Martínez-Laiz et al.,  
593 2020). Therefore, the potential efficacy of identification to high taxonomic levels in  
594 some monitoring programmes does not replace by any means the information obtained  
595 at the species level, which is paramount for basic and applied ecological studies (de  
596 Oliveira et al., 2020).

597

#### 598 *4.4. Global assessment*

599 The quality assessment applied in the present study and developed specifically  
600 for marinas was based on six selected ecological indicators (MEPI, BCI, Taxa Richness,  
601 H', biotic indices and nematode:copepod ratio), encompassing five of the eleven  
602 descriptors of MSFD: D1 (Biological diversity), D2 (NIS), D5 (Human-induced  
603 eutrophication), D6 (Sea floor integrity) and D8 (Concentrations of contaminants) (Berg  
604 et al., 2015; Piroddi et al., 2015). The assessment was useful for discriminating among  
605 marinas, even when they were closely located. The evaluation simultaneously combined  
606 abiotic and biotic information, integrating chemical pollution, NIS risk and attributes of  
607 the macro- and meiofaunal assemblages.

608 Regarding taxa richness in the assessment method, identification to the species  
609 level is advisable, but this study showed that other categories such as genus, family or  
610 even order could provide similar ecological intervals (see Fig. 8 and Table S10). Indeed,  
611 values of these four taxonomic categories were significantly correlated ( $r > 0.9$ ,  $p < 0.01$ )  
612 (Table S11). Therefore, this global assessment for marinas can be conducted whenever  
613 the macrofauna is, at least identified to the order level.

614 In recent years, a variety of biotic indices dealing with the structure of  
615 macrobenthic communities have been proposed to address the objectives of European  
616 Directives (e.g., Marine Strategy Framework Directive -MSFD- 2008/56/EC, EU  
617 Biodiversity Strategy to 2020 and Water Framework Directive -WFD- 2000/60/EC).  
618 Indeed, biotic indices have been recently demonstrated to be suitable for assessing the  
619 ecological status of Mediterranean ports and marinas (Dimitrou et al., 2020).

620 Although a unified protocol within the EU has not yet been adopted (Dimitriou  
621 et al., 2020) and there is no agreement on what index or indices should be used by  
622 environmental managers to establish benthic quality (Borja et al., 2015), the most used  
623 ones are AMBI (AZTI's Marine Biotic Index; Borja et al., 2000), M-AMBI  
624 (multivariate AMBI; Muxika et al., 2007) and BENTIX (Simboura and Zenetos, 2002).

625 The MEDOCC index was an adaptation of the AMBI for the Mediterranean area  
626 (Pinedo et al., 2015). All these indices rely on the taxonomic identification of species.  
627 Recently, a new index based on identification to the family level, i.e. BENFES, was  
628 developed (Sánchez-Moyano et al., 2017). The application of these indices in marinas  
629 revealed important differences in the ecological status assigned (Fig. 8). BENFES was  
630 the most favourable (most marinas classified as good). Conversely, BENTIX was  
631 clearly more restrictive (most marinas qualified as bad or poor), coinciding with the  
632 results obtained by Dimitrou et al. (2020). BENTIX did not correlate with any other  
633 index, nor with other selected ecological indicators, so its use in marinas, at least in the  
634 Southern Iberian Peninsula, is discouraged. MEDOCC was the only index correlating  
635 with environmental pollution (MEPI), so its calculation in marinas can be useful.  
636 Therefore, we recommend using the biotic indices in marinas with caution and,  
637 whenever possible, calculating several to obtain mean scores and reduce potential biases  
638 in the quality assessment.

639 The selected meiofaunal indicator for assessment was the nematode:copepod  
640 ratio, which is relatively easy to undertake in a marina. Undoubtedly, considering the  
641 difficulty and time-consuming process of taxonomic identification of nematodes and  
642 copepods, the use of taxonomic sufficiency is one of the most attractive features of this  
643 ratio (Rubal et al., 2019). Hence, this index has become a common method for  
644 evaluating organic enrichment and chemical pollution (Rohal et al., 2020). A higher  
645 ratio is usually associated to chemical and organic pollution (Raffaelli and Mason,  
646 1981), because copepods, specifically harpacticoids, are more sensitive to pollutants  
647 than nematodes (Elarbaoui et al., 2015). In fact, Moreno et al. (2008) found a significant  
648 correlation between nematodes and concentrations of environmental contaminants in  
649 tourist marinas of the Mediterranean Sea. The present study revealed that the index  
650 discriminates well among marinas. It correlated with other biotic indices and, most  
651 importantly, with the environmental pollution levels as defined by the MEPI.  
652 Accordingly, we consider that the measure of nematode:copepod ratio in marinas can be  
653 an adequate proxy for environmental pollution.

654 Concerning exotic species, recreational boating is currently considered a  
655 significant vector for the introduction and secondary spread of NIS, especially at local  
656 scales (e.g. Ros et al., 2013; Ashton et al., 2014; Foster et al., 2016; Ferrario et al.,  
657 2017; Martínez-Laiz et al., 2018, 2019; Ulman et al., 2019). In the present study, we

658 used the Biocontamination Index (BCI) for marinas. Alternatively, a more recent index,  
659 ALEX, has also been proposed to assess the impacts of alien species on benthic  
660 communities (Çinar and Bakir, 2014). While BCI is based on presence and abundance  
661 of alien species *sensu lato*, ALEX does not take into account abundances and its  
662 calculation requires classification of alien species as ‘causal’, ‘established’ or  
663 ‘invasive’. However, these categories are hard to assign when considering small  
664 invertebrates in marinas. For this reason, we have not calculated ALEX in the present  
665 study; instead, we consider BCI to be more appropriate for marinas. Despite marinas  
666 being deemed ‘hot spots’ for exotics, the number of alien species inhabiting soft  
667 bottoms was remarkably low, with only 4 NIS (all crustaceans, 3 peracarids and 1  
668 decapod) out of 166 species. It should be noted though, that the native range of more  
669 than a third of the reported taxa is unknown (their status remains undetermined or  
670 cryptogenic), which highlights the lack of baseline information and taxonomic  
671 uncertainty for many of these small mobile taxa. In any case, the low number of alien  
672 species reported here contrasts considerably with the high number of NIS (both mobile  
673 and sessile taxa) inhabiting floating pontoons (e.g. Martínez-Laiz et al., 2018; Tempesti  
674 et al., 2020). In fact, higher numbers of NIS in hard substrates in comparison with soft  
675 bottoms have been reported by other authors (Ruiz et al., 2009; Jimenez et al., 2018).  
676 Our results confirm this pattern in marinas and suggest that exploring NIS only in  
677 sediments is not enough to properly characterize biopollution levels in recreational  
678 harbours. The contrasting invasion pattern observed between hard and soft-bottom  
679 habitats also illustrates the importance of sampling across a variety of habitats (Jiménez  
680 et al., 2018).

681

#### 682 4.5. Towards Blue-flag marinas

683 A suitable management of marinas and ports should be a priority in the context  
684 of implementation of MWF and United Nations (UN) sustainability goals 2030. One  
685 of these goals includes marine life and aims to reduce pollution in coastal waters by  
686 increasing scientific knowledge and developing research capacities and transference of  
687 marine technology. Therefore, monitoring anthropogenic pressure and ecological  
688 quality of marinas should be an important issue for local authorities (D’Alessandro et  
689 al., 2020). The assessment of quality status can allow for design of standards when  
690 assigning “sustainable quality seals” to those marinas with better values of ecological

691 indicators. Environmental quality is, indeed, one of the main criteria to assign a Blue  
692 Flag to a marina (<http://www.banderaazul.org/puertos-y.embarcaciones-2020>, accessed  
693 8 August 2020). In our study, the marinas at P. América, La Línea and Motril reached  
694 the ‘good’ status according to the global environmental assessment based on soft-  
695 bottom habitats. This assessment based on sediment indicators could provide a tool for  
696 marinas to be awarded a Blue Bag.

697 To reduce environmental impact and increase biological diversity, the design of  
698 new marinas should allow water exchange by building more openings or having a  
699 through-flow design to facilitate exchange with the adjacent open waters (Guerra-García  
700 and García-Gómez 2004a,b,c, 2005a,b; Ng et al., 2019). In fact, marinas that are well  
701 designed, with adequate structural complexity and good water quality, can even  
702 function as nurseries for juvenile fish (Bouchoucha et al., 2016) and small invertebrates  
703 (pers. observ.).

704 Marinas may support a rich and diverse marine biodiversity and are easily  
705 visible to the public (Toh et al., 2017). Pontoons are full of sessile and mobile colourful  
706 fauna representing different phyla, and can be visited at any time by walking (no need  
707 for snorkeling or SCUBA diving). Beside hard substrates, the bottom of marinas usually  
708 corresponds to muddy sediments that can easily be sampled with small grabs for macro-  
709 and meiofauna. These manageable grabs can be effortlessly hand-carried and deployed  
710 from the pontoon in the marina without the need of a boat or any motorized lift system.  
711 For all these reasons, marinas become suitable enclaves to develop teaching activities  
712 on sampling methods in hard and soft bottoms, citizenship education and biodiversity  
713 conservation programmes as well as training courses (e.g. NIS identification), among  
714 others. Furthermore, by using social perception surveys and media programmes, boat  
715 owners can become aware of the marine life inhabiting pontoons and sediments, and get  
716 involved in reaching more ecologically sustainable marinas.

717

## 718 **5. Conclusions**

719

720 The present study reveals that the proposed method based on selected abiotic and biotic  
721 indicators [Marinas Environmental Pollution Index (MEPI), Biocontamination Index  
722 (BCI), macrofaunal biotic indices (AMBI, M-AMBI, BENTIX, MEDOCC and  
723 BENFES), macrofaunal taxa richness, Shannon-Wiener’s diversity, and

724 nematode:copepod index], can be useful for integrative assignment of the ecological  
725 status of marinas. Although the consideration of several indicators is advisable in terms  
726 of scientific integrity, it could be questioned that this would be user-friendly and easy to  
727 apply for management stakeholders. As scientists, we must encourage policy makers,  
728 governments and funding agencies to invest in rigorous scientific studies to properly  
729 address specific monitoring guidelines before making any decision. Alternatively, we  
730 must provide easy tools to facilitate this work, so a reasonable commitment must be  
731 reached with a proper balance between scientific integrity and a manageable approach.  
732 Although our proposed method involves several parameters and indicators, it can be  
733 flexible according to the available data. For example, taxa can be identified to family  
734 and order instead of species, and MEPI can be adjusted based on the environmental data  
735 available (see Guerra-García et al., 2021). Similarly, species richness can be  
736 alternatively substituted by the number of genera, family or orders. Values of the  
737 nematode:copepod index are not difficult to calculate and, regarding biotic indices, the  
738 score can be calculated on the basis of the indices available, and some of them do not  
739 require species identification. The only index that requires identification at the species  
740 level is the BCI, since it needs the correct identification of exotic species. But, if  
741 necessary, under time and budget constraints, the global score can be done by  
742 eliminating some of the markers, such as BCI, and extrapolating the score to the new  
743 intervals. Future studies must be conducted to test the method proposed across different  
744 biogeographical and seasonal scales.

745

#### 746 **Declaration of competing interest.**

747 We declare no conflict of interest.

748

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756

757 **Appendix A. Supplementary data**

758 Supplementary data related to this article can be found online at  
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760

761

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1149

### 1150 **Figure legends**

1151 Fig. 1. Map of the study area showing the location of the eight studied marinas.

1152

1153 Fig. 2. Flowchart showing the methodology of the assessment method proposed in the  
1154 present study.

1155

1156 Fig. 3. A) Abundance of the three main macrofaunal phyla (Annelida, Arthropoda and  
1157 Mollusca) in each marina; “others” include remaining phyla. B) Taxa richness for each  
1158 considered taxonomic level (species, genus, family, order, class and phylum). C)  
1159 Species richness of the three main phyla. Values represent the mean (A–C) and standard  
1160 error (B–C) (n=3) per marina.

1161

1162 Fig. 4. A) Non-parametric multidimensional scaling (MDS) showing the relationship  
1163 among locations (Atlantic vs Mediterranean) and marinas according to macrofauna  
1164 separately for the six considered taxonomic levels (species, genus, family, order, class  
1165 and phyla). B) Second stage MDS considering all macrofaunal taxonomic levels.

