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1 **Transparent Exopolymer Particle (TEP) distribution and in situ**
2 **prokaryotic generation across the deep Mediterranean Sea and nearby**
3 **North East Atlantic Ocean**

4
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7
8 Keywords: transparent exopolymer particles, prokaryotes, organic carbon, open ocean,
9 Mediterranean Sea

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26 **Abstract**

27

28 Transparent exopolymer particles (TEP) play a key role in ocean carbon export and
29 structuring microbial habitats, but information on their distribution across different ocean
30 basins and depths is scarce, particularly in the dark ocean. We measured TEP vertical
31 distribution from the surface to bathypelagic waters in an east-to-west transect across the
32 Mediterranean Sea (MedSea) and the adjacent North Eastern Atlantic Ocean (NEA), and
33 explore their physical and biological drivers. TEP ranged from 0.6 to 81.7 $\mu\text{g XG eq L}^{-1}$,
34 with the highest values in epipelagic waters above the deep chlorophyll maximum, and in
35 areas near the Gibraltar and Sicily Straits. TEP were significantly related to particulate
36 organic carbon (POC) in all basins and depth layers (epipelagic vs. deep), but the
37 contribution of TEP to POC was higher in the NEA (85%, 79% and 67% in epi-, meso- and
38 bathypelagic waters, respectively) than in the MedSea (from 53% to 62% in epipelagic
39 waters, and from 45% to 48% in meso- and bathypelagic waters), coinciding with higher
40 carbon to nitrogen particulate organic matter ratios in the NEA. The TEP connectivity
41 between epipelagic waters and mesopelagic waters was less straightforward than between
42 mesopelagic waters and bathypelagic waters, with a 23% and 55 % of the variance in the
43 relationship between layers explained respectively. Prokaryotes were found to be a likely
44 net source of TEP as inferred by the significant direct relationship observed between
45 prokaryotic heterotrophic abundance and TEP. This assumption was confirmed using
46 experimental incubations, where prokaryotes produced TEP in concentrations ranging from
47 0.7 (Western Mediterranean, bathypelagic) to 232 (Western Mediterranean, mesopelagic)
48 $\mu\text{g XG eq. L}^{-1} \text{ day}^{-1}$.

49

50 **Keywords:** transparent exopolymer particles, particulate organic carbon, prokaryotes,
51 Mediterranean Sea, dark ocean, biological carbon pump

52

53 **Introduction**

54

55 Transparent Exopolymer particles (TEP) are polysaccharide-rich microgels (from
56 ~0.4 to > 200 μm) with an ubiquitous distribution in the ocean, where they play a crucial
57 role in transferring carbon from the dissolved to the particulate pool (Alldredge et al. 1993).
58 On the one hand, TEP are sticky (Engel 2000; Passow and Alldredge 1995a), and can thus
59 promote particle aggregation leading to their downward export when they are ballasted
60 (Passow 2002b; Wurl et al. 2011), enhancing the biological carbon pump. On the other
61 hand, these particles are porous and of low density and, consequently, may reduce the
62 sinking rates of aggregates enriched in TEP. They can even float or ascend through the
63 water column (Azetsu-Scott and Passow 2004; Mari et al. 2017), counteracting the
64 downward export of particulate organic matter and affecting air-sea gas exchange after their
65 accumulation in the surface microlayer (Wurl et al. 2011).

66

67 TEP net accumulation across marine environments is determined by the quality and
68 quantity of the polymers released by microorganisms (Gogou and Repeta 2010), their
69 degradation pathways (Taylor et al. 2014) and the environmental conditions promoting
70 abiotic self-assembly of these polymers (Alldredge et al. 1993; Berman-Frank et al. 2016;
71 Mari et al. 2005; Mopper et al. 1995). TEP were first described in cultures and natural
72 proliferations of phytoplankton (Alldredge et al. 1993). Thenceforth, phytoplankton have
73 been considered to be the main TEP source in the euphotic layer, also because high TEP
74 concentrations have been regularly observed associated with phytoplankton blooms (Hong
75 et al. 1997; Mari and Kjørboe 1996) and regions where algal biomass increased due to
76 favourable growth conditions (Prieto et al. 2006). In contrast to highest TEP absolute
77 values, relatively elevated TEP concentrations in relation to chlorophyll or primary
78 production values may also be expected in oligotrophic areas since a high TEP formation
79 per unit cell occurs under nutrient-depleted conditions and as a consequence of
80 phytoplankton carbon overflow (Bar-Zeev et al., 2009; 2011).

81

82 In contrast to the unequivocal role of phytoplankton as a TEP source; the role of
83 heterotrophic prokaryotes on TEP dynamics is less straightforward. Prokaryotes may act
84 both as TEP sinks via colonization and degradation of the particles (Mari and Kjørboe
85 1996; Taylor et al. 2014) or as sources (Ortega-Retuerta et al. 2010; Passow 2002a), and
86 interact with phytoplankton promoting TEP production (Gärdes et al. 2011; Gärdes et al.
87 2012). Heterotrophic prokaryotes release extracellular polymers during their growth, by
88 producing mucous capsules (Decho 1990), or modifying the stickiness of the polymers
89 (Rochelle-Newall et al. 2010; Van Oostende et al. 2013), contributing to the assembly of
90 dissolved polymers into larger sized particles.

91

92 While all published studies to date have looked at TEP-prokaryote interactions in
93 the euphotic layer or in laboratory experiments, there is no information on the role of
94 heterotrophic prokaryotes on TEP formation in the dark ocean. In contrast to surface
95 waters, the deep waters are enriched in inorganic nutrients and microbial metabolism is
96 highly dependent on the downward flux of organic carbon from surface waters (Arístegui et
97 al. 2009). Meso- and bathypelagic prokaryotes mostly sit on particles and are apparently
98 well adapted to a particle-attached lifestyle (Baltar et al. 2009; Herndl and Reinthaler
99 2013). During sedimentation the particles evolve and it is well known that changes in

100 element stoichiometry (Engel et al. 2015; Radic et al. 2005) or the nature of the available
101 organic matter (Koch et al. 2014; Ogawa et al. 2001) affect the prokaryotic production of
102 TEP. Therefore, quantification of the net rates of TEP generation by prokaryotes in the
103 deep ocean will help to better understand their contribution to the global carbon cycle and,
104 principally, to particulate organic carbon (POC) flux attenuation.
105

106 The Mediterranean Sea is an oligotrophic ecosystem characterized by surface
107 phosphorus limitation, particularly accentuated in the Levantine basin (Krom et al. 1991;
108 Thingstad et al. 2005). This semi-enclosed sea is connected to the Northeast Atlantic Ocean
109 through the highly dynamic and productive region of the Strait of Gibraltar, characterized
110 by a two-layer system with an upper Atlantic layer inflowing into the Mediterranean Sea,
111 and Mediterranean water outflowing at depth (Gascard and Richez 1985; Lacombe and
112 Richez 1982). Frequent events of large mucus aggregates, preceded by periods with
113 elevated TEP levels, have been observed in some regions of the Mediterranean Sea (i.e.
114 coastal Adriatic and Aegean Seas, Radic et al., 2005; Danovaro et al., 2009). However,
115 these local studies might not be representative of the entire basin and only a handful of
116 studies have documented the variability of TEP concentrations in surface (Garcia et al.,
117 2002; Ortega-Retuerta et al., 2010, 2016) and mesopelagic waters (Bar-Zeev et al., 2011;
118 Prieto et al., 2006; Weinbauer et al., 2013), showing variable TEP accumulations both
119 spatially and with depth.
120

121 Here we present a detailed and comprehensive vertical distribution of TEP across
122 the Mediterranean Sea, from the eastern to the western basins, including the nearby
123 Northeast Atlantic Ocean. We discuss TEP distribution in relation with water masses
124 circulation and mixing, from the surface down to the dark sea, including for the first time
125 the Mediterranean Sea bathypelagic waters. We also explore the different potential physical
126 and biological factors that might drive TEP concentrations in the deep Mediterranean Sea,
127 and quantify experimentally their production by heterotrophic prokaryotes.
128

129 **2. Materials and Methods**

130

131 **2.1. Study site and sampling strategy**

132

133 Sampling was carried out during the HOTMIX 2014 cruise on board the *R/V*
134 *Sarmiento de Gamboa*, from 29th April to 28th May 2014. A total of 29 stations were
135 sampled along a Mediterranean Sea section from East to West, also extending to the
136 adjacent subtropical Northeast Atlantic Ocean (NEA) reaching the Canary Islands (Figure
137 1). Detailed information about the distributions of salinity, potential temperature and
138 chlorophyll *a* concentrations as well as the water masses intercepted during the cruise is
139 provided in Martínez-Pérez et al. (2017). Samples were collected using a rosette sampler
140 holding 24 Niskin bottles (12 L each), coupled to a Seabird SBE 9-11 plus conductivity-
141 temperature–pressure probe (CTD), complemented with a SBE43 oxygen sensor and a
142 SeaTech fluorometer. Up to 13 depths were sampled covering the entire water column,
143 from 3 m down to 10 m above the seafloor.

144

145 Samples from the epipelagic layer (surface water; down to 200 m) were
146 systematically collected at four depths: 3 m, the depth receiving 20% of the surface
147 photosynthetically active radiation, the depth of the deep chlorophyll maximum (DCM),
148 and between 10 m and 45 m below the DCM. The depth of the DCM was determined after
149 visual inspection of the vertical profiles of chlorophyll *a* (Chl *a*) fluorescence.

