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## Meteorological and climatic variability influences anthropogenic microparticle content in the stomach of the European anchovy *Engraulis encrasicolus* --Manuscript Draft--

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<b>Abstract:</b>	<p>Meteorological and climatic phenomena affect oceanographic characteristics and, consequently, anthropogenic microparticle aggregation. The same phenomena influence the ecology of pelagic fish, but whether there is a connection between meteorological and climatic characteristics and microparticle ingestion remains unknown. In the NW Mediterranean during the springs of 2011-2014, the incidence of contaminated European anchovies (35±17%) and microparticle abundance in the stomach content (0.46±0.25 microparticles ind<sup>-1</sup>) may have owed to higher concentrations of microparticles due to hydrodynamism. Year 2011 showed a higher fragment contribution (60±17%). The statistical analysis indicated a link between fragment abundance and climatic characteristics, with low North Atlantic Oscillation index values for the previous cold season indicating the transport of water from the polluted Tyrrhenian Sea. Low-density microplastic (polyethylene and polypropylene) was found, a selection due to the pelagic behaviour of anchovy. Fibre abundance remained quite constant throughout the 4-year period, pointing to diffused input not dependent on meteorological forcing. In 2012, anchovies were subjected to bottom-up limitation, due to adverse meteorological forcing (high early spring temperatures, low rainfall). The anchovies mainly ingested fibres through less energy-expensive filter-feeding. Therefore, meteorological and climatic forcing regulates microparticle intake by fish and should be considered for pollution mitigation.</p>
<b>Response to Reviewers:</b>	<p>Editor:</p> <p>Hydrobiologia attempts to strictly follow the rules of nomenclature, with the correct use of parentheses around species authorships. As such, the parentheses around the author and year of <i>Engraulis japonicum</i> on line 277 should not be present, according to FishBase, <a href="https://www.fishbase.se/summary/Engraulis-japonica.html">https://www.fishbase.se/summary/Engraulis-japonica.html</a> and art 51.3 of ICZN, <a href="http://iczn.anasp.org/wiki/Article51">http://iczn.anasp.org/wiki/Article51</a></p> <p>Please, check and amend all species names used in the text.</p>

Only two species were cited, we corrected the authorship following FishBase

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2 **of the European anchovy *Engraulis encrasicolus*.**

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26

27 **Abstract**

28 Meteorological and climatic phenomena affect oceanographic characteristics and, consequently,  
29 anthropogenic microparticle aggregation. The same phenomena influence the ecology of pelagic fish, but  
30 whether there is a connection between meteorological and climatic characteristics and microparticle  
31 ingestion remains unknown. In the NW Mediterranean during the springs of 2011-2014, the incidence of  
32 contaminated European anchovies ( $35\pm 17\%$ ) and microparticle abundance in the stomach content ( $0.46\pm 0.25$   
33 microparticles  $\text{ind}^{-1}$ ) may have owed to higher concentrations of microparticles due to hydrodynamism. Year  
34 2011 showed a higher fragment contribution ( $60\pm 17\%$ ). The statistical analysis indicated a link between  
35 fragment abundance and climatic characteristics, with low North Atlantic Oscillation index values for the  
36 previous cold season indicating the transport of water from the polluted Tyrrhenian Sea. Low-density  
37 microplastic (polyethylene and polypropylene) was found, a selection due to the pelagic behaviour of  
38 anchovy. Fibre abundance remained quite constant throughout the 4-year period, pointing to diffused input  
39 not dependent on meteorological forcing. In 2012, anchovies were subjected to bottom-up limitation, due to  
40 adverse meteorological forcing (high early spring temperatures, low rainfall). The anchovies mainly ingested  
41 fibres through less energy-expensive filter-feeding. Therefore, meteorological and climatic forcing regulates  
42 microparticle intake by fish and should be considered for pollution mitigation.

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46 **Key words:** European anchovy, anthropogenic microparticles, meteorological and climatic influence,  
47 Ligurian Sea, NW Mediterranean

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49

## 50 **Introduction**

51 In the Mediterranean Sea, the European anchovy *Engraulis encrasicolus* (Linnaeus, 1758) represents,  
52 together with sardine, an important percentage of the pelagic fish captured for human nutrition (Leonart &  
53 Maynou, 2003). Therefore, any alteration in the anchovy stock (i.e. due to overfishing, climatic changes and  
54 pollution) can directly influence the health as well as the economic dimension of human society.

55 One of the emerging pollution threats to pelagic fish and to ecosystems in general is the increasing  
56 concentration of anthropogenic microparticles (dimension lower than 5 mm; Arthur et al., 2009) in the water  
57 column (Eriksen et al., 2014; Deudero & Alomar, 2015; van Sebille et al., 2015; Belzagui et al., 2020).

58 These materials can have different morphologies, but microplastic fragments and fibres in particular have  
59 been the focus of previous research, the latter being synthetic or natural and coming from clothes and textiles  
60 (Laedwig et al., 2015; Remy et al., 2015). All these anthropogenic microparticles are potential pollutant  
61 carriers, given that they are composed of materials that are chemically modified and stained (Turner, 2019).

62 In addition, they can absorb hydrophobic and metal compounds and play an important role in the  
63 environmental distribution of these substances (Sillanpää & Sainio, 2017; Rios-Fuster et al., 2019; Yu et al.,  
64 2019; Enfrin et al., 2020). Microparticles may be ingested by large and small metazoans, by active predation  
65 or via passive filtration (Boerger et al., 2010; Davison & Asch, 2011). Beyond potential mechanical damage  
66 (Cedervall et al., 2012; Pedá et al., 2016), microparticles can exert a chemical, toxic action through releasing  
67 of all the toxic substances in the organisms that ingest them (Lima et al., 2020). The threat is real, given that  
68 anthropogenic microparticle ingestion has already been observed in commercial pelagic fish such as  
69 anchovies (Neves et al., 2015; Nadal et al., 2016; Tanaka & Takada, 2016; Jovanović, 2017; Compa et al.,  
70 2018; Rios-Fuster et al., 2019). Nevertheless, the real effects are not yet totally understood (Remy et al.,  
71 2015; Ferreira et al., 2019).

72 The origin, distribution, accumulation and availability of microparticles have been related to the origin of  
73 surface water masses, to surface currents and to the influence of near-surface winds (Lebreton et al., 2017).

74 Lima et al. (2021) have proposed a global model of microfibre accumulation based on the main  
75 oceanographic properties (current velocity and direction, surface sea temperature and salinity, wind speed).

76 The relevant role of sea temperature and salinity has been observed, confirming the results of other local

77 approaches (Lusher et al., 2015; Kanhai et al., 2018). Furthermore, convergence areas have been identified  
78 where accumulation is enhanced (van Sebille et al., 2015; Jiang et al., 2020).