1166

1167 Fig. 5. A) Non-parametric multidimensional scaling (MDS) showing the relationship  
1168 among locations (Atlantic vs Mediterranean) and marinas according to macrofauna  
1169 (separately for Annelida, Arthropoda and Mollusca). B) Second stage MDS for the three  
1170 main phyla and the total macrofauna community.

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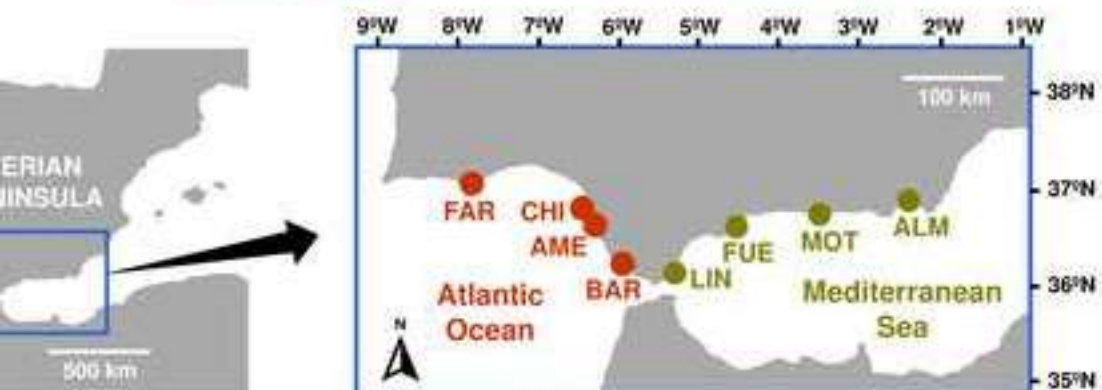
1172 Fig. 6. A) Nematode:copepod ratio and abundance of the main meiofaunal taxa  
1173 measured in each marina. B) Non-parametric multidimensional scaling (MDS) showing  
1174 the relationship among locations (Atlantic vs Mediterranean) and marinas according to  
1175 meiofauna (higher taxa abundances).

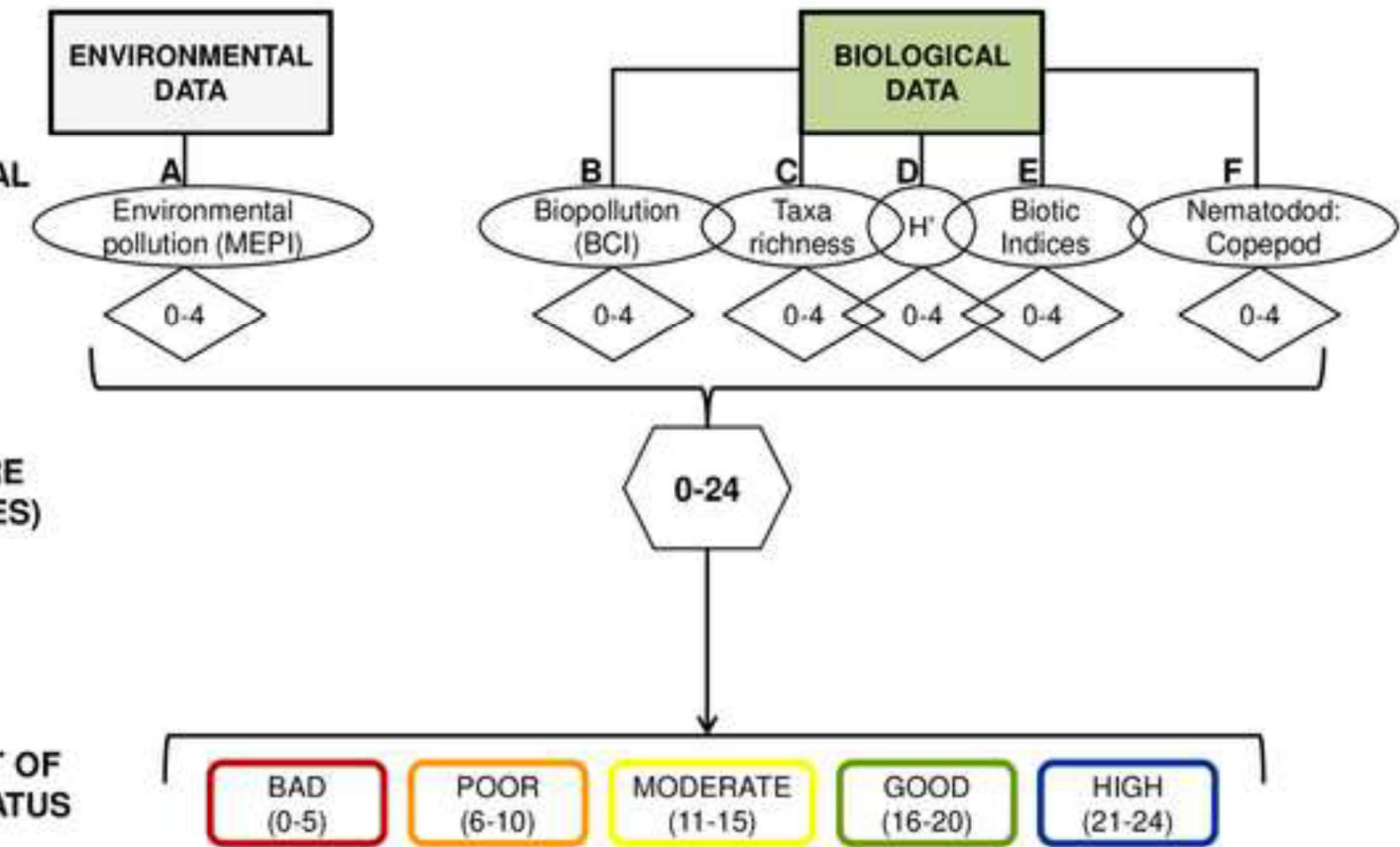
1176

1177 Fig. 7. Graphic representation of the dbRDA showing ordination of marinas and  
1178 variables with respect to the first two axes. Only significant variables according to the  
1179 DistLM (see Table 5) are included.

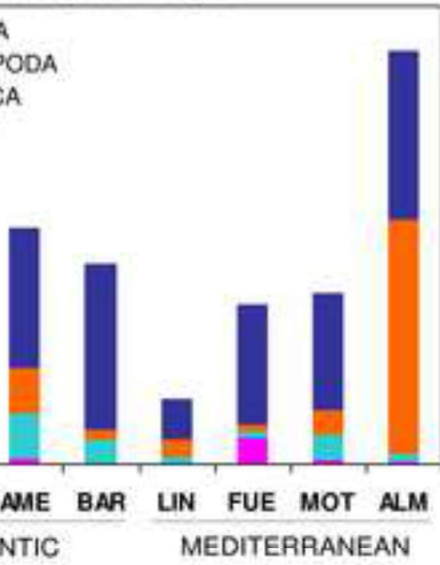
1180

1181 Fig. 8. Global assessment of ecological quality status of the marinas from southern  
1182 Iberian Peninsula based on six selected indicators: Marinas Environmental Pollution  
1183 Index -MEPI-, Biocontamination Index -BCI-, macrofaunal taxa richness, Shannon-  
1184 Wiener's diversity ( $H'$ ), macrofaunal biotic indices and meiofaunal nematode:copepod  
1185 ratio. Total score for each marina (0-24) is based on the sum of the scores for each  
1186 indicator.