150

151 Sampling depths in the meso- and bathypelagic layers were decided on the basis of
152 the full-depth potential temperature, salinity and dissolved oxygen profiles to ensure that all
153 the water masses of the Mediterranean Sea and their respective mixing zones were sampled.
154 More specifically, in the mesopelagic layer of the Eastern Mediterranean (EM) we focused
155 our sampling efforts on the intermediate waters, including the Levantine Intermediate
156 Water (LIW) and the Cretan Intermediate Water (CIW), which occupy the water column
157 from 200 to 400 m depth. The core of the LIW can be easily characterised by its absolute
158 salinity maximum. In the Western Mediterranean (WM), modified LIW and Winter
159 Intermediate water (WIW) occupy the water column from 200 to 600 m depth. The
160 modified LIW can be characterised not only by its salinity maximum but also by its
161 absolute oxygen minimum. Finally, the bathypelagic layer is occupied by Eastern (EMDW)
162 and Western (WMDW) Mediterranean Deep waters. Both the EMDW and WMDW are in
163 fact composites of different varieties formed at different sites and/or under different
164 conditions. In the Eastern Mediterranean, EMDW can be of Adriatic or Aegean origin and
165 the waters of Adriatic origin form with different salinity and temperature depending on the
166 climate conditions at the time of formation. The same is applicable to the WMDW where
167 different varieties can be found depending on the proportions of Atlantic Water (AM)
168 and/or LIW at the time of WMDW formation in the Gulf of Lions. In the North Eastern
169 Atlantic NEA Ocean, we focused on three water masses and their mixing horizons: North
170 Atlantic Central Water (NACW) between 200 to 750 m depth, Mediterranean Water (MW)
171 from 750 to 1500 m depth, and North Atlantic Deep Water (NADW) from >1500 m. For a
172 detailed description of the water masses during the cruise, see Catalá et al. (2018)
173 (Supplementary Figure 1) for the Mediterranean water masses and Catalá et al. (2015) for
174 the NEA water masses.

175

176

177 *2.2. Analytical procedures*

178

179 *2.2.1. TEP*

180

181 Transparent exopolymer particles (TEP) concentrations were measured using the
182 colorimetric alcian blue method (Passow and Alldredge 1995b). Duplicate or triplicate
183 samples (0.4 – 2 L) were filtered through 0.4 μm polycarbonate filters (25 mm diameter,
184 Poretics) and the TEP retained on the filters were stained with 0.5 ml of a 0.02 % solution
185 of alcian blue (Sigma) in 0.06 % acetic acid (pH 2.5). The stained filters were frozen at -
186 80°C until analysis in the laboratory (for less than 1 month). The alcian blue-stained TEP
187 were extracted from the thawed filters adding 80 % sulphuric acid and the absorbance was
188 measured at 787 nm in 1 cm path disposable polystyrene cuvettes using ultrapure water as
189 blanks. Three blanks were performed for each batch of samples filtered every day
190 (including staining and freezing in parallel to the samples). Each solution of alcian blue was
191 calibrated using a fresh standard solution of xanthan gum. The coefficient of variation of
192 the replicates was ~ 17 %. TEP concentration was expressed as micrograms of xanthan
193 gum equivalents per liter ($\mu\text{g XG eq L}^{-1}$). To estimate TEP carbon content in our dataset,
194 and with the aim of comparing with particulate organic carbon (POC) concentration, we
195 used the canonical conversion factor of 0.75 $\mu\text{g C} / \mu\text{g XG eq}$ proposed in the literature
196 (Engel and Passow 2001).

197 *2.2.2. Particulate organic carbon (POC)*

198

199 Samples for Particulate Organic Carbon (POC) were determined after filtering 2-4 L
200 of the water samples through combusted (450°C for 12 h) Whatman GF/F filters (25 mm
201 diameter and 0.7 μm nominal pore size). These filters were stored frozen (-20 °C) until
202 processed. In the laboratory, the filters were thawed and dried overnight at 65 °C in a
203 desiccator under HCl fumes to remove carbonates and, then, dried overnight in a desiccator
204 with silica gel. The POC and PON analyses were performed by high-temperature (900 °C)
205 combustion in an elemental analyzer (Perkin Elmer 2400 CHN).

206

207 *2.2.3. Dissolved Oxygen (O₂), Inorganic Nitrogen (DIN) and Phosphorus (DIP)*

208

209 Samples for O₂ determination were collected in flared neck iodine calibrated flasks and
210 measured using a Winkler potentiometric method adapted from Langdon (2010). Samples
211 for inorganic nutrient analysis were collected in 50 mL polyethylene bottles and kept in the
212 dark at 4 °C until analysis on board. Nitrate, phosphate and silicate concentrations were
213 determined using a Skalar segmented flow autoanalyzer SAN++ following the colorimetric
214 methods of Grasshoff et al. (1999).

215

216 *2.2.4. Dissolved organic carbon (DOC)*

217

218 DOC samples were filtered through pre-combusted (450°C for 12 h) Whatman GF/F
219 filters in an all-glass filtration system under positive pressure of high purity N₂ and
220 collected into pre-combusted glass ampoules, acidified with phosphoric acid (final pH < 2),
221 sealed and stored at 4°C until analysis. Samples below 150 m were not filtered. These

222 samples were analyzed by high-temperature catalytic oxidation on a Shimadzu TOC-V total
223 organic carbon analyzer. Potassium hydrogen phthalate (99.95–100.05%, p.a., Merck) was
224 used to calibrate the system daily. The precision of the equipment was $\pm 1 \mu\text{mol L}^{-1}$. The
225 accuracy was checked daily with the DOC reference materials provided by D. A. Hansell
226 (University of Miami, USA).

227 228 2.2.5. *Chlorophyll a (Chl a) concentration* 229

230 For Chl-*a*, 500 mL of sea water were sampled and filtered through 25 mm Whatman GF/F
231 filters under low vacuum pressure. The filters were kept frozen at -20°C until analysis.
232 Before chlorophyll *a* determination, pigments were extracted using 10 mL of 90% acetone
233 at 4°C in the dark for 24 h. Extracts were then measured fluorometrically, before and after
234 acidification, by means of a Turner Designs bench fluorometer 10-AU, previously
235 calibrated with pure chlorophyll *a* (Sigma Chemical), following Holm-Hansen et al. (1965).
236

237 2.2.6 *Picophytoplankton abundance* 238

239 *Prochlorococcus* and *Synechococcus* type cyanobacteria and small photosynthetic
240 eukaryotic cells (picoeukaryotes) were enumerated with a FACScalibur (Becton and
241 Dickinson) flow cytometer. Samples of about 1 mL were analyzed in fresh material 30-60
242 min after retrieval. Phytoplankton groups were identified by their signatures in a plot of
243 side scatter (SSC) versus red (FL3) and orange (FL2) fluorescence. Samples were run at
244 60 mL min^{-1} . A suspension of yellow-green $1 \mu\text{m}$ latex beads ($\sim 10^5$ beads mL^{-1}) was added
245 as an internal standard (Polysciences, Inc.). Pigmented nanoeukaryotes (2-20 μm) were
246 counted on fresh samples with a Cytobuoy cytometer (Dubelaar and Gerritzen 2000),
247 provided with flow-imaging. Samples (about 3 ml) were analyzed *in vivo* for 7 min at a
248 flow rate of $300 \mu\text{L min}^{-1}$.
249

250 2.2.7. *Prokaryotic heterotrophic abundance (PHA)* 251

252 PHA was measured by flow cytometry (Gasol and del Giorgio, 2000). Aliquots of 1.5 mL
253 were fixed with 2% of paraformaldehyde, deep-frozen in liquid nitrogen and then stored at
254 -80°C until analysis, a few hours after collection. The samples were stained with SYBR
255 Green I and run through a FACScalibur cytometer fitted with a laser emitting at 488 nm. A
256 suspension of yellow-green $1 \mu\text{m}$ latex beads was added as an internal standard
257 (Polyscience Inc). The flow rate was determined volumetrically after every 10 samples.

258 2.2.8. *Prokaryotic heterotrophic Production (PHP)* 259

260 PHP rates were estimated from ^3H -Leucine (specific activities = 112 Ci mmol^{-1})
261 incorporation into proteins (Kirchman et al., 1985) and using the microcentrifugation
262 protocol proposed by Smith and Azam (1992). Three replicates (1.2 ml) and two
263 trichloroacetic acid (TCA)-killed blanks in microcentrifuge tubes were added L-[4, 5- ^3H]
264 leucine at 20 nM. Samples and blanks were incubated (for 3 to 15 h) at *in situ* temperatures.
265 Incubations were stopped by adding 50% TCA. Subsequently, the samples were
266 centrifuged twice (10 min. and 14000 r.p.m.) and rinsed with 5% TCA. Scintillation
267 cocktail (1 mL Optisafe HiSafe) was added, and after 24 h, the samples were counted in a

268 liquid scintillation counter. Leucine incorporation rates ($\text{pmol Leu L}^{-1} \text{ h}^{-1}$) were converted
269 into carbon ($\mu\text{g C l}^{-1} \text{ d}^{-1}$) by using a theoretical factor of $1.55 \text{ kg C mol Leu}^{-1}$ (Simon and
270 Azam, 1989), assuming negligible isotope dilution.

