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1 **Title**

2 Microlitter pollution in the marine environment and preliminary evidences of *in vitro* cytotoxic
3 effects on two Mediterranean commercial fish species

4

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18 **Abstract**

19 Marine litter, which is composed mainly of plastics, is recognized as one of the most serious
20 threats to marine ecosystems and a global environmental concern. Microplastics (MPs)
21 densities were estimated in all environmental compartments: marine organisms are highly
22 exposed to and ingest them, resulting in disruption of biological functions. Ecotoxicological
23 approaches have also started elucidating the potential severity of MPs in controlled laboratory
24 studies, but the commercially-available and pristine materials employed hardly reflect the
25 actual composition of the environmental litter, which can be contaminated by chemical
26 pollutants or biological agents. Building on the lack of research employing marine
27 environmental MPs or microlitter as a whole, we characterized the quantity and quality of litter
28 in the coastal epipelagic and in the digestive tract of two commercially-relevant fish species,
29 and exposed primary cell cultures of mucosal and lymphoid organs to marine microlitter. A
30 concentration of 0.30 ± 0.02 microlitter items m^{-3} was found in the water column of the Northern
31 Tyrrhenian sea. μFT-IR analysis revealed that particles of plastic origin, namely
32 polypropylene, HDPE and polyamide, were present in 100% and 83.3% of *M. merluccius* and
33 *M. barbatus* stomachs, respectively, which overall ingested 14.67 ± 4.10 and 5.50 ± 1.97
34 items. Microlitter was confirmed as a vector of bacteria, fungi and flagellates. Lastly, and for
35 the first time, the apical end-point of viability was significantly reduced in splenic cells exposed
36 *in vitro* to two microlitter conditions. Considering the role of the spleen in the mounting of
37 adaptive immune responses, our results warrant more in-depth investigations for clarifying the
38 actual susceptibility of the biota to anthropogenic microliter.

39

40 **Keywords**

41 Marine microlitter; Bioindicators; Cytotoxicity; *In vitro* approaches; Primary cell cultures;
42 Biological agents

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43 **Highlights**

44 - 0.30 ± 0.02 microlitter items m^{-3} was found in the coastal epipelagic Northern
45 Tyrrhenian sea
46 - 14.67 ± 4.10 and 5.50 ± 1.97 items were retrieved from hake and mullet stomach
47 contents
48 - A subsample of the ingested microlitter was of plastic origin
49 - Microlitter was validated as a carrier of bacteria, fungi and flagellates
50 - Splenic cells exposed to both microlitter conditions for 72 hours suffered cytotoxicity

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51 **Figure/Table captions**

52 **Fig. 1** Map of sampling sites - Study area with indications of 250 μm net tows site locations, haul route
53 and bathymetry.

54 **Fig. 2** A-E Microlitter collected from water column - Some examples of microlitter particles collected
55 from the water column by 250 μm net tows (SUP1-SUP4).

56 **Fig. 3** Quali-quantitative characterization of anthropogenic litter collected from the water column - A)
57 Particle density of the micro- and macrolitter fractions per net tow. Average litter density is reported as
58 mean ± SE. B) Cumulative microlitter density per type per net tow. C) Size class distribution per
59 microlitter shape type. D-F) Percentage of color abundance per microlitter shape type.

60 **Fig. 4** Microlitter retrieved from the digestive tract of fish - A) White fragment from *M. barbatus*. B) Black
61 filament from *M. merluccius*. C) Green fragment from *M. merluccius*.

62 **Fig. 5** Quali-quantitative characterization of microlitter retrieved from fish stomach contents - A)
63 Microlitter particle abundance distribution per species (log y scale). Whiskers plotted according to the
64 Tukey method. B) Cumulative particle abundance per type. C) Size class distribution per microlitter
65 shape type per species. D-G) Percentage of color abundance per microlitter shape type per species.

66 **Fig. 6** μFT-IR spectra - Spectra of randomly selected representative samples of microlitter retrieved
67 from fish stomach content. Matching with reference substance as per the Open Specy open source
68 database. r: Pearson's correlation coefficient as measure of linear correlation between data sets.

69 **Fig. 7** A-D Microlitter as a vector of biological agents - Examples of microorganisms observed in cell
70 suspensions following a 72-hour incubation period with microlitter. A) European hake splenic cell,
71 negative control. B-D) Grey, white and black arrowheads indicate bacilliform bacteria, unicellular fungi
72 and flagellates, respectively. Scale bar: 10 μm.

73 **Fig. 8** Quantification of ATP levels as a proxy of cell viability - ATP data distribution per species, time,
74 organ and treatment. Statistical significance as per one-way ANOVA followed by Tukey's HSD *post hoc*
75 test. *: $p < 0.05$

76 **Fig. 9** Comparison of microlitter densities retrieved from representative scientific literature with data
77 herein presented. Color-coded boxes indicate means ± SD items m^{-3}.

78 **Table 1** Sampling details - Summary of the experimental campaign with hauls and net tows details.
79 Time is expressed as UTC/GMT +2:00.

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80 **Table 2** Full statistical details of the rank-based nonparametric Kruskal-Wallis tests performed on
81 microbial counts per species and organ. ns: non significant.
82 **Table S1** Water column microlitter abundance and density (items m^{-3}) per color per net tow.
83 **Table S2** Water column microlitter size classes per particle type per net tow.
84 **Table S3** Full statistical details of the one way ANOVA tests performed on cytotoxic data of A)
85 *Merluccius merluccius* and B) *Mullus barbatus*, per time and organ. DFn: degrees of freedom in the
86 numerator; DFd: degrees of freedom in the denominator; F: test statistic for ANOVA; ges: generalized
87 eta squared.

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88 ## 1. Introduction

89 Coastal areas are subject to an exponential increase in population density and the
90 development of impacting human activities, e.g. industries, tourism, recreational activities,
91 fishing and aquaculture. As a consequence, they can be affected by both sporadic and
92 continuous pollution events, with consequences on all compartments, and are thus considered
93 "hotspots" of contamination (Cole et al., 2011).

94 Marine litter, defined as "any anthropogenic manufactured, or processed solid material
95 (regardless of size) discarded, disposed of, or abandoned in the environment, including all
96 materials discarded into the sea, on the shore, or brought indirectly to the sea by rivers,
97 sewage, storm water, waves, or winds" (UNEP, 2016), is one of the most serious threats to
98 marine ecosystems and a global environmental concern. It includes glass, metal, cardboard
99 and textiles items (Löhr et al., 2017) as well as anthropogenic particles produced by industrial
100 activities (e.g., coal-fired power plants) and transport emissions (Piazzolla et al., 2020) but
101 Tekman et al. (2021) revealed that plastic accounts for the 66-79% of the global litter
102 composition.

103 Annual global plastic production accounted for 348 million tonnes in 2018 (Association of
104 Plastic Manufacturers, 2018): about 1.3-3.1% of these (5-12 million tonnes year-1) reach the
105 Oceans (Jambeck et al., 2015), but the total amount of floating plastic was estimated at 0.3
106 million tons (van Sebille et al., 2015). In addition to primary microplastics (MPs), i.e. particles
107 that are purposefully manufactured of microscopic sizes < 5 mm, the vast majority of marine
108 litter is subject to degradation by abiotic (UV radiation, mechanical abrasion, temperature) and
109 biotic (microbiological depolymerization) agents, resulting into secondary MPs (Ru et al., 2020;
110 Thompson et al., 2004). Their chemico-physical properties, such as type of polymer, density,
111 size, shape, internal geometry and color, influence their transport, buoyancy and sinking as
112 well as rates of ingestion and removal by aquatic organisms (Kowalski et al., 2016; Nguyen et
113 al., 2020; Shim et al., 2018).

114 Due to their small size, MPs are bioavailable for a variety of taxa (e.g. Cole et al., 2013;
115 Gomiero et al., 2018; Lusher et al., 2013; Pittura et al., 2018) and can either be mistaken with

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116 or selectively chosen instead of food (Clark et al., 2016; Moore, 2008), with demonstrated
117 impacts. Once ingested, MPs can affect biological functions and tissue integrity of marine
118 organisms (Cole et al., 2015; Pedà et al., 2016; Sussarellu et al., 2016). Moreover, MPs can
119 be potential carriers of pollutants (Amelia et al., 2021; Guo and Wang, 2019) and can be
120 colonized by microbial pathogens, transferring them along the trophic web (Caruso, 2019;
121 Casabianca et al., 2019). Due to its geographical and oceanographical features, the
122 Mediterranean Sea is regarded as an accumulation zone for marine litter, with marine litter
123 densities comparable to those of the five subtropical gyres (Cózar et al., 2015; UNEP/MAP,
124 2015; Van Sebille et al., 2020).

125 Ecotoxicological and physiological impacts of MPs have not received as much attention and,
126 to the best of our knowledge, no data exist about cytotoxic effects caused by field-collected
127 MPs to cell cultures from fish mucosal and lymphoid organs. The aim of the present study was
128 hence to characterize microlitter abundance in a coastal area of the Northern Tyrrhenian Sea
129 (Italy) in the water column and in the digestive tracts of selected fish species, as well as to
130 evaluate the biological contamination and the potential *in vitro* cytotoxicity of environmentally-
131 collected microlitter particles on fish primary cell cultures. The European hake *Merluccius*
132 *merluccius* (Linnaeus, 1758) and the red mullet *Mullus barbatus* (Linnaeus, 1758) were
133 chosen as models based on biological features, commercial relevance, abundance in the
134 sampling area and their suitability as small-scale plastic pollution bioindicators.

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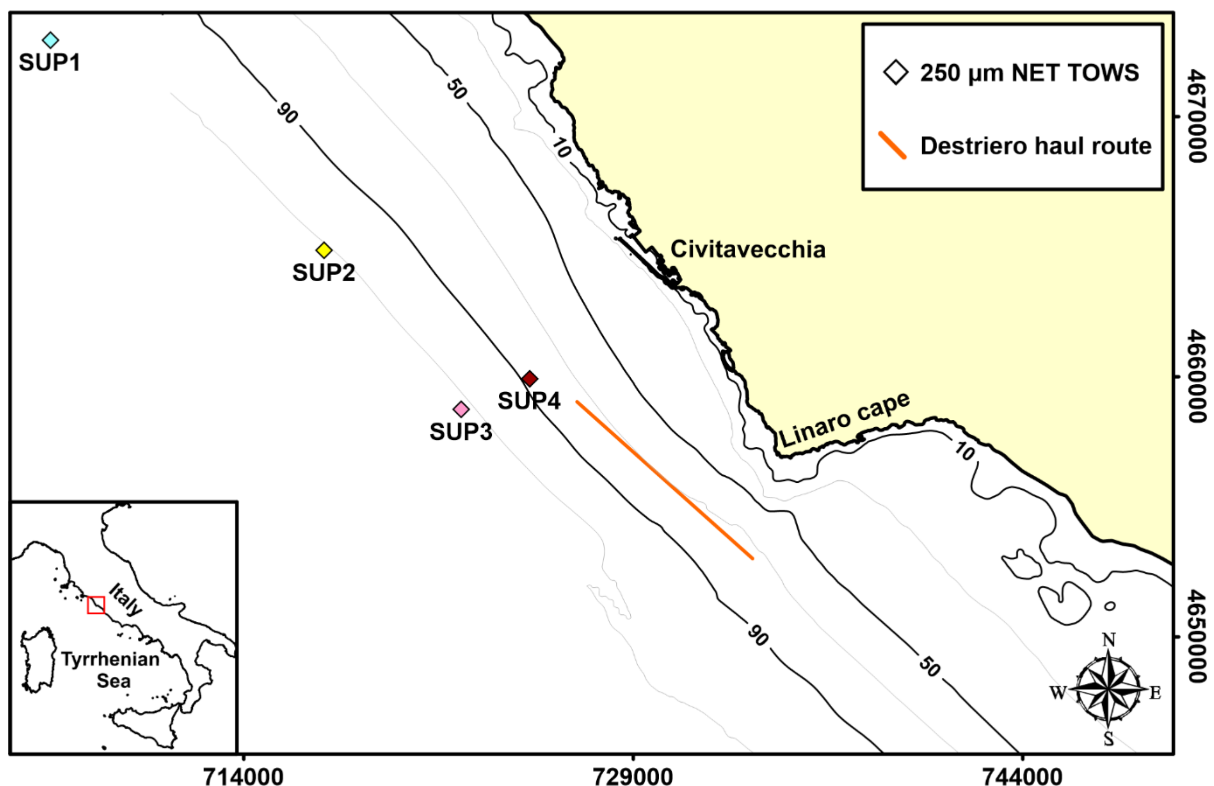
135 ## 2. Material & Methods

136 ### a. Study area

137 The research was conducted in the Northern Tyrrhenian Sea (Italy), FAO's General Fisheries
138 Commission for the Mediterranean (GFCM) Geographical Sub-Area 9 (GSA 9). The
139 experimental campaign fell within the physiographical unit (PU) M. Argentario – Cape Linaro,
140 and included the coastal platform that extends from Santa Severa (42.01676 N, 11.95604 E)
141 to the Tarquinia coastal area (42.22243 N, 11.70495 E). More details about bathymetry and
142 sediment type found in the study area can be found in Mancini et al. (2021).