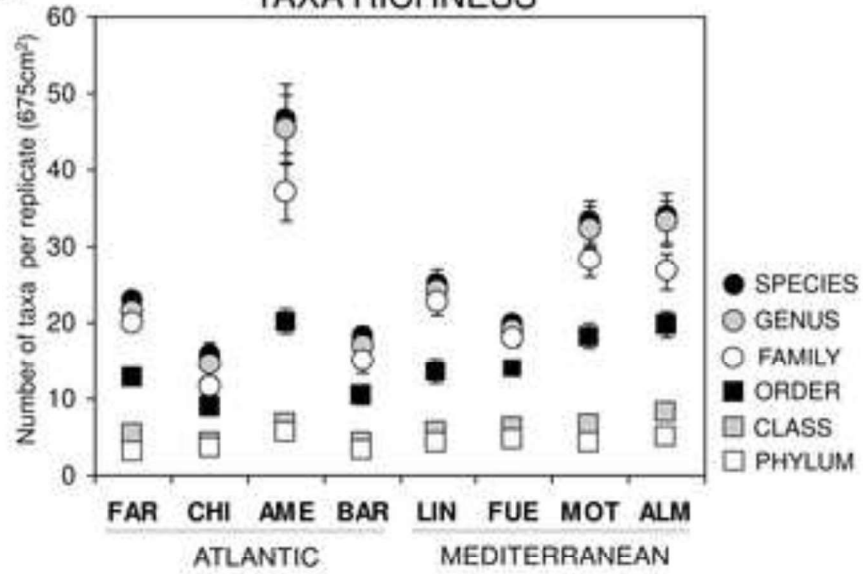


### ABUNDANCE

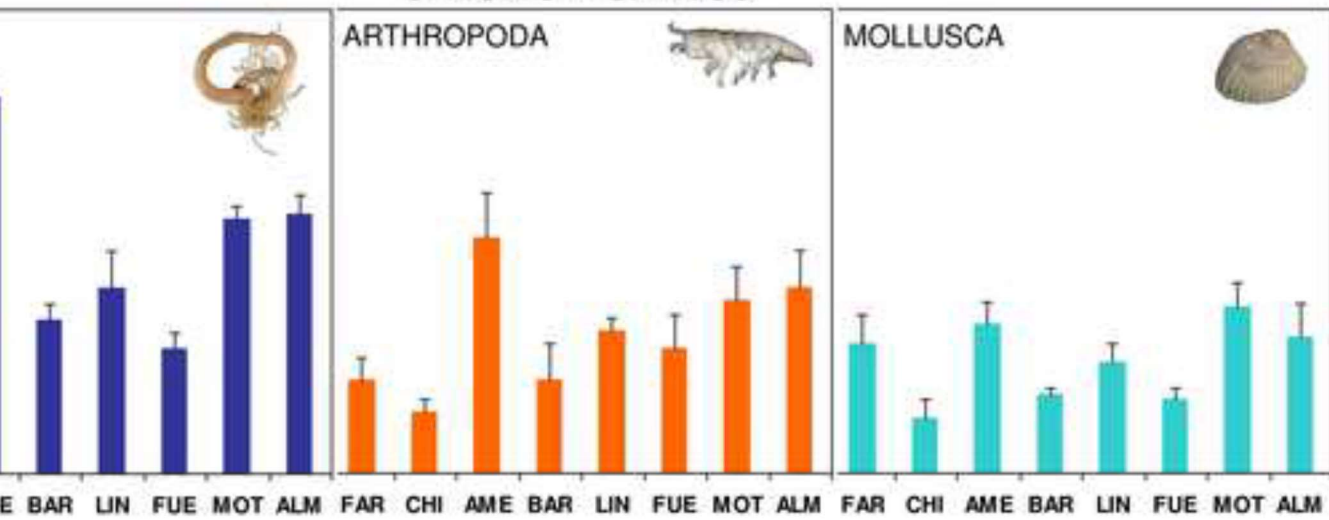


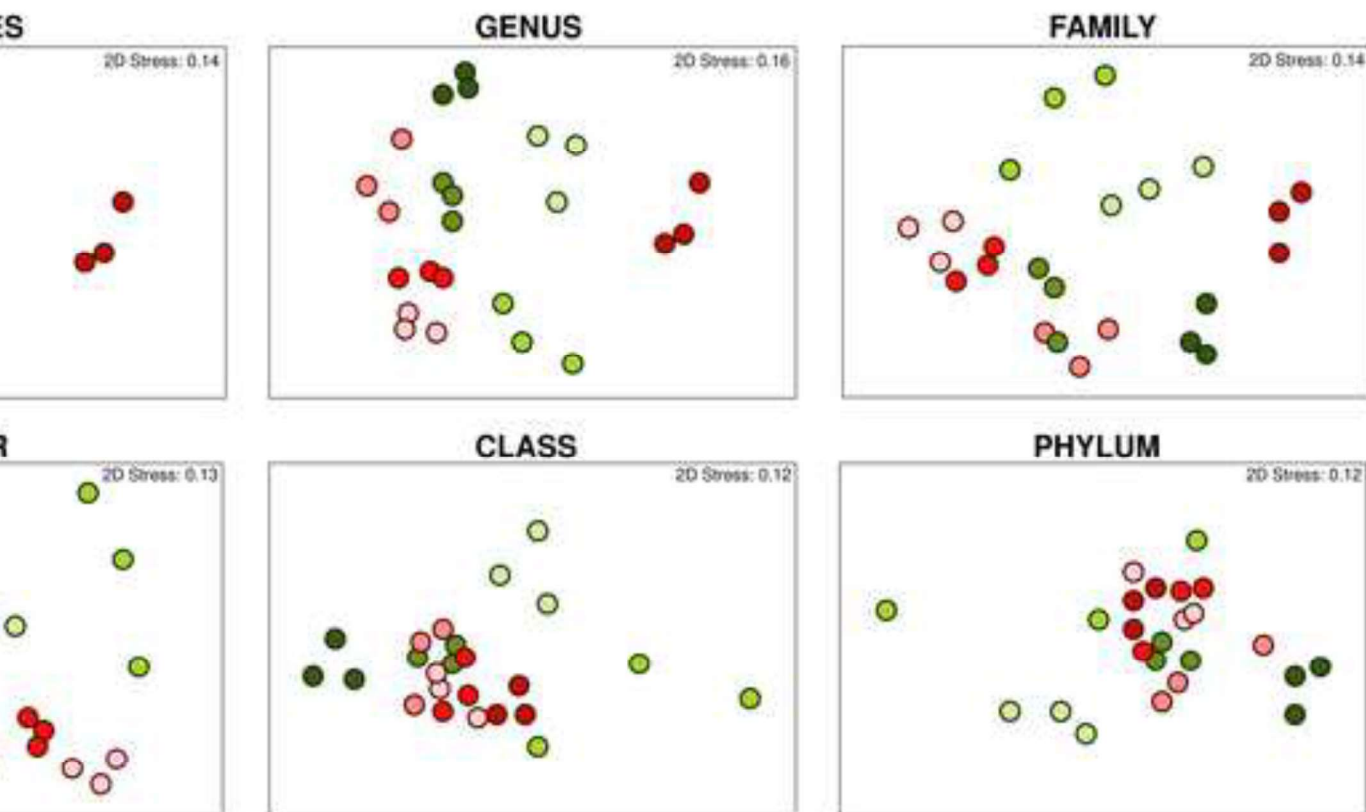
### B

### TAXA RICHNESS

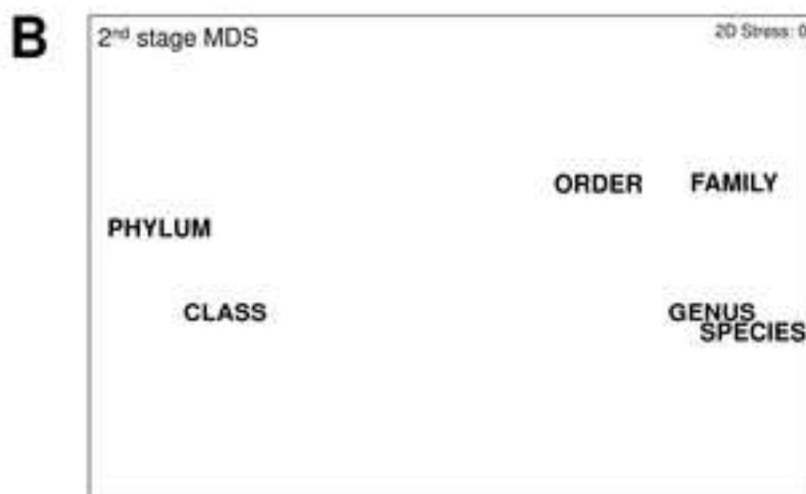


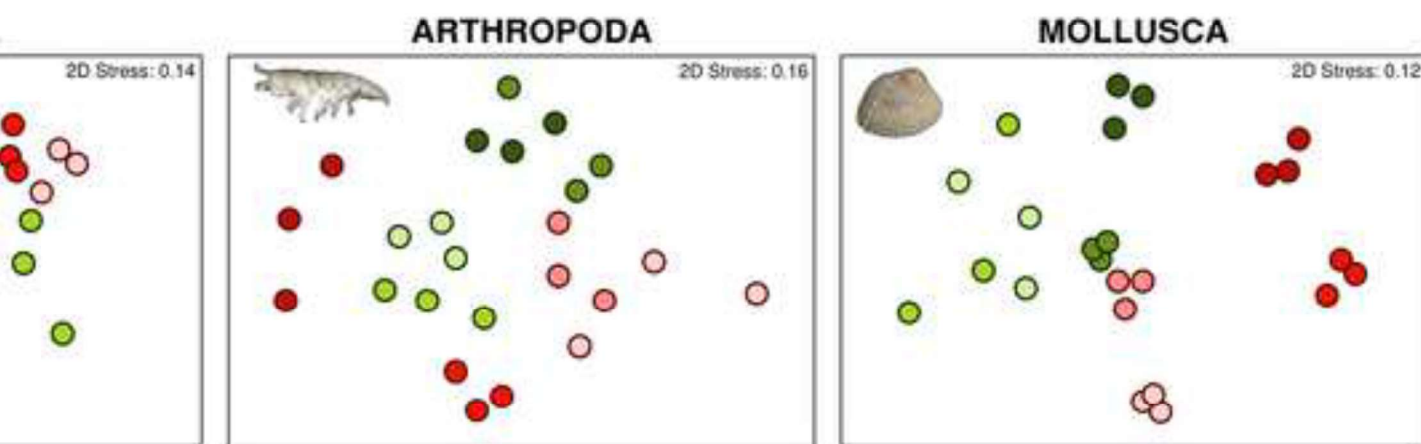
### SPECIES RICHNESS





MARINAS  
A  
AMÉRICA  
E  
ANEAN MARINAS  
A  
ROLA  
A





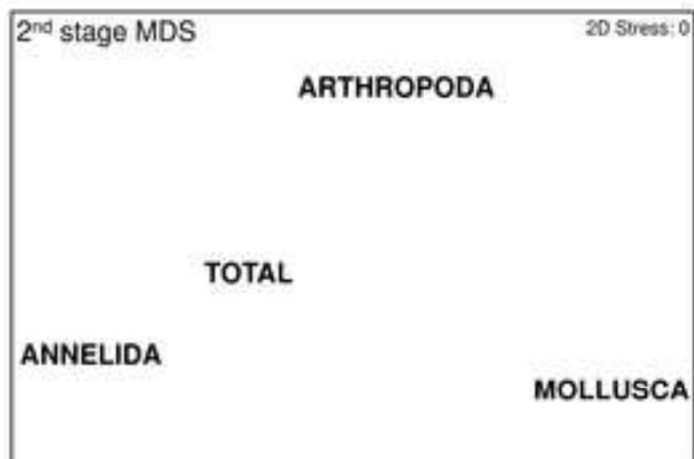
RINAS

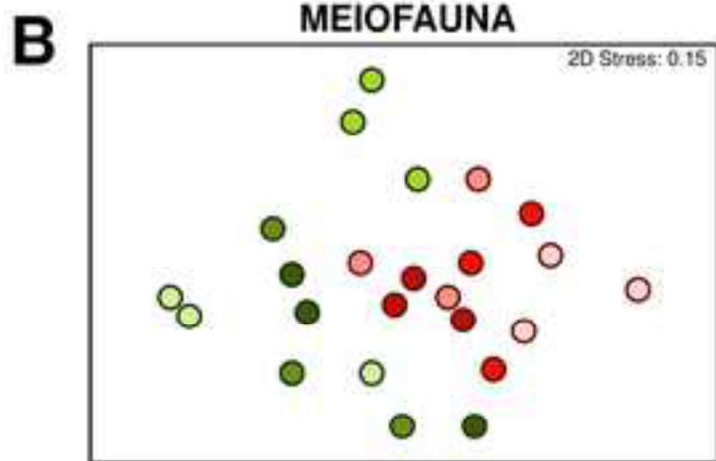
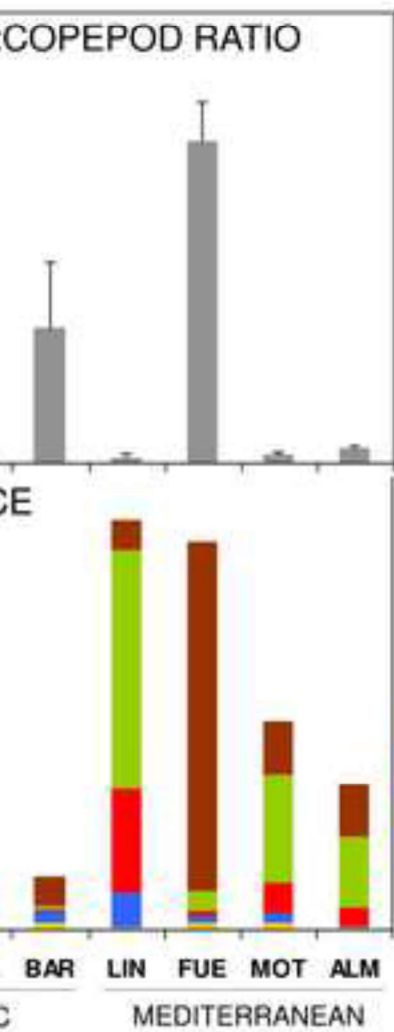
ÉRICA

AN MARINAS

A

**B**



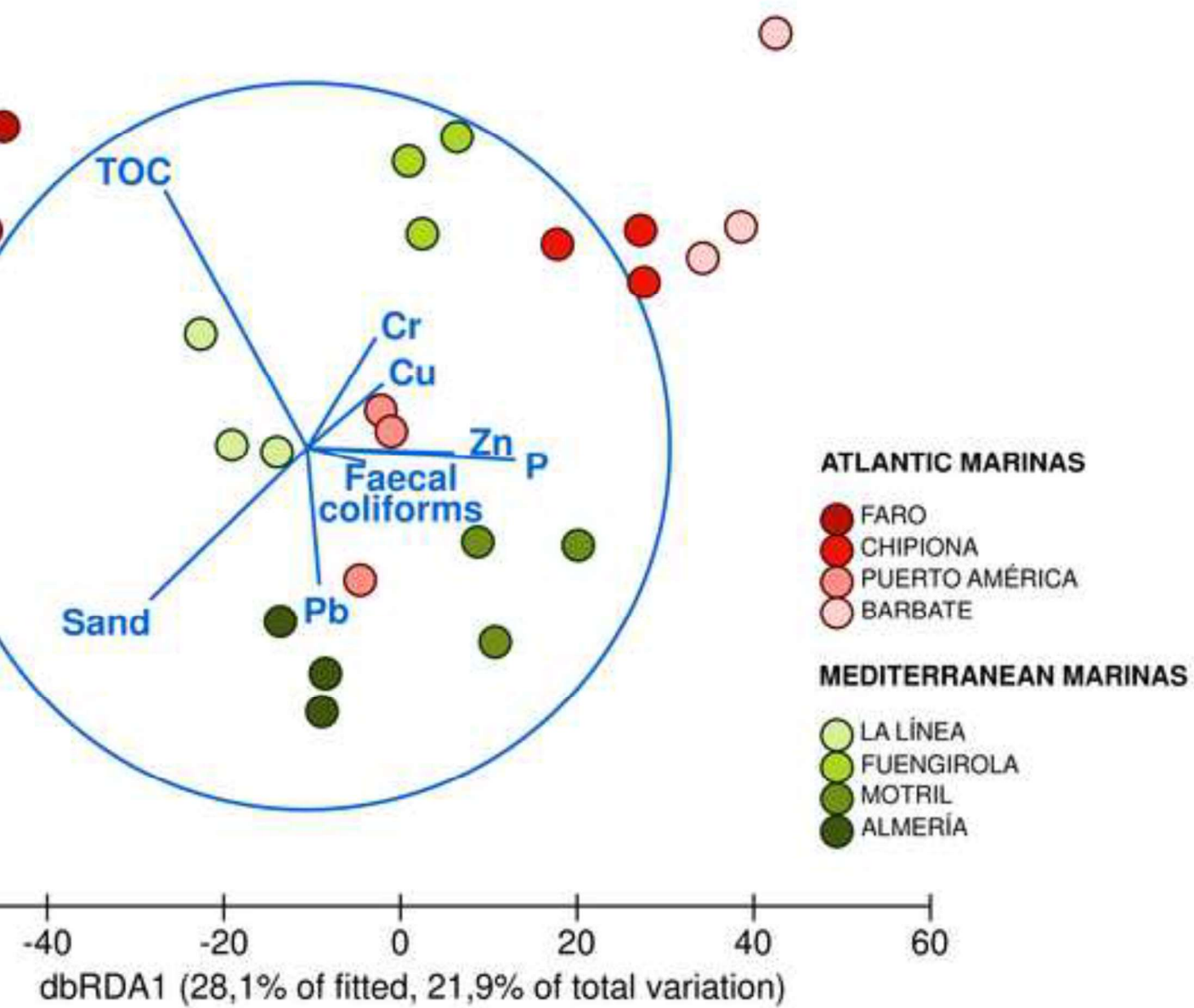


**ATLANTIC MARINAS**

- FARO (FAR)
- CHIPIONA (CHI)
- PUERTO AMÉRICA (AME)
- BARBATE (BAR)