271

272 2.2.9. Optimum multiparameter (OMP) water mass analysis

273

274 Catalá et al. (2018) developed an OMP water mass analysis that allowed computing the
275 contribution of the 19 deep water types identified during the HOTMIX 2014 cruise to every
276 water sample (Supplementary Figure 1). The process included clustering the samples into
277 mixing groups and creating an over-determined system of linear mixing equations for
278 volume, potential temperature, salinity, $\text{NO} (= \text{O}_2 + R_N \cdot \text{NO}_3; \text{ with } R_N = 9.4 \text{ mol O}_2 \text{ mol N}^{-1})$
279 and silicate that was solved in a non-negative least-squares sense for each mixing group.
280 In the Western Mediterranean, the shallowest mesopelagic water type considered was the
281 Atlantic Water (AW) that enters the Mediterranean Sea across the Strait of Gibraltar. Below
282 the AW the Winter Intermediate Water (WIW), formed in the slope of the Gulf of Lions
283 and the Balearic Sea, and the Eastern Intermediate Water (EIW), which comes from the
284 Eastern Mediterranean through the Strait of Sicily, were identified. The bathypelagic layer
285 was occupied by five varieties of Western Mediterranean Deep Water (WMDW), formed in
286 the Gulf of Lions. In the shallow Eastern Mediterranean, Modified Atlantic Water (MAW)
287 in the Strait of Sicily and Levantine Surface Water (LSW) in the Levantine basin were the
288 main water masses observed. The intermediate layer was occupied by the Levantine (LIW)
289 and Cretan (CIW) intermediate waters. In the bathypelagic layer, five varieties of the
290 Eastern Mediterranean Deep Water (EMDW) could be observed, one of Aegean origin
291 (EMT) and four of Adriatic origin (Supplementary Figure 1).

292

293 2.2.10. Estimation of water mass archetype concentrations

294

295 Once the water mass proportions were calculated (see section 2.2.9), water-mass weighted
296 average values of any variable N for each water mass (N_i), hereinafter “archetype values”,
297 were calculated as:

298

$$299 N_i = \frac{\sum_j x_{ij} \cdot N_j}{\sum_j x_{ij}}$$

300

301 where x_{ij} is the proportion of water mass i in sample j and N_j is the value of variable N in
302 sample j . N_j is also called archetype value of N in water mass j , and retains the variability
303 of N due to mixing and large scale biogeochemical processes from the formation area of the
304 water mass to the study site (Álvarez-Salgado et al. 2013; Catalá et al. 2018).

305

306 The standard deviation of the archetype value of N for each water mass, SDN_i , was
307 calculated as:

308

$$309 SDN_i = \frac{\sqrt{\sum_j x_{ij} \cdot (N_j - N_i)^2}}{\sum_j x_{ij}}$$

310

311

312

313 **2.3. Prokaryotic TEP generation experiments**

314

315 To quantify TEP formation by prokaryotes in the Mediterranean Sea, we performed
316 a set of re-growth culture experiments using water from the well-contrasted Eastern and
317 Western Mediterranean basins at different depths in a total of four incubations, two located
318 in the Eastern basin and two located in the Western basin (Figure 1). Those using EIW
319 were carried out in October 2013 during a previous test cruise between Barcelona and the
320 island of Majorca. Water was incubated using triplicate 2 L Nalgene bottles, for 6 days,
321 under *in situ* temperature conditions and in the dark. All used material was acid-washed and
322 rinsed with ultrapure water prior to its use. The samples were pre-filtered through 1 μm
323 filters (using 0.1N HCl pre-washed Preflow capsule filters, Pall Corporation), to remove
324 large particles and grazers, and were used as inoculum of natural microbial populations.
325 The water samples were subsequently filtered by 0.2 μm (using sterile Whatman Polycap
326 cartridge filters). These fractions were mixed, and homogenized, with 75% of 0.2 μm
327 filtered seawater + 25% of microbial inocula. Prokaryotic heterotrophic abundance (PHA)
328 and production (PHP) were monitored during the course of the experiments (6 days) while
329 the concentrations of TEP were determined at the onset and the end of the experiments.

330

331 **2.4. Statistical Analyses**

332

333 We used non-parametric Mann-Whitney U tests to compare differences in TEP and
334 other variables among ocean basins. Depth-averaged data were calculated using the
335 conventional trapezoid method. Linear regression analyses were used to investigate the
336 decreases in TEP and POC concentration with depth, and reduced major axis (RMA)
337 regressions were used to explore the environmental drivers of TEP variability in meso- and
338 bathypelagic water masses, and the relationship between TEP increases and PHA and PHP
339 increases along the incubations. All variables were \log_{10} transformed prior to analyses to
340 facilitate comparison between slopes. All these analyses were conducted using the lmodel2
341 package in the R software (Legendre 2014).

342

343 Results

344

345 *3.1. Environmental conditions*

346

347 Sea surface temperature ranged from 16.3°C at station 17 (near the island of
348 Corsica, Western Mediterranean basin) to 19.3 °C at station 28 in the North Eastern
349 Atlantic, and all visited stations were thermally stratified (Supplementary Figure 2A). Deep
350 waters were increasingly warmer from West to East: waters >1000 m had on average $6.7 \pm$
351 4.4 °C in the North Eastern Atlantic basin, 13.2 ± 0.6 °C in the Western Mediterranean
352 basin, and 14.0 ± 0.9 °C in the Eastern Mediterranean basin (Supplementary Figure 2A).
353 Salinity increased from West to East in all depth layers. A layer of high salinity (>39),
354 indicative of the presence of LIW, was located in the Eastern Mediterranean basin from the
355 surface to 400 m at station 1 (the Easternmost station) and became deeper (between 100
356 and 600 m) near the Sicily Strait, becoming mixed with other water masses in the Western
357 Mediterranean basin (Supplementary Figure 2B). Salinity in the bathypelagic layer was on
358 average 35.4 ± 0.45 at the North Eastern Atlantic, 38.5 ± 0.17 at the Western Mediterranean
359 basin and 38.8 ± 0.45 at the Eastern Mediterranean basin (Supplementary Figure 2B).

360

361 The DCM was located deeper in the Eastern Mediterranean (up to 130 m) than in
362 the Western Mediterranean (50-77m) and North Eastern Atlantic basin (60-86 m),
363 according to the West to East increase in oligotrophy (Figure 2). The Chl *a* concentration in
364 these maxima ranged from 0.13 (station 1, Eastern basin) to $0.92 \mu\text{g L}^{-1}$ (Station 23,
365 Alboran Sea, Western Mediterranean basin). Particulate organic carbon (POC)
366 concentrations ranged from 0.1 to $8.6 \mu\text{mol L}^{-1}$ (Supplementary Table 1) and significantly
367 decreased with depth (log-log regression, $r^2 = 0.62$, p -value < 0.001; supplementary Figure
368 3). These decreases in POC; described with the slopes of the log-log regressions between
369 depth and POC, were $-0.33 (\pm 0.02)$ in the Eastern Mediterranean, $-0.35 (\pm 0.02)$ in the
370 Western Mediterranean and $-0.31 (\pm 0.02)$ in the Northeastern Atlantic. The C:N ratios of
371 particulate organic matter were significantly higher (Anova, $p < 0.005$) in the Atlantic
372 Ocean than in both Mediterranean basins, particularly in the bathypelagic layer
373 (supplementary Table 1)

374

375 *3.2. TEP geographical distribution*

376

377 TEP concentrations in the epipelagic layer increased from East to West (Table 1,
378 Figure 2) and showed significant differences (Mann-Whitney U test) between all basins in
379 the epipelagic layer ($p < 0.01$). In the meso- and bathypelagic layers, although TEP also
380 increased from East to West (Table 1), the values were significantly higher in the North
381 Atlantic Ocean ($p < 0.05$). However, although more TEP were observed in the Western
382 than in the Eastern Mediterranean basins (Table 1), the differences were not significant.
383 The highest TEP values were found at stations 22, 23 and 25, close to the Strait of Gibraltar
384 in the upper 50 m ($63.0 \pm 7.0 \mu\text{g XG eq L}^{-1}$), and the lowest TEP values ($< 2 \mu\text{g XG eq L}^{-1}$)
385 were observed in the easternmost stations (station 1 between 500 and 1500 m, Figure 2).
386 Significant and positive relationships were found between depth-averaged TEP
387 concentrations in the mesopelagic and in the epipelagic layers (Figure 3A; slope= $0.78 \pm$
388 0.13 , $r^2 = 0.23$, $p < 0.01$, $n = 29$) and between depth-averaged TEP in the bathypelagic with

389 respect to that in the mesopelagic layer (Figure 3B; slope= 0.80 ± 0.11 , $r^2= 0.55$, $p<0.001$,
390 $n=26$), indicating a connection between layers. The explained variance for the mesopelagic-
391 epipelagic relationship was lower than for the bathypelagic-mesopelagic relationship,
392 suggesting that between the epipelagic and mesopelagic layers other pathways that release
393 or remove TEP are affecting the distributions.
394