143 ### b. Experimental campaign

144 The experimental campaign took place on October 23rd 2020 and extended over a 14-hour
145 period (03:00 - 17:00 UTC/GMT +2:00). Four 3-hour long fishing hauls aimed at macrolitter
146 sampling and four 15-minute long horizontal tows with a 250 μm mesh size net aimed at
147 microlitter sampling were performed at 102-115 m depth and sea surface, respectively (Fig.
148 2). Table 1 reports time of start, coordinates and depth per each fishing haul and tow. Meteo-
149 marine conditions are reported as per the World Meteorological Organization sea state coding.



150

151 Fig. 1

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152 **Table 1**

Hauls						250 µm net tows					
Start time	**Start coordinates**	**End coordinates**	**Start depth**	**End depth**	**Macrolitter items Km^{-2} (>1, < 450 cm)**	**Start time (sample #)**	**Start coordinates**	**End coordinates**	**Microlitter items m^{-3} (>0.25, <5 mm)**	**Macrolitter items m^{-3} (>0.5, < 6 cm)**	**Meteo-marine conditions**
04:50	42°05.690 N 11°38.785 E	42°10.888 N 11°30.065 E	105	114	400	07:35 (SUP1)	42°10.883N 11°30.070E	42°10.570N 11°30.470E	0.29	0.008	Calm (rippled)
08:00	42°10.480 N 11°30.704 E	42°06.390 N 11°37.467 E	110	102	312.5	11:15 (SUP2)	42°06.349N 11°37.538E	42°05.789N 11°38.128E	0.23	0.01	Smooth (wavelets)
12:10	42°07.135 N 11°35.469 E	42°03.040 N 11°41.115 E	115	105	363.64	14:05 (SUP3)	42°02.957N 11°41.231E	42°02.4230N 11°41.919E	0.32	0.013	Smooth (wavelets)
14:26	42°02.761 N 11°41.678 E	42°03.383 N 11°42.977 E	108	110	2125	16:45 (SUP4)	42°03.554N 11°43.158E	42°03.522N 11°43.884E	0.35	0.023	Sligth

153

154 **c. Qualitative and quantitative characterization of microlitter in the water**
155 **column**

156 The 250 µm net was equipped with a metered line, a non-filtering cod-end and a flow meter.
157 Upon deployment, utmost care was taken to ensure the net stretched out correctly. Each tow
158 lasted for 15 minutes. Upon retrieval, the content of the net cod-end was transferred to 500 ml
159 containers and stored at 4 °C until transported to the laboratory. Each sample (SUP1- was
160 visually sorted 5 ml at a time using a stereomicroscope (Leica 8APO). Microlitter was classified
161 in terms of shape (i.e. filament, fragment, film), color and size (A: 250 < x < 500 µm; B: 500 <
162 x < 1000 µm; C: 1000 < x < 3000 µm; D: 3000 < x < 5000 µm; E: x > 5000 µm).
163 Microlitter abundances are presented as items m^{-3} of seawater ± standard error (SE).

164 **d. Fish sampling**

165 Fish specimens were collected in the same fishing area (October 30th 2020, 01:11:00 PM haul
166 start time, start coordinates 41°59.669 N 11°49.214 E, end coordinates 42°03.0410 N
167 11°44.4621 E) by means of the bottom trawl net typically known as "volantina" geared with a
168 cod-end mesh size of 50 mm diamond and a vertical opening of 4 m (Sala et al., 2013). *M.*
169 *merluccius* and *M. barbatus* specimens were within the 17.3-20.2 cm and 10.5-12.7 cm total
170 length ranges, respectively: they were employed for the quali-quantitative characterization of
171 microlitter in stomach contents (n=6 per species) and for cytotoxicity assays (n=3 per species).
172 Immediately following the opening of the net, specimens of similar within-species sizes were
173 sorted and immediately transferred in ice and kept refrigerated until lab processing.

174

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175 **e. Characterization of microlitter quantity and quality in fish stomach**
176 **contents**
177 In the laboratory, fish stomachs were sampled and preserved in 75% ethanol until processing.
178 Stomach contents were then placed in a Petri dish and visually sorted using a
179 stereomicroscope (Leica 8APO) to classify microlitter particles in terms of shape, size and
180 color, using the same classification as in section 2c. The microlitter found in the stomach
181 contents was maintained in 50 ml falcon tubes with 0.22 μm-filtered water. Microlitter
182 abundances are presented as mean particles per species ± standard error (items ± SE).
183 **f. μFT-IR analysis**
184 To classify the chemical composition of the microlitter retrieved from fish stomach contents, a
185 representative subsample was analyzed by using Fourier-transform infrared
186 microspectroscopy (μFT-IR). The experiments were performed at the DAFNE Laboratory of
187 INFN (Frascati, Italy) in transmission mode, using a Bruker Hyperion 3000 FTIR microscope
188 equipped with a Globar IR source, a broadband beamsplitter (KBr) and a mercury-cadmium
189 telluride (MCT) detector; the beam size was set at 20x20 μm. Spectroscopic analysis yielded
190 absorbance spectra, which were analysed by using the Open Specy open source database
191 (Cowger et al., 2021) with the Pearson's correlation coefficient as measure of the linear
192 correlation between the data sets. Spectra visualization and overlay were achieved with
193 SpectraGryph v1.2 using the peak normalization method (i.e. each spectrum highest peak
194 within the visible area was set to 1).
195 **g. Assessment of microlitter cytotoxic effects on fish primary cell cultures**
196 Marine microlitter obtained from the same sampling location of fish were stored in 50 ml falcon
197 tubes in 0.22 μm-filtered sea water until employed for cytotoxicity assays. They were dried,
198 randomly selected and counted under a stereo microscope (average item weight 160 μg).
199 Isolation and cultivation of fish primary cells were performed according to published standard
200 procedure. Gills (G), head kidney (HK) and spleen (SPL) from *M. merluccius* and *M. barbatus*
201 specimens (n=3) were removed and immersed in cold Hanks Balanced Salt Solution without
202 calcium and magnesium (HBSS), previously adjusted for appropriate sea water osmolarity

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203 (355 mOsm Kg^{-1}) with 3M NaCl. Cells were obtained by pushing organs with a plastic pestle
204 in cold HBSS through 100 and 40 µm nylon mesh strainers and washing by centrifugation (10
205 min, 400 g, 4 °C. Subsequently, cells were resuspended in sterile L-15 (Leibovitz) medium
206 containing 10% heat-inactivated fetal calf serum (FCS, Gibco) and antibiotics (penicillin–
207 streptomycin, Gibco). Cells were counted in a Neubauer chamber and adjusted to a
208 concentration of $5x10^5$ cells ml^{-1} in L-15 medium. Six hundred microliters of the cellular
209 suspensions were cultured at 15°C and exposed to 4 and 20 field-collected microlitter
210 particles, representing the “Low” and “High” conditions respectively, with the lowest microlitter
211 concentration being in line with future modelled estimates (Isobe et al., 2019). Sterility was
212 ensured in all microliter and cell preparation phases by working under laminar flow cabinet.
213 Cells were treated for 2 and 72 hours at 15°C with gentle rotary shaking to ensure a continuous
214 contact with microlitter particles. Because the bottom trawl net was not equipped with any
215 temperature sensor, the incubation temperature was selected according to the near real-time
216 numerical model MEDSEA_ANALYSISFORECAST_PHY_006_013 (Clementi et al., 2021),
217 resolving for variable “sea_water_potential_temperature_at_sea_floor (bottomT)” using
218 sampling location, date and depth as input. A negative control consisting of cells incubated at
219 same conditions without microliter was tested. Three technical replicates per biological sample
220 were used in all experimental groups. Intracellular ATP value, as a proxy of cell
221 viability/cytotoxicity (Schoonen et al., 2005), was then quantitatively evaluated using the
222 ATPlite assay (PerkinElmer) in 96 well plates following the manufacturer’s instructions: 50 µl
223 of cell lysis and 50 µl substrate solutions were added to 100 µl cell suspensions per replicate
224 and shaken for 5 min. Resulting homogenates were transferred to opaque well plates
225 (OptiPlate-96, PerkinElmer) and luminescence was measured using a microplate reader
226 (Wallac Victor2, PerkinElmer), following a 10-minute dark adaptation period.

227 **h. Validation of microlitter as a carrier of biological agents**

228 Following a 72-hour incubation of primary cultures with microlitter following an alike
229 experimental design as above, 10 µl of cell suspension from each experimental group was
230 qualitatively observed under a Zeiss microscope equipped with a colour 8 video camera

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231 (AxioCam MRC) and a software package (KS 300 and AxioVision). Multiple sets of
232 photographs at random frames were taken per each experimental group and total counts of
233 microorganisms were quantified over a 100.000 μm^2 area by an operator unaware of
234 treatments.

235 **i. Data analysis, visualization and statistics**

236 Stomach content particle abundance was tested for statistical significance between species
237 using an independent samples t-test with the null hypothesis of equal population means
238 between groups. Datasets were checked for normality and homoscedasticity. A log-
239 transformation was applied to meet the normal distribution assumption.
240 The relation among species and ingestion of microlitter particle types by color was examined
241 with a chi-square test on a two-way contingency table. The null hypothesis assumed no
242 association between variables. Results are reported as χ^2_{df}=test statistic.
243 Cytotoxicity data, grouped by species, time, organ and treatment, were tested for statistical
244 significance using a one-way ANOVA with the null hypothesis of equal population means
245 among groups, followed by a Tukey's HSD *post-hoc* test in case the main effect of the models
246 was significant. Datasets were checked for normality and homoscedasticity.
247 Microbial count was analyzed with the rank-based nonparametric Kruskal-Wallis test because
248 datasets, many of which were zero-inflated, did not meet the assumptions for parametric
249 testing. The null hypothesis was that samples were drawn from the same population or from
250 populations whose medians did not differ.
251 A comparison of microlitter densities retrieved from representative scientific literature was
252 visualized as mean±SD items m^{-3} with the R "forestplot" package v2.0.1. Data reported in other
253 units than items m^{-3} was excluded from the analysis. Studies were organized hierarchically by
254 location and year, and box size was set to constant.

255 **j. Ethics statement**

256 Ethical review and approval was not required for this study because animals were sampled
257 from the marine environment in strict compliance of the provisions of Directive 2010/63/EU on

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258 the protection of animals used for scientific purposes, and were not subject to any
259 experimental manipulation.