**MEDITERRANEAN MARINAS**

- LA LÍNEA (LIN)
- FUENGIROLA (FUE)
- MOTRIL (MOT)
- ALMERÍA (ALM)



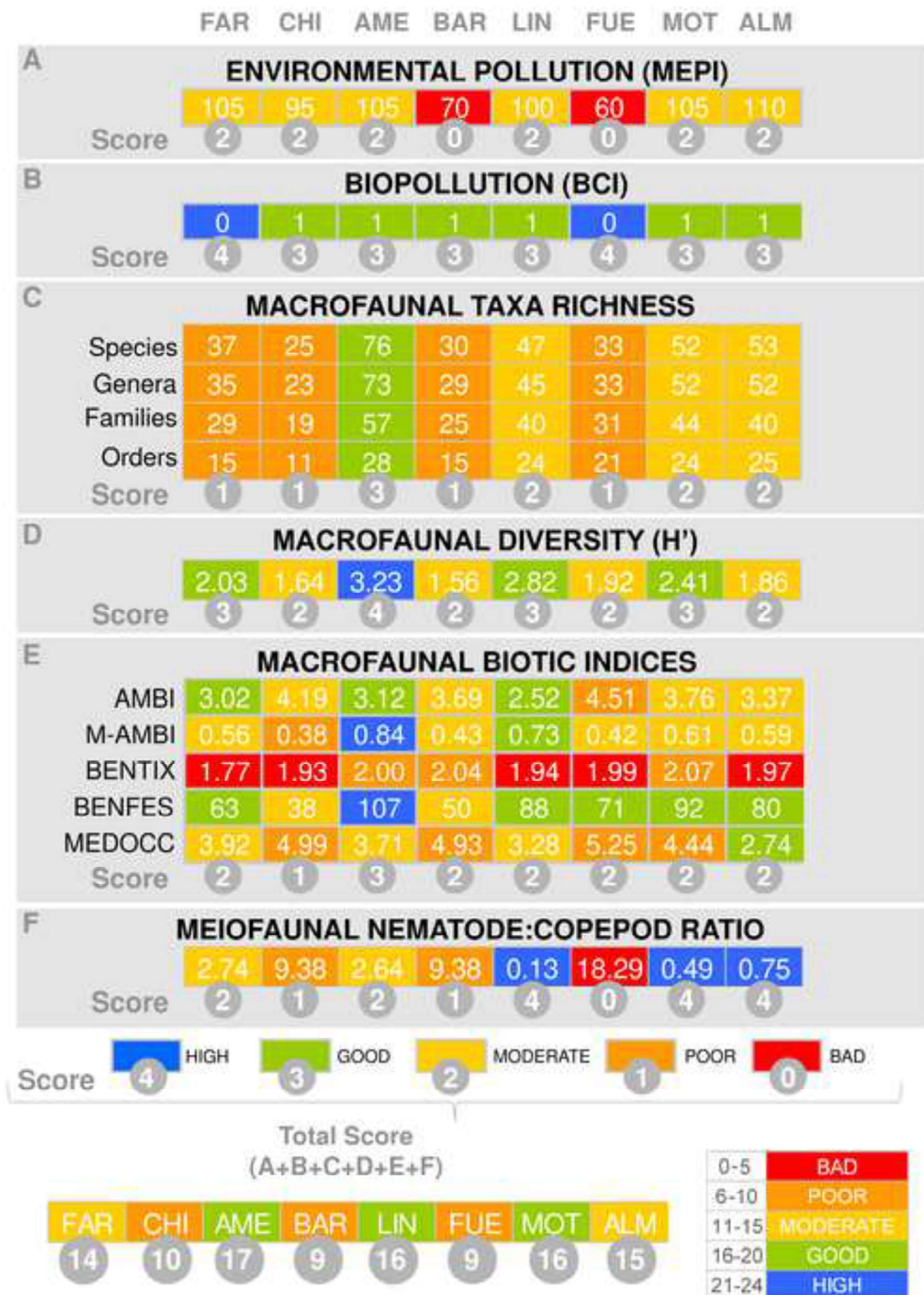


Table 1. List of macrofaunal species with average abundance higher than 1% of the total abundance at each marina. Dark blue: 1-100 ind/m<sup>2</sup>; medium blue: 101-1000 ind/m<sup>2</sup>; light blue: 1001-10,000 ind/m<sup>2</sup>. The whole list of species and abundances per replicate are detailed in Table S1 and S2 (Supplementary material). FAR: Faro, CHI: Chipiona, AME: Puerto América, BAR: Barbate, LIN: La Línea, FUE: Fuengirola, MOT: Motril, ALM: Almería.

	FAR	CHI	AME	BAR	LIN	FUE	MOT	ALM
<b>Phylum Annelida</b>								
Class Clitellata								
Oligochaeta undet.	Light blue						Dark blue	
Class Polychaeta								
<i>Aphelocheata multibranchis</i> (Grube, 1863)			Light blue					
<i>Aphelocheata marioni</i> (Saint-Joseph, 1894)		Dark blue	Light blue	Medium blue	Medium blue	Dark blue	Dark blue	Medium blue
<i>Capitella capitata</i> (Fabricius, 1780)	Medium blue	Medium blue	Medium blue			Medium blue	Medium blue	Dark blue
<i>Chaetozone gibber</i> Woodham & Chambers, 1994		Dark blue	Dark blue			Medium blue	Light blue	
<i>Chaetozone</i> sp.			Dark blue					
<i>Cirriiformia tentaculata</i> (Montagu, 1808)			Medium blue		Medium blue			Light blue
<i>Cirrophorus furcatus</i> (Hartman, 1957)		Medium blue	Light blue	Light blue	Light blue	Light blue	Light blue	Medium blue
<i>Cossura</i> cf. <i>pygodactylata</i> Jones, 1956		Medium blue				Light blue	Light blue	
<i>Euclymene oerstedii</i> (Claparède, 1863)	Light blue		Light blue		Light blue			Light blue
<i>Fabricia stellaris</i> (Müller, 1774)	Light blue			Medium blue	Light blue	Light blue	Light blue	
<i>Galathowenia oculata</i> (Zachs, 1923)								Dark blue
<i>Heteromastus filiformis</i> (Claparède, 1864)		Light blue	Medium blue				Light blue	Light blue
<i>Kirkegaardia dorsobranchialis</i> (Kirkegaard, 1959)			Medium blue					Light blue
<i>Lumbrineris latreilli</i> Audouin & Milne Edwards, 1833			Medium blue		Light blue			
<i>Melinna palmata</i> Grube, 1870	Light blue		Medium blue					
<i>Neanthes acuminata</i> (Ehlers, 1868)	Medium blue				Medium blue			
<i>Nephtys hombergii</i> Savigny in Lamarck, 1818	Light blue			Light blue		Light blue	Light blue	Light blue
<i>Notomastus latericeus</i> Sars, 1851	Medium blue		Light blue					
<i>Paradoneis lyra</i> (Southern, 1914)	Medium blue							
<i>Prionospio</i> sp.		Medium blue	Light blue		Light blue			
<i>Pseudopolydora</i> sp.				Light blue	Light blue		Medium blue	Medium blue
<i>Sabellaria spinulosa</i> (Leuckart, 1849)							Light blue	
<i>Scoloplos armiger</i> (Müller, 1776)			Light blue		Light blue		Light blue	Light blue
<i>Sternaspis scutata</i> (Ranzani, 1817)			Medium blue					
<i>Streblospio benedicti</i> Webster, 1879	Light blue							
<b>Phylum Arthropoda</b>								
Class Malacostraca								
Order Amphipoda								
<i>Ampelisca</i> sp.			Light blue					
<i>Aora</i> sp.		Light blue		Light blue	Light blue			
<i>Gammarella fucicola</i> (Leach, 1814)	Light blue				Light blue	Light blue		
<i>Leptocheirus longimanus</i> Ledoyer, 1973			Light blue		Light blue			
<i>Medicorophium runcicorne</i> (Della Valle, 1893)		Medium blue		Light blue	Light blue			
<i>Medicorophium</i> sp.			Light blue	Light blue	Light blue			
<i>Monoculodes</i> sp.				Light blue	Light blue			
<i>Pariambus typicus</i> (Krøyer, 1844)					Light blue		Medium blue	
<i>Phtisica marina</i> Slabber, 1769			Light blue		Medium blue		Light blue	Light blue
<i>Pseudolirius kroyeri</i> (Haller, 1879)								Light blue
<i>Stenothoe monoculoides</i> (Montagu, 1813)	Light blue							
Order Cumacea								
<i>Cumella</i> ( <i>Cumella</i> ) <i>pygmaea</i> G.O. Sars, 1865					Light blue			
<i>Iphinoe tenella</i> Sars, 1878		Light blue	Light blue		Dark blue	Light blue		Light blue
Order Isopoda								
<i>Kupellonura mediterranea</i> Barnard, 1925			Light blue				Light blue	Dark blue
Order Tanaidacea								
<i>Apsuopsis latreillii</i> (Milne Edwards, 1828)			Medium blue				Light blue	Dark blue
<b>Phylum Mollusca</b>								
Class Bivalvia								
<i>Abra alba</i> (W. Wood, 1802)			Light blue		Light blue	Light blue	Light blue	
<i>Abra nitida</i> (O.F. Müller, 1776)		Light blue	Light blue	Medium blue				
<i>Abra tenuis</i> (Montagu, 1803)	Medium blue	Medium blue	Light blue					
<i>Acanthocardia paucicostata</i> (G.B. Sowerby, 1834)				Medium blue				
<i>Corbula gibba</i> (Olivi, 1972)			Medium blue		Light blue		Medium blue	Light blue
<i>Loripes orbiculatus</i> Poli, 1795	Light blue		Medium blue		Light blue	Light blue	Light blue	Medium blue
<i>Nucula nitidosa</i> Winckworth, 1930			Light blue	Light blue	Light blue	Light blue	Light blue	Light blue
<i>Parvicardium exiguum</i> (Gmelin, 1791)				Medium blue	Light blue	Light blue	Medium blue	
<i>Solen marginatus</i> Pulteney, 1799				Light blue	Light blue			
<i>Spisula subtruncata</i> (da Costa, 1778)					Light blue	Light blue	Light blue	Light blue
<i>Thyasira flexuosa</i> (Montagu, 1803)			Light blue			Light blue		
Class Gastropoda								
<i>Tritia nitida</i> (Jeffreys, 1867)	Light blue		Light blue					
<b>Phylum Nematoda undet.</b>								
			Light blue		Light blue	Dark blue	Light blue	Light blue
<b>Phylum Nemertea undet.</b>								
		Light blue	Light blue	Light blue	Light blue		Light blue	Light blue

Table 2. Summary of results of the two-way ANOVAs for number of species, total macrofaunal abundance and Shannon-Wiener's diversity, and two-way PERMANOVA for macrofaunal community (species abundances). Lo: Location (Atl: Atlantic, Med: Mediterranean); Ma(Lo): Marina nested within location (FAR: Faro, CHI: Chipiona, AME: Puerto América, BAR: Barbate, LIN: La Línea, FUE: Fuengirola, MOT: Motril, ALM: Almería). df: degrees of freedom, MS: mean squares, MC: Montecarlo, p: level of significance. \*:  $p < 0.05$ , \*\*\*:  $p < 0.001$ , n.s. not significant. PERMDISP results for the factors Lo and Ma(Lo) are also included.