395 The TEP depth-profiles showed consistently higher concentrations in the epipelagic
396 layer than in deeper waters (Figure 4). Within the epipelagic waters, TEP peaks were
397 always shallower than the deep chlorophyll maxima, and were located at the surface in 13
398 out of 29 stations (located in the Levantine basin, the Ionic sea and the Gibraltar and Sicily
399 straits) and between 40 and 50 m in 15 out of 29 stations (mainly in the Western
400 Mediterranean and Eastern North Atlantic Ocean). TEP concentrations in the upper 200 m
401 were maxima and ranged from 5.3 to 81.7 $\mu\text{g XG eq l}^{-1}$, with a median value of 30.9 $\mu\text{g XG}$
402 eq L^{-1} ($n = 121$). By contrast, TEP concentrations in deep waters (≥ 200 m down to bottom
403 depth) showed very constant values, with an average of $6.64 \pm 0.02 \mu\text{g XG eq L}^{-1}$ ($n = 221$;
404 Figure 4). Considering all data, the decline of TEP with depth could be described by the
405 log-log regression $\text{Log}_{10} \text{TEP } (\mu\text{g XG eq L}^{-1}) = -0.40 (\pm 0.03) \text{Log}_{10} \text{depth (m)} + 1.97 (\pm$
406 $0.09)$ ($n = 342$, $r^2 = 0.60$, $p\text{-value} < 0.001$). The slopes of the regression lines were different
407 between the two Mediterranean basins and the adjacent Atlantic Ocean: The Atlantic slope
408 (-0.33 ± 0.03) was significantly lower than those of the Mediterranean basins (-0.42 ± 0.02
409 and -0.40 ± 0.03 in the Eastern and Western Mediterranean respectively).
410

411 The TEP contribution to POC (expressed as a percentage) ranged from 3.3% to
412 $>100\%$ and was higher in surface waters (up to 200 m depth) than in the meso- and
413 bathypelagic waters (Table 1). This percentage was particularly high at the surface (3 m)
414 and in subsurface waters (between 3 m and the DCM). Among basins, %POC values were
415 consistently higher in the Northeast Atlantic than in the Mediterranean Sea (Table 1).
416 However, no marked differences in these % were observed between meso- and
417 bathypelagic waters (Table 1).
418

419 ***3.3. Environmental drivers of TEP distribution in the epipelagic and in the deep*** 420 ***Mediterranean Sea***

421
422 To test which were the main variables driving TEP distribution in the epipelagic and
423 in the meso- and bathypelagic Mediterranean Sea, we used RMA regression analyses
424 between TEP and a complex suite of environmental variables. TEP were not significantly
425 related to Chl *a* concentration. However, we observed significant positive correlation
426 between TEP and O_2 concentration, and between TEP and specific groups of phytoplankton
427 (*Synechococcus*, pico- and nanophytoplankton, Table 2) and prokaryotic heterotrophic
428 abundance (PHA) and production (PHP). Also negative relationships between dissolved
429 inorganic nutrients and TEP were observed (Table 2). None of the correlations was
430 particularly strong.
431

432 In the meso- and bathypelagic layers, RMA regressions showed significant
433 relationships between the archetype TEP values in the water masses intercepted during the
434 cruise (supplementary Figure 1) and archetype values of log-transformed temperature,
435 dissolved organic carbon (DOC), particulate organic carbon (POC), and prokaryote

436 abundance (PHA) and production (PHP, Table 3). In addition, the regression equations
437 between archetypal temperature and TEP (Figure 5A) and between archetypal DOC and
438 TEP (Figure 5B) were significantly ($p < 0.01$) different between the Eastern and Western
439 basins (Table 3). In contrast, Archetypal TEP were also significantly and positively related
440 to archetypal POC (with $r^2 = 0.97$, Figure 5C) and archetypal prokaryotic abundance (PHA,
441 $r^2 = 0.94$, Figure 5D) and archetypal production (PHP, $r^2 = 0.76$, Table 3). In these analyses,
442 the intercepts and slopes of the regression equations were similar among basins (Table 3).
443 Comparing between layers, the POC-TEP relationships showed similar slopes in the
444 epipelagic and deep layers (Table 2, Table 3), while the in PHA-TEP relationships the slope
445 was significantly lower in the deep than in the epipelagic layer (Table 2, Table 3, Figure 6).
446

447 **3.4. Prokaryotic TEP generation in experimental incubations**

448 Prokaryotic heterotrophic abundance (PHA) increased from 2 (Experiment with
449 WMDW) to 85-fold (EIW) during the incubations (Table 4). Prokaryotic heterotrophic
450 production (PHP) increased over time in all incubations and reached the plateau after three
451 days, with time- integrated PHP values of between 8.71 and 14.14 $\mu\text{g C L}^{-1}$ for all the
452 experiments (Table 4). TEP concentrations increased in all incubations, with production
453 rates (ΔTEP) ranging from 0.7 (WMDW experiment) to 232.2 (EIW experiment) $\mu\text{g XG eq}$
454 $\text{L}^{-1} \text{d}^{-1}$ (Table 4). The TEP production rates (ΔTEP) were significantly and positively related
455 to the increases in prokaryotic heterotrophic abundance ΔPHA ($r^2 = 0.99$, p -value < 0.007 ,
456 $n = 4$). The slope of the log-log regression equation between PHA and TEP observed in the
457 experiments (1.25 ± 0.09) was similar to that observed in situ in epipelagic waters ($1.24 \pm$
458 0.19), but significantly higher than the one observed in deep waters (0.62 ± 0.04) (Figure
459 6).
460

461 **4. Discussion**

462
463 We present here the first basin-wide and full-depth dataset of TEP concentrations in
464 the open Mediterranean Sea. We observed consistent vertical profiles along the Eastern-
465 Western transect across the Mediterranean Sea and adjacent North Eastern Atlantic Ocean,
466 with higher TEP concentrations in the upper water column, being maximum above the
467 DCM depth, and lower and more uniform from 200 m down to the bottom (Figures 2 and
468 3). Stations close to the Straits of Gibraltar and Sicily exhibited the highest TEP
469 concentrations through the water column (68.4 and 69.8 $\mu\text{g XG eq L}^{-1}$, Figure 3). The range
470 of TEP concentrations observed in this study (0.6 - 81.7 $\mu\text{g XG eq L}^{-1}$) was comparable to
471 the values reported in some Mediterranean Sea regions (Table 5), such as the similar survey
472 carried out by Ortega-Retuerta et al. (2010) in epipelagic waters of the Mediterranean Sea,
473 in the region of the Strait of Gibraltar (Prieto et al. 2006), the Catalan Sea and Balearic Seas
474 (Iuculano et al. 2017; Ortega-Retuerta et al. 2018; Ortega-Retuerta et al. 2017) or the
475 Aegean Sea (Parinos et al. 2017). In other ocean areas, such as higher latitudes in the
476 Northeast Atlantic Ocean (Engel 2004; Harlay et al. 2010; Leblanc et al. 2009), the
477 oligotrophic western North Pacific Ocean (Kodama et al. 2014), or the Southern Ocean
478 (Ortega-Retuerta et al. 2009b) values within the range of the present study have also been
479 observed. In contrast, up to ten times higher epipelagic TEP concentrations were reported
480 in studies conducted in the waters of the Strait of Gibraltar (Prieto et al. 2006), and in the
481 ultraoligotrophic eastern Mediterranean basin (Bar-Zeev et al. 2011).

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In deep waters, TEP concentrations were usually below $10 \mu\text{g XG eq L}^{-1}$. There are only a handful of studies reporting TEP concentrations in the deep (> 200 m depth) Mediterranean Sea (Table 5): in the mesopelagic waters of the Strait of Gibraltar (Prieto et al. 2006), the Eastern Mediterranean (Bar-Zeev et al. 2011) and the Northwest Mediterranean (Ortega-Retuerta et al. 2017; Weinbauer et al. 2013). Only Bar-Zeev et al. (2011) observed higher (up to 80 times higher) values in the Eastern basin than the TEP range obtained in this study. The published information on TEP concentrations in other deep oceans basins is also scarce. Our concentrations are within the ranges of those published from the Pacific and Arctic Oceans by Wurl et al. (2011) but lower than those published by Yamada et al. (2017) from the Pacific Ocean and by Cisternas-Novoa et al. (2015) from the Sargasso Sea.

The TEP profiles in the surface waters of our study presented maximum values at depths located between the surface and the DCM. Previous studies in open waters have reported similar vertical profiles during the stratification period (Bar-Zeev et al. 2011; Kodama et al. 2014; Ortega-Retuerta et al. 2010; Ortega-Retuerta et al. 2017; Prieto et al. 2006; Wurl et al. 2011). These previous studies and our observations thus establish that this vertical pattern, with higher TEP concentrations in the epipelagic layer decreasing with depth, is very common at least during seasonal stratification, even at the beginning of the stratified season, when this study was carried out.

The significant relationships found between the depth-integrated TEP values in the epipelagic and the meso- and bathypelagic waters (Figure 4) suggest that the downward flux of particulate carbon, via TEP, into the deep waters must be important. Particularly robust were the relationships between meso- and bathypelagic layers, suggesting that the export of TEP from meso- to bathypelagic waters should be more efficient than that from epi- to mesopelagic waters. Another alternative explanation is that in the epipelagic layer there are TEP sinks that are absent or are relatively less important in deeper layers, such as photodegradation (Ortega-Retuerta et al. 2009a), emission to the atmosphere as primary aerosols (Orellana et al. 2011), or uptake and degradation by zooplankton (Ling and Alldredge 2003; Passow and Alldredge 1999; Taylor et al. 2014), which are more abundant and exhibit higher activity rates in epipelagic waters.