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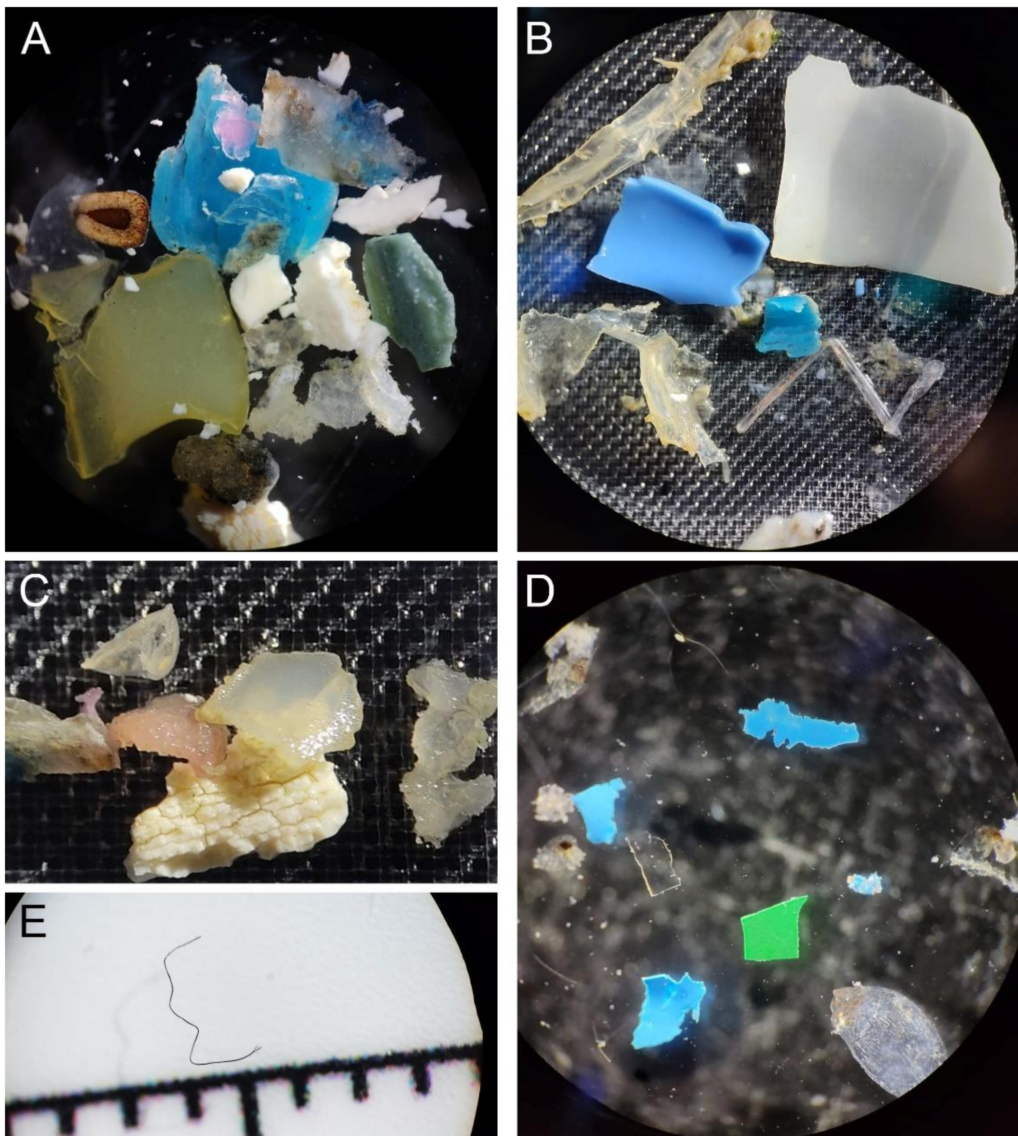
260 ## 3. Results

261 ### a. Qualitative and quantitative characterization of microlitter in the water
262 column

263 Anthropogenic marine litter mainly of plastic origin and in the form of filaments, fragments and
264 films was found in all water samples taken in the Civitavecchia area, i.e. SUP1-SUP4 (Fig. 2,
265 Table 1). Both microlitter (250 μm < x < 5 mm) and macrolitter (5 mm < x < 6 cm) categories
266 were identified.



267

268 **Fig. 2**

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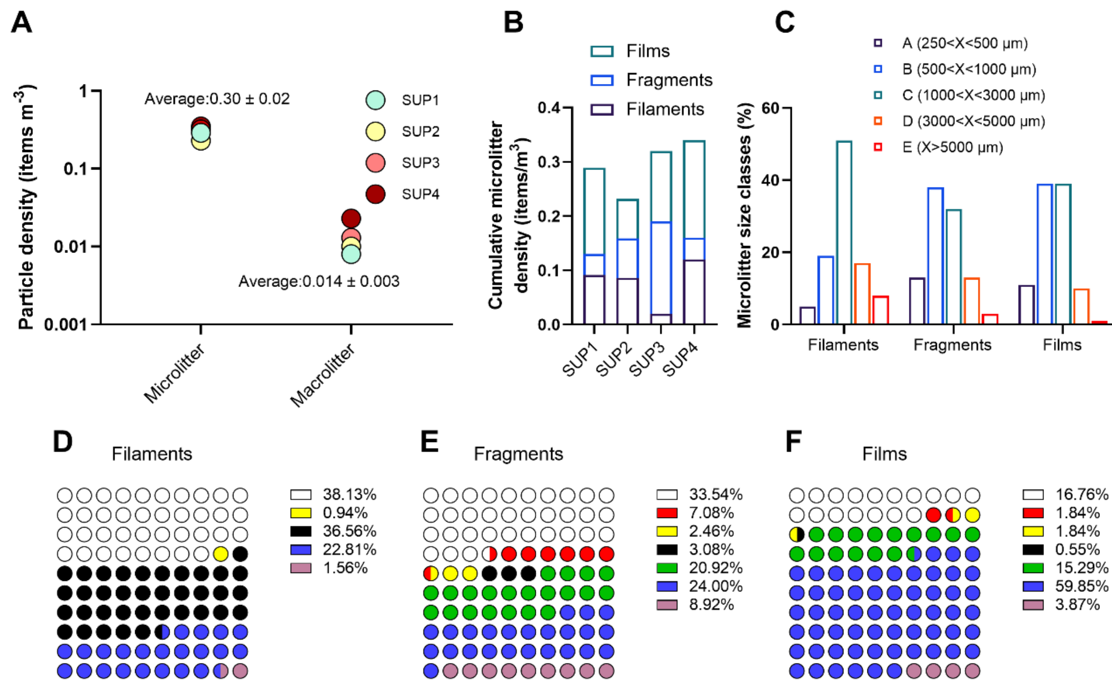
269 SUP4 and SUP2 had the highest and lowest microlitter particle densities with 0.35 and 0.23
270 items m^{-3}, respectively (Table 1). SUP4 and SUP 1 had the highest and lowest macrolitter
271 particle densities with 0.023 and 0.008 items m^{-3}, respectively (Table 1). Their average
272 abundance among all samples was 0.30 ± 0.02 items m^{-3} and 0.014 ± 0.003 items m^{-3} (Fig.
273 3A).



274

275 **Fig. 3**

276

277 All following data refer to the microlitter fraction. With regards to particle shape, filaments,
278 fragments and films were observed. In particular, the highest and lowest concentration of
279 filaments were found in SUP4 (0.12 items m^{-3}) and SUP3 (0.02 items m^{-3}), respectively; the
280 highest and lowest concentration of fragments were found in SUP3 (0.17 items m^{-3}) and SUP1
281 (0.039 items m^{-3}), and the highest and lowest concentration of films was found in SUP4 (0.18
282 items m^{-3}) and SUP2 (0.07 items m^{-3}) (Fig. 3B and Table S1).
283 The vast majority (51%) of filaments belonged to size class C and a smaller population (5%)
284 to size class A (5%). Fragments fell for the most part within size class B (38%), while size
285 class E was the least represented (3%). Films mostly belonged to size classes B and C (39%),
286 while size class E was found in the 1% of cases (Fig. 3C and Table S2).

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287 Filaments from all tows were mostly white (38%) and black (37%); less frequent colors in terms
288 of abundance were blue (23%) and yellow (1%). Fragments were more chromatically
289 diversified: while most of them were white (34%), blue and green fragments were found with
290 a percentage of 24% and 21%, respectively; red (7%), black (3%) and yellow (2%) fragments
291 were less abundant. Films were mostly blue (60%), followed by white (17%) and green (15%);
292 yellow (2%) and red (2%) films were less frequent (Fig. 3D-F and Table S1).

293 **b. Characterization of quantity, quality and chemical composition of**
294 **microlitter in fish stomach content**

295 Microlitter particles were found in 100% *Merluccius merluccius* specimens and in 5 out of 6
296 (83.3%) *Mullus barbatus* (Fig. 4).

297

298 **Fig. 4**

299

300 A higher abundance of microlitter was found in the stomach contents of hake (14.67 ± 4.10
301 items) than mullet (5.50 ± 1.97 items), but the difference between group means was not
302 statistically significant (t_{10}=2.045, p=0.068) (Fig. 5A).

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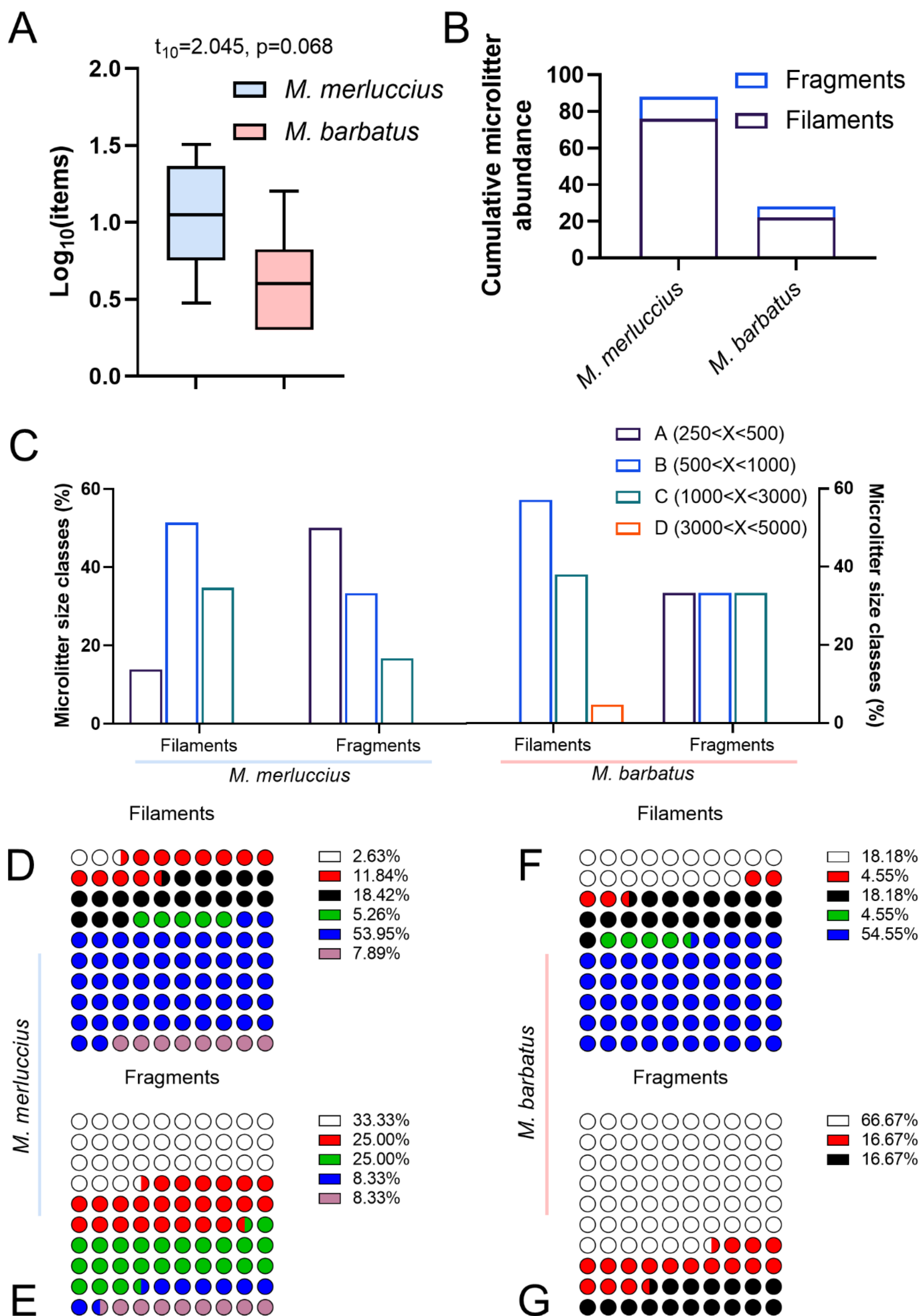

303

304 **Fig. 5**

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305 The retrieved microlitter was classified as filaments and fragments, and no films were found.
306 Filaments were the most abundant shape type, with 76/88 particles (83.36%) in hake and
307 22/28 (78.57%) in mullet, respectively (Fig. 5B).
308 *M. merluccius* ingested mostly filaments and fragments in the B and A size ranges,
309 respectively. The most represented filament size class in *M. barbatus* stomach contents was
310 B , while fragments were equally assigned to the three size classes (Fig. 5C).
311 Filaments found in the stomach contents of *M. merluccius* were generally blue (53.95%) and
312 black (18.42%) followed by red, green, white and other colored types (all below 12%). Blue,
313 black and green filaments were found in similar percentages also in *M. barbatus* (54.55%,
314 18.18% and 4.55%, respectively). Microlitter fragments in hake and mullet were mostly white
315 (33% and 66.67%, respectively) and red (25% and 16.67%, respectively); however, green,
316 blue and other colored-fragments were retrieved only from hake, while black fragments were
317 only found in mullet (Fig. 5D-G). Differences in microlitter stomach content by color between
318 species was not statistically significant either for filaments ($X^2_{(5)}$=9.38, p=0.094) or fragments
319 ($X^2_{(5)}$=5.63, p=0.34).
320 A representative subsample of item types found within stomach contents of both species was
321 analyzed by μFT-IR. Particles of plastic origin, namely polypropylene, HDPE and polyamide
322 (87%, 72% and 58% match with corresponding reference spectra, respectively), were
323 identified (Fig. 6 A-C).

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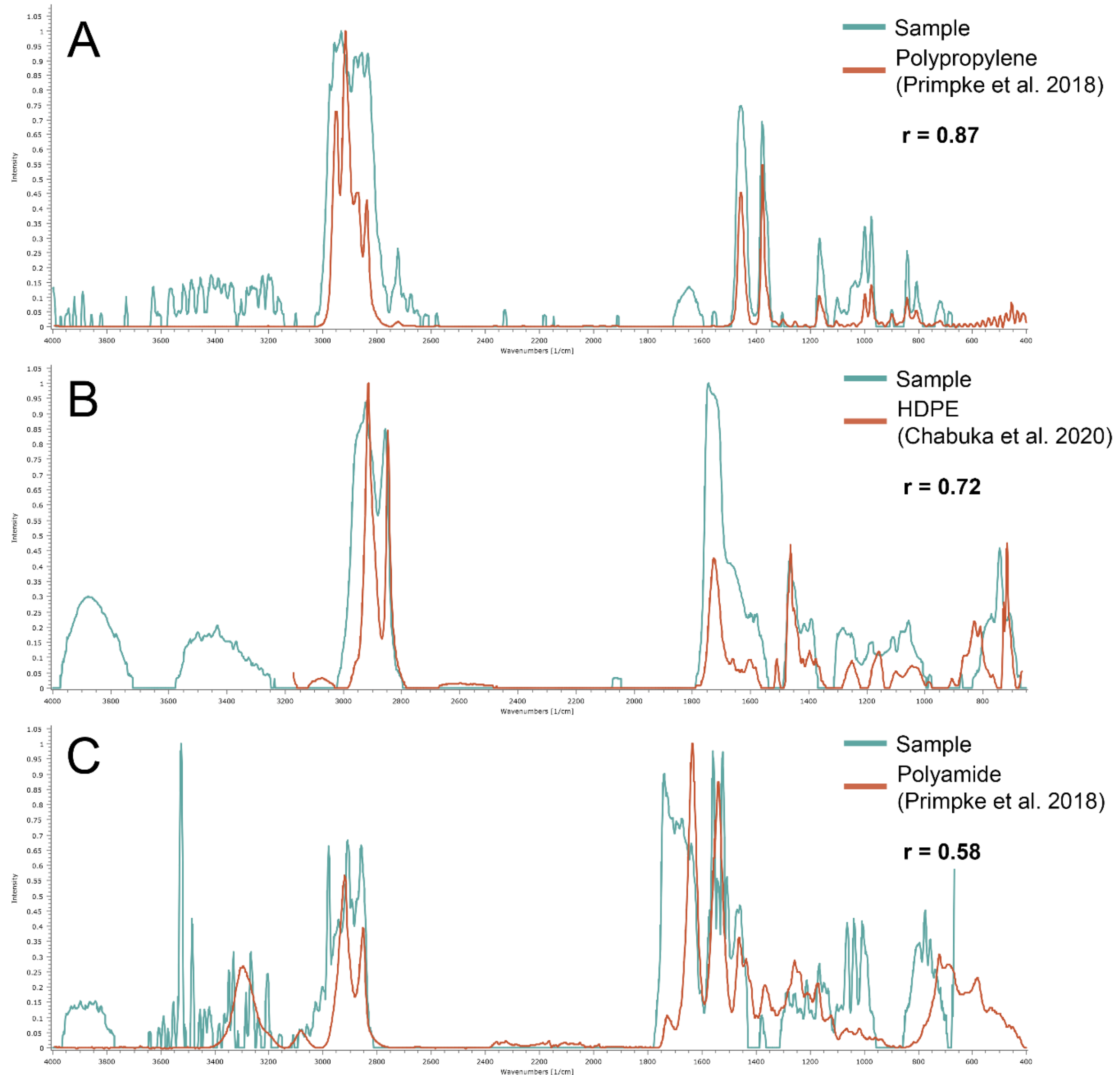

324

325 **Fig. 6**

326

327 **c. Microlitter as carrier of biological agents to *M. merluccius* and *M.***
328 ***barbatus* cells**

329 To evaluate the presence of microorganisms and consequently validate the role of microlitter
330 as carrier of biological agents, 10 µl cell suspension from all cultures following a 72-hour
331 incubation were qualitatively assessed by optical microscopy. Numerous bacilliform bacteria
332 (grey arrowheads), unicellular fungi (white arrowheads) and flagellates (black arrowheads)
333 were observed in microlitter-exposed samples (Fig. 7 B-D) but not in the controls (Fig. 7 A).

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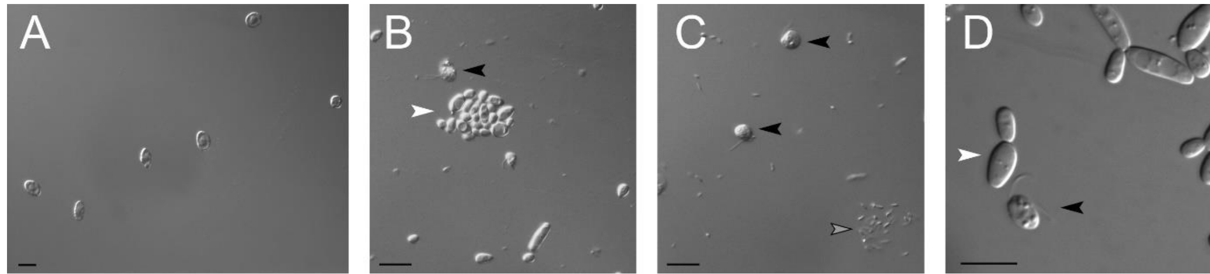
334 

335 **Fig. 7**

336

337 Their abundance was quantified over a 37.000 μm^2 area and normalized to 100.000 μm^2 area
338 per species, organ and microlitter concentration. A variable degree of biological contamination
339 was found in conditioned primary cultures of both species (Table 2).

340 **Table 2**

		Mean microbial count			SE microbial count			95% CI microbial count [L;U]			Statistical test		
Species	**Organ**	ctrl	low	high	ctrl	low	high	ctrl	low	high	H	p	
M. merluccius	Gills	0	269	241.2	0	143.8	57.95	0;0	-349.8;887.8	-8.14;490.5	5.609	0.068	ns
	Head kidney	0	14.4	23.31	0	7.00	3.91	0;0	-15.79;44.48	6.49;40.13	6.161	0.025	*
	Spleen	0	8.07	48.42	0	5.6	34.97	0;0	-16.02;32.16	-102.0;198.9	5.162	0.1	ns
M. barbatus	Gills	0	0	18.83	0	0	4.11	0;0	0;0	1.15;36.51	7.624	0.036	*
	Head kidney	0	0	17.04	0	0	5.88	0;0	0;0	-8.26;42.34	7.624	0.036	*
	Spleen	0	5.38	23.31	0	5.38	13.92	0;0	-17.77;28.53	-36.58;83.21	4.587	0.107	ns

341

342 For the hake, a significant effect of microlitter concentration on microbial counts was found
343 only in HK primary cultures (H statistics=6.16, p=0.025). For the mullet, statistical significance
344 was evidenced for microbial counts in G and HK (H statistics=7.62, p=0.036).

345 **d. Microlitter cytotoxicity in *M. merluccius* and *M. barbatus* cells**

346 Microlitter cytotoxicity was evaluated based on the viability of cells from G, HK and SPL
347 following a 2- and a 72-hour long incubation with two environment-sampled microlitter
348 concentrations (Fig. 8).

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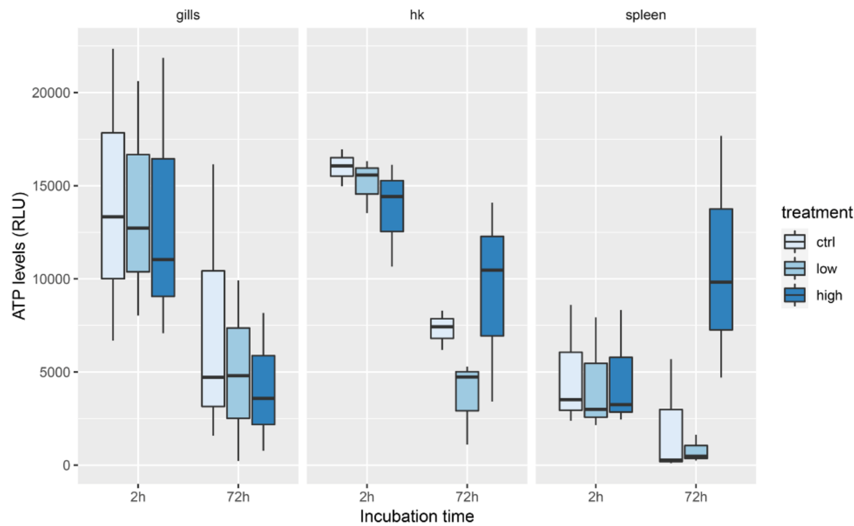
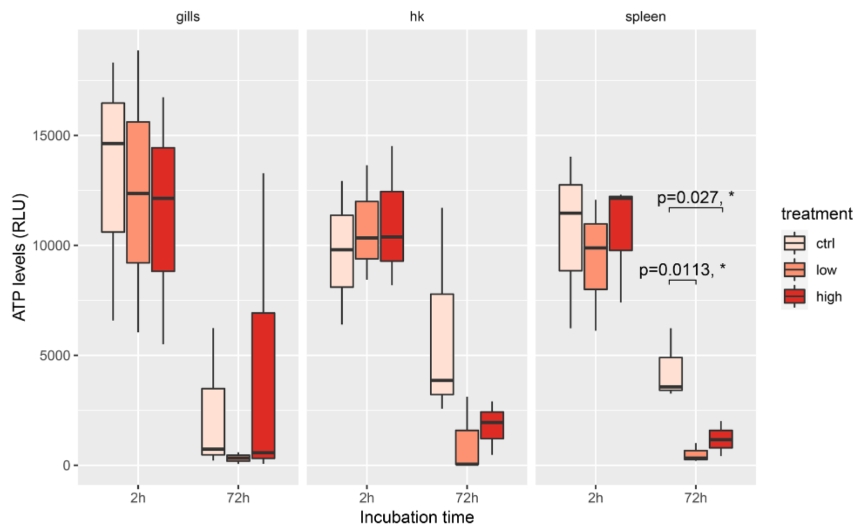
M. merluccius



M. barbatus



349

350 **Fig. 8**

351

352 Exposure to microlitter did not induce any statistically significant decrease in intracellular ATP
353 following the short incubation time in either species or organ (Table S3a-b).
354 In *M. merluccius*, microlitter induced a decrease in median cell viability after a 72-hour
355 incubation in primary G cultures at the high concentration and in both HK and SPL cultures at
356 the low concentration. The linear model fit to splenic cell culture data following the 72-hour
357 exposure explained the 62.3% of ATP levels variation, even though differences among
358 experimental groups were slightly non-significant (p=0.054) (Table S3a).
359 In *M. barbatus* primary cultures from all organs, the median intracellular ATP levels in the Low
360 and High groups were lower than those of respective controls. Such a decrease revealed a

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361 statistically significant main effect of microlitter concentration on splenic cells ($F(2,6) = 10.8$, p
362 $=0.01$), with the overall treatment effect explaining almost 80% of ATP levels variation of the
363 model ($\eta 2 = 0.783$) (Table S3b). Pairwise comparisons between control and the “Low” and
364 “High” groups were statistically significant (p adjusted = 0.0113 and 0.027, respectively) (Fig.
365 8).

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366 **4. Discussion**

367 In this work we applied a multidisciplinary approach combining oceanographical,
368 spectroscopical, cellular and microscopical methods to characterize the quality and quantity
369 of microlitter particles in the coastal epipelagic water column and in the digestive tract of two
370 commercially-valuable Mediterranean fish species; we also preliminarily addressed the
371 cytotoxic potential of field-collected microlitter on primary cultures of cells extracted from
372 mucosal (gills) and lymphoid (head kidney and spleen) organs.

373

374 The marine litter causes multiple environmental, economic, social, political and cultural
375 impacts (Barboza et al., 2019; Galgani et al., 2019; GESAMP, 2015; UNEP, 2014), especially
376 to the health and functioning of organisms and ecosystems (Corinaldesi et al., 2021; Garcia-
377 Vazquez et al., 2018; Rios et al., 2007). At the European level, such pollutant was included
378 among the 11 qualitative descriptors of the Marine Strategy Framework Directive upon which
379 the quality of the marine environment is assessed (European Parliament, 2008/56/EC). Since
380 2004, when the term microplastic was coined, extensive research has demonstrated the
381 ubiquity of plastic pollution in several matrices such as beaches (Fortibuoni et al., 2021;
382 Prevenios et al., 2018), sediments (Piazzolla et al., 2020; Renzi et al., 2018) and seawater
383 (Atwood et al., 2019; Capriotti et al., 2021) - regardless of how remote they are (Cincinelli et
384 al., 2017; Lusher et al., 2015). Microlitter was retrieved from all water samples taken within
385 the framework of the PISCES project in a much higher (~20-fold) average concentration (0.30
386 ± 0.02) than litter particles > 5 mm (0.014 ± 0.003 items/m^3). Keeping in mind the
387 environmental and biological severity of litter < 5 mm, our results are in good agreement with
388 microlitter concentrations reported from other areas of the Mediterranean Sea, Yellow Sea
389 and oceanic waters (Baini et al., 2018; Cincinelli et al., 2017; Collignon et al., 2012; Constant
390 et al., 2018; Cózar et al., 2015; de Lucia et al., 2018, 2014; Expósito et al., 2021; Fagiano et
391 al., 2022; Fossi et al., 2012, 2016; Kazour et al., 2019; Lusher et al., 2015; Panti et al., 2015;
392 van der Hal et al., 2017; Zhang et al., 2017; Zhao et al., 2014) (Fig. 9), suggesting that even
393 surveys that are not extensive in either duration or sample sizes can reliably capture the extent

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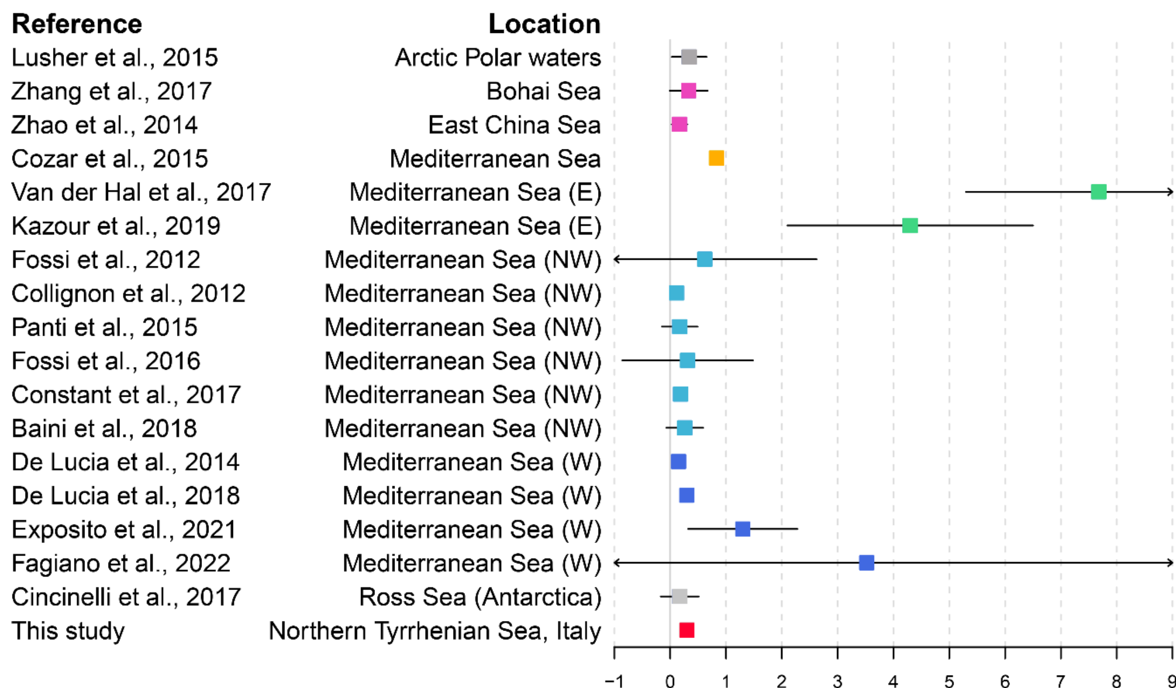
394 of microlitter pollution. This is auspicable to minimize the impact of research-related
395 anthropogenic activities. An exception was represented by the Eastern Mediterranean Sea,
396 which appears to be much more polluted than the western basin. We must highlight that data
397 dispersion could not be quantified from Cózar et al. (2015) and Constant et al. (2018) as only
398 as mean items m^{-3} were reported, and that data from Vasilopoulou et al. (2021) was discarded
399 because of non-informative results (41.31±112.05 mean±SD items m^{-3} - SD could be back-
400 calculated from standard error because a sample size was clearly indicated by authors).



401

402 **Fig. 9**

403

404 The relationship between the marine biota and microlitter was so far mostly evaluated by
405 ingestion rates (e.g. Rios-Fuster et al., 2019; Savoca et al., 2019). Here we confirmed that
406 both our target species ingest plastic materials that range in size from 250 to 3000 μm and
407 are represented by polypropylene, high density polyethylene and polyamide items (Chabuka
408 and Kalivas, 2020; Primpke et al., 2018). Benthic macrolitter in the area was recently
409 described quali-quantitatively, and the most abundant categories were attributed to plastic
410 (Mancini et al., 2021). We call the attention on the fact that the same chemistry was also
411 demonstrated for items sampled from marine sediments (Piazzolla et al., 2020) and in the

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412 atmosphere (Lucci et al., 2021) of the same area, pointing to the high and pervasive dispersion
413 of anthropogenic litter in multiple environmental compartments.
414
415 MPs are thought to be mistaken for or even purposefully chosen instead of food (Clark et al.,
416 2016; Ling et al., 2017) probably also depending on their color (Du et al., 2021; Wright et al.,
417 2013). The presence of both shape types in the stomach of *M. merluccius* and *M. barbatus*
418 and the lack of statistically significant differences based on the chromatic factor support the
419 idea that microlitter may be ingested non-selectively by these two species, even though a
420 biomagnification origin cannot be excluded. Hake and mullet were chosen as experimental
421 models for a variety of reasons: on one hand they are among the most targeted demersal fish
422 species by the Mediterranean deep-sea fisheries and the two most fished target species in
423 the shallow area of the coastal sector (Sabatella et al., 2017; Tiralongo et al., 2021); on the
424 other, they are regarded as bioindicators in coastal marine ecosystems and display a benthic
425 feeding behavior in part of (juvenile *M. merluccius*) or throughout their lifespan (*M. barbatus*)
426 (Carrozzi et al., 2019; Esposito et al., 2014). Moreover, some authors already described the
427 occurrence of MPs in these two species (Atamanalp et al., 2021; Avio et al., 2015; Bellas et
428 al., 2016; Digka et al., 2018; Giani et al., 2019; Mancuso et al., 2019) and MPs were
429 demonstrated to abound in superficial sediments in the study area (Piazzolla et al., 2020).
430
431 A lower number of studies aimed at also elucidating physiological impacts exposed fish to
432 pristine commercially-available MPs under controlled laboratory conditions. Their
433 bioavailability was demonstrated and effects such as altered feeding behaviour, metabolic
434 disorders, energy depletion, growth impairment, delayed development, compromised immune
435 response, reproduction and lifespan were reported (Botterell et al., 2019; Espinosa et al.,
436 2019, 2017; Guerrera et al., 2021; Mazurais et al., 2015; Rios-Fuster et al., 2021; Sendra et
437 al., 2021; Yong et al., 2020).
438 Recently, beach-sampled microlitter was employed in *in vivo* experiments on the European
439 sea bass *Dicentrachus labrax* (Zitouni et al., 2021) and medaka *Oryzias latipes* (Pannetier et

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440 al., 2020) to investigate survival, development, uptake, oxidative stress and genotoxicity
441 following the administration of a microlitter-spiked feed. Their results showed the ability of
442 environmental microplastics to i) accumulate in fish organs, ii) significantly affect the activity
443 of enzymes involved in the antioxidant defense system and iii) induce DNA damages following
444 acute exposures. HK primary cultures were also employed to define the impacts of non-
445 environmental MPs on the abundance and antibody response of B lineage cells in rainbow
446 trout (Zwollo et al., 2021): a lower rate of B cell development together with reduced expression
447 of Ig heavy chain genes were found, suggesting that not only innate but also adaptive immunity
448 may be threatened by MPs.
449 Despite some similarities with the three just-mentioned studies may be perceived, we must
450 highlight that no other research had ever investigated the apical cytotoxic event in primary cell
451 cultures derived from select fish mucosal and lymphoid organs following their exposure to
452 microlitter that had been collected in the same water column from where animals originated
453 (search conducted on Web of Science on October 24, 2021). We believe that our results,
454 obtained in an attempt to bridge the fields of biological oceanography and experimental
455 toxicology, are biologically significant because i) microlitter particles and fish specimens
456 originated from the same sampling site, ii) microlitter cytotoxicity was measured by the well-
457 established and unambiguous direct luciferase-based quantification of cellular ATP (Cree and
458 Andreotti, 1997; Mahto et al., 2010) iii) primary cultures were obtained from organs that are
459 critical for ensuring immune barrier and competency and iv) the strategy suitability for testing
460 for MP toxicity was overall demonstrated and recently reviewed in details (Revel et al., 2021).
461 In addition, fish have been increasingly established as experimental models in the fields of
462 biomedical sciences and toxicology because they share many similarities with higher
463 vertebrates immunology-wise (Miccoli et al., 2021; Scapigliati et al., 2018).
464
465 It was known that MPs are a carrier of biological agents (Amaral-Zettler, 2019; Kiessling et al.,
466 2015), and our data confirm this. Because a dedicated experiment aimed at molecular
467 taxonomy could not be set up due to limited microlitter availability, flagellates were attempted

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468 a classification on phenotypic properties. Based on flagellar features and because they are
469 extremely common in marine plankton, where they can be found free-swimming or attached
470 to bacterial mats or other surfaces, we suggest that flagellates belong to *Paraphysomonas*
471 sp., *Spumella* sp. or aloricate Bicosoecida. The fate of MPs in the water column and sediments
472 can be influenced by microbes (Rogers et al., 2020). Once the microlitter is ingested, its
473 associated microorganisms may colonize the gastrointestinal tract of the host, possibly
474 affecting its welfare: in fact, harmful microorganisms, including potential human and animal
475 pathogens, were found associated to litter (Zettler et al., 2013) and, according to Zwollo et al.
476 (2021), serious consequences may arise due to the reduced ability to respond adequately to
477 pathogens because of suboptimal humoral immune responses.
478
479 Taking into account cytotoxicity data (Fig. 8) and the lack of statistically different microbial
480 counts observed in *M. barbatus* spleen cultures (Table 2), splenic cell subpopulations
481 appeared to be the most sensitive to microlitter exposures among all investigated organs. No
482 further reduction in ATP levels were seen in the High compared to the Low condition,
483 suggesting that such a pollutant can impact cell viability also at low concentrations that are in
484 line with modelled estimates over the next three decades. These results are concerning
485 because spleen, together with thymus and kidney, is the major lymphoid organ of teleosts
486 where adaptive immune responses are generated (Flajnik, 2018; Zapata et al., 2006). It is
487 important to highlight that neither the physiological endpoints reported in the large majority of
488 scientific literature nor our results herein presented provide insight into the molecular
489 mechanisms underlying microlitter toxicity pathways; rather, they inform about apical events
490 manifested either by the whole organism or primary cell cultures, respectively. However, the
491 novelty of our approach was to provide data on a lower, possibly more predictive, level of
492 biological organization (cellular vs. organismal) by means of so-called New Approach
493 Methodologies, which heavily rely on *in vitro* testing. This is compliant with the 3Rs principle
494 in animal testing in addition to having been validated by the latest internationally-agreed test

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495 guidelines (OECD, 2021) and supported by regulatory toxicology roadmaps (e.g. EPA's
496 strategic vision).
497
498 ## 5. Conclusion
499 In conclusion, the present study has characterized the anthropogenic litter in the coastal
500 epipelagic Northern Tyrrhenian Sea and the digestive tract of commercially-relevant fish
501 species, validated the microliter fraction as a carrier of biological agents and, for the first time,
502 demonstrated that splenic cell viability is negatively affected following exposure to such a
503 contaminant. Future investigations with larger sample sizes, cell cultures from additional
504 organs, either primary or continuous, and more in-depth methodological approaches are
505 warranted for clarifying possible differences in susceptibility of the biota to anthropogenic
506 microliter.

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507 **6. Author contribution**

508 AM: Conceptualization, Funding acquisition, Data curation, Formal analysis, Visualization,
509 Supervision, Project administration, Writing - original draft, Writing - review.
510 EM: Conceptualization, Funding acquisition, Investigation, Writing - Review & Editing.
511 PRS: Methodology, Investigation, Writing - original draft, Writing - Review & Editing.
512 GDV: Methodology, Resources, Writing - Review & Editing.
513 GS: Resources, Supervision, Writing - Review & Editing.
514 SP: Supervision, Writing - Review & Editing
515

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528 ## 8. References

529 Amaral-Zettler, L., 2019. Plastics: Colonization and Degradation, in: Reference Module in Life Sciences. Elsevier. doi:10.1016/B978-0-12-809633-
530 8.90685-X
531 Amelia, T.S.M., Khalik, W.M.A.W.M., Ong, M.C., Shao, Y.T., Pan, H.-J., Bhubalan, K., 2021. Marine microplastics as vectors of major ocean
532 pollutants and its hazards to the marine ecosystem and humans. Prog. Earth Planet. Sci. 8, 12. doi:10.1186/s40645-020-00405-4
533 Association of Plastic Manufacturers, 2018. Plastics - the Facts 2018 - An analysis of European plastics production, demand and waste data.
534 Atamanalp, M., Köktürk, M., Uçar, A., Duyar, H.A., Özdemir, S., Parlak, V., Esenbuğa, N., Alak, G., 2021. Microplastics in Tissues (Brain, Gill,
535 Muscle and Gastrointestinal) of Mullus barbatus and Alosa immaculata. Arch. Environ. Contam. Toxicol. 81, 460–469.
536 doi:10.1007/s00244-021-00885-5
537 Atwood, E.C., Falcieri, F.M., Piehl, S., Bochow, M., Matthies, M., Franke, J., Carniel, S., Sclavo, M., Laforsch, C., Siegert, F., 2019. Coastal
538 accumulation of microplastic particles emitted from the Po River, Northern Italy: Comparing remote sensing and hydrodynamic modelling
539 with in situ sample collections. Mar. Pollut. Bull. 138, 561–574. doi:10.1016/j.marpolbul.2018.11.045
540 Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish
541 tissues: First observations in commercial species from Adriatic Sea. Mar. Environ. Res. 111, 18–26. doi:10.1016/j.marenvres.2015.06.014
542 Baini, M., Fossi, M.C., Galli, M., Caliani, I., Campani, T., Finoia, M.G., Panti, C., 2018. Abundance and characterization of microplastics in the
543 coastal waters of Tuscany (Italy): The application of the MSFD monitoring protocol in the Mediterranean Sea. Mar. Pollut. Bull. 133, 543–
544 552. doi:10.1016/J.MARPOLBUL.2018.06.016
545 Barboza, L.G.A., Cózar, A., Gimenez, B.C.G., Barros, T.L., Kershaw, P.J., Guilhermino, L., 2019. Macroplastics Pollution in the Marine
546 Environment, in: Shepperd, C. (Ed.), World Seas: An Environmental Evaluation. Elsevier, pp. 305–328. doi:10.1016/B978-0-12-805052-
547 1.00019-X
548 Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from
549 the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109, 55–60. doi:10.1016/j.marpolbul.2016.06.026
550 Botterell, Z.L.R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C., Lindeque, P.K., 2019. Bioavailability and effects of microplastics on
551 marine zooplankton: A review. Environ. Pollut. 245, 98–110. doi:10.1016/j.envpol.2018.10.065
552 Capriotti, M., Cocci, P., Bracchetti, L., Cottone, E., Scandiffio, R., Caprioli, G., Sagratini, G., Mosconi, G., Bovolin, P., Palermo, F.A., 2021.
553 Microplastics and their associated organic pollutants from the coastal waters of the central Adriatic Sea (Italy): Investigation of adipogenic
554 effects in vitro. Chemosphere 263, 128090. doi:10.1016/j.chemosphere.2020.128090
555 Carrozzi, V., Di Lorenzo, M., Massi, D., Titone, A., Ardizzone, G., Colloca, F., 2019. Prey preferences and ontogenetic diet shift of European hake
556 Merluccius merluccius (Linnaeus, 1758) in the central Mediterranean Sea. Reg. Stud. Mar. Sci. 25, 100440.
557 doi:10.1016/j.rsma.2018.100440
558 Caruso, G., 2019. Microplastics as vectors of contaminants. Mar. Pollut. Bull. 146, 921–924. doi:10.1016/j.marpolbul.2019.07.052
559 Casabianca, S., Capellacci, S., Giacobbe, M.G., Dell'Aversano, C., Tartaglione, L., Varriale, F., Narizzano, R., Risso, F., Moretto, P., Dagnino, A.,
560 Bertolotto, R., Barbone, E., Ungaro, N., Penna, A., 2019. Plastic-associated harmful microalgal assemblages in marine environment.
561 Environ. Pollut. 244, 617–626. doi:10.1016/j.envpol.2018.09.110
562 Chabuka, B.K., Kalivas, J.H., 2020. Application of a Hybrid Fusion Classification Process for Identification of Microplastics Based on Fourier
563 Transform Infrared Spectroscopy. Appl. Spectrosc. 74, 1167–1183. doi:10.1177/0003702820923993
564 Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Fossi, M.C., Corsolini, S., 2017. Microplastic in the
565 surface waters of the Ross Sea (Antarctica): Occurrence, distribution and characterization by FTIR. Chemosphere 175, 391–400.
566 doi:10.1016/j.chemosphere.2017.02.024
567 Clark, J.R., Cole, M., Lindeque, P.K., Fileman, E., Blackford, J., Lewis, C., Lenton, T.M., Galloway, T.S., 2016. Marine microplastic debris: a
568 targeted plan for understanding and quantifying interactions with marine life. Front. Ecol. Environ. 14, 317–324. doi:10.1002/fee.1297
569 Clementi, E., Aydogdu, A., Goglio, A.C., Pistoia, J., Escudier, R., Drudi, M., Grandi, A., Mariani, A., Lyubartsev, V., Lecci, R., Cretí, S., Coppini,
570 G., Masina, S., Pinardi, N., 2021. Mediterranean Sea Physical Analysis and Forecast (CMEMS MED-Currents, EAS6 system) (Version 1)

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571 [Data set]. Copernicus Monitoring Environment Marine Service (CMEMS).
572 doi:10.25423/CMCC/MEDSEA_ANALYSISFORECAST_PHY_006_013_EAS6
573 Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The Impact of Polystyrene Microplastics on Feeding, Function and
574 Fecundity in the Marine Copepod Calanus helgolandicus. Environ. Sci. Technol. 49, 1130–1137. doi:10.1021/es504525u
575 Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic Ingestion by Zooplankton.
576 Environ. Sci. Technol. 47, 6646–6655. doi:10.1021/es400663f
577 Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: A review. Mar. Pollut. Bull.
578 62, 2588–2597. doi:10.1016/j.marpolbul.2011.09.025
579 Collignon, A., Hecq, J.H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western
580 Mediterranean Sea. Mar. Pollut. Bull. doi:10.1016/j.marpolbul.2012.01.011
581 Constant, M., Kerherve, P., Sola, J., Sanchez-Vidal, A., Canals, M., Heussner, S., 2018. Floating Microplastics in the Northwestern Mediterranean
582 Sea: Temporal and Spatial Heterogeneities, in: M., C., Di Pace, E., Errico, M., Gentile, G., Montarsolo, A., Mossotti, R. (Eds.),
583 Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea. pp. 9–15. doi:10.1007/978-3-319-71279-
584 6_2
585 Corinaldesi, C., Canensi, S., Dell'Anno, A., Tangherlini, M., Di Capua, I., Varrella, S., Willis, T.J., Cerrano, C., Danovaro, R., 2021. Multiple
586 impacts of microplastics can threaten marine habitat-forming species. Commun. Biol. 4, 1–13. doi:10.1038/s42003-021-01961-1
587 Cowger, W., Steinmetz, Z., Gray, A., Munno, K., Lynch, J., Hapich, H., Primpke, S., De Frond, H., Rochman, C., Herodotou, O., 2021.
588 Microplastic Spectral Classification Needs an Open Source Community: Open Specy to the Rescue! Anal. Chem. 93, 7543–7548.
589 doi:10.1021/acs.analchem.1c00123
590 Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, J.Á., Irigoien, X., Duarte, C.M., 2015. Plastic Accumulation in the
591 Mediterranean Sea. PLoS One 10, e0121762. doi:10.1371/journal.pone.0121762
592 Cree, I.A., Andreotti, P.E., 1997. Measurement of cytotoxicity by ATP-based luminescence assay in primary cell cultures and cell lines. Toxicol.
593 Vitr. 11, 553–556. doi:10.1016/S0887-2333(97)00060-X
594 de Lucia, G.A., Caliani, I., Marra, S., Camedda, A., Coppa, S., Alcaro, L., Campani, T., Giannetti, M., Coppola, D., Cicero, A.M., Panti, C., Baini,
595 M., Guerranti, C., Marsili, L., Massaro, G., Fossi, M.C., Matiddi, M., 2014. Amount and distribution of neustonic micro-plastic off the
596 western Sardinian coast (Central-Western Mediterranean Sea). Mar. Environ. Res. 100, 10–16. doi:10.1016/j.marenvres.2014.03.017
597 de Lucia, G.A., Vianello, A., Camedda, A., Vani, D., Tomassetti, P., Coppa, S., Palazzo, L., Amici, M., Romanelli, G., Zampetti, G., Cicero, A.M.,
598 Carpentieri, S., Di Vito, S., Matiddi, M., 2018. Sea water contamination in the Vicinity of the Italian minor islands caused by microplastic
599 pollution. Water (Switzerland) 10. doi:10.3390/w10081108
600 Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., Zeri, C., 2018. Microplastics in mussels and fish from the Northern Ionian Sea. Mar.
601 Pollut. Bull. 135, 30–40. doi:10.1016/j.marpolbul.2018.06.063
602 Du, S., Zhu, R., Cai, Y., Xu, N., Yap, P.-S., Zhang, Yunhai, He, Y., Zhang, Yongjun, 2021. Environmental fate and impacts of microplastics in
603 aquatic ecosystems: a review. RSC Adv. 11, 15762–15784. doi:10.1039/D1RA00880C
604 Espinosa, C., Cuesta, A., Esteban, M.Á., 2017. Effects of dietary polyvinylchloride microparticles on general health, immune status and
605 expression of several genes related to stress in gilthead seabream (Sparus aurata L.). Fish Shellfish Immunol. 68, 251–259.
606 doi:10.1016/j.fsi.2017.07.006
607 Espinosa, C., Esteban, M.Á., Cuesta, A., 2019. Dietary administration of PVC and PE microplastics produces histological damage, oxidative
608 stress and immunoregulation in European sea bass (Dicentrarchus labrax L.). Fish Shellfish Immunol. 95, 574–583.
609 doi:10.1016/j.fsi.2019.10.072
610 Esposito, V., Andaloro, F., Bianca, D., Natalotto, A., Romeo, T., Scotti, G., Castriota, L., 2014. Diet and prey selectivity of the red mullet, Mullus
611 barbatus (Pisces: Mullidae), from the southern Tyrrhenian Sea: the role of the surf zone as a feeding ground. Mar. Biol. Res. 10, 167–178.
612 doi:10.1080/17451000.2013.797585
613 Expósito, N., Rovira, J., Sierra, J., Folch, J., Schuhmacher, M., 2021. Microplastics levels, size, morphology and composition in marine water,

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614 sediments and sand beaches. Case study of Tarragona coast (western Mediterranean). Sci. Total Environ.
615 doi:10.1016/j.scitotenv.2021.147453
616 Fagiano, V., Alomar, C., Compa, M., Soto-Navarro, J., Jordá, G., Deudero, S., 2022. Neustonic microplastics and zooplankton in coastal waters
617 of Cabrera Marine Protected Area (Western Mediterranean Sea). Sci. Total Environ. 804, 150120.
618 doi:10.1016/J.SCITOTENV.2021.150120
619 Flajnik, M.F., 2018. A cold-blooded view of adaptive immunity. Nat. Rev. Immunol. 18, 438–453. doi:10.1038/s41577-018-0003-9
620 Fortibuoni, T., Amadesi, B., Vlachogianni, T., 2021. Composition and abundance of macrolitter along the Italian coastline: The first baseline
621 assessment within the european Marine Strategy Framework Directive. Environ. Pollut. 268, 115886. doi:10.1016/j.envpol.2020.115886
622 Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., Caliani, I., Minutoli, R., Lauriano, G., Finoia, M.G., Rubegni, F.,
623 Panigada, S., Bérubé, M., Urbán Ramírez, J., Panti, C., 2016. Fin whales and microplastics: The Mediterranean Sea and the Sea of
624 Cortez scenarios. Environ. Pollut. 209, 68–78. doi:10.1016/j.envpol.2015.11.022
625 Fossi, M.C., Panti, C., Guerranti, C., Coppola, D., Giannetti, M., Marsili, L., Minutoli, R., 2012. Are baleen whales exposed to the threat of
626 microplastics? A case study of the Mediterranean fin whale (Balaenoptera physalus). Mar. Pollut. Bull. 64, 2374–2379.
627 doi:10.1016/j.marpolbul.2012.08.013
628 Galgani, L., Beiras, R., Galgani, F., Panti, C., Borja, A., 2019. Editorial: Impacts of Marine Litter. Front. Mar. Sci. 6. doi:10.3389/fmars.2019.00208
629 Garcia-Vazquez, E., Cani, A., Diem, A., Ferreira, C., Geldhof, R., Marquez, L., Molloy, E., Perché, S., 2018. Leave no traces – Beached marine
630 litter shelters both invasive and native species. Mar. Pollut. Bull. 131, 314–322. doi:10.1016/j.marpolbul.2018.04.037
631 GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment, IMO/FAO/UN. ed, Rep. Stud.
632 GESAMP.
633 Giani, D., Baini, M., Galli, M., Casini, S., Fossi, M.C., 2019. Microplastics occurrence in edible fish species (Mullus barbatus and Merluccius
634 merluccius) collected in three different geographical sub-areas of the Mediterranean Sea. Mar. Pollut. Bull. 140, 129–137.
635 doi:10.1016/j.marpolbul.2019.01.005
636 Gomiero, A., Strafella, P., Pellini, G., Salvalaggio, V., Fabi, G., 2018. Comparative Effects of Ingested PVC Micro Particles With and Without
637 Adsorbed Benzo(a)pyrene vs. Spiked Sediments on the Cellular and Sub Cellular Processes of the Benthic Organism Hediste
638 diversicolor. Front. Mar. Sci. 5. doi:10.3389/fmars.2018.00099
639 Guerrera, M.C., Aragona, M., Porcino, C., Fazio, F., Laurà, R., Levanti, M., Montalbano, G., Germanà, G., Abbate, F., Germanà, A., 2021. Micro
640 and Nano Plastics Distribution in Fish as Model Organisms: Histopathology, Blood Response and Bioaccumulation in Different Organs.
641 Appl. Sci. 11, 5768. doi:10.3390/app11135768
642 Guo, X., Wang, J., 2019. The chemical behaviors of microplastics in marine environment: A review. Mar. Pollut. Bull. 142, 1–14.
643 doi:10.1016/j.marpolbul.2019.03.019
644 Isobe, A., Iwasaki, S., Uchida, K., Tokai, T., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat.
645 Commun. 10, 1–3. doi:10.1038/s41467-019-08316-9
646 Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into
647 the ocean. Science (80-.). 347, 768–771. doi:10.1126/science.1260352
648 Kazour, M., Jemaa, S., Issa, C., Khalaf, G., Amara, R., 2019. Microplastics pollution along the Lebanese coast (Eastern Mediterranean Basin):
649 Occurrence in surface water, sediments and biota samples. Sci. Total Environ. doi:10.1016/j.scitotenv.2019.133933
650 Kiessling, T., Gutow, L., Thiel, M., 2015. Marine Litter as Habitat and Dispersal Vector, in: Marine Anthropogenic Litter. Springer International
651 Publishing, Cham, pp. 141–181. doi:10.1007/978-3-319-16510-3_6
652 Kowalski, N., Reichardt, A.M., Waniek, J.J., 2016. Sinking rates of microplastics and potential implications of their alteration by physical,
653 biological, and chemical factors. Mar. Pollut. Bull. 109, 310–319. doi:10.1016/j.marpolbul.2016.05.064
654 Ling, S.D., Sinclair, M., Levi, C.J., Reeves, S.E., Edgar, G.J., 2017. Ubiquity of microplastics in coastal seafloor sediments. Mar. Pollut. Bull. 121,
655 104–110. doi:10.1016/j.marpolbul.2017.05.038
656 Löhr, A., Savelli, H., Beunen, R., Kalz, M., Ragas, A., Van Belleghem, F., 2017. Solutions for global marine litter pollution. Curr. Opin. Environ.

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657 Sustain. 28, 90–99. doi:10.1016/j.cosust.2017.08.009
658 Lucci, F., Della Ventura, G., Piazzolla, D., Venettacci, C., Terribili, A., La Bella, C., Conte, A., Scanu, S., Bonamano, S., Radica, F., Marcelli, M.,
659 2021. Development and testing for a low-cost device for airborne PM monitoring: the port of Civitavecchia (Italy) during COVID-19
660 lockdown. Atmos. Environ. under Submiss.
661 Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the
662 English Channel. Mar. Pollut. Bull. 67, 94–99. doi:10.1016/j.marpolbul.2012.11.028
663 Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-
664 surface samples. Sci. Rep. 5, 14947. doi:10.1038/srep14947
665 Mahto, S.K., Chandra, P., Rhee, S.W., 2010. In vitro models, endpoints and assessment methods for the measurement of cytotoxicity. Toxicol.
666 Environ. Health Sci. 2, 87–93. doi:10.1007/BF03216487
667 Mancini, E., Miccoli, A., Piazzolla, D., Saraceni, P.R., Lezzi, M., Tiralongo, F., Bonifazi, A., Picchietti, S., Marcelli, M., 2021. Macrozoobenthic
668 fauna associated with benthic marine litter (Northern Tyrrhenian Sea, Italy) and first report of two bryozoan species in Italian waters. Reg.
669 Stud. Mar. Sci. 47, 101912. doi:10.1016/j.rsma.2021.101912
670 Mancuso, M., Savoca, S., Bottari, T., 2019. First record of microplastics ingestion by European hake Merluccius merluccius from the Tyrrhenian
671 Sicilian coast (Central Mediterranean Sea). J. Fish Biol. 94, 517–519. doi:10.1111/jfb.13920
672 Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A., Zambonino-
673 Infante, J., 2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Mar.
674 Environ. Res. 112, 78–85. doi:10.1016/j.marenvres.2015.09.009
675 Miccoli, A., Buonocore, F., Picchietti, S., Scapigliati, G., 2021. The sea bass Dicentrarchus labrax as a marine model species in immunology :
676 Insights from basic and applied research. Aquac. Fish. doi:10.1016/j.aaf.2021.09.003
677 Moore, C.J., 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environ. Res. 108, 131–139.
678 doi:10.1016/j.envres.2008.07.025
679 Nguyen, T.H., Tang, F.H.M., Maggi, F., 2020. Sinking of microbial-associated microplastics in natural waters. PLoS One 15, e0228209.
680 doi:10.1371/journal.pone.0228209
681 OECD, 2021. Test No. 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay, OECD Guidelines for the Testing of Chemicals, Section
682 2. OECD, Paris. doi:10.1787/C66D5190-EN
683 Pannetier, P., Morin, B., Le Bihanic, F., Dubreil, L., Clérandeau, C., Chouvellon, F., Van Arkel, K., Danion, M., Cachot, J., 2020. Environmental
684 samples of microplastics induce significant toxic effects in fish larvae. Environ. Int. 134, 105047. doi:10.1016/j.envint.2019.105047
685 Panti, C., Giannetti, M., Baini, M., Rubegni, F., Minutoli, R., Fossi, M.C., 2015. Occurrence, relative abundance and spatial distribution of
686 microplastics and zooplankton NW of Sardinia in the Pelagos Sanctuary Protected Area, Mediterranean Sea. Environ. Chem. 12, 618.
687 doi:10.1071/EN14234
688 Pedà, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., Perdichizzi, A., Romeo, T., Maricchiolo, G., 2016. Intestinal alterations in
689 European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environ. Pollut.
690 doi:10.1016/j.envpol.2016.01.083
691 Piazzolla, D., Cafaro, V., de Lucia, G.A., Mancini, E., Scanu, S., Bonamano, S., Piermattei, V., Vianello, A., Della Ventura, G., Marcelli, M., 2020.
692 Microlitter pollution in coastal sediments of the northern Tyrrhenian Sea, Italy: microplastics and fly-ash occurrence and distribution.
693 Estuar. Coast. Shelf Sci. doi:10.1016/j.ecss.2020.106819
694 Pittura, L., Avio, C.G., Giuliani, M.E., D'Errico, G., Keiter, S.H., Cormier, B., Gorbi, S., Regoli, F., 2018. Microplastics as Vehicles of
695 Environmental PAHs to Marine Organisms: Combined Chemical and Physical Hazards to the Mediterranean Mussels, Mytilus
696 galloprovincialis. Front. Mar. Sci. 5. doi:10.3389/fmars.2018.00103
697 Prevenios, M., Zeri, C., Tsangaris, C., Liubartseva, S., Fakiris, E., Papatheodorou, G., 2018. Beach litter dynamics on Mediterranean coasts:
698 Distinguishing sources and pathways. Mar. Pollut. Bull. 129, 448–457. doi:10.1016/j.marpolbul.2017.10.013
699 Primpke, S., Wirth, M., Lorenz, C., Gerdts, G., 2018. Reference database design for the automated analysis of microplastic samples based on

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700 Fourier transform infrared (FTIR) spectroscopy. Anal. Bioanal. Chem. 410, 5131–5141. doi:10.1007/s00216-018-1156-x
701 Renzi, M., Blašković, A., Bernardi, G., Russo, G.F., 2018. Plastic litter transfer from sediments towards marine trophic webs: A case study on
702 holothurians. Mar. Pollut. Bull. 135, 376–385. doi:10.1016/j.marpolbul.2018.07.038
703 Revel, M., Roman, C., Châtel, A., 2021. Is cell culture a suitable tool for the evaluation of micro- and nanoplastics ecotoxicity? Ecotoxicology 30,
704 421–430. doi:10.1007/s10646-021-02355-z
705 Rios-Fuster, B., Alomar, C., Compa, M., Guijarro, B., Deudero, S., 2019. Anthropogenic particles ingestion in fish species from two areas of the
706 western Mediterranean Sea. Mar. Pollut. Bull. 144, 325–333. doi:10.1016/j.marpolbul.2019.04.064
707 Rios-Fuster, B., Arechavala-Lopez, P., García-Marcos, K., Alomar, C., Compa, M., Álvarez, E., Julià, M.M., Solomando Martí, A., Sureda, A.,
708 Deudero, S., 2021. Experimental evidence of physiological and behavioral effects of microplastic ingestion in Sparus aurata. Aquat.
709 Toxicol. 231, 105737. doi:10.1016/j.aquatox.2020.105737
710 Rios, L.M., Moore, C., Jones, P.R., 2007. Persistent organic pollutants carried by synthetic polymers in the ocean environment. Mar. Pollut. Bull.
711 54, 1230–1237. doi:10.1016/j.marpolbul.2007.03.022
712 Rogers, K.L., Carreres-Calabuig, J.A., Gorokhova, E., Posth, N.R., 2020. Micro-by-micro interactions: How microorganisms influence the fate of
713 marine microplastics. Limnol. Oceanogr. Lett. 5, 18–36. doi:10.1002/lol2.10136
714 Ru, J., Huo, Y., Yang, Y., 2020. Microbial Degradation and Valorization of Plastic Wastes. Front. Microbiol. 11. doi:10.3389/fmicb.2020.00442
715 Sabatella, E.C., Colloca, F., Coppola, G., Fiorentino, F., Gambino, M., Malvarosa, L., Sabatella, R., 2017. Key Economic Characteristics of Italian
716 Trawl Fisheries and Management Challenges. Front. Mar. Sci. 4. doi:10.3389/fmars.2017.00371
717 Sala, A., Conides, A., De Carlo, F., Klaoudatos, D., Grech, D., Lucchetti, A., Mayans, A., Notti, E., Paci, N., Salom, S., Sartor, P., Sbrana, M.,
718 Soler, I., Spedicato, M.T., Virgili, M., 2013. Final project report Technical specifications of Mediterranean trawl gears (myGears).
719 doi:10.13140/RG.2.2.24528.76804
720 Savoca, S., Capillo, G., Mancuso, M., Bottari, T., Crupi, R., Branca, C., Romano, V., Faggio, C., D'Angelo, G., Spanò, N., 2019. Microplastics
721 occurrence in the Tyrrhenian waters and in the gastrointestinal tract of two congener species of seabreams. Environ. Toxicol. Pharmacol.
722 67, 35–41. doi:10.1016/j.etap.2019.01.011
723 Scapigliati, G., Fausto, A.M., Picchietti, S., 2018. Fish Lymphocytes: An Evolutionary Equivalent of Mammalian Innate-Like Lymphocytes? Front.
724 Immunol. 9, 1–8. doi:10.3389/fimmu.2018.00971
725 Schoonen, W.G.E.J., de Roos, J.A.D.M., Westerink, W.M.A., Débiton, E., 2005. Cytotoxic effects of 110 reference compounds on HepG2 cells
726 and for 60 compounds on HeLa, ECC-1 and CHO cells. Toxicol. Vitr. 19, 491–503. doi:10.1016/j.tiv.2005.01.002
727 Sendra, M., Pereiro, P., Yeste, M.P., Mercado, L., Figueras, A., Novoa, B., 2021. Size matters: Zebrafish (Danio rerio) as a model to study toxicity
728 of nanoplastics from cells to the whole organism. Environ. Pollut. 268, 115769. doi:10.1016/j.envpol.2020.115769
729 Shim, W.J., Hong, S.H., Eo, S., 2018. Marine Microplastics: Abundance, Distribution, and Composition, in: Zeng, E. (Ed.), Microplastic
730 Contamination in Aquatic Environments. Elsevier, pp. 1–26. doi:10.1016/B978-0-12-813747-5.00001-1
731 Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau,
732 C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene
733 microplastics. Proc. Natl. Acad. Sci. 113, 2430–2435. doi:10.1073/pnas.1519019113
734 Tekman, M.B., Gutow, L., Macario, A., Haas, A., Walter, A., Bergmann, M., 2021. LITTERBASE [WWW Document]. Alfred Wegener Inst.
735 Helmholtz Cent. Polar Mar. Res. URL https://litterbase.awi.de/litter_detail
736 Thompson, R.C., Olsen, Y., Mitchell, R.C., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russel, A.E., 2004. Lost at Sea: Where Is All
737 the Plastic? Science (80-.). 304, 838–838. doi:10.1126/science.1094559
738 Tiralongo, F., Mancini, E., Ventura, D., de Malherbe, S., Paladini de Mendoza, F., Sardone, M., Arciprete, R., Massi, D., Marcelli, M., Fiorentino,
739 F., Minervini, R., 2021. Commercial catches and discards composition in the central Tyrrhenian Sea: a multispecies quantitative and
740 qualitative analysis from shallow and deep bottom trawling. Mediterr. Mar. Sci. 22, 521–531. doi:10.12681/mms.25753
741 UNEP/MAP, 2015. Marine litter assessment in the Mediterranean Sea. Athens (Greece).
742 UNEP, 2016. Marine plastic debris and microplastics - Global Lessons and Research to Inspire Action and guide policy change, UN.

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743 UNEP, 2014. Valuing Plastics: The Business Case for Measuring, Managing and Disclosing Plastic Use in the Consumer Goods Industry, UN.
744 van der Hal, N., Ariel, A., Angel, D.L., 2017. Exceptionally high abundances of microplastics in the oligotrophic Israeli Mediterranean coastal
745 waters. Mar. Pollut. Bull. doi:10.1016/j.marpolbul.2016.12.052
746 Van Sebille, E., Aliani, S., Law, K.L., Maximenko, N., Alsina, J.M., Bagaev, A., Bergmann, M., Chapron, B., Chubarenko, I., Cózar, A.,
747 Delandmeter, P., Egger, M., Fox-Kemper, B., Garaba, S.P., Goddijn-Murphy, L., Hardesty, B.D., Hoffman, M.J., Isobe, A., Jongedijk, C.E.,
748 Kaandorp, M.L.A., Khatmullina, L., Koelmans, A.A., Kukulka, T., Laufkötter, C., Lebreton, L., Lobelle, D., Maes, C., Martinez-Vicente, V.,
749 Morales Maqueda, M.A., Poulain-Zarcos, M., Rodríguez, E., Ryan, P.G., Shanks, A.L., Shim, W.J., Suaria, G., Thiel, M., Van Den Bremer,
750 T.S., Wichmann, D., 2020. The physical oceanography of the transport of floating marine debris. Environ. Res. Lett. doi:10.1088/1748-
751 9326/ab6d7d
752 van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F., Law, K.L., 2015.
753 A global inventory of small floating plastic debris. Environ. Res. Lett. 10, 124006. doi:10.1088/1748-9326/10/12/124006
754 Vasilopoulou, G., Kehayias, G., Kletou, D., Kleitou, P., Triantafyllidis, V., Zotos, A., Antoniadis, K., Rousou, M., Papadopoulos, V., Polykarpou, P.,
755 Tsiamis, G., 2021. Microplastics Investigation Using Zooplankton Samples from the Coasts of Cyprus (Eastern Mediterranean). Water
756 2021, Vol. 13, Page 2272 13, 2272. doi:10.3390/W13162272
757 Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: A review. Environ. Pollut. 178,
758 483–492. doi:10.1016/j.envpol.2013.02.031
759 Yong, C., Valiyaveettil, S., Tang, B., 2020. Toxicity of Microplastics and Nanoplastics in Mammalian Systems. Int. J. Environ. Res. Public Health
760 17, 1509. doi:10.3390/ijerph17051509
761 Zapata, A., Diez, B., Cejalvo, T., Gutiérrez-De Frías, C., Cortés, A., 2006. Ontogeny of the immune system of fish. Fish Shellfish Immunol. 20,
762 126–136. doi:10.1016/j.fsi.2004.09.005
763 Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “Plastisphere”: Microbial Communities on Plastic Marine Debris. Environ. Sci.
764 Technol. 47, 7137–7146. doi:10.1021/es401288x
765 Zhang, W., Zhang, S., Wang, J., Wang, Y., Mu, J., Wang, P., Lin, X., Ma, D., 2017. Microplastic pollution in the surface waters of the Bohai Sea,
766 China. Environ. Pollut. 231, 541–548. doi:10.1016/j.envpol.2017.08.058
767 Zhao, S., Zhu, L., Wang, T., Li, D., 2014. Suspended microplastics in the surface water of the Yangtze Estuary System, China: First observations
768 on occurrence, distribution. Mar. Pollut. Bull. 86, 562–568. doi:10.1016/j.marpolbul.2014.06.032
769 Zitouni, N., Bousserrhine, N., Missawi, O., Boughattas, I., Chèvre, N., Santos, R., Belbekhouche, S., Alphonse, V., Tisserand, F., Balmassiere, L.,
770 Dos Santos, S.P., Mokni, M., Guerbej, H., Banni, M., 2021. Uptake, tissue distribution and toxicological effects of environmental
771 microplastics in early juvenile fish Dicentrarchus labrax. J. Hazard. Mater. 403. doi:10.1016/j.jhazmat.2020.124055
772 Zwollo, P., Quddos, F., Bagdassarian, C., Seeley, M.E., Hale, R.C., Abderhalden, L., 2021. Polystyrene microplastics reduce abundance of
773 developing B cells in rainbow trout (Oncorhynchus mykiss) primary cultures. Fish Shellfish Immunol. 114, 102–111.
774 doi:10.1016/j.fsi.2021.04.014

775