<b>ANOVAs</b>		<b>Number of species</b>			<b>Total Abundance</b>			<b>Shannon-Wiener Diversity</b>		
<b>Source of variation</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>	<b>MS</b>	<b>F</b>	<b>p</b>	<b>MS</b>	<b>F</b>	<b>p</b>
Lo	1	24.0000	0.06	0.8098 n.s.	630.37	0.01	0.9412 n.s.	0.0030	0.04	0.8462 n.s.
Ma(Lo)	6	379.5278	20.29	0.0000***	106706.59	13.64	0.0000***	0.0739	15.34	0.0000***
Residual	16	18.7083			7824.75			0.0048		
Total	23									
Cochran's test			C=0.4031 n.s.			C=0.2631 n.s.			C=0.5662 $p < 0.05$	
Transformation			None			None			Sqrt (x+1)	
SNK tests:										
	Lo	Atl=Med			Atl=Med			Atl=Med		
	Ma(Lo)	Atl: (FAR=CHI=BAR)<AME Med: LIN=FUE, LIN<MOT, LIN=ALM, FUE<(MOT=ALM)			Atl: FAR=CHI=AME=BAR Med: LIN<FUE, LIN=MOT, LIN<ALM, (FUE=MOT)<ALM			Atl: (FAR=CHI=BAR)<AME Med: LIN>FUE, LIN=MOT, LIN>ALM, FUE<MOT, FUE=ALM, MOT=ALM		
<b>PERMANOVA</b>		<b>Macrofaunal community</b>				<b>PERMDISP</b>				
<b>Source of variation</b>	<b>df</b>	<b>MS</b>	<b>Pseudo-F</b>	<b>p(MC)</b>	<b>Unique permutations</b>	<b>Deviations from centroid</b>				
Lo	1	8574.9	1.0052	0.4327 n.s.	35	F=2.4940, $p=0.1599$ n.s.				
Ma(Lo)	6	8531.0	10.894	0.0001***	9876	F=5.0242, $p=0.1406$ n.s.				
Residual	16	783.1								
Total	23									
Pair-wise tests:										
	Lo	Atl=Med								
	Ma(Lo)	Atl: FAR≠CHI≠AME≠BAR Med: LIN≠FUE≠MOT≠ALM								



Table 4. Summary results of the two-way ANOVAs for number of meiofaunal taxa, total meiofaunal abundance and nematode:copepod ratio, and two-way PERMANOVA for meiofaunal community (based on higher taxa abundances). Lo: Location (Atl: Atlantic, Med: Mediterranean); Ma(Lo): Marina nested within location (FAR: Faro, CHI: Chipiona, AME: Puerto América, BAR: Barbate, LIN: La Línea, FUE: Fuengirola, MOT: Motril, ALM: Almería). df: degrees of freedom, MS: mean squares, MC: Montecarlo, p: level of significance. \*:  $p < 0.05$ , \*\*\*:  $p < 0.001$ , n.s. not significant. PERMDISP results for the factors Lo and Ma(Lo) are also included.

ANOVAs		Number of taxa			Total Abundance			Nematode:copepod ratio		
Source of variation	df	MS	F	p	MS	F	p	MS	F	p
Lo	1	0.6667	0.67	0.4454 n.s.	279.1699	13.27	0.0108*	13.1092	0.10	0.7594 n.s.
Ma(Lo)	6	1.0000	1.50	0.2405 n.s.	21.0432	1.23	0.2405 n.s.	127.6104	9.36	0.0002***
Residual	16	0.6667			17.0490			13.6349		
Total	23									
Cochran's test			C=0.2500 n.s.			C=0.5966 $p < 0.05$			C=0.5053 n.s.	
Transformation			None			Sqrt (x+1)			None	
SNK tests:										
	Lo		Atl=Med			Atl<Med			Atl=Med	
	Ma(Lo)		Atl: FAR=CHI=AME=BAR Med: LIN=FUE=MOT=ALM			Atl: FAR=CHI=AME=BAR Med: LIN=FUE=MOT=ALM			Atl: FAR=CHI=AME=BAR Med: FUE>(LIN=MOT=ALM)	
PERMANOVA		Meiofaunal community				Unique permutations		PERMDISP		
Source of variation	df	MS	Pseudo-F	p (MC)			Deviations from centroid			
Lo	1	4776.6	3.3032	0.0246*	35		F=4.7188, $p=0.0541$ n.s.			
Ma(Lo)	6	1446.1	2.9222	0.0003***	9903		F=1.3130, $p=0.7926$ n.s.			
Residual	16	494.8								
Total	23									
Pair-wise tests:										
	Lo		Atl≠Med							
	Ma(Lo)		Atl: FAR≠CHI≠BAR, FAR=AME, CHI=AME, AME=BAR Med: FUE≠(LIN=MOT=ALM)							

Table 5. Results of DistLM (distance-based linear modelling) analysis. The variables explaining macrofaunal community (species abundances) are included. Table shows significance levels for each predictor variable and the proportion of variation explained. Only variables with significant results ( $p < 0.05$ ) in marginal tests are shown. Overall best models are also included. AIC: Akaike information criterion; SS (trace): sum of squares of sequential test, Prop.: proportion of variability explained by each factor (in the marginal tests without coaction of factors), TOC: Total Organic Carbon, F. colif.: faecal coliforms, \*:  $p < 0.05$ , \*\*\*:  $p < 0.001$ .

Variables	SS (trace)	Pseudo-F	p	Prop. (%)
P	13181	4.91	0.0001***	18.2
Sand	12057	4.40	0.0001***	16.7
TOC	11571	4.19	0.0001***	16.0
Zn	11121	3.99	0.0002***	15.3
Cr	10068	3.56	0.0004***	13.9
Cu	9833	3.46	0.0001***	13.6
F. colif.	6916	2.33	0.0156*	9.5
Pb	6880	2.31	0.0122*	9.5

AIC	R <sup>2</sup>	Variables
180.46	0.73	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Cu, Pb
180.60	0.75	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Cu, Pb, Zn
180.73	0.73	Sand, Silt, TOC, P, F. colif., Cr, Cu, Pb, Zn

Table 6. Summary results of BIO-ENV and DistLM analysis for macrofauna (separately for each of six considered meiofauna (higher taxa). The variables included in the best BIO-ENV model are shaded. Significance levels for each variable are shown in the right column. Only variables with significant results ( $p < 0.05$ ) in marginal tests are shown. TOC: Total Organic Carbon, Hydr.: Hydrated metal oxides. Rho: correlation coefficient, p: p-value. n.s.: not significant, \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ .

			BIO-ENV														
	Rho	p	Sand	Silt	TOC	P	Hydr.	F.colif.	Cd	Cr	Cu	Pb	Zn	Sand	Silt	TOC	P
<b>Macrofauna:</b>																	
<b>Species</b>	0.820	0.002**												***		***	***
<b>Genus</b>	0.802	0.006**												***		***	***
<b>Family</b>	0.777	0.006**												***		**	***
<b>Order</b>	0.742	0.007**												***		***	***
<b>Class</b>	0.411	0.710 n.s.													*	**	
<b>Phylum</b>	0.385	0.870 n.s.														***	
<b>Annelida</b>	0.797	0.010**												***		***	***
<b>Arthropoda</b>	0.594	0.090 n.s.												***		***	**
<b>Mollusca</b>	0.591	0.220 n.s.												**		*	**
<b>Meiofauna</b>	0.630	0.130 n.s.												**			

## SUPPLEMENTARY MATERIAL

### ECOLOGICAL QUALITY ASSESSMENT OF MARINAS: AN INTEGRATIVE APPROACH COMBINING BIOLOGICAL AND ENVIRONMENTAL DATA

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Table S1. Previous studies addressing sediments (biotic and abiotic data) from worldwide marinas.

References	Topics addressed
Albanis et al., 2002	Antifouling biocides in Greek marinas
Batista-Andrade et al., 2018	Antifouling biocides in marinas from Panama Canal
Biselli et al., 2020	Antifouling biocides in marinas from North and Baltic Sea, Germany
Bowman et al., 2003	Antifouling biocides in Brighton Marina, UK
Boxall et al., 2000	Antifouling biocides in Orwell and Hamble marinas, UK
Briant et al., 2013	Trace elements and butyltin concentrations in Port Camargue marina, France
Cassi et al., 2008	Antifouling biocides in French marinas
Chatzinikolaou et al., 2018 (sta. C1, E1-E3, H1)	Sediment pollutants and macrobenthic biodiversity in touristic harbours of Italy, Greece and Tunisia
Covazzi Harriague et al., 2012	Macro and meiofaunal communities, and environmental data of the touristic harbour of Rapallo, Italy
Dimitriou et al., 2020	Benthic indices and ecological assessment in touristic harbours of Italy, Greece and Tunisia
Egardt et al., 2017	Biocides and trace metals in Swedish marinas
Estacio et al., 1997 (stations 1-7)	Macrobenthic communities and environmental data of Saladillo harbour, Algeciras, Southern Spain
García et al., 2020	Antifouling biocides in marinas of SW Spain
Gatidou et al., 2007	Antifouling biocides in Shoreham Harbour and Brighton Marina, UK
Gómez et al., 2017	Atlas of susceptibility to pollution of 320 Spanish marinas
Gómez et al., 2019	Environmental risks of 320 Spanish marinas
Guerra-García et al., 2004 a,b,c (sta 1 and 2)	Polychaetes, Crustaceans and Molluscs of marina of Ceuta, northern Africa
Guerra-García and García-Gómez, 2005 a,b (sta 1 and 2)	Macrobenthic communities and environmental data of marina of Ceuta, northern Africa
Guerra-García et al., in press	Development of MEPI (Marinas Environmental Pollution Index) based on environmental data of marinas from Southern Spain
Hinke and Zaidi, 2007	Trace metals in marinas from Virgin Islands, USA
Jupp et al., 2017	Heavy metals and hydrocarbon pollution in Oman marinas
Kenworthy et al., 2018	Pollutants in Brest marinas, France
Mali et al., 2017 (suppl. material)	Heavy metals and PHAs in marinas of the Apulia region, Italy
Martínez and Barceló, 2001	Antifouling biocides in marinas from Catalonia, NE Spain
McGee et al., 1995 (sta. BR1-BR4)	Sediment contamination and biological effects in a Chesapeake Bay marina
Moreira et al., 2005 (sta. 19, 20)	Mollusc assemblages and sediment characteristics of Baiona marina, NW Spain
Moreira et al., 2010	Temporal dynamics (one year) of the benthic assemblage of Baiona marina, NW Spain
Moreno et al., 2009	Nematode response to metal and organic enrichment, including PHAs in touristic marinas of Ligurian Sea, Italy
Neira et al., 2017	PHAs in San Diego Bay marinas, USA
Neira et al., 2018	PCBs in recreational marina of Southern California, USA
Norén et al., 2020	Integrated assessment of management strategies for metal-contaminated dredged sediments
Sapozhnikova et al., 2013	Antifouling biocides in California marinas, USA
Sedano et al., 2014 a,b	Meiofaunal communities from Marina del Este, Southeastern Spain
Shakkas et al. 2002	Antifouling biocides in Greek marinas
Sim et al., 2015	Sediment contaminants and infauna in marinas from Clyde Estuary, Australia
Tamburini et al., 2020	Sediment pollutants and prokaryotic biodiversity in touristic harbours of Italy, Greece and Tunisia
Thomas et al., 2000, 2001, 2002	Antifouling biocides in UK marinas
Viana et al., 2009	Antifouling biocides in marinas from São Luís Island, Brazil
Voudrias and Smith, 1986	Hydrocarbon pollution in marinas from USA
Zhang et al., 2019	Spatiotemporal characterization of metals in harbours, including marinas from Nova Scotia, Canada
Zhou, 2008	Antifouling biocides in marinas of Southern UK

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Table S2. Average abundance of the macrofaunal species found during the present study. Dark blue: 1-100 ind/m<sup>2</sup>; medium blue: 101-1000 ind/m<sup>2</sup>; light blue: 1001-10,000 ind/m<sup>2</sup>. Status of each species is indicated (N: Native, I: Introduced, C: Cryptogenic, U: Undetermined). Information of the status taken from Gofas and Zenetos, 2003; Çinar, 2013; López and Richter, 2017; Marchini and Cardeccia, 2017; Bonifazi et al., 2018; Martínez-Laiz et al., 2018; WORMS (<http://www.marinespecies.org/>). FAR: Faro, CHI: Chipiona, AME: Puerto América, BAR: Barbate, LIN: La Línea, FUE: Fuengirola, MOT: Motril, ALM: Almería.