79 The Mediterranean Sea has been indicated as an area of microparticle accumulation (Eriksen et al., 2014;  
80 Suaria et al., 2016; Lima et al., 2021) due to its morphology and high human density. In the NW  
81 Mediterranean (specifically the Ligurian Sea), hydrodynamic structures such as frontal areas and gyres may  
82 play a role in microparticle accumulation (Collignon et al., 2012; Fossi et al., 2012; Bainsi et al., 2018). In the  
83 Ligurian Sea, sea surface temperature and salinity, as well as the speed and direction of currents are  
84 regulated by large-scale phenomena. The North Atlantic Oscillation (NAO) is the dominant mode,  
85 responsible for atmospheric behaviour throughout the year (Marshall et al., 2002). The NAO, especially  
86 during the winter/cold season, determines the volume of waters that enter from the southernmost Tyrrhenian  
87 Sea (Astraldi et al., 1999; Vignudelli et al., 1999). Given the link between climatic and meteorological  
88 characteristics (as summarised in the NAO index) and the circulation and oceanographic properties of the  
89 Ligurian Sea, an influence of NAO on microparticle density and accumulation is likely. Therefore, it is  
90 probable that anchovies in the Ligurian Sea ingest microparticles depending on the climatic-meteorological  
91 conditions, although this relationship is yet to be defined.

92 This work aimed to gather information on the relationship between the climatic and meteorological  
93 characteristics and the microparticle content of anchovy, in terms of abundance, morphology (fibre or  
94 fragment) of microparticles found in anchovy stomachs and incidence of contaminated individuals. The  
95 study focused on the spring period because this is crucial for anchovy. In fact, from March to May the fish  
96 suddenly increase in dimension and gonad maturity and begin their reproductive period, requiring additional  
97 food (Politikos et al., 2011; Bonanno et al., 2016; Compa et al., 2018). Consequently, they are at greater  
98 threat of ingesting non-natural microparticles.

99

100

## 101 **Materials and Methods**

### 102 *Study area and sampling*

103 In the Ligurian Sea, the hydrodynamic characteristics are mainly controlled by two main currents (Fig. 1):  
104 the East Corsica Current (ECC, flowing northwards between Italy and the island of Corsica, carrying

105 relatively warm waters), and the West Corsica Current (WCC, flowing northwards but off the western coast  
106 of Corsica, characterised by colder waters) (Astraldi et al., 1999). The velocity of these currents changes  
107 seasonally, being linked to the broader climatic characteristics (NAO) and, locally, to the different coasts'  
108 morphological characteristics and meteorological forcing.

109 Sampling was performed along the eastern Ligurian coast (Fig. 1). In the sampling area, the bottom depth  
110 ranged between 55 m and 120 m. A commercial fishing boat (27 m in length, gross tonnage 94 tonnes, power  
111 397 kW) was equipped with purse seine fishing gear (net dimensions: 800 m in length and 120 m in height,  
112 capture was performed along the entire water column depending on bottom depth, 14 mm mesh size). The  
113 purse seine was placed in the sea during the night (from midnight to 4:30 a.m.), to collect all the fish  
114 attracted by an artificial light on the boat.

115 Samples were collected during the springs (March, April, and May) of 2011 to 2014, although in 2014,  
116 sampling was carried out only in March and April. Depending on the wind-wave conditions, during each  
117 month, samples were collected on a minimum of 1 to a maximum of 5 sampling dates. On each sampling  
118 date,  $16 \pm 4$  individuals (median value 15 individuals; see Appendix-Table 1 for details) were randomly  
119 isolated, their characteristics were determined and their stomach content was analysed to find any  
120 microparticle they had ingested, as described below.

121

#### 122 *Laboratory analyses*

123 All fish were acquired directly from fishers within 5 h after their capture, stored in a clean cooler box and  
124 brought to the laboratory within the next 2 h.

125 All the analyses undertaken are described in a previous study (Capone et al., 2020). Briefly, the fish were  
126 measured to the nearest 0.5 cm (total length), and weighed (wet weight,  $\pm 0.1$  g with a Kern PCB electronic  
127 balance). Fulton's condition factor was calculated using the following formula:  $K=100 * \text{weight} / \text{total}$   
128  $\text{length}^3$  (Froese, 2006). Gonads were removed to determine the gonadosomatic index (GSI), calculated as the  
129 wet weight contribution of gonads to the wet weight of the specimen minus its gonad weight.

130 Each fish's stomach content was transferred to a Petri dish, where its stomach walls were carefully washed  
131 with 70% ethanol, after cutting longitudinally with a micro-dissecting lancet. This material was used for the  
132 identification of microparticles, following previous protocols (Lusher et al., 2013; Nadal et al., 2016; Alomar

133 & Deudero, 2017). A dissecting microscope (Zeiss Stemi DV4) at 8× to 32 × magnification was used to  
134 differentiate microparticles from other natural materials. Before use, all tools used for sample processing and  
135 sorting were carefully cleaned with ethanol and checked under the dissecting microscope. The sample  
136 treatment and analysis were performed in a room specifically used for these analyses, to avoid  
137 contamination. A white 100% cotton lab coat was always worn during all analysis procedures and clean filter  
138 paper was placed next to the sample during stomach dissection and microscopic analysis, to evaluate any air-  
139 borne contamination. After analysing each stomach, the filter paper was observed with the same microscope  
140 and the fibres found were subtracted to the results of the stomach analysis. Generally, these fibres were blue,  
141 and were found only occasionally.

142 All microparticles not resembling natural materials (namely those that were mouldable, with consistent  
143 thickness, and that did not break when pressed with forceps; Bellas et al., 2016) were counted. Their  
144 morphological type (fragment or fibre) was recorded. Fragments were measured with a Leica Z16 APO  
145 stereomicroscope equipped with Leica Application Suite software.

146 All fragments were analysed with transmission Fourier Transform Infrared (FT-IR) spectroscopy (4000–700  
147  $\text{cm}^{-1}$  PerkinElmer Spectrum 65) using commercial and custom-made spectral databases for microplastic  
148 identification. By contrast, fibres were too small to be analysed with this method.

149

#### 150 *Climatic and meteorological variables*

151 In the NW Mediterranean Sea, the NAO is the dominant mode, responsible for atmospheric behaviour  
152 throughout the year (Marshall et al., 2002) and regulating long-term sea surface temperature and circulation  
153 (Vignudelli et al., 1999; Bolle, 2002). Astraldi et al. (1999) have shown an inverse correlation between the  
154 ECC's water transport and winter NAO index values. In the winter/cold season, the NAO exerts a strong  
155 influence on the NW Mediterranean's ecological dynamics (Fernández de Puellas et al., 2007): enhanced  
156 vertical mixing of the water column enables a proper nutrient supply to reach the euphotic zone (Fernández  
157 de Puellas & Molinero, 2007) and favours the production of the organisms that are trophic targets for pelagic  
158 fish. Following these observations, the monthly NAO index values were obtained from  
159 <https://www.cpc.ncep.noaa.gov>. The winter/cold season averages were obtained from  
160 <https://crudata.uea.ac.uk/cru/data/nao/values.htm>, e.g the October-March NAO index average and the



161 December-February NAO index average. The monthly NAO index values (www.cpc.ncep.noaa.gov) from  
162 October to December were averaged to obtain a further NAO index mean value, given that in the study area  
163 the spring season, during which phytoplanktonic production occurs, can start from early February (Misic et  
164 al., 2011).

165 Air temperature was recorded at the meteorological station of Genova Sestri Ponente, while rainfall data  
166 were obtained from the meteorological station of the Department of Chemical, Civil and Environmental  
167 Engineering (University of Genova). In this study, the latter data are presented as the sum of the rainfall of  
168 the 4 days before each sampling, to consider the time between a rain event and the arrival of the continental  
169 water at sea.