Noticeably, while the decrease in TEP with depth was similar in both Mediterranean Sea basins, it was lower in the Atlantic Ocean, as shown by a lower slope in the depth-TEP log-log relationship (Figure 2). This would suggest that, in the Atlantic Ocean, TEP is more efficiently transferred to deep waters than in the Mediterranean Sea. Although POC and TEP transfer efficiencies are still to be quantified by particle flux measurements, which were not performed in our study, this observation might help explain the relatively higher C:N molar ratios of particulate organic matter in the deep Atlantic basin (10.7 ± 2.0) than in the deep Mediterranean basin (9.1 ± 3.4 and 8.9 ± 0.8 in the Eastern and Western Mediterranean basins respectively, Supplementary Table 1), as TEP are known to be relatively enriched in carbon (Mari et al. 2001). The underlying reasons for this difference across oceans are yet unclear, but we could point to different non-exclusive mechanisms: Higher TEP uptake and TEP remineralization rates in the Mediterranean than in the

528 Atlantic would yield lower export efficiency. Although this fact is unknown for the TEP
529 pool specifically, dissolved organic matter remineralization rates are higher in the deep
530 Mediterranean Sea than in other ocean basins (Hansell et al. 2012; Santinelli et al. 2010),
531 likely due to the about 10°C higher temperature of the meso- and deep Mediterranean Sea
532 waters compared with the world oceans. Also TEP production and cycling depends on the
533 in situ microbial community composition (Engel et al. 2017) and environmental drivers
534 such as nitrogen vs. phosphorus availability (Engel et al. 2015; Gärdes et al. 2012).
535

536 The percentage of POC that could be attributed to TEP in our study varied between
537 4 and >100% (Table 1) and was on average 75% of POC in the epipelagic Mediterranean
538 Sea but 50% of POC in the deep Mediterranean layers. These values are in the upper range
539 of previously published studies in the Atlantic Ocean (Engel 2004; Malpezzi et al. 2013),
540 higher than in the West Coast of India (Bhaskar and Bhosle 2006), but lower than those
541 measured in the Arctic Ocean (Yamada et al. 2015; Yamada et al. 2017), the Pacific Ocean
542 (Yamada et al. 2017) or in the Eastern Mediterranean Sea (Bar-Zeev et al. 2011). Estimates
543 of TEP contribution to the POC pool must be taken with caution, because in some of our
544 samples the %TEP/POC was higher than 100%, a value impossible by definition, yet a fact
545 that has been previously observed in Mediterranean Sea studies (Bar-Zeev et al. 2011;
546 Parinos et al. 2017). The different cut-off filters used (GF/F for POC, 0.4 µm for TEP)
547 make the comparisons difficult since small TEP are particularly abundant (Passow 2002b).
548 In any case, the used invariant standard conversion factor from XG to TEP-C was
549 calculated from phytoplankton cultures or waters enriched in phytoplankton (Engel and
550 Passow 2001) but not present in our study. In situ TEP, particularly in the deep ocean, are
551 likely derived from other sources, and their specific composition and properties, including
552 their carbon content, is likely different than that in surface samples. Additional studies are
553 definitely needed to obtain accurate estimations of TEP carbon content in contrasting
554 locations and environmental scenarios, the deep ocean in our case.
555

556 Looking at the variability between ocean basins, the most remarkable feature is the
557 higher contribution of TEP to the POC pool in the Atlantic Ocean: 79% in mesopelagic
558 Atlantic waters opposed to 47% in mesopelagic Mediterranean Waters, and 67% in
559 bathypelagic Atlantic waters, opposed to bathypelagic Mediterranean waters (48% and 45%
560 in the E and W basins, Table 1). This difference likely reflects changes in POC export and
561 the efficiency of the biological carbon pump between oceans. The decrease in TEP with
562 depth was paralleled by the decrease in POC with depth: Both TEP and POC decreases
563 with depth were lower in the Atlantic Ocean than in the Mediterranean Sea (supplementary
564 Figure 1). This could be caused by the higher TEP contribution to POC in the Atlantic
565 Ocean, that could enhance particle aggregation due to the high TEP stickiness (Logan et al.
566 1995).
567

568 In epipelagic waters, the uncoupling of Chl *a* and TEP distribution in our study was
569 in line with previous observations in the Mediterranean Sea (Bar-Zeev et al. 2011; Ortega-
570 Retuerta et al. 2010; Ortega-Retuerta et al. 2018; Ortega-Retuerta et al. 2017). This may
571 reflect the inaccuracy of Chl *a* concentration as an estimator of phytoplankton biomass, as
572 C:Chl *a* ratios vary in the water column due to e.g. photo-acclimation. In fact, we observed,
573 that the highest TEP concentrations were generally shallower than the deep chlorophyll
574 maxima, in agreement with Ortega-Retuerta et al. (2017). Moreover, the relatively good

575 relationship between TEP and O₂ suggests that primary production, not measured during
576 the HOTMIX 2014 cruise, could be a better predictor of TEP distribution than Chl *a* in line
577 with the results of Ortega-Retuerta et al. (2017). TEP distribution in the epipelagic was
578 better predicted by the abundance of *Synechococcus*, micro- and nanoplankton. Although
579 this is the first time a significant relationship between *Synechococcus* and TEP is shown,
580 *Synechococcus* are known to produce aggregates (Deng et al. 2015). Conversely,
581 prokaryotic abundance and production were related to TEP, as previously observed in the
582 epipelagic Mediterranean Sea (Ortega-Retuerta et al. 2010). However, the relatively low
583 explained variance in all these relationships illustrates the complexity of mechanisms
584 driving TEP distributions and thus the difficulty in establishing a single predictor for TEP
585 occurrence in the Mediterranean Sea.

586
587 The RMA regression analyses between archetype TEP and temperature or DOC
588 showed different relationships between these variables in the two Mediterranean basins
589 (Figure 5A-B, Table 3). In general, water masses from the Western Mediterranean were
590 relatively more enriched in TEP compared to DOC than those from the Eastern basin. This
591 observation supports the fact that a higher percentage of oxygen demand is due to DOC in
592 the Eastern than in the Western basin (Catalá et al. 2018). This difference in relative TEP
593 concentrations (i.e. more TEP in water masses of similar temperatures) suggests that TEP
594 in the Mediterranean Sea were not conservatively distributed with the different water
595 masses (i.e. the main mechanisms transporting TEP to the ocean interior are not convection
596 and/or deep water formation events).

597
598 Alternatively, the significant relationships between TEP and POC could be
599 described using similar equations in the two Mediterranean basins (Figure 5C), so TEP
600 could be similarly predicted by POC changes in both Mediterranean sub-basins. This
601 suggests that TEP are distributed and cycled following pathways similar to those affecting
602 bulk POC, i.e. particulate matter is mostly generated in surface layers and a fraction is
603 exported downward to the deep Mediterranean Sea. This fraction is apparently similar for
604 TEP and POC, as indicated by the similar slopes of POC-TEP relationships in epipelagic
605 and deep waters (Table 2, Table 3). Our observations contrast with previous studies that
606 show evidence of different export efficiencies of TEP and the overall POC pool in other
607 ocean areas (Hamanaka et al. 2002; Mari et al. 2017).

608
609 TEP distribution in the meso- and bathypelagic waters are also well predicted by
610 prokaryotic heterotrophic abundance (PHA, Table 3 and Figure 5D). Weinbauer et al.
611 (2013) also observed significant relationships between heterotrophic prokaryotes and TEP
612 concentrations in the twilight zone of the NW Mediterranean Sea, though not comparable to
613 our study since they measured TEP microscopically. Although conditions for prokaryotes
614 in the deep ocean are less favourable than in the epipelagic, and microbes grow at lower
615 rates (Aristegui et al. 2009), deep ocean prokaryotes are also known to release gel-
616 forming polysaccharides potentially generating TEP (Bar-Zeev et al. 2011).

617
618 The role of prokaryotes as a significant TEP source in the deep Mediterranean Sea
619 was experimentally confirmed, as elevated amounts of TEP were generated following
620 prokaryotic growth during all the incubations we performed, particularly in the mesopelagic
621 waters assayed. This is, to our knowledge, the first experimental evidence of prokaryotic

622 TEP generation in deep ocean waters. Increases in TEP could be predicted from prokaryotic
623 growth, with rather similar per cell TEP generation rates in the four incubations. The
624 experimental generation rates presented similar slopes than the in situ PHA-TEP
625 relationship in epipelagic waters. Therefore, supporting previous evidences (Ortega-
626 Retuerta et al. 2010), we can conclude that TEP generation is the dominant process
627 determining the prokaryote-TEP relationship, so changes in prokaryote abundances would
628 be accompanied by similar changes in TEP as observed in the experiments. However, the
629 lower slope in the PHA-TEP relationship in the deep than in the epipelagic or in the
630 experiments suggests that prokaryotes in this layer do not only produce TEP but might also
631 consume and degrade TEP derived from other sources, likely those exported from the upper
632 layers. In this line, previous studies have shown intense prokaryote colonization of TEP in
633 the deep ocean (Bar-Zeev et al. 2011; Bochsansky et al. 2016).

634

635 **Conclusions**

636

637 We present the first basin-wide distribution of TEP in the Mediterranean Sea. We
638 conclude that TEP distribution and cycling patterns in the Mediterranean Sea are different
639 from those in the Atlantic Ocean, reflected in a more limited connectivity of TEP from the
640 epipelagic layer to the dark ocean -probably due to higher remineralization rates in the
641 Mediterranean Sea, but with higher TEP relative content in particles in the Atlantic Ocean.
642 This could translate into different POC export efficiencies among the different basins. In
643 situ measurements of particle fluxes in these ocean basins are needed to confirm this
644 hypothesis. Combining in situ data and experiments, we suggest that TEP sinking from
645 upper layers and in situ production by prokaryotes, mostly drive TEP spatial distribution in
646 Mediterranean meso- and bathypelagic layers.

647

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649

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659

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662

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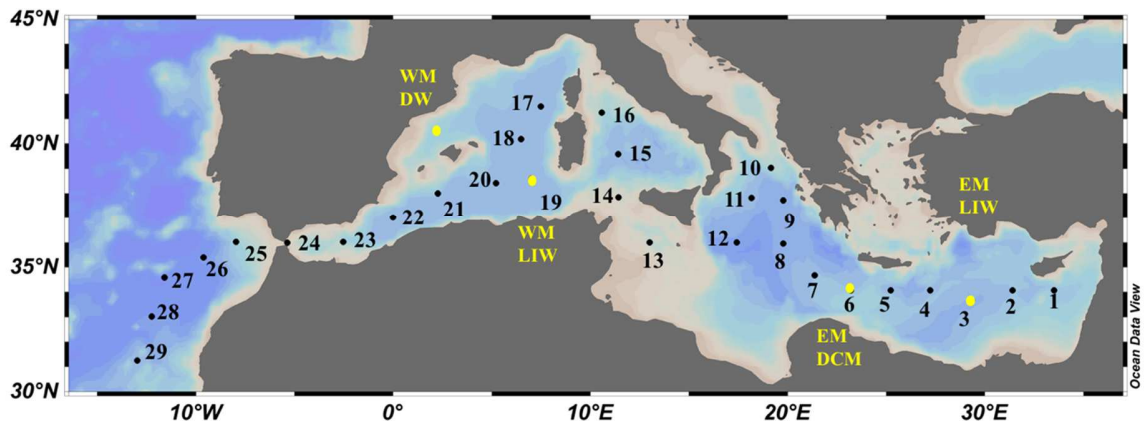
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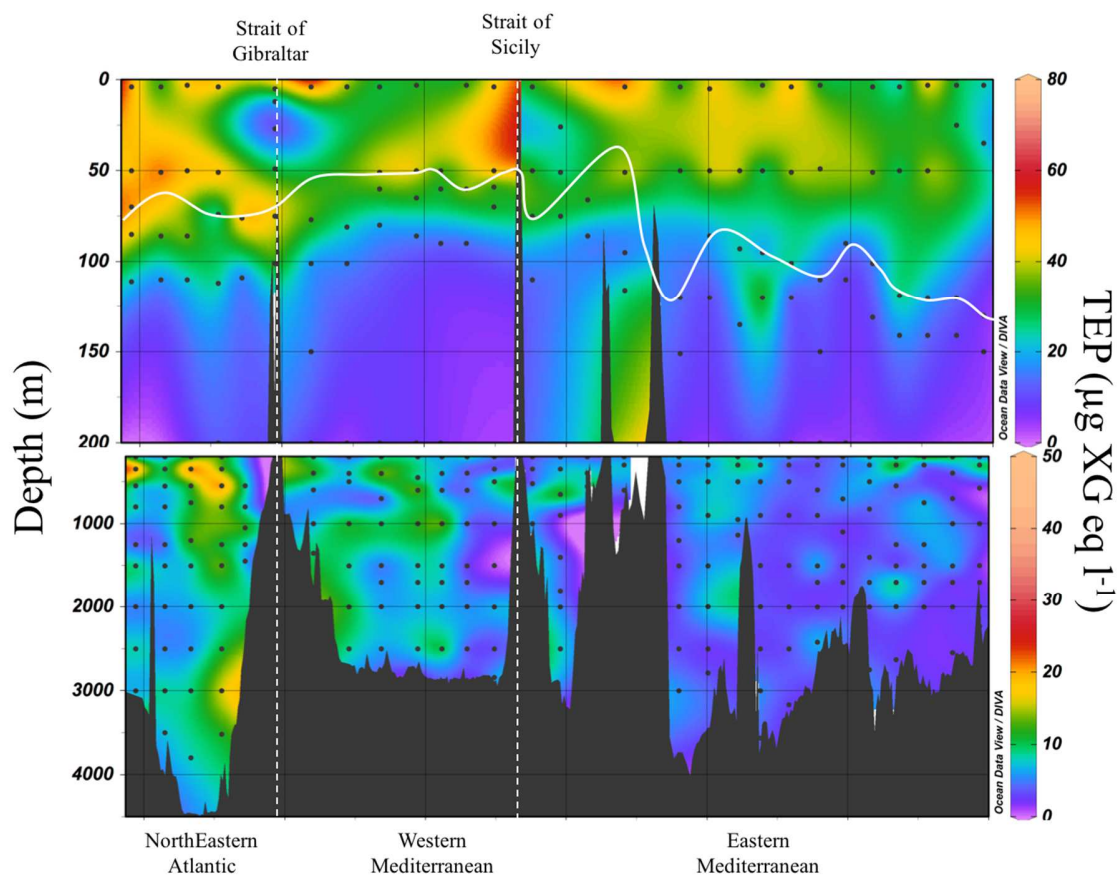
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1 **Figure 1.** Map showing the sampling stations (numbers from 1 to 29) during the HOTMIX
 2 2014 cruise. The strait of Sicily separates the Eastern (stations 1 to 13) from the Western
 3 (stations 14 to 15) basins; while the strait of Gibraltar separates the Mediterranean Sea from
 4 the subtropical Northeast Atlantic Ocean (stations 25 to 29). Yellow dots represent the
 5 locations where the experimental incubations were performed (EM LIW: Eastern
 6 Mediterranean Levantine Intermediate Water. EM DCM: Eastern Mediterranean deep
 7 chlorophyll maximum water. EIW: Western Mediterranean Levantine Intermediate Water.
 8 WM DW: Western Mediterranean Deep Water. This last experiment was performed in
 9 October 2013 during a previous test cruise between Barcelona and the island of Majorca
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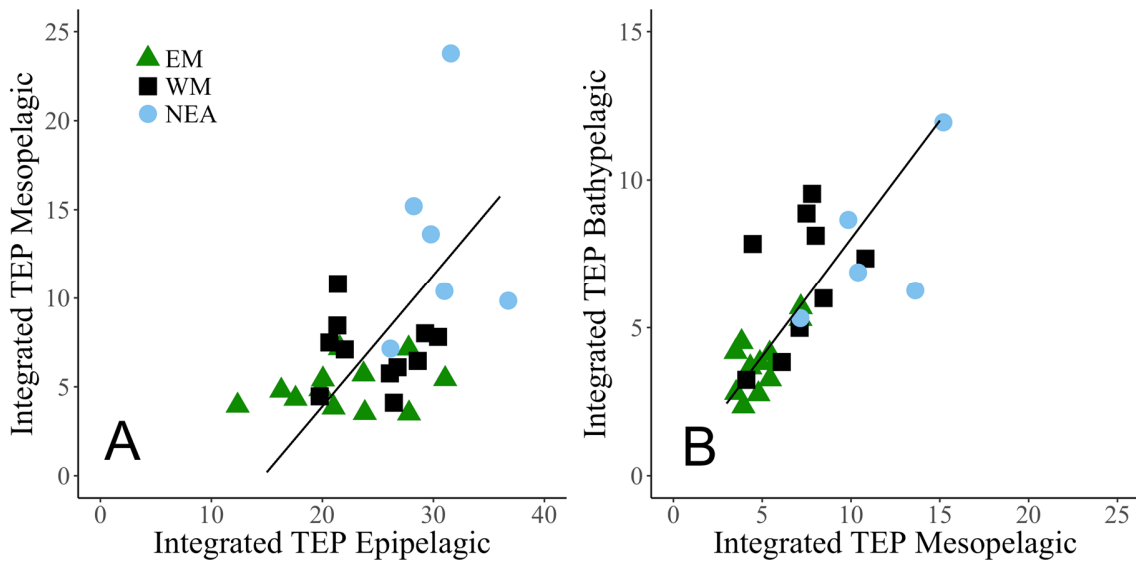
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15 **Figure 2.** Distribution of transparent exopolymeric particles (TEP) during the HOTMIX
 16 2014 cruise across the Mediterranean Sea and the Northeast Atlantic Ocean. White dashed
 17 lines indicate the approximate boundaries between ocean basins. Black dots correspond to
 18 the sampled stations and depths. The continuous white line in the upper panel indicates the
 19 depth of the deep chlorophyll maximum.
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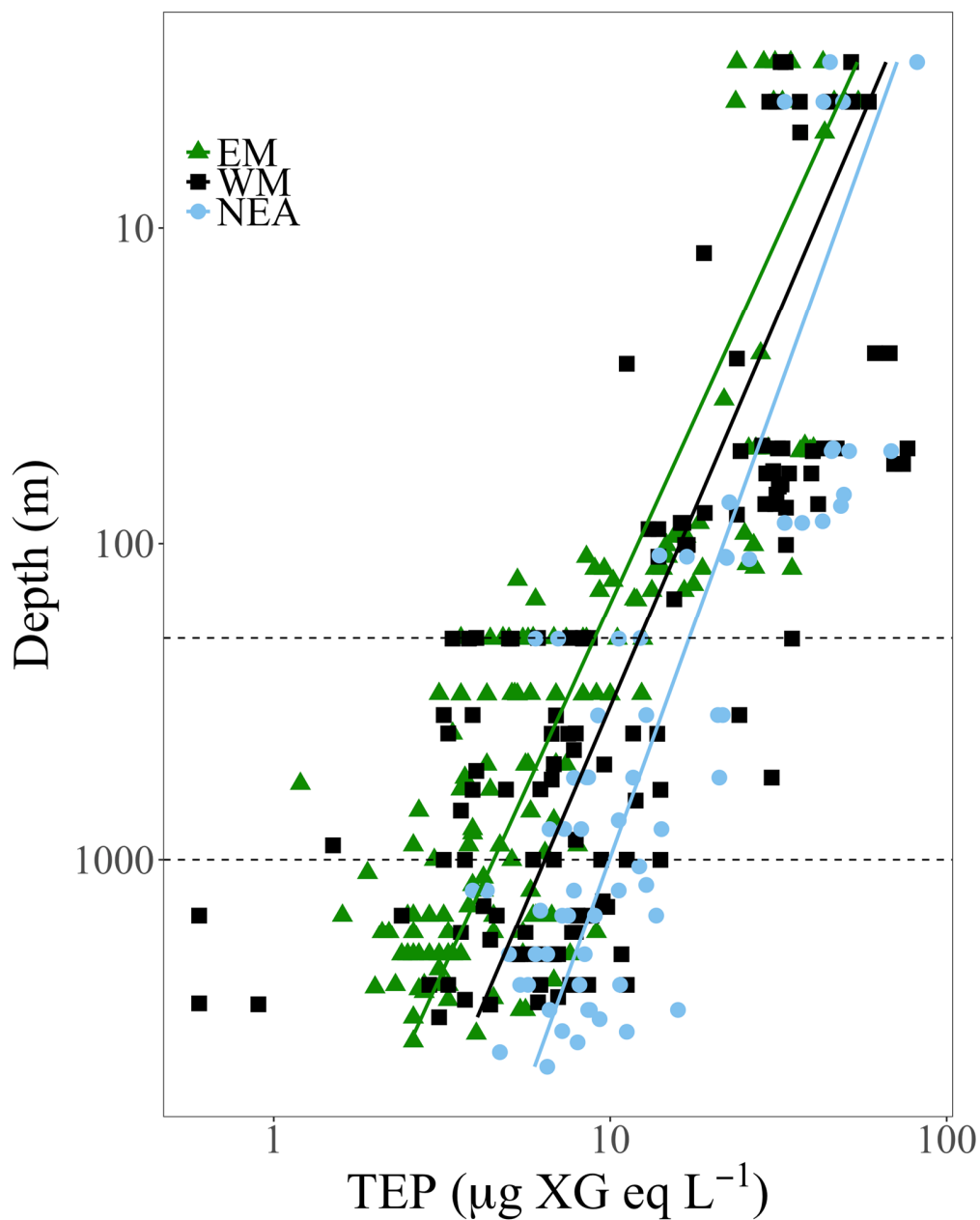
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23 **Figure 3.** Scatter plots showing RMA regressions between depth-integrated transparent
 24 exopolymer particles (TEP) concentration in mesopelagic vs. epipelagic waters (a) and in
 25 bathypelagic vs. mesopelagic waters (b). The slopes of the regression lines are 0.78 ± 0.13
 26 for the epi-meso relationship and 0.80 ± 0.11 for the meso-bathy relationship. (EM:
 27 Eastern Mediterranean, green triangles. WM: Western Mediterranean, black squares. NEA:
 28 Northeastern Atlantic Ocean, light blue circles)



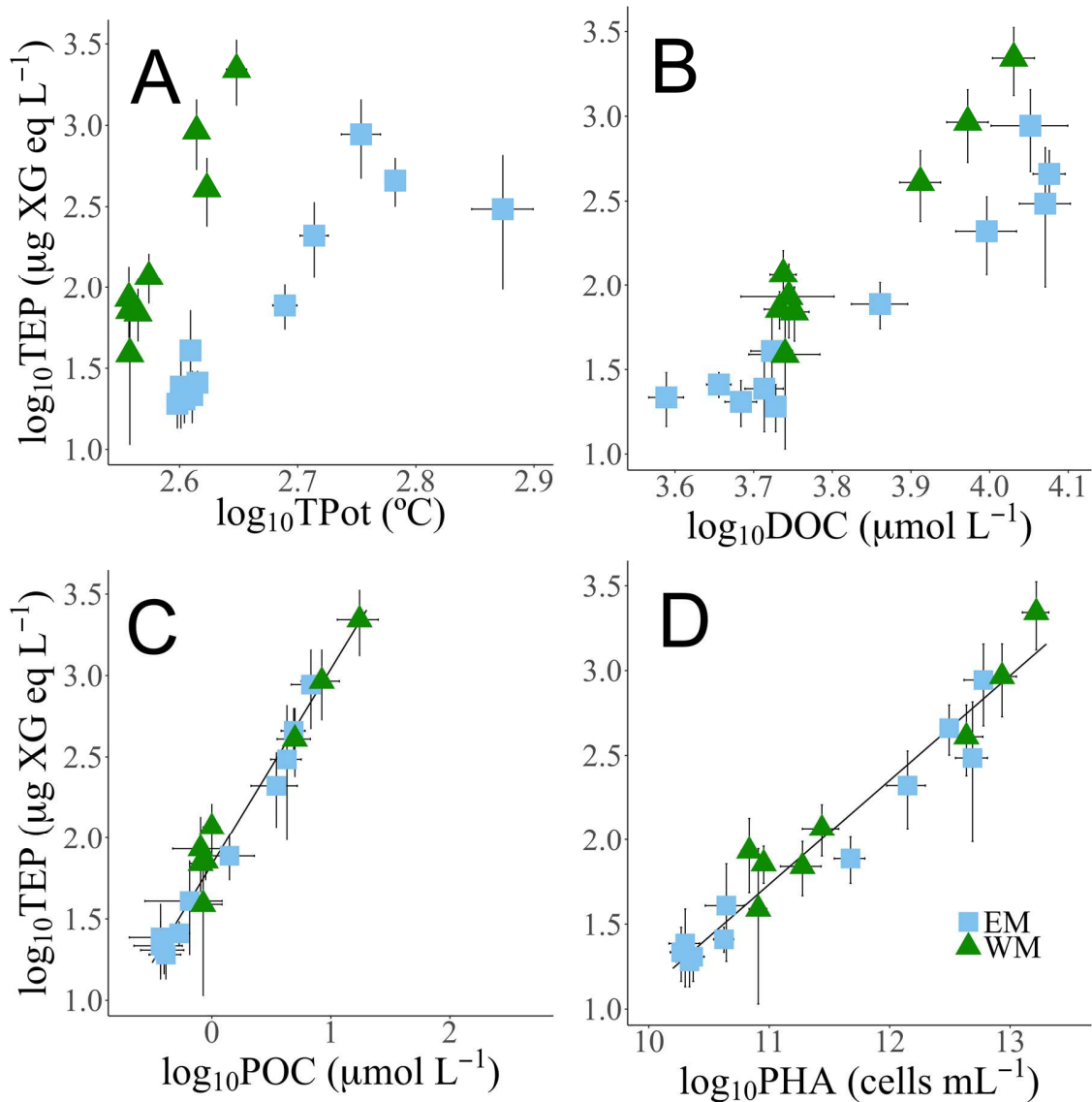
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34 **Figure 4.** Depth profiles of transparent exopolymer particle (TEP) concentration in the
35 three ocean basins sampled in this study. Horizontal dashed lines at 200 m and 1000 m
36 separate the three depth layers (epipelagic, mesopelagic, bathypelagic). The different
37 symbols discriminate between ocean basins (EM: Eastern Mediterranean, green triangles.
38 WM: Western Mediterranean, black squares. NEA: Northeastern Atlantic Ocean, light blue
39 circles)
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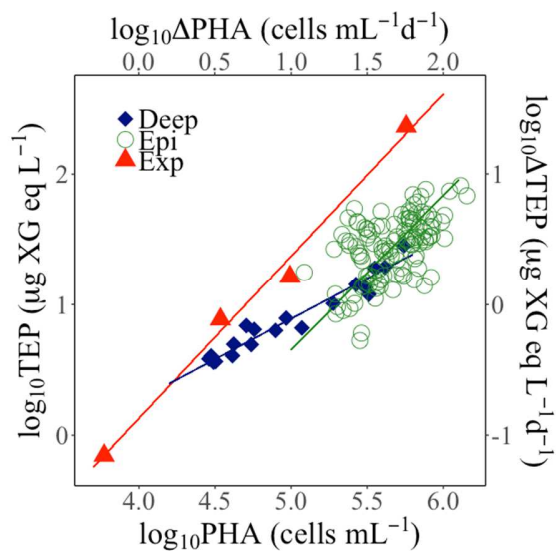
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45 Figure 5. Scatter plots showing log-log RMA regression analyses between archetype values
 46 of temperature (TPot, A), dissolved organic carbon (DOC, B), particulate organic carbon
 47 (POC, C), and prokaryotic heterotrophic abundance (PHA, D), and archetype TEP
 48 concentrations in the meso- and bathypelagic water masses of the Mediterranean Sea. The
 49 regression equations are presented in Table 3. Green triangles: Eastern Mediterranean
 50 basin. Light blue squares: Western Mediterranean basin.
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53

54 **Figure 6.** Log-log RMA relationships between prokaryotic heterotrophic abundance (PHA) and transparent exopolymer particles (TEP) in epipelagic waters (Epi, open green circles),
 55 and deep waters (Deep, dark blue diamonds) and between daily increases in PHA (Δ PHA) and increases in TEP (Δ TEP) in the experimental incubations (Exp, red triangles).
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61 Table 1. Average (\pm Standard Error) and ranges (in brackets) of TEP concentrations (μg
 62 XG eq l^{-1}) and the fraction of TEP as particulate organic carbon pool (%POC) for each
 63 depth layer (epi-, meso and bathypelagic waters) in the different ocean basins. We used a
 64 constant conversion factor of 0.75 $\mu\text{gC } \mu\text{gXG eq}^{-1}$ taken from the literature (Engel and
 65 Passow 2001).
 66

Basin	Variable	Epipelagic	Mesopelagic	Bathypelagic
Eastern Med	TEP	25.1 \pm 0.25 (5.3-54.6)	5.7 \pm 0.05 (1.2-12.5)	3.8 \pm 0.03 (1.6-9.1)
Western Med		34.8 \pm 0.34 (11.2-76.5)	8.6 \pm 0.16 (1.5-34.7)	5.9 \pm 0.08 (0.6-11.2)
NE Atlantic		40.2 \pm 0.79 (14.0-81.7)	11.4 \pm 0.28 (6.0-21.6)	8.2 \pm 0.10 (3.9-15.9)
Eastern Med	%POC	62.0 \pm 0.5 (14.8-144.8)	47.2 \pm 0.6 (8.6-157.9)	47.5 \pm 0.8 (8.6-256.4)
Western Med		53.1 \pm 0.5 (19.5-147.1)	46.9 \pm 0.8 (12.9-197.6)	44.7 \pm 0.8 (3.3-137.7)
NE Atlantic		85.2 \pm 1.6 (27.2-139.2)	78.7 \pm 3.0 (33.5-182.8)	66.7 \pm 1.2 (22.1-169.1)

67

68 Table 2. Results of log-log RMA regression analysis between temperature (Temp), salinity
 69 (Sal), dissolved inorganic phosphorus (DIP) and nitrogen (DIN), particulate organic carbon
 70 (POC), abundances of different phytoplankton groups, dissolved oxygen concentration (O₂)
 71 prokaryotic heterotrophic abundance (PHA) and production (PHP) as independent variables
 72 and TEP (independent variable) in epipelagic waters along the HOTMIX 2014 cruise. r²=
 73 explained variance; p= level of significance
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 75

Dep.Var	Independent Var.	Intercept	slope	r ²	p	n
TEP	Temp	-3.88 ± 2.89	2.57 ± 0.03	0.06	0.009	119
	Sal	50.25 ± 10.14	-12.91 ± 0.06	0.16	< 0.001	119
	DIP	1.91 ± 0.27	-0.31 ± 0.06	0.22	< 0.001	114
	DIN	2.93 ± 0.06	-0.20 ± 0.04	0.25	< 0.001	108
	POC	1.81 ± 0.19	1.36 ± 0.17	0.38	< 0.001	116
	Chl <i>a</i>				ns	
	<i>Prochlorococcus</i>				ns	
	<i>Synechococcus</i>	0.73 ± 0.32	0.30 ± 0.05	0.26	< 0.001	118
	Picophytoplankton	-4.01 ± 1.97	0.98 ± 0.26	0.14	< 0.001	119
	Nanophytoplankton	1.15 ± 0.33	0.49 ± 0.08	0.29	< 0.001	119
	Microphytoplankton	1.61 ± 0.64	0.49 ± 0.19	0.08	0.002	119
	Cryptophytes				ns	
	O ₂	-60.59 ± 9.42	11.76 ± 1.73	0.31	< 0.001	119
	PHA	-12.82 ± 2.50	1.24 ± 0.19	0.28	< 0.001	119
	PHP	4.46 ± 0.30	0.62 ± 0.17	0.14	< 0.001	114

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 77

78 Table 3. Results of log-log RMA regression analyses between archetype values of
 79 temperature (temp), dissolved organic carbon (DOC), particulate organic carbon (POC),
 80 heterotrophic prokaryotic abundance (PHA) and prokaryotic heterotrophic production
 81 (PHP) and archetype values of transparent exopolymer particles (TEP) in meso- and
 82 bathypelagic waters of the Mediterranean Sea, both merged and split into Eastern and
 83 Western basins. r^2 = explained variance; p = level of significance
 84
 85

Dep. Var.	Ind. Var	All basins (n=19)				Eastern basin (n=11)				Western basin (n=8)			
		r^2	p	Intercept \pm SE	Slope \pm SE	r^2	p	Intercept \pm SE	Slope \pm SE	r^2	p	Intercept \pm SE	Slope \pm SE
TEP	Temp	0.25	0.03	-19.96 \pm 17.65	8.33 \pm 6.69	0.78	<0.001	-16.07 \pm 3.98	6.70 \pm 1.49	0.93	<0.001	-42.45 \pm 6.60	17.28 \pm 2.56
“	DOC	0.79	<0.001	-13.31 \pm 2.10	4.01 \pm 0.55	0.91	<0.001	-10.93 \pm 1.55	3.34 \pm 0.41	0.95	<0.001	-17.00 \pm 4.89	5.04 \pm 0.61
“	POC	0.97	<0.001	1.84 \pm 0.01	1.21 \pm 0.06	0.98	<0.001	1.79 \pm 0.01	1.21 \pm 0.07	0.96	<0.001	1.91 \pm 0.04	1.14 \pm 0.12
“	PHA	0.94	<0.001	-5.05 \pm 0.46	0.62 \pm 0.04	0.96	<0.001	-4.72 \pm 0.55	0.58 \pm 0.05	0.94	<0.001	-5.12 \pm 1.01	0.63 \pm 0.08
“	PHP	0.76	<0.001	4.15 \pm 1.26	0.77 \pm 0.12	0.80	<0.001	4.43 \pm 0.52	0.90 \pm 0.19	0.78	0.004	3.85 \pm 0.49	0.62 \pm 0.19

86

87 Table 4. Average, and standard deviation (in brackets), of the daily increments in
 88 prokaryotic heterotrophic abundance (Δ PHA), time-integrated prokaryotic heterotrophic
 89 production (PHP), the average bacterial specific growth rates (SGR) and the daily TEP
 90 generation rates (Δ TEP).
 91

Experiment	ΔPHA (x 10⁷ cells L⁻¹ d⁻¹)	PHP_{int} (μg C L⁻¹)	SGR (d⁻¹)	ΔTEP (μg XG eq L⁻¹ d⁻¹)
Western Mediterranean <i>-Deep water</i>	0.59 (\pm 0.07)	-	0.13	0.7 (\pm 0.1)
Western Mediterranean <i>-Levantine intermediate water</i>	57.05 (\pm 0.25)	14.14	0.71	232.2 (\pm 14.6)
Eastern Mediterranean <i>-DCM water</i>	3.43 (\pm 0.26)	8.97	0.17	7.7 (\pm 3.0)
Eastern Mediterranean <i>-Levantine intermediate water</i>	9.81 (\pm 0.71)	8.71	0.69	16.4 (\pm 5.0)

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95 Table 5. TEP concentrations in the Mediterranean Sea published in the literature.
96

Region	Depth (m)	Month	Year	TEP ($\mu\text{g XG eq l}^{-1}$)	Chl ($\mu\text{g l}^{-1}$)	POC ($\mu\text{mol l}^{-1}$)	Ref
Levantine basin	surface	March/Jul/Sep	2008/2009	116-420	0.04-0.07	13.8	(Bar-Zeev et al. 2011)
“	dcm	“	“	48-189	<0.32	“	“
“	>300	“	“	83-386	“	“	“
North Western	4-200	May-June	2012	4.9-54.2	0.10-0.65	3.7-7.2	(Ortega-Retuerta et al. 2017)
“	200-2300	May-June	2012	5.2-19.0	“	“	“
North Western	surface	3 years	2012-2014	11.3-289.1	0.15-1.21	5.4-24.0	(Ortega-Retuerta et al. 2018)
Coastal NW (rocky shore)	surface	3 years	2012-2015	4.6-90.6	0.02-0.54	“	(Iuculano et al. 2017)
Coastal NW (seagrass litter)	surface	3 years	2012-2015	26.8-1878.4	0.02-0.54	“	“
North Western	20-50 (dcm)	1 year	1999-2000	0.35-.2 x10 ⁷ *	<2.9	3.70-10.35	(Beauvais et al. 2003)
North Western	300m	1 year	2008-2009	0.52-1.4 x10 ⁶ *	“	“	(Weinbauer et al. 2013)
Alboran Sea Inshore	0-70	June-July	1997	507-560	“	“	(Prieto et al. 2006)
Alboran Sea offshore	“	“	“	25-121	“	“	“
East-West transect	0-200	May	2007	4.5-94.3	0-1.78	“	(Ortega-Retuerta et al. 2010)
Aegean Sea	0-100	October	“	15.4-81.4	0.08-0.30	2.15-5.84	(Parinos et al. 2017)
“	“	March	“	39.1-188	0.04-0.60	1.64-9.75	“
“	“	July	“	31.7-156	0.04-0.50	2.01-7.00	“
Coastal Aegean	0-5	June-Jul/Jan-Feb	2003-2004	208-441	0.5-1.6	0.28-1.28	(Scoullou et al. 2006)
Coastal Adriatic	0-37	1 year	1999-2002	4-14800	>1	“	(Radic et al. 2005)
East-West transect	0-200	May	2014	5.6-76.5	0.13-0.92	0.10-8.60	This study
“	201-1000	May	2014	1.2-34.7	“	0.26-5.37	“
“	1001-bottom	May	2014	0.6-11.2	“	0.13-1.62	“

* TEP analysed by microscopy counting

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