	<b>STATUS</b>	<b>FAR</b>	<b>CHI</b>	<b>AME</b>	<b>BAR</b>	<b>LIN</b>	<b>FUE</b>	<b>MOT</b>	<b>ALM</b>
<b>Phylum Annelida</b>									
Class Clitellata	U	Medium Blue	Light Blue	Light Blue	Light Blue	Light Blue	Dark Blue	Light Blue	Light Blue
Oligochaeta undet.	U	Medium Blue	Light Blue	Light Blue	Light Blue	Light Blue	Dark Blue	Light Blue	Light Blue
Class Polychaeta									
<i>Ampharete santillani</i> Parapar et al., 2017	N		Light Blue						
<i>Amphitrite</i> sp.	U	Light Blue							
<i>Aonides oxycephala</i> (Sars, 1862)	N								Light Blue
<i>Aphelo chaeta marioni</i> (Saint-Joseph, 1894)	C	Dark Blue	Light Blue	Light Blue	Light Blue	Light Blue	Dark Blue	Dark Blue	Dark Blue
<i>Aphelo chaeta multibranchis</i> (Grube, 1863)	N		Light Blue						
<i>Aponuphis bilineata</i> (Baird, 1870)	N		Light Blue						
<i>Aricidea (Acmira) catherinae</i> (Laubier, 1967)	N								
<i>Branchiomm a</i> sp.	U								
<i>Capitella capitata</i> (Fabricius, 1780)	C	Medium Blue	Medium Blue	Medium Blue			Medium Blue	Medium Blue	Dark Blue
<i>Chaetozone gibber</i> Woodham & Chambers, 1994	N	Dark Blue		Dark Blue			Light Blue	Light Blue	
<i>Chaetozone</i> sp.	U			Dark Blue					
<i>Cirriformia tentaculata</i> (Montagu, 1808)	N					Light Blue			Light Blue
<i>Cirrophorus furcatus</i> (Hartman, 1957)	N	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue
<i>Cos sura</i> cf. <i>pygo dactylata</i> Jones, 1956	U	Light Blue					Light Blue	Light Blue	
<i>Diopatra</i> sp.	N								
<i>Drilonereis filum</i> (Claparède, 1868)	N							Light Blue	
<i>Euchymene oers tedii</i> (Claparède, 1863)	C	Light Blue		Light Blue					Light Blue
<i>Eum ida sanguinea</i> (Ørsted, 1843)	C								Light Blue
<i>Eunice</i> sp.	U								
<i>Fabricia stellaris</i> (Müller, 1774)	N	Light Blue			Light Blue	Light Blue	Light Blue	Light Blue	
<i>Galathowenia oculata</i> (Zachs, 1923)	N								Dark Blue
<i>Glycera tridactyla</i> Schmarda, 1861	N		Light Blue	Light Blue	Light Blue				
<i>Hamothoe</i> sp.	U								
<i>Heteromastus filiformis</i> (Claparède, 1864)	C		Light Blue	Light Blue				Light Blue	Light Blue
<i>Kirkegaardia dorsobranchialis</i> (Kirkegaard, 1959)	C		Light Blue	Light Blue				Light Blue	Light Blue
<i>Lagis koreni</i> Malmgren, 1866	N		Light Blue	Light Blue	Light Blue				
<i>Leio chone leiopygos</i> (Grube, 1860)	N								Light Blue
<i>Lumbrineris latreilli</i> Audouin & Milne Edwards, 1833	N			Light Blue		Light Blue			
<i>Magelona alleni</i> Wilson, 1958	N								
<i>Magelona minuta</i> Eliason, 1962	N								
<i>Melinna palmata</i> Grube, 1870	N	Light Blue		Light Blue					
<i>Naineris laevigata</i> (Grube, 1855)	N						Light Blue		
<i>Neanthes acuminata</i> (Ehlers, 1868)	N	Light Blue				Light Blue			
<i>Nephtys hombergii</i> Savigny in Lamarck, 1818	N	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue
<i>Nereididae</i> undet.	U								Light Blue
<i>Notomastus latericeus</i> Sars, 1851	N	Light Blue		Light Blue					
<i>Paradoneis armata</i> Glémarec, 1966	N								
<i>Paradoneis hya</i> (Southern, 1914)	N	Light Blue							
<i>Parexogone hebes</i> (Webster & Benedict, 1884)	N					Light Blue			
<i>Perkinsyllis anophthalma</i> (Capaccioni & San Martín, 1990)	N	Light Blue							
<i>Pholo e inomata</i> Johnston, 1839	N								Light Blue
<i>Pista cristata</i> (Müller, 1776)	N			Light Blue					
<i>Podarkeopsis capensis</i> (Day, 1963)	C	Light Blue							
<i>Polycirrus</i> sp.	U			Light Blue					
<i>Polydora</i> sp.	U							Light Blue	
<i>Priono spio</i> sp.	U		Light Blue	Light Blue	Light Blue	Light Blue			
<i>Pseudopolydora</i> sp.	U							Light Blue	Light Blue
<i>Sabellaria spinulosa</i> (Leuckart, 1849)	N								Light Blue
<i>Sabellidae</i> undet.	U								Light Blue
<i>Schistomeringos rudolphi</i> (Delle Chiaje, 1828)	C			Light Blue			Light Blue		
<i>Scoloplos armiger</i> (Müller, 1776)	N					Light Blue	Light Blue	Light Blue	Light Blue
<i>Serpulidae</i> undet.	U				Light Blue	Light Blue	Light Blue	Light Blue	Light Blue
<i>Sigambra parva</i> (Day, 1963)	C		Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue
<i>Spionidae</i> sp. 1	U					Light Blue			
<i>Spionidae</i> sp. 2	U	Light Blue				Light Blue			
<i>Stemaspis scutata</i> (Ranzani, 1817)	C			Light Blue					
<i>Streblospio benedicti</i> Webster, 1879	N	Light Blue							
<i>Syllidia armata</i> Quatrefoages, 1866	N		Light Blue						
<i>Syllis gracilis</i> Grube, 1840	C			Light Blue					
<i>Terebellides stroemii</i> Sars, 1835	C					Light Blue			





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Table S3 (Cont.)

## Atlantic marinas (cont.)

PHYL.	CLASS	ORDER	FAMILY	SPECIES	FAR 1	FAR 2	FAR 3	CHI 1	CHI 2	CHI 3	AME
ANN	Polychaeta	Sabellida	Serpulidae	<i>Serpulidae</i> undet.	0	0	0	0	0	0	0
ANN	Polychaeta	Phyllodocida	Pilargidae	<i>Sigambra parva</i>	0	0	0	0	0	0	0
ANN	Polychaeta	Spionida	Spionidae	<i>Spionidae</i> sp. 1	0	0	0	0	0	0	0
ANN	Polychaeta	Spionida	Spionidae	<i>Spionidae</i> sp. 2	1	0	0	0	0	0	0
ANN	Polychaeta	Terebellida	Sternaspidae	<i>Sternaspis scutata</i>	0	0	0	0	0	0	0
ANN	Polychaeta	Spionida	Spionidae	<i>Streblospio benedicti</i>	0	0	0	0	3	0	0
ANN	Polychaeta	Phyllodocida	Hesionidae	<i>Syllidia armata</i>	0	0	0	0	0	0	0
ANN	Polychaeta	Phyllodocida	Syllidae	<i>Syllis gracilis</i>	0	0	0	0	0	0	0
ANN	Hexanauplia	Harpacticoida		Order Harpacticoida undet.	0	0	1	0	0	0	0
ANN	Hexanauplia	Cyclopoida		Order Cyclopoida undet.	0	0	0	0	0	0	0
ANN	Malacostraca	Amphipoda	Ampeliscidae	<i>Ampelisca</i> sp.	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Amphilochidae	<i>Amphilochus neapolitanus</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Aoridae	<i>Aora</i> sp.	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Corophiidae	<i>Apocorophium acutum</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Caprellidae	<i>Caprella scaura</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Dexaminidae	<i>Dexamine spinosa</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Maeridae	<i>Elasmopus</i> sp.	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Ischyroceridae	<i>Erichthonius</i> sp.	2	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Nuanuidae	<i>Gammarella fucicola</i>	0	0	1	1	7	0	0
ART	Malacostraca	Amphipoda	Iphimediidae	<i>Iphimedia</i> sp.	0	0	0	0	1	0	0
ART	Malacostraca	Amphipoda	Ischyroceridae	<i>Jassa slatteryi</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Corophiidae	<i>Leptochirus longimanus</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Leucothoidae	<i>Leucothoe oboa</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Corophiidae	<i>Medicorophium runcicorne</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Corophiidae	<i>Medicorophium</i> sp.	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Aoridae	<i>Microdeutopus</i> sp.	2	0	0	1	1	0	0
ART	Malacostraca	Amphipoda	Oedicerotidae	<i>Monoculodes</i> sp.	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Caprellidae	<i>Phthisica marina</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Oedicerotidae	<i>Pontocrates arenarius</i>	0	0	0	0	0	0	0
ART	Malacostraca	Amphipoda	Stenothoidae	<i>Stenothoe monoculoides</i>	0	1	0	0	0	0	0
ART	Malacostraca	Cumacea	Bodotriidae	<i>Eocuma</i> sp.	0	0	0	0	0	0	0
ART	Malacostraca	Cumacea	Bodotriidae	<i>Iphinoe tenella</i>	0	0	0	0	0	0	0
ART	Malacostraca	Decapoda	Alpheidae	<i>Alpheus pontederiae</i>	0	0	0	0	0	0	0
ART	Malacostraca	Decapoda	Alpheidae	<i>Athanas nitescens</i>	0	0	0	0	0	0	0
ART	Malacostraca	Decapoda	Varunidae	<i>Brachynothus</i> sp.	0	0	0	0	0	0	0
ART	Malacostraca	Decapoda	Carcinidae	<i>Carcinus maenas</i>	0	0	0	0	0	0	0
ART	Malacostraca	Decapoda	Crangonidae	<i>Philocheras bispinosus</i>	0	0	0	0	0	0	0
ART	Malacostraca	Isopoda	Anthuridae	<i>Cyathura carinata</i>	0	1	0	2	0	0	0
ART	Malacostraca	Isopoda	Sphaeromatidae	<i>Dynamene edwardsi</i>	0	0	0	0	0	0	0







Table S3 (Cont.)

## Mediterranean marinas (cont.)

PHYL.	CLASS	ORDER	FAMILY	SPECIES	LIN 1	LIN 2	LIN 3	FUE 1	FUE 2	FUE 3	MOT												
MOL	Bivalvia	Cardiida	Semelidae	<i>Abra alba</i>	1	1	0	0	1	0	3	1	0	0	0	1	0	0	0	1	0	1	0
MOL	Bivalvia	Venerida	Veneridae	<i>Chamelea gallina</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Bivalvia	Myida	Corbulidae	<i>Corbula gibba</i>	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	9	7
MOL	Bivalvia	Cardiida	Tellinidae	<i>Fabulina fabula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Bivalvia	Galeommatida	Lasacidae	<i>cf. Hemilepton nitidum</i>	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0
MOL	Bivalvia	Galeommatida	Lasacidae	<i>Kurtiella bidentata</i>	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
MOL	Bivalvia	Nuculanida	Nuculanidae	<i>Lembulus pella</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Bivalvia	Lucinida	Lucinidae	<i>Loripes orbiculatus</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
MOL	Bivalvia	Cardiida	Tellinidae	<i>Moerella distorta</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Bivalvia	Mytilida	Mytilidae	<i>Mytilidae undet.</i>	0	0	0	0	0	0	1	0	0	0	0	2	0	2	12	2	0	4	0
MOL	Bivalvia	Nuculida	Nuculidae	<i>Nucula nitidosa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
MOL	Bivalvia	Cardiida	Cardiidae	<i>Parvicardium exiguum</i>	0	0	0	0	1	0	1	0	0	0	1	1	1	0	0	0	0	0	5
MOL	Bivalvia	Cardiida	Tellinidae	<i>Serratina serrata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
MOL	Bivalvia	Solemyida	Solemyidae	<i>Solemya togata</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Bivalvia	Adapedonta	Solenidae	<i>Solen marginatus</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Bivalvia	Venerida	Mactridae	<i>Spisula subtruncata</i>	4	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
MOL	Bivalvia	Lucinida	Thyasiridae	<i>Thyasira flexuosa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Bivalvia	Venerida	Veneridae	<i>Veneridae undet.</i>	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Bivalvia	Venerida	Veneridae	<i>Venus verrucosa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
MOL	Gastropoda	Pylopulmonata	Pyramidellidae	<i>Eulimella acicula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Gastropoda	Cephalaspidea	Retusidae	<i>cf. Retusa umbilicata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Gastropoda	Neogastropoda	Nassariidae	<i>Tritia cuvierii</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
MOL	Gastropoda	Neogastropoda	Nassariidae	<i>Tritia neritea</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Scaphopoda	Dentaliida	Dentaliidae	<i>Antalis novemcostata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MOL	Scaphopoda	Dentaliida	Fustiariidae	<i>Fustiaria rubescens</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEM				Phyllum Nematoda undet.	0	1	0	0	0	0	1	0	0	53	3	48	6	16	0	0	1	4	2
NET				Phyllum Nemertea undet.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
PHO				Phyllum Phoronida undet.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0

Table S4. Summary of the two-way ANOVA results for number of species of Annelida, Arthropoda and Mollusca (A), and for global taxa richness for each taxonomical level (species, genera, families, orders, classes and phyla) (B). Lo: Location (Atl: Atlantic, Med: Mediterranean); Ma(Lo): Marina nested with location (FAR: Faro, CHI: Chipiona, AME: Puerto América, BAR: Barbate, LIN: La Línea, FUE: Fuengirola, MOT: Motril, ALM: Almería). df: degrees of freedom, MS: mean squares, p: level of significance. \*: p<0.05, \*\*: p<0.01, \*\*\*: p <0.001, n.s. not significant.