170

#### 171 *Statistical analyses*

172 The results for each sampling date (n=32) were used to perform the statistical analyses.

173 Pearson's correlation coefficient was used to test the significance of the relationships among variable trends  
174 (STATISTICA software).

175 A multivariate analysis was performed to highlight similarities between groups of samplings. The data for  
176 each variable were previously normalised (subtracting the mean value and dividing by the standard deviation  
177 calculated for the entire data set for each variable). A principal component analysis was performed using the  
178 PRIMER 6 $\beta$  programme package (Clarke & Warwick, 2001) on the normalised values of the NAO index  
179 (winter and monthly), air temperatures (minimum and maximum air temperature for each sampling date,  
180 Tmin and Tmax, respectively) and rainfall (cumulative value for the 4 days before each sampling), to  
181 characterise the environmental scenario for each sampling date. Analysis of similarities (ANOSIM) was  
182 carried out on the same variables using the PRIMER 6 $\beta$  programme package. ANOSIM is an approximate  
183 analogue of the standard univariate analysis of variance (ANOVA) test, which allows testing of the null  
184 hypothesis that there are no differences between groups of samples specified by the levels of a single factor.  
185 ANOSIM was applied on similarity matrices, created using the Euclidean distances on the normalised data of  
186 the climatic (winter NAO index and monthly NAO index) and meteorological (Tmin, Tmax and rainfall)  
187 variables.

188 Brodgar software (Brodgar 2.5.6 package, 2011, Highland Statistics Ltd.) was used to perform a redundancy  
189 analysis (RDA) (Zuur et al., 2007). The analysis was applied on the normalised data of all the samplings, to  
190 evaluate whether the microparticles (represented by the abundance of fibres and fragments per individual and  
191 by the incidence of contaminated individuals) and the fish characteristics (length, weight, Fulton's condition  
192 factor, and GSI) were influenced by the climatic features indicated by the winter NAO index averages, the  
193 monthly NAO index, the meteorological features represented by the cumulative rainfall in the 4 days before  
194 sampling and the atmospheric temperature on the day of sampling (min and max). To test the order of  
195 importance of the explanatory variables, an automated forward selection model was applied. In particular,  
196 the 'conditional effects' that show the increase in the total sum of the eigenvalues after including a new  
197 variable during the forward selection were calculated. Finally, a permutation test was applied (number of  
198 permutations: 499) to test the null hypothesis that the explained variation is larger than a random  
199 contribution.

200

## 201 **Results**

### 202 *Climatic and meteorological characteristics*

203 The climatic and meteorological data (winter/cold season and monthly NAO index, temperature and rainfall)  
204 were analysed using a multivariate PCA. The results (PC1 explaining 45.8%, PC2 24.9%) (Fig. 2)  
205 highlighted the different climatic and meteorological characteristics of the years.

206 Year 2011 was characterised by lower winter/cold season NAO index values and higher monthly NAO index  
207 values (Fig. 3). Year 2013 and, especially, years 2012 and 2014, showed an opposite distribution on the plot.  
208 Years 2012 and 2014 showed a lower scattering along PC2 (dominated by air temperature values). In 2014,  
209 the distribution along PC2 owed to the absence of May sampling. In 2012, the distribution along PC2  
210 highlighted the peculiar characteristics of the year (Fig. 4). The Tmin of March 2012 ( $11.0 \pm 0.5^\circ\text{C}$ ) was  
211 higher than in the other years (2011:  $8.7 \pm 1.0^\circ\text{C}$ , 2013:  $7.5 \pm 0.6^\circ\text{C}$ , 2014:  $8.2^\circ\text{C}$ ). On the contrary, 2012  
212 showed the lowest Tmin among the years in April ( $7.6^\circ\text{C}$ ), the other years reaching values higher than  $12^\circ\text{C}$ .  
213 The same dynamic was seen in May 2012 ( $12.4 \pm 0.7^\circ\text{C}$ ), with the other years showing values higher than  
214  $14^\circ\text{C}$ .

215 ANOSIM performed on years (sample statistic global R 0.587; significance level of sample statistic 0.1%)  
216 (Table 1) confirmed both that year 2011 differed from the other years and that there was a difference  
217 between 2012 and 2013.

218 Years 2011 and 2013 showed the highest peaks of rainfall (over 60 mm for the 4 days before the sampling)  
219 in March and April, while the other years showed lower maxima, with 2012 reaching a maximum of 20 mm  
220 in April and May and 2014 only 5 mm in April (Fig. 4).

221

#### 222 *Anchovy characteristics and microparticles*

223 The data pertaining to anchovy characteristics and microparticles as well as the relationships between them  
224 are presented and discussed in a previous study (Capone et al., 2020). Nevertheless, a summary may be  
225 useful in this study; moreover, all the data for the 32 samplings are reported in Appendix -Table 1.

226 Fig. 5 reports the general trends over time of the anchovies' main characteristics. The markers report the  
227 average for each month in the 4 years (bars denote standard deviation), while lines represent a descriptive  
228 (not statistical) trend for each year. In nearly every case, 2011 showed the highest values, namely longer (on  
229 average  $12.5\pm 0.9$  cm) and heavier anchovies (on average  $12.9\pm 3.4$  g) and higher Fulton's factor ( $0.65\pm 0.03$ )  
230 and sexual development (GSI on average  $1.2\pm 1.3\%$ ). In general, the increase in the length and the weight of  
231 the fish was higher from March to April, and lower from April to May. However, year 2012 showed an  
232 initial hindrance to growth, which was partially recovered in May, although the values were lower than the  
233 other years. The GSI trends increased sharply for 2011 and 2014, while they were similar in March and April  
234 2012 and 2013 and increased only in May. Fulton's factor showed a peculiar 2014 trend, with a decrease in  
235 April.

236 Microparticles were found in  $24\pm 10\%$  (March 2011) to  $53\pm 28\%$  (May 2012) of the analysed individuals,  
237 with an average value for all the observations of  $35\pm 17\%$ . Fig. 6 reports the fibre and fragment abundance  
238 per individual. All the data $\pm$ SD for the 32 samplings are reported in Appendix - Table 1. The contribution of  
239 the two microparticle types was largely dominated by fibres in 2012 and 2014 (on average  $92\pm 17\%$  and  
240  $98\pm 4\%$ , respectively) and in 2013 ( $64\pm 39\%$ ). By contrast, the year 2011 showed a dominance of fragments  
241 ( $60\pm 17\%$ ).

242 Fibres were the most abundant (Fig. 6), with values of up to  $1.17 \pm 0.41$  fibres  $\text{ind}^{-1}$  (18 May 2012). Year 2011  
243 showed the lowest values, with maxima of  $0.27 \pm 0.46$  fibres  $\text{ind}^{-1}$  (2 April). Fairly low values were also seen  
244 in year 2013 (maximum of  $0.59 \pm 0.80$  fibres  $\text{ind}^{-1}$  on 1 March).

245 Fragments showed opposite trends (Fig. 6), with higher values in 2011 (up to  $0.43 \pm 0.76$  fragment  $\text{ind}^{-1}$  on 9  
246 May) compared to the other years (maxima on 25 April 2013 of  $0.17 \pm 0.39$  fragment  $\text{ind}^{-1}$ ). Fragments were  
247 observed on all the 2011 sampling dates, in 67% of the 2013 sampling dates, in 33% of the 2012 sampling  
248 dates and only once (25%) in 2014. The FT-IR analyses for 2011 identified the composition of 41 fragments,  
249 while 3 were not recognised as plastic, but as organic materials such as cellulose. Polyethylene was the most  
250 represented plastic polymer (71% in March, 87% in April, and 73% in May), while the other recognised  
251 fragments were composed of polypropylene. The FT-IR analyses on the few fragments in the other years  
252 confirmed the dominance of polyethylene.

253

#### 254 *Relationships of anchovy characteristics and microparticles with climatic and meteorological characteristics*

255 The results of the RDA performed using fish characteristics and ingested microparticle features as response  
256 variables and climatic and meteorological characteristics as explanatory variables are reported in Table 2 and  
257 Fig. 7. The RDA explained 52% of the variance (axis 1: 30%, axis 2: 15%). The response variables were  
258 mainly influenced by the variability of the December-February NAO index average and the minimum air  
259 temperature.

260 Table 3 reports the results of the analysis performed to ascertain whether the climatic and meteorological  
261 variables correlated with the fish characteristics and ingested microparticles. The correlations between the  
262 climatic and meteorological variables and the fish characteristics were positive for temperature, in  
263 accordance with the increases in dimensions and sexual maturation during the year. No correlations were  
264 found for rainfall. All significant correlations of the NAO index averages with the fish characteristics were,  
265 instead, negative, while the monthly NAO index showed no significant correlations.

266 The incidence of contaminated fish was not linked to meteorological or climatic variables.

267 Higher values of the NAO index averages were related to a larger number of fibres per individual and to a  
268 lower number of fragments per individual.

269

## 270 **Discussion**

271 The mean frequency of individuals containing one or more items was variable, from a minimum of 13% to a  
272 maximum of 100% (averaging all the observation  $35\pm 17\%$ ), with an average of  $0.46\pm 0.25$  items  $\text{ind}^{-1}$  ( $0.13$ -  
273  $1.18$  items  $\text{ind}^{-1}$ ). These values are higher than the results obtained for *E. encrasicolus* along the Spanish  
274 Mediterranean coast by Compa et al. (2018), who found a frequency of 15% (7-27%) and from 0.07 to 0.33  
275 microparticles per individual; and also higher than the data reported by Rios-Fuster et al. (2019) for the same  
276 area (frequency of 3% and  $0.03\pm 0.16$  items  $\text{ind}^{-1}$ ). However, our results are lower than the 77% found in the  
277 highly polluted Tokyo Bay for *Engraulis japonicum* Temminck & Schlegel 1846 (on average 2.3 items  $\text{ind}^{-1}$ )  
278 (Tanaka & Takada, 2016).

279 It has been reported that the presence and concentration of microparticles in seawater influences these items'  
280 abundance in the stomach content (Bellas et al., 2016; Nadal et al., 2016; Setälä et al., 2018). We have no  
281 data related to microparticle availability in seawater during the sampled years, but previous observations  
282 related to the surface layer have highlighted the presence of an accumulation site for microplastics in the  
283 eastern coastal Ligurian Sea, where our sampling was performed (Fossi et al., 2012). This area has also been  
284 confirmed as a "hot spot" for microplastics by Collignon et al. (2012), due to its peculiar hydrodynamic  
285 characteristics and wind regime. Therefore, the greater microparticle abundance in anchovies from the  
286 present study than in those of the Spanish coast (Compa et al., 2018) may have owed to accumulation and the  
287 higher availability of microparticles in the eastern part of the coastal NW Mediterranean.

288 We found major differences in terms of microparticle type among years (fragments dominating for  $60\pm 17\%$   
289 in 2011 vs fibres reaching the highest contribution of  $84\pm 27\%$  in the other years). This indicates that  
290 anchovies may be subjected to different types of microparticle contamination, despite being captured in the  
291 same area.

292 Several authors have observed that fibres are more abundant in the marine environment than fragments  
293 (Claessens et al., 2011; Barrows et al., 2018; Zhu et al., 2018) and that fish can feed on these anthropogenic  
294 microparticles (Lusher et al., 2013; Ferreira et al., 2019). Neves et al. (2015) found that 65.8% of the  
295 ingested microplastics in commercial fish off Portuguese coasts were fibres and 34.2% were fragments.  
296 Fibre's contribution to the total items found in stomachs during the 2012-2014 period identified in the  
297 present study is similar to the 83% observed by Compa et al. (2018) for *E. encrasicolus*. The abundance of

298 ingested fibres ind<sup>-1</sup> was in general similar among the years, despite the lower values of 2011. The quite  
299 constant fibre abundance per individual observed in the present study is in agreement with previous  
300 observations. In fact, it has been observed that fibres are largely distributed in the water column, especially  
301 offshore (Enders et al., 2015; Lima et al., 2021) and that the abundance of microparticles in the stomach  
302 content depends on their concentration in seawater (Bellas et al., 2016; Nadal et al., 2016; Setälä et al.,  
303 2018).

304 The microparticle content of the fish stomach did not correlate with local meteorological conditions such as  
305 rainfall. Especially for fibres, the continental inputs via river outfalls and urban wastewaters have been  
306 indicated as the main sources to the sea (Browne et al., 2011; Lima et al., 2014; Hartline et al., 2016;  
307 Lebreton et al., 2017). Rainfall exerts a positive influence on these sources, favouring fibre inputs by  
308 increasing soil drainage and river flow into the sea. Nevertheless, the coastal circulation often transports the  
309 fibres offshore (Lima et al., 2021), thus limiting the significance of a direct correlation between fibre  
310 concentrations and continental water inputs, as well as fibre ingestion by fish. In addition, the Ligurian  
311 region does not have large rivers and the cities are rather small compared to other areas, for instance, on the  
312 Tuscany coast or along the rivers that flow into the Tyrrhenian Sea (Arno in Tuscany and Tevere in Lazio).  
313 A limited local continental input of contaminated waters may explain the absence of correlation between the  
314 ingested fibre abundance and rainfall events, indicating continuous and diffused accumulation processes.

315 The fragment number per individual was higher in 2011 than in the other years, suggesting that the  
316 dominance of fragments in this year was probably an anomaly. ANOSIM indicated a separation between the  
317 environmental conditions of 2011 and of the other years. Furthermore, the fragment number negatively  
318 correlated to the NAO index averages, and the December-February NAO index average was the most  
319 significant among all the explanatory variables of the RDA.

320 The NAO index can be considered a good proxy for long-term series on environmental variables, because it  
321 records the overall physical variability, integrating local climatic changes (Drinkwater et al., 2003). Astraldi  
322 et al. (1999) have shown an inverse correlation between the ECC's water transport and the winter NAO  
323 index. With a negative NAO index, cold and dry air masses from the polar regions prevail, generating an  
324 increase in the flow of warmer water across the Corsica Channel from the Tyrrhenian Sea. The mean values  
325 of the winter/cold season NAO index of 2010-2011 were the lowest among the studied years, below -1. This

326 threshold is considered a critical value as, whenever the NAO index drops below this limit, the water  
327 transport through the Corsica channel is significantly increased, while for higher values, it diminishes  
328 considerably (Vignudelli et al., 1999). Bainsi et al. (2018) found in the Northern Tyrrhenian Sea (Tuscany  
329 waters) a significant concentration of microplastics, especially fragments, at offshore sampling sites (> 10  
330 km from the coast) in winter and spring season, with an accumulation in the gyre generated at the boundary  
331 between the northern Tyrrhenian Sea and the Ligurian Sea. A more intense flux of the ECC, correlated with  
332 the constantly negative NAO index values of winter 2010 and January 2011 (-0.88), may have favoured the  
333 increased transport of such microplastic fragments, composed mainly of polyethylene (>66%) and  
334 polypropylene (28%), the most used polymers (Gago et al., 2018). A similar composition was found in the  
335 stomach content of the anchovies of the present study (76% and 24% for polyethylene and polypropylene,  
336 respectively). The pelagic behaviour of anchovy (Rumolo et al., 2016) may play a role in the chemical  
337 composition of the microparticles found in their stomachs. In fact, pelagic fish ingest particles that float in  
338 the water column given their low density, such as polyethylene and polypropylene (characterised by densities  
339 of 0.89-0.98 g cm<sup>-3</sup> and 0.85-0.92 g cm<sup>-3</sup>, respectively; Enders et al., 2015).

340 In the NW Mediterranean, environmental characteristics determine the establishment of phyto- and zoo-  
341 planktonic blooms (Lacroix et al., 2001; Fernández de Puelles et al., 2007), and the abundance of plankton in  
342 turn determines the success of anchovy development (Bonanno et al., 2014). Thus, cold and wet early spring  
343 conditions, which increase the nutrient availability to phytoplankton due to water column mixing (Fernández  
344 de Puelles & Molinero, 2007) and continental inputs (Pesce et al., 2018), favour anchovy growth and  
345 reproductive development in the early spring.

346 Therefore, the higher fragment abundance per individual of 2011 owed not only to the potentially higher  
347 availability of fragments carried by the northward current from the Tyrrhenian Sea, but also to the better  
348 conditions of the individuals (higher Fulton's factor values representing the status of the fish: the higher the  
349 value, the better the fish's condition). These body conditions sustained the active particle-feeding mode,  
350 given that this can ensure a high energy gain after capture, despite the considerable energetic effort of  
351 predation (James et al., 1989). However, the active predator behaviour of healthy organisms may expose  
352 them to a higher intake of fragments that resemble the morphological characteristics of some crustaceans,  
353 molluscs, or other zooplankton (Boerger et al., 2010; de Sá et al., 2015).

354 By contrast, March 2012 was quite warm and dry and the NAO index values were always positive from  
355 October to January. The anchovies were smaller and sexual maturation increased only in May, because of a  
356 bottom-up limitation induced by the climatic and meteorological conditions. Smaller and lighter anchovies  
357 may prefer the less energy-expensive filter-feeding mode, passively collecting fibres together with  
358 phytoplankton and small zooplankton.

359

## 360 **Conclusions**

361 In the Ligurian Sea, the European anchovy is subjected to microparticle intake, given that one third of the  
362 analysed specimens were found to contain fibres and/or fragments. Fragments showed different values from  
363 the fairly stable fibre content in the individuals' stomachs, indicating that the same species in the same area  
364 may be affected differently by microparticles. In our study, climatic variables were found to exert an effect  
365 on the contamination of fish by microparticles, on the one hand by potentially increasing the northward  
366 transport of contaminated waters from the Tyrrhenian Sea, while on the other hand by favouring the good  
367 status of individuals, allowing them to perform the energy-expensive bite-predation feeding of fragments.  
368 Thus, the dominance of fibres or fragments in the individuals depended on oceanographic and  
369 meteorological characteristics, highlighting the close link between different global processes and ecosystem  
370 alterations (anthropogenic microparticle ingestion).

371

372

373

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377

## 378 **Conflicts of interest /Competing interests**

379 The authors declare that they have no conflict of interest nor competing interests.

380

## 381 **Data availability**

382 The data supporting the findings of this study are available within the article and its supplementary  
383 information file.



384

385 **Code availability**

386 Not applicable

387

388 **Authors' contributions**

389 All authors contributed to the study conception and design. Material preparation, data collection and analysis  
390 were performed by Alessandro Capone and Mario Petrillo. The first draft of the manuscript was written by  
391 Cristina Misic and all authors commented on previous versions of the manuscript. All authors read and  
392 approved the final manuscript.

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573

574

575 **Captions to figures**

576 **Fig.1.** NW Mediterranean: Ligurian Sea and northern Tyrrhenian Sea. Arrows denote the Eastern Corsica  
577 Current (ECC), the Western Corsica Current (WCC) and the Ligurian Current (Lig. C). The black star  
578 indicates the area where the samplings took place.

579 **Fig. 2.** PCA for the climatic variables (monthly NAO index and NAO index averages) and meteorological  
580 variables (minimum air temperature of the sampling day: Tmin, maximum air temperature of the  
581 sampling day: Tmax, cumulative rainfall for the 4 days before sampling: rainfall) in the NW  
582 Mediterranean for the springs of 2011-2014. The 32 observations (sampling days) are reported with  
583 different markers: 2011 full black circle, 2012 empty triangle, 2013 full black square, 2014 empty circle.

584 **Fig. 3.** Values of the NAO index. A: average values for the cold/winter period before each sampled spring  
585 (data from <https://crudata.uea.ac.uk/cru/data/nao/values.htm>). B: monthly NOA index values (data from:  
586 [www.cpc.ncep.noaa.gov](http://www.cpc.ncep.noaa.gov)) for the sampled months.

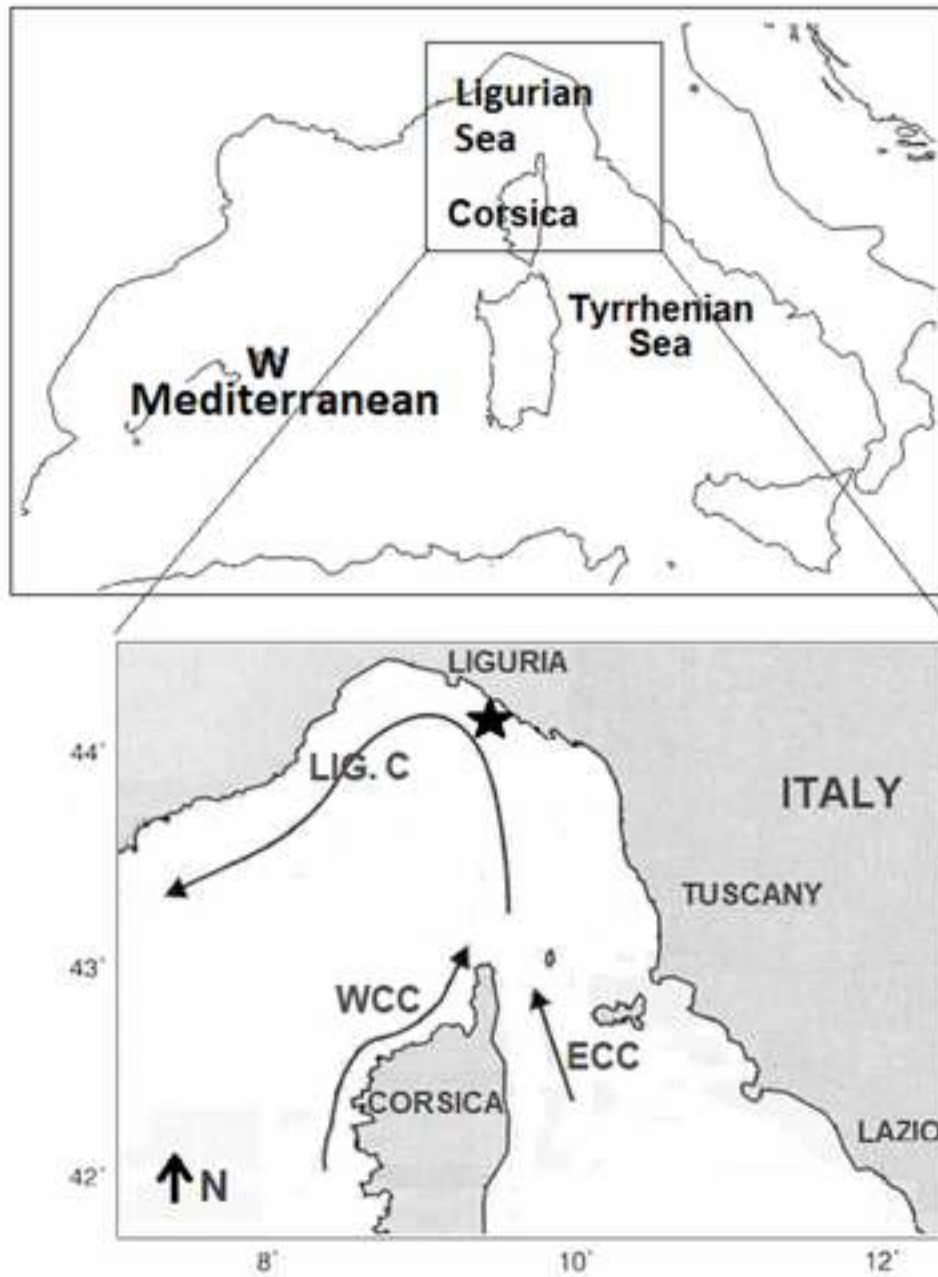
587 **Fig. 4.** Meteorological variable trends in the period 2011-2014 in the NW Mediterranean. Minimum air  
588 temperature of the sampling day: Tmin, maximum air temperature of the sampling day: Tmax, cumulative  
589 rainfall for the 4 days before sampling: rain.

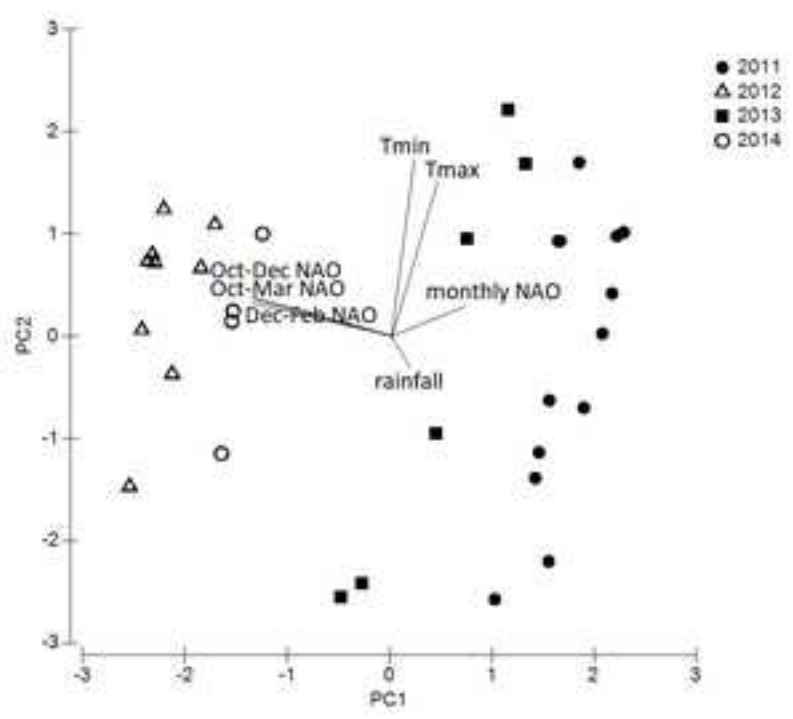
590 **Fig. 5.** Anchovy characteristics (A: length, B: weight, C: Fulton's condition factor, and D: gonadosomatic  
591 index GSI) averaged for each month in the four years. Bars denote standard deviations, only positive  
592 standard deviations are reported to simplify the figures. Lines are descriptive trends of each variable for  
593 each year.

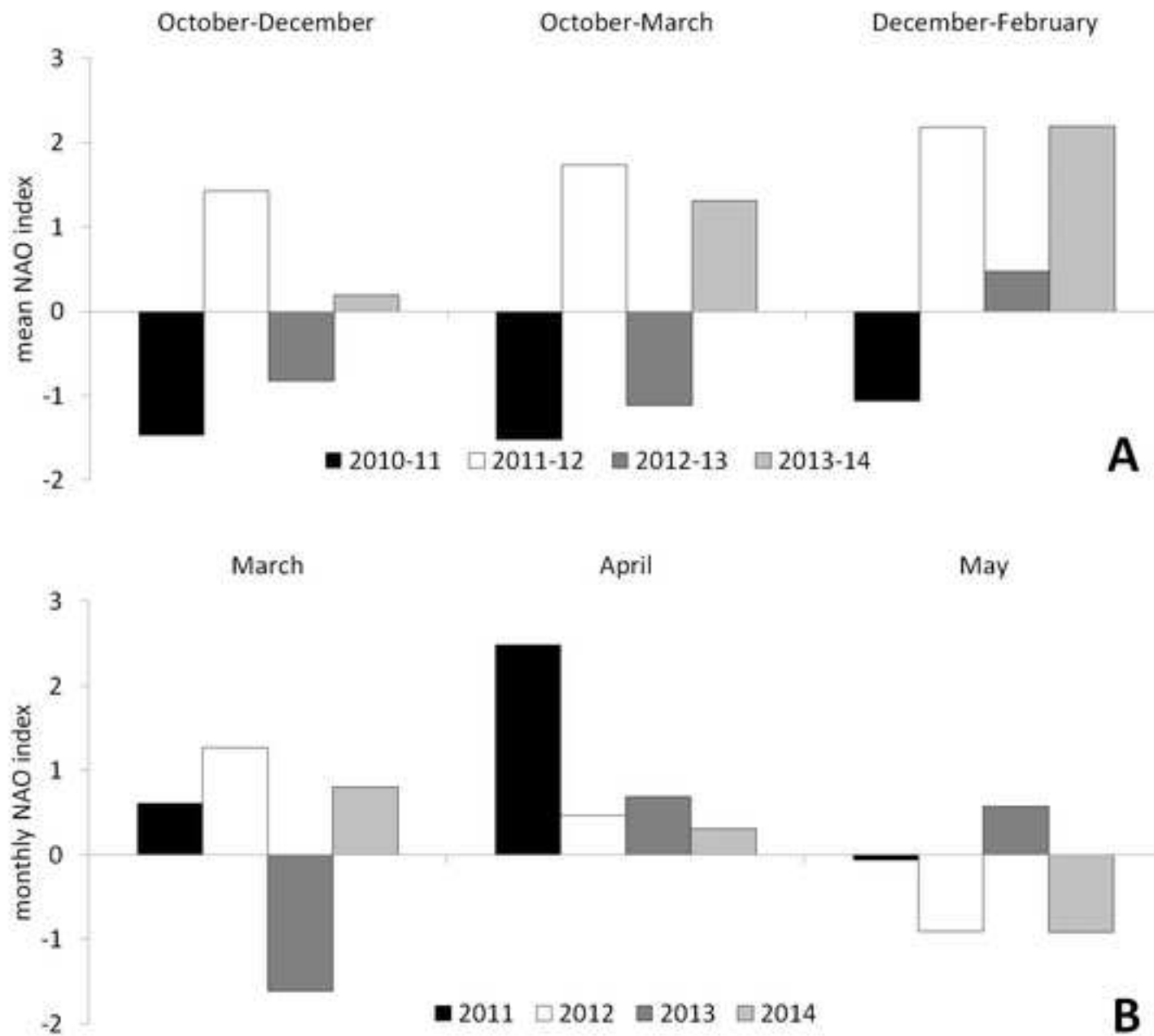
594 **Fig. 6.** Fibre and fragment abundances per individual in the 32 sampling dates. To simplify the figure,  
595 standard deviations are not reported for each sampling date, but they are available in Appendix - Table 1.

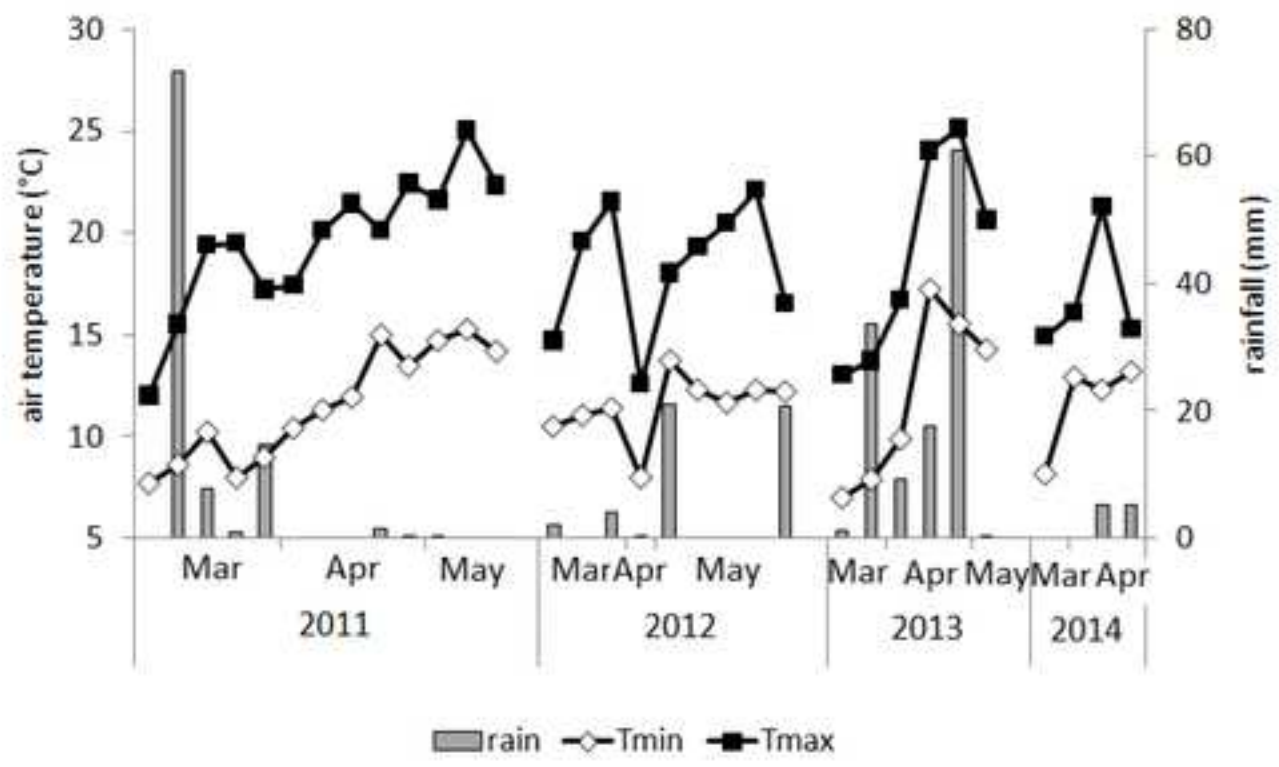
596 **Fig. 7.** Triplot for the RDA results. The explanatory variable vectors (monthly NAO index, winter/cold  
597 season NAO index averages, Tmin, Tmax, and rainfall) are reported with bold lines. The response  
598 variable vectors (incidence of contaminated fish, fibres per individual, fragments per individual, fish  
599 length and weight, Fulton's condition factor, and gonadosomatic index GSI) are reported with thin lines.  
600 The 32 observations (sampling days) are reported with different markers: 2011 full black circle, 2012  
601 empty triangle, 2013 full black square, 2014 empty circle.

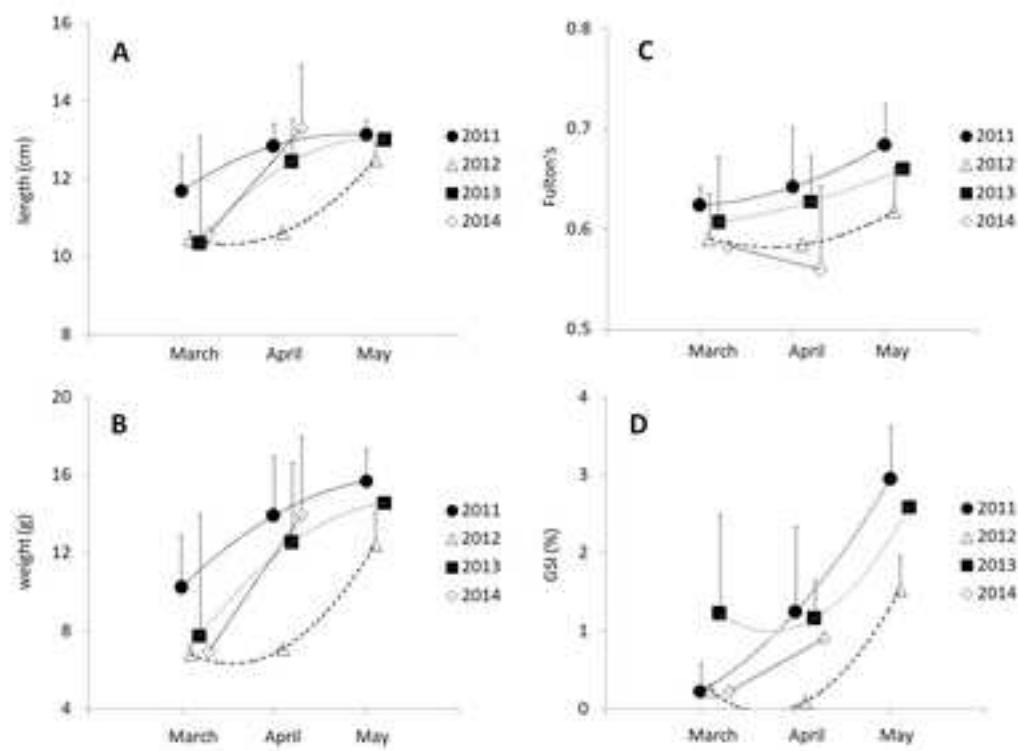


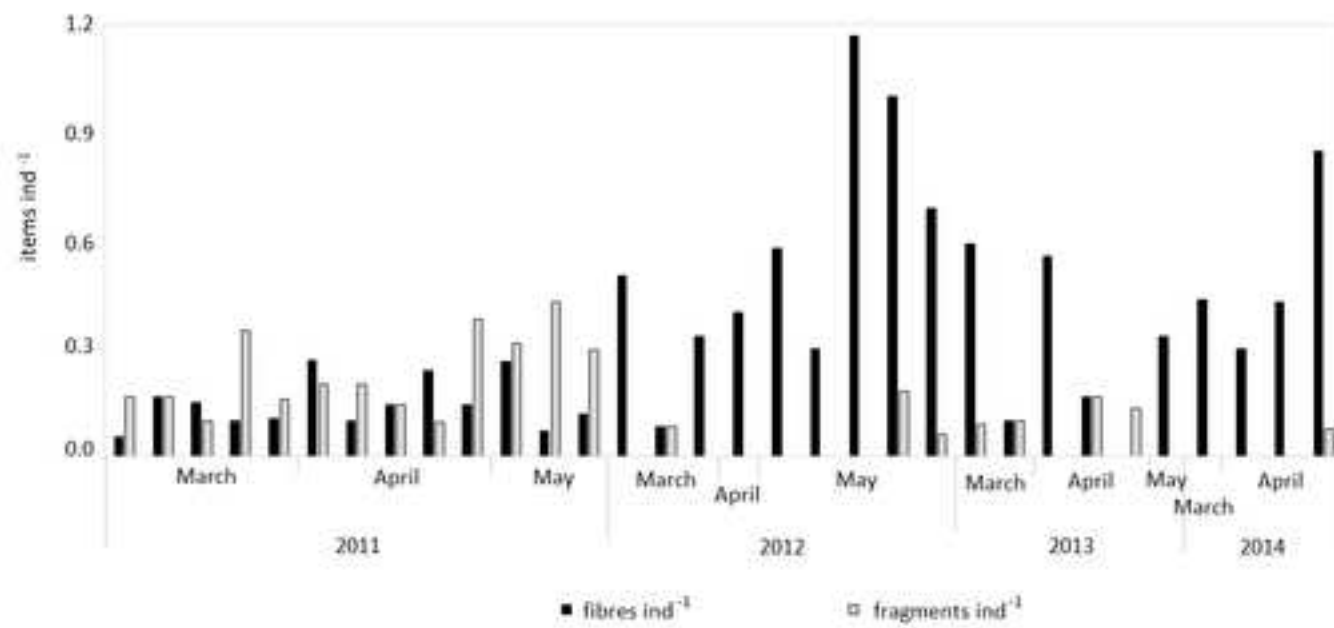












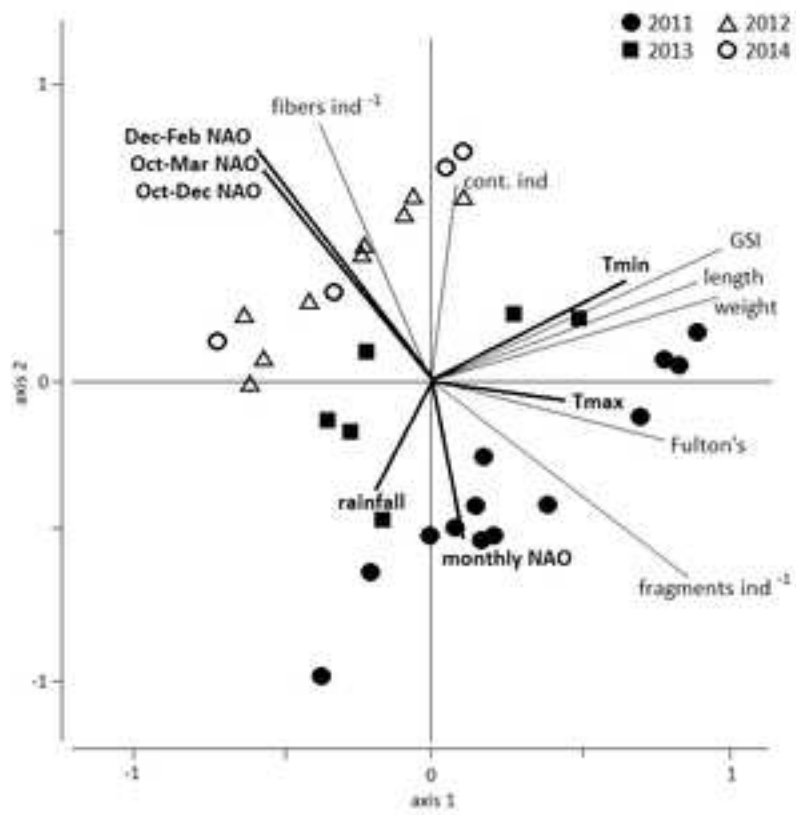


Table 1. ANOSIM pairwise tests among the years 2011-2014 for the environmental variables in the NW Mediterranean.

years	R statistic	significance level (%)
2011, 2012	0.870	0.1
2011, 2013	0.304	1.3
2011, 2014	0.692	0.3
2012, 2013	0.759	0.1
2012, 2014	0.057	28.7
2013, 2014	0.250	8.6



Table 2. Results of RDA applied on fish characteristics and ingested microparticle characteristics (response variables) using environmental characteristics as explanatory variables. Bold values indicate those explanatory variables whose variability significantly ( $p < 0.01$ ) influence the variability of the response variables.

explanatory variables	increase total sum of eigenvalues after including new variable	F statistic and P-values of conditional effects	
		F statistic	P-value
<b>December-February NAO index</b>	<b>0.20</b>	<b>7.336</b>	<b>0.002</b>
<b>Tmin</b>	<b>0.15</b>	<b>6.485</b>	<b>0.002</b>
rainfall	0.05	2.757	0.052
Tmax	0.05	2.388	0.076
monthly NAO index	0.04	1.930	0.112
October-March Nao index	0.01	0.469	0.750
October-December NAO index	0.02	0.865	0.436

Table 3. Significant correlations of the environmental variables (NAO index, air temperature and rainfall) with the fish characteristics (length, weight, Fulton's condition factor, and gonadosomatic index GSI) and the ingested microparticles (proportion of contaminated fish, number of fibres and fragments per individual). N=32, bold p<0.01, dash: not significant.

	length	weight	Fulton's	GSI	% cont. fish	fibre ind <sup>-1</sup>	fragment ind <sup>-1</sup>
monthly NAO index	-	-	-	-	-	-	-
October-December NAO index	-	-	-0.36	-	-	<b>0.63</b>	<b>-0.64</b>
December-February NAO index	-	-0.35	<b>-0.48</b>	-0.36	-	<b>0.59</b>	<b>-0.75</b>
October-March NAO index	-	-	-0.44	-0.35	-	<b>0.59</b>	<b>-0.65</b>
rainfall	-	-	-	-	-	-	-
Tmin	<b>0.54</b>	<b>0.51</b>	-	<b>0.60</b>	-	-	-
Tmax	-	-	-	0.38	-	-	0.43



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**Supplementary Material**  
Misic et al Table1 appendix.docx