**A**

Source of variation	df	Number of annelid species			Number of arthropod species			Number of mollusc species		
		MS	F	p	MS	F	p	MS	F	p
Lo	1	4.17	0.06	0.8113 n.s.	22.04	0.74	0.4225 n.s.	0.67	0.03	0.8727n.s.
Ma(Lo)	6	66.97	17.86	0.0000***	29.76	3.57	0.0194*	23.86	5.97	0.0020**
Residual	16	3.75			8.33			4.00		
Total	23									
Cochran's test			C=0.4000 n.s.			C=0.2600 n.s.			C=0.2917 n.s.	
Transformation			None			None			None	
SNK tests										
Lo		Atl=Med			Atl=Med			Atl=Med		
Ma(Lo)		Atl: (FAR=CHI=BAR)<AME Med: (MOT=ALM)>LIN>FUE			Atl: (FAR=CHI=BAR)<AME Med: LIN=FUE=MOT=ALM			Atl: (FAR=CHI=BAR)<AME Med: LIN=FUE=ALM, FUE<MOT, LIN=MOT=ALM		

**B**

Source of variation	df	Number of species			Number of genera			Number of families		
		MS	F	p	MS	F	p	MS	F	p
Lo	1	24.00	0.06	0.8098 n.s.	42.67	0.12	0.7453 n.s.	51.04	0.23	0.6492 n.s.
Ma(Lo)	6	379.52	20.29	0.0000***	368.78	21.85	0.0000***	222.88	20.42	0.0000***
Residual	16	18.71			16.87			10.92		
Total	23									
Cochran's test			C=0.4031 n.s.			C=0.4321 n.s.			C=0.4466 n.s.	
Transformation			None			None			None	
SNK tests										
Lo		Atl=Med			Atl=Med			Atl=Med		
Ma(Lo)		Atl: (FAR=CHI=BAR)<AME Med: LIN=FUE, LIN<MOT LIN=ALM, FUE<(MOT=ALM)			Atl: (FAR=CHI=BAR)<AME Med: (LIN=FUE)<(MOT=ALM)			Atl: (FAR=CHI=BAR)<AME Med: (LIN=FUE)<(MOT=ALM)		

Source of variation	df	Number of orders			Number of classes			Number of phyla		
		MS	F	p	MS	F	p	MS	F	p
Lo	1	66.67	1.31	0.2955 n.s.	9.38	2.57	0.1603 n.s.	2.04	0.90	0.3790 n.s.
Ma(Lo)	6	50.78	11.95	0.0000***	3.65	3.51	0.0208*	2.26	3.88	0.0139*
Residual	16	4.25			1.04			0.58		
Total	23									
Cochran's test			C=0.3039 n.s.			C=0.2800 n.s.			C=0.5000 n.s.	
Transformation			None			None			None	
SNK tests										
Lo		Atl=Med			Atl=Med			Atl=Med		
Ma(Lo)		Atl: (FAR=CHI=BAR)<AME Med: (LIN=FUE)<(MOT=ALM)			Atl: (FAR=CHI=BAR)<AME Med: LIN=FUE=MOT=ALM			Atl: (FAR=CHI=BAR)<AME Med: LIN=FUE=MOT=ALM		

Table S5. Summary of the two-way PERMANOVA results for Annelida, Arthropoda and Mollusca (at species level) (A) and for total macrofaunal community of each considered taxonomic level (species, genus, family, order, class, phylum) (B). Lo: Location (Atl: Atlantic, Med: Mediterranean); Ma(Lo): Marina nested with location (FAR: Faro, CHI: Chipiona, AME: Puerto América, BAR: Barbate, LIN: La Línea, FUE: Fuengirola, MOT: Motril, ALM: Almería). df: degrees of freedom, MS: mean squares, MC: Montecarlo, p: level of significance. \*\*\*:  $p < 0.001$ , n.s. not significant.

**A**

Source of variation	df	Annelida			Arthropoda			Mollusca		
		MS	Pseudo-F	p (MC)	MS	Pseudo-F	p (MC)	MS	Pseudo-F	p (MC)
Lo	1	6692.7	0.7945	0.5615 n.s.	8538.4	0.9668	0.4712 n.s.	16313.0	1.9178	0.0961 n.s.
Ma(Lo)	6	8424.0	12.716	0.0001***	8831.7	5.4811	0.0001***	8506.1	9.581	0.0001***
Residual	16	662.4			494.8			887.8		
Total	23				1611.3					
Pair-wise tests										
	Lo	Atl=Med			Atl=Med			Atl=Med		
	Ma(Lo)	Atl: FAR≠CHI≠AME≠BAR			Atl: FAR≠CHI≠(AME=BAR)			Atl: FAR≠CHI≠AME≠BAR		
		Med: LIN≠FUE≠MOT≠ALM			Med: LIN≠FUE≠MOT≠ALM			Med: (LIN=FUE=MOT)≠ALM		

**B**

Source of variation	df	Species			Genus			Family		
		MS	Pseudo-F	p (MC)	MS	Pseudo-F	p (MC)	MS	Pseudo-F	p (MC)
Lo	1	8574.9	1.0052	0.4327 n.s.	9172.5	1.195	0.3110 n.s.	5552.2	0.8856	0.5197
Ma(Lo)	6	8531	10.894	0.0001***	7675.8	9.996	0.0001***	6269.5	9.3464	0.0001***
Residual	16	783.1			767.9			670.7		
Total	23									
Pair-wise tests										
	Lo	Atl=Med			Atl=Med			Atl=Med		
	Ma(Lo)	Atl: FAR≠CHI≠AME≠BAR			Atl: FAR≠CHI≠AME≠BAR			Atl: FAR≠CHI≠AME≠BAR		
		Med: LIN≠FUE≠MOT≠ALM			Med: LIN≠FUE≠MOT≠ALM			Med: LIN≠FUE≠MOT≠ALM		
Source of variation	df	Order			Class			Phylum		
		MS	Pseudo-F	p (MC)	MS	Pseudo-F	p (MC)	MS	Pseudo-F	p (MC)
Lo	1	3651.7	0.9081	0.4845 n.s.	2023.0	1.2739	0.2951 n.s.	1454.3	1.453	0.2480 n.s.
Ma(Lo)	6	4021.0	9.503	0.0001***	1588.1	7.7824	0.0001***	1000.9	6.8949	0.0001***
Residual	16	423.1			204.1			145.2		
Total	23									
Pair-wise tests										
	Lo	Atl=Med			Atl=Med			Atl=Med		
	Ma(Lo)	Atl: FAR≠CHI≠AME≠BAR			Atl: FAR≠(CHI=AME=BAR)			Atl: FAR≠(CHI=AME=BAR)		
		Med: LIN≠FUE≠MOT≠ALM			Med: LIN≠FUE≠MOT≠ALM			Med: (LIN=FUE=MOT)≠ALM		

Table S6. Abundance of meiofaunal taxa per replicate (10 cm<sup>2</sup>). Three replicates (1, 2, 3) per marina were studied. FAR: Barbate, LIN: La Línea, FUE: Fuengirola, MOT: Motril, ALM: Almería.

	FAR			CHI			AME			BAR			LIN			FUE			MOT		ALM	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	1	2
ACARI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	-
AMPHIPODA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
COPEPODA	6	22	14	3	3	2	35	10	8	3	5	-	22	342	244	22	11	16	32	9	-	-
CUMACEA	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
FORAMINIFERA	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KINORHYNCHA	3	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MOLLUSCA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
NAUPLII	5	6	11	10	12	1	16	8	-	-	3	-	22	69	175	5	6	1	3	4	-	-
NEMATODA	29	42	44	8	32	35	47	22	71	36	15	24	15	25	37	406	151	339	10	1	-	-
OSTRACODA	-	-	-	-	-	-	2	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
PYCNOGONIDA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
POLYCHAETA	4	4	4	3	10	10	10	9	6	17	12	4	1	32	57	3	12	1	2	4	-	-
ROTIFERA	-	6	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SARCOMASTIGOPHORA	-	-	-	-	-	-	-	-	-	3	5	5	-	-	-	-	3	2	2	2	-	-
TANAIDACEA	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
TURBELLARIA	-	-	1	5	12	10	5	1	1	-	-	2	-	1	-	4	6	-	-	-	-	-

Table S7. Environmental variables measured in each marina (3 replicates). TOC: Total Organic Carbon, N: total Nitrogen, Kjeldahl method, FAR: Faro, CHI: Chipiona, AME: Puerto América, BAR: Barbate, LIN: La Línea, FUE: Fuengirola, MOT: Motril, ALM: Almería.

**Atlantic marinas**

	<b>FAR 1</b>	<b>FAR 2</b>	<b>FAR 3</b>	<b>CHI 1</b>	<b>CHI 2</b>	<b>CHI 3</b>	<b>AME 1</b>	<b>AME 2</b>	<b>AME 3</b>	<b>BAR 1</b>	<b>BAR 2</b>	<b>BAR 3</b>
Sand (%)	55.0	52.7	56.6	4.0	5.0	2.5	60.7	56.8	13.6	17.9	4.5	6.4
Silt (%)	28.9	29.8	26.1	34.4	34.0	32.5	16.1	21.7	35.8	18.2	21.4	23.7
Clay (%)	16.1	17.5	17.3	61.6	61.0	65.4	23.2	21.5	50.6	63.9	74.1	69.9
TOC (%)	2.64	2.91	3.80	1.40	1.51	1.35	0.91	0.99	2.15	1.88	2.18	2.07
N (%)	0.13	0.15	0.18	0.13	0.14	0.13	0.09	0.08	0.18	0.14	0.16	0.15
P (ppm)	388.09	429.46	380.66	925.46	931.37	893.36	527.12	571.53	856.06	1016.37	1212.61	1112.78
S (ppm)	11251.58	10523.38	11605.33	2026.03	2260.33	1495.59	1787.70	2283.34	5968.11	3277.29	3172.06	3123.43
Hydrocarbons (ppm)	2000	1600	4300	4600	300	400	3400	1200	1200	1800	200	2500
Faecal coliforms (cfu/g)	50	200	50	850	1600	1300	50	50	350	2000	1500	950
Irganol (ppb)	0.00	0.00	3.93	2.01	0.00	2.57	0.00	0.00	1.85	6.70	7.52	5.86
As (ppm)	10.58	12.23	13.54	18.71	16.13	17.37	6.77	10.30	13.20	12.75	17.91	19.79
Cd (ppm)	0.07	0.43	0.03	0.21	0.11	0.09	0.25	0.18	0.18	0.08	0.30	0.00
Co (ppm)	3.40	3.79	3.35	8.52	8.72	8.73	4.37	4.96	8.25	9.09	11.21	10.69
Cr (ppm)	20.75	24.40	22.37	47.97	51.10	49.86	23.99	24.85	51.13	74.32	88.57	82.79
Cu (ppm)	51.52	69.13	65.90	92.03	95.95	91.56	41.85	41.99	136.83	119.65	153.06	135.14
Ni (ppm)	11.35	13.19	11.86	25.05	25.54	25.93	10.78	11.65	23.26	28.02	32.17	30.08
Pb (ppm)	18.91	15.24	13.07	34.35	34.78	36.43	39.38	14.86	47.73	17.75	36.13	16.89
Zn (ppm)	59.80	71.37	70.64	151.63	153.34	140.14	153.28	82.19	152.65	141.72	191.69	165.94

**Mediterranean marinas**

	<b>LIN 1</b>	<b>LIN 2</b>	<b>LIN 3</b>	<b>FUE 1</b>	<b>FUE 2</b>	<b>FUE 3</b>	<b>MOT 1</b>	<b>MOT 2</b>	<b>MOT 3</b>	<b>ALM 1</b>	<b>ALM 2</b>	<b>ALM 3</b>
Sand (%)	20.5	55.1	50.6	26.5	9.5	21.6	23.4	37.1	75.2	82.2	76.7	72.6
Silt (%)	42.0	25.2	26.2	46.8	63.9	38.5	71.6	59.8	24.0	16.4	21.1	25.3
Clay (%)	37.5	19.7	23.2	26.7	26.6	39.9	5.0	3.1	0.8	1.4	2.2	2.1
TOC (%)	2.33	1.58	1.77	4.10	3.10	2.83	0.97	1.00	0.84	0.65	0.76	0.89
N (%)	0.13	0.21	0.14	0.19	0.24	0.20	0.07	0.07	0.07	0.08	0.15	0.05
P (ppm)	624.99	583.39	638.00	1453.42	1105.56	936.14	1074.95	1068.04	1039.58	647.86	750.74	708.84
S (ppm)	9768.70	4676.37	5982.53	7810.35	6800.35	6354.08	1523.83	1562.98	1400.23	1097.45	1158.91	1238.93
Hydrocarbons (ppm)	1600	9600	8200	1700	1400	2800	2400	3900	7400	1700	2200	2600
Faecal coliforms (cfu/g)	650	1000	1400	2000	800	350	50	1000	650	1500	1100	1300
Irganol (ppb)	1.96	0.00	2.09	0.00	0.00	0.61	0.00	0.00	10.93	2.12	1.98	0.00
As (ppm)	12.79	8.23	5.54	20.50	23.62	25.08	18.37	17.87	13.14	17.64	13.76	17.11
Cd (ppm)	0.72	0.43	0.32	0.24	0.34	0.48	0.21	0.34	0.43	0.26	0.49	0.24
Co (ppm)	5.91	3.47	4.57	15.92	18.83	19.10	9.85	10.22	8.70	7.47	6.75	7.54
Cr (ppm)	77.71	47.01	57.99	113.27	117.17	115.14	15.71	16.94	11.80	8.83	6.17	7.07
Cu (ppm)	56.89	67.29	83.31	187.55	145.51	129.78	136.93	82.87	53.89	51.94	51.49	70.64
Ni (ppm)	51.37	29.54	34.93	193.71	219.43	220.67	24.72	24.08	22.48	17.42	16.23	18.86
Pb (ppm)	25.48	19.46	21.80	44.47	34.02	32.16	33.69	33.66	28.98	32.95	40.87	43.06
Zn (ppm)	83.83	78.14	111.00	216.99	167.48	190.35	153.26	136.70	118.10	122.86	125.46	151.94

Table S8. Results of DistLM (distance-based linear modelling) analysis for macrofauna. The variables explaining macrofaunal community (based on identifications at genus, family, order, class and phylum level) are included. Analyses were conducted also for Annelida, Arthropoda and Mollusca separately. Table shows significance levels for each predictor variable and the proportion of variation explained for any faunal assemblage. Only variables with significant results ( $p < 0.05$ ) in marginal tests are shown. Overall best models are also included. AIC: Akaike information criterion; SS (trace): sum of squares of sequential test, Prop.: proportion of variability explained by each factor (in the marginal tests without coaction of factors), TOC: Total Organic Carbon, F. colif.: faecal coliforms, \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ .

### Genus level:

Variables	SS (trace)	Pseudo-F	P	Prop. (%)
Sand	11610	4.56	0.0001***	17.2
TOC	19888	4.26	0.0001***	16.3
P	12213	4.85	0.0001***	18.1
F. colif.	5994	2.14	0.0237*	8.9
Cr	9902	3.78	0.0004***	14.7
Cu	9197	3.46	0.0001***	13.6
Pb	6924	2.51	0.0085**	10.3
Zn	10559	4.08	0.0002***	15.6

AIC	R <sup>2</sup>	Variables
178.10	0.74	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Cu, Pb
178.43	0.76	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Cu, Pb, Zn
178.53	0.74	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Cu, Zn

### Family level:

Variables	SS (trace)	Pseudo-F	P	Prop. (%)
Sand	10788	5.50	0.0001***	20.0
TOC	7438	3.52	0.0015**	13.8
P	11115	5.71	0.0001***	20.6
F. colif.	4759	2.13	0.0299*	8.8
Cr	9834	4.91	0.0001***	18.2
Cu	9118	4.48	0.0003***	16.9
Pb	4076	1.80	0.0691*	7.5
Zn	8916	4.36	0.0002***	16.5

AIC	R <sup>2</sup>	Variables
172.45	0.74	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Cu, Pb
172.63	0.74	Sand, Silt, TOC, P, F. colif., Cr, Cu, Pb, Zn
172.67	0.72	Sand, Silt, TOC, P, F. colif., Cr, Cu, Pb

Table S8 (Cont.)

**Order level:**

Variables	SS (trace)	Pseudo-F	P	Prop. (%)
Sand	6958	5.54	0.0001***	20.1
TOC	5759	4.40	0.0007***	16.7
P	6760	5.35	0.0001***	19.5
F. colif.	3185	2.23	0.0308*	9.2
Cr	7978	6.61	0.0001***	23.1
Cu	6308	4.91	0.0004***	18.3
Pb	3315	2.33	0.0237*	9.6
Zn	5560	4.21	0.0004***	16.1

AIC	R <sup>2</sup>	Variables
160.94	0.73	Sand, Silt, TOC, P, F. colif., Cr, Cu, Pb
161.09	0.75	Sand, Silt, TOC, P, F. colif., Cr, Cu, Pb, Zn
161.23	0.77	Sand, TOC, P, Hydrocarbons, F. colif., Irgarol, Cr, Cu, Pb, Zn

**Class level:**

Variables	SS (trace)	Pseudo-F	P	Prop. (%)
Silt	1472	2.42	0.0464*	9.9
TOC	3968	8.05	0.0001**	26.8
Cr	2833	5.20	0.0006***	19.1
Cu	1445	2.38	0.0466*	9.7

AIC	R <sup>2</sup>	Variables
142.34	0.73	Sand, Silt, TOC, P, Hydrocarbons, F. colif., Irgarol, Cr, Zn
142.40	0.69	Sand, Silt, TOC, F. colif., Cr, Pb, Zn
142.51	0.71	Sand, TOC, P, F. colif., Irgarol, Cr, Pb, Zn

**Phylum level:**

Variables	SS (trace)	Pseudo-F	P	Prop. (%)
TOC	1918	5.36	0.0007***	19.6
Cr	1710	4.66	0.0021**	17.5
Pb	1069	2.70	0.0334*	10.9

AIC	R <sup>2</sup>	Variables
134.27	0.69	Sand, TOC, P, F.colif, Irgarol, Cr, Pb, Zn
134.29	0.66	Sand, Silt, TOC, F. colif., Cr, Pb, Zn
134.59	0.71	Sand, TOC, P, F. colif., Irgarol, Cr, Cu, Pb, Zn

Table S8 (Cont.)

**ANNELIDA:**

Variables	SS (trace)	Pseudo-F	P	Prop. (%)
Sand	12493	4.97	0.0003***	18.4
TOC	12793	5.11	0.0001***	18.8
P	15331	6.42	0.0001***	22.6
F. colif.	8001	2.94	0.0078**	11.8
Cr	9182	3.44	0.0029**	13.5
Cu	11905	4.68	0.0001***	17.6
Pb	7164	2.59	0.0157*	10.6
Zn	12664	5.05	0.0001***	18.7

AIC	R <sup>2</sup>	Variables
177.55	0.75	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Cu, Pb
177.93	0.72	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Pb
178.04	0.76	Sand, Silt, TOC, P, F. colif., Irgarol, Cd, Cr, Cu, Zn

**ARTHROPODA:**

Variables	SS (trace)	Pseudo-F	P	Prop. (%)
Sand	9755	2.76	0.0006***	11.1
TOC	10945	3.15	0.0002***	12.5
P	9071	2.55	0.0013**	10.3
Cr	10769	3.09	0.0005***	12.3
Pb	8166	2.27	0.0067*	9.4
Zn	8124	2.26	0.0072**	9.3

AIC	R <sup>2</sup>	Variables
191.66	0.62	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Pb
191.84	0.58	Sand, Silt, TOC, P, F. colif., Cr, Pb
192.07	0.64	Sand, Silt, TOC, P, F. colif., Irgarol, Cr, Pb, Zn

**MOLLUSCA:**

Variables	SS (trace)	Pseudo-F	P	Prop. (%)
Sand	11913	3.76	0.0011**	14.6
TOC	7692	2.29	0.0283*	9.4
P	9638	2.94	0.0062**	11.8
Cd	10108	3.11	0.0033**	12.4
Cr	11254	3.52	0.0019**	13.8
Cu	7916	2.36	0.00272*	9.7
Zn	6949	2.05	0.0488*	8.5

AIC	R <sup>2</sup>	Variables
183.92	0.73	Sand, Silt, TOC, P, F. colif., Cr, Cu, Pb, Zn
184.00	0.70	Sand, Silt, TOC, P, F. colif., Cr, Cu, Zn
184.06	0.75	Sand, Silt, TOC, P, F. colif., Cd, Cr, Cu, Pb, Zn

Table S9. Results of DistLM (distance-based linear modelling) analysis for meiofauna. The variables explaining meiofaunal community (based on identifications at high taxonomic levels) are included. Table shows significance levels for each predictor variable and the proportion of variation in the community explained. Only variables with significant results ( $p < 0.05$ ) in marginal tests are shown. Overall best models are also included. AIC: Akaike information criterion; SS (trace): sum of squares of sequential test, Prop.: the proportion of variability which explained each factor (in the marginal tests without coaction of factors), TOC: Total Organic Carbon, F. colif.: faecal coliforms, \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

Variables	SS (trace)	Pseudo-F	P	Prop. (%)
Sand	3656	4.54	0.0012**	17.1
Hydrocarbons	2998	3.59	0.0053**	14.0
Cd	2929	3.49	0.0059**	13.7
Cu	2148	2.46	0.0333*	10.0

AIC	R <sup>2</sup>	Variables
157.62	0.62	Sand, Silt, TOC, F. colif., Cd, Cr, Cu, Zn
157.76	0.65	Sand, Silt, TOC, F. colif., Cd, Cr, Cu, Pb, Zn
157.86	0.65	Sand, TOC, Hydrocarbons, F. colif., Cd, Cr, Cu, Pb, Zn

Table S10. Intervals established to assign ecological quality status for taxa richness (species, genus, family and order level), Shannon-Wiener diversity ( $H'$ ) and the nematode:copepod ratio (Nem:Cop). This information has been used to conduct the global assessment (see Fig. 7).

Taxa richness				$H'$	Nem:Cop	
Species	Genera	Families	Orders			
<25	<20	<15	<10	<1.0	>15	BAD
25-44	20-44	15-34	10-22	1.0-1.4	7-15	POOR
45-74	45-69	35-49	23-27	1.5-1.9	3-6	MODERATE
75-100	70-80	50-60	28-32	2.0-3.0	1-2	GOOD
>100	>80	>60	>32	>3.0	<1	HIGH

Table S11. Spearman correlations among ecological indicators selected for global assessment (see Fig. 7). \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

	MEPI	BCI	Species	Genera	Families	Orders	H'	AMBI	M-AMBI	BENTIX	BENFES	MEDOCC
MEPI												
BCI												
Species	0.781*											
Genera	0.761*		0.994**									
Families			0.934**	0.958**								
Orders			0.952**	0.939**	0.939**							
H'			0.714*	0.755*	0.814**	0.663*						
AMBI												
M-AMBI			0.881**	0.898**	0.886**	0.843**	0.833**	-0.690*				
BENTIX												
BENFES			0.905**	0.934**	0.994**	0.916**	0.857**		0.905**			
MEDOCC	-0.805**		-0.762*	-0.719*		-0.687*		-0.810**	-0.762*			
Nem:Cop	-0.675*		-0.683*	-0.699*	-0.699*			-0.647*	-0.814**		-0.683*	-0.802**

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**Declaration of interests**

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: