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1 **Seasonality of amino acid enantiomers and microbial community at**  
2 **MOLA time series in the NW Mediterranean**

3  
4  
5 **Highlights**

- 6  
7
- 8 • Ship-based time series can be combined with remote sensing to close  
9 sampling gaps.
  - 10 • Phytoplankton spring bloom occurs between February and May in  
11 NW Mediterranean Sea.
  - 12 • Phytoplankton and river discharge affect the seasonality of L-DHAA  
13 concentrations.
  - 14 • Accumulation of d-DHAA suggests bacterial processing of labile DOC  
15 in summer.
  - 16 • DOM-microbe interactions are most apparent in lineages associated  
17 with blooms
- 18

**Seasonality of amino acid enantiomers and microbial community at  
MOLA time series in the NW Mediterranean**

Anabel von Jackowski <sup>1</sup>, Nawal Bouchachi <sup>1</sup>, Quentin-Boris Barral <sup>2</sup>, Paul Labatut <sup>3</sup>, Barbara Marie <sup>1</sup>, Olivier Crispi <sup>1</sup>, Karine Escoubeyrou <sup>3</sup>, Charles-Hubert Paulin <sup>3</sup>, Celine Dimier <sup>4</sup>, Josephine Ras <sup>5</sup>, Alexander Hayward <sup>6</sup>, Eva Ortega Retuerta <sup>1</sup>

1-CNRS/Sorbonne Université, UMR7621 Microbial Oceanography Laboratory, Banyuls sur Mer, France

2-CNRS/Université de Toulouse, UMR5566 Laboratory of Space Geophysical and Oceanographic Studies, Toulouse, France

3-Oceanographic Observatory of Banyuls sur Mer (OOB), Banyuls sur Mer, France

4-CNRS/Sorbonne Université, Institut de la Mer de Villefranche (IMEV), Villefranche-sur-Mer, France

5-CNRS/Sorbonne Université, UMR 7093 Laboratoire d'Océanographie de Villefranche (LOV), Villefranche, France

6-Danish Meteorological Institute, København, Denmark

\* Corresponding author:

Anabel von Jackowski [vonjackowski@obs-banyuls.fr](mailto:vonjackowski@obs-banyuls.fr)

Eva Ortega Retuerta, [ortegaretuerta@obs-banyuls.fr](mailto:ortegaretuerta@obs-banyuls.fr)

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1 **Abstract**

2 Seasonality in light, temperature, and nutrient availability are well-known to  
3 regulate phytoplankton blooms and the bacterioplankton community. During the  
4 spring bloom, phytoplankton release biomolecules as part of the dissolved organic  
5 matter (DOM) pool exploited by the bacterioplankton. Here, we investigated the  
6 seasonal variability of phytoplankton biomass, enantiomers of dissolved  
7 hydrolyzable amino acids (DHAA), bacterioplankton abundances and community  
8 composition at the Microbial Observatory Laboratory Arago (MOLA) in the NW  
9 Mediterranean Sea from 2019 to 2021. Phytoplankton biomass estimated from  
10 pigment biomarkers suggests a spring bloom succession from cryptophytes,  
11 haptophytes, and prasinophytes in March to diatoms in April. The spring bloom  
12 coincided with a 50% increase in L-enantiomers of DHAA and an increase in  
13 bacterial abundance. After the spring bloom, elevated concentrations of D-  
14 enantiomers of DHAA and gamma-aminobutyric acid suggest bacterial processing  
15 of labile biomolecules contributed to the seasonal accumulation of dissolved  
16 organic carbon (DOC). Linking organic molecules with the free-living  
17 bacterioplankton community showed a seasonal succession of niches and substrate  
18 regimes. The parallel analysis of DOM and bacterioplankton community provides  
19 an important baseline for bacteria-substrate relationships over the seasonal cycle  
20 in the northwestern Mediterranean Sea.

21

## 22 1 Introduction

23 Marine dissolved organic matter (DOM) is a diverse mixture of compounds that  
24 account for 662 Pg of organic carbon and play a critical role in global  
25 biogeochemical cycles (Hansell, 2013). A single water sample can contain up to  
26 20,000 molecular formulas, most of which remain uncharacterized to date (Riedel  
27 and Dittmar, 2014; Wagner et al., 2020). The characterizable fraction of DOM is  
28 frequently described based on its physical properties, i.e., size, and/or  
29 characterizing major biochemical groups, such as amino acids, to determine the  
30 bioavailability, diversity, and origin. Specifically, amino acids can be used to  
31 estimate the labile, semi-labile, and refractory DOC reservoirs (Davis and Benner,  
32 2007).

33 DOM sources and sinks can be estimated using L- and D-enantiomers of  
34 amino acids. The main sources of marine DOM are phytoplankton biomass that  
35 produce L-enantiomers of amino acids, which are essential building blocks for all  
36 living organisms (Jørgensen et al., 1999). DOM is remineralized by heterotrophic  
37 bacterioplankton (i.e., archaea and bacteria), which are enriched in D-enantiomers  
38 as key constituents of the peptidoglycan, capsules, lipopolysaccharides,  
39 siderophores, and antimicrobial peptides (Park and Strominger, 1957; Hancock,  
40 1960; Lam et al., 2009). Additionally, most bacterioplankton preferentially use L-  
41 over D-enantiomers of amino acids (Azúa et al., 2014; Zhang and Sun, 2014). Some  
42 cultural representatives do use D-amino acids, with a preference for D-Alanine, D-  
43 Glx, D-Asx, and D-Serine over D-Methionine, D-Valine, and D-Leucine, which are  
44 assumed to be less bioavailable (Zhang et al., 2016). Therefore, tracing the  
45 evolution of both pools of enantiomers in dissolved hydrolyzable amino acids

46 (DHAA) can provide information on whether the main DOM sources are  
47 phytoplanktonic versus bacterial origin.

48 DOM bioavailability varies seasonally and controls the bacterioplankton  
49 community. Organic carbon is predominantly derived from phytoplankton blooms  
50 that provide pulses of labile and semi-labile compounds, which sustain bacterial  
51 carbon and energy requirements during a given year (Piontek et al., 2011; Liu et  
52 al., 2020, 2022; von Jackowski et al., 2022). Upon the decay of the spring  
53 phytoplankton bloom, members of the *Roseobacter* clade (class:  
54 Alphaproteobacteria) and Flavobacteria (class: Bacteroidetes) typically dominate  
55 the bacterioplankton community in early successional stages (Pinhassi and  
56 Hagström, 2000; Teeling et al., 2016; Bunse and Pinhassi, 2017). In summer,  
57 SAR86 (class: Gammaproteobacteria) and SAR11 (class: Alphaproteobacteria)  
58 become dominant clades in the bacterioplankton community (Alderkamp et al.,  
59 2006; Alonso-Saez et al., 2007; Salter et al., 2015). Simultaneously, semi-labile  
60 DOM compounds tend to accumulate since they resist rapid bacterial turnover and  
61 possibly remineralized during fall or exported to the deep ocean during wintertime  
62 convective mixing (Carlson et al., 1994; von Jackowski et al., 2020).

63 In the NW Mediterranean Sea, abiotic factors control the seasonal niche  
64 preferences of the autotrophic and heterotrophic community (Salter et al., 2015;  
65 Galand et al., 2018; Lambert et al., 2019). Seasonal changes in the DOM pool are  
66 driven by the interplay between bacterioplankton during the spring bloom and low  
67 salinity water (LSW, PSU < 37.9) events in summer (Laghdass et al., 2010;  
68 Gonzalez et al., 2019). Additionally, the release of phytoplankton-derived  
69 transparent exopolymer particles (TEP) have been linked to a peak in bacterial

70 extracellular enzyme activities resulting in an accumulation of recalcitrant  
71 dissolved organic carbon (DOC) in the euphotic zone (0-200 m) during the summer  
72 stratification period (Copin-Montégut and Avril, 1993; Romera-Castillo et al.,  
73 2010; Vila-Reixach et al., 2012; Jones et al., 2013; Ortega-Retuerta et al., 2018). It  
74 is possible that the seasonal accumulation of recalcitrant DOC causes a  
75 malfunctioning of the microbial loop in summer to late fall (Thingstad et al., 1997;  
76 Sánchez-Pérez et al., 2020). To understand seasonal DOC accumulation and its  
77 factors in regulating bacterioplankton community patterns, we conducted a 3-year  
78 survey at the Microbial Observatory Laboratory Arago (MOLA) in the Gulf of Lion,  
79 northwestern Mediterranean Sea (NWMED). We hypothesized that the  
80 pronounced seasonal variability of DHAA regulates the bioavailability, which has  
81 consequences for microbial loop dynamics. Assessing relationships between  
82 microbes (i.e., phytoplankton and bacterioplankton) and the DOM pool over the  
83 seasonal cycle is important to understanding carbon cycling and substrate regimes  
84 in the NW Mediterranean Sea.

85

## 86 **2 Material and Methods**

### 87 **2.1 *Sampling Site***

88 The “Microbial Observatory Laboratory Arago” (MOLA) time series station is  
89 located at the edge of the continental shelf in the NW Mediterranean Sea  
90 (42°27'205"N - 3°32'565"E) (Fig. 1). MOLA core parameters are sampled almost  
91 every month since 2004 and the time series was incorporated into the  
92 Mediterranean Ocean Observing System for the Environment (MOOSE) research  
93 infrastructure in 2010. At MOLA, the conductivity, temperature, and depth sensor

94 (CTD) is equipped with two temperature probes, two conductivity probes, and a  
95 Digiquarz pressure sensor. Seawater is collected using 12-L Niskin bottles to  
96 sample nine discrete depths: 5 m, 20 m, 40 m, 80 m, 120 m, 150 m, 200 m, 300 m,  
97 and 500 m.

98 MOLA was sampled 20 times for MOOSE core parameters and additional  
99 microbial parameters between 2019 and 2021 (Supplementary Table 1). In brief,  
100 MOOSE core parameters include the concentration of oxygen determined with  
101 titration (Winkler, 1888), inorganic nutrients determined on a continuous flow  
102 nutrient analyzer (AA3HR AutoAnalyzer, Bran-Luebbe, Ireland), chlorophyll-a  
103 (chl-a) extracted from GF/F filters (Whatman, USA) with acetone and determined  
104 fluorometrically (Evans et al., 1987), particulate organic carbon and nitrogen  
105 determined on a CHNS-O Elemental Analyzer (Sharp, 1974), and bacterial cell  
106 counts determined using flow cytometry (Cytoflex, Beckman Coulter, USA) and  
107 converted into abundances using the software FlowJo (v7.6, USA). The protocols  
108 for the core parameters and data management are available on the MOOSE  
109 website (<https://www.moose-network.fr/>). Additional microbial parameters  
110 included pigment-based chemotaxonomy, dissolved organic carbon (DOC), total  
111 dissolved nitrogen (TDN), total dissolved phosphorus (TDP), dissolved  
112 hydrolyzable amino acids (DHAA), and bacterioplankton community composition  
113 using the 16S rRNA marker gene.

114

## 115 **2.2 Characterization of *DOM and Amino Acid Enantiomers***

116 Seawater for DOM was filtered through a double-layer of combusted GF/F filters  
117 (450°C for 8 hours, Whatman, USA), equivalent to 0.2 µm pore-size, and collected



118 at all MOLA depths between 5-500 m. Samples for DOC were fixed with 10  $\mu$ L  
119 phosphoric acid (pH < 2), and measured using high-temperature catalytic  
120 oxidation (Sugimura and Suzuki, 1988; Qian and Mopper, 1996). Samples for TDN  
121 and TDP were submitted to persulfate wet-oxidation (Pujo-Pay and Raimbault,  
122 1994) and using inorganic nutrients were then used to derive dissolved organic  
123 nitrogen and phosphorus (DON and DOP) concentration.

124 Samples for DHAA were collected at 5 and 500 m. Seawater was filtered  
125 through a double-layer of combusted GF/F filters (Whatman, USA) and collected  
126 in combusted glass vials and frozen (-20°C). Samples and blanks using ultrapure  
127 water were analyzed following the protocol by Escoubeyrou and Tremblay (2014).  
128 In brief, hydrolysis (110°C for 20 h) with hydrochloric acid (30% Suprapur, Merck  
129 Millipore, USA) under vacuum and o-phthaldialdehyde derivatization with either  
130 N-Isobutyryl-L-Cysteine (Sigma-Aldrich, USA) for the L-DHAA reagent run or N-  
131 Isobutyryl-D-Cysteine for D-DHAA reagent run separated enantiomeric amino  
132 acids and achiral amino acids by reversed-phase high-performance liquid  
133 chromatography (HPLC). The HPLC (UltiMate 3000, Thermo Fisher Scientific,  
134 USA) was equipped with a Gemini C18 column (Phenomenex, USA), an  
135 autosampler, and a fluorescence detector (excitation at 335 nm, emission at 450  
136 nm). A gradient of the organic phase (methanol, acetonitrile) and phosphate buffer  
137 was used with a constant flow rate of 0.8 mL per minute (Escoubeyrou and  
138 Tremblay, 2014). DHAA identification and quantification in unknown samples  
139 were achieved by comparing the retention times and peak areas with those of  
140 standard amino acid solutions and procedural blanks were subtracted from final  
141 concentrations. Asparagine and Glutamine were deaminated during the

142 hydrolysis and were quantified as Aspartic acid (Asx) and Glutamic acid (Glx).  
143 Overall, 13 L-DHAA were detected: Alanine, Arginine, Asx, Glx, Histidine,  
144 Isoleucine, Leucine, Lysine, Phenylalanine, Serine, Threonine, Tyrosine, and  
145 Valine. A total of 6 D-DHAA were detected: Alanine, Asx, Glx, Leucine, Serine,  
146 and Valine. Additionally, we detected Beta-Alanine, Glycine, and Gamma-  
147 aminobutyric acid (GABA).

148

### 149 ***2.3 Phytoplankton Pigment Analysis***

150 Seawater (1-2 L) for pigment-based chemotaxonomy was collected at 5 m, filtered  
151 onto combusted GF/F filters (Whatman, USA), and stored frozen (-20°C). The  
152 filters were extracted using 3 mL methanol (100%) for two hours, disrupted by  
153 sonication, and clarified by vacuum filtration through GF/F filters. The extracts  
154 were quantified using reversed-phase HPLC (Agilent Technologies, USA), after  
155 Ras et al. (2008). Phytoplankton taxonomy was assessed from pigment  
156 concentrations (Table 1) and converted into class abundances and pigment-to-chl-  
157 a ratios (Supplementary Table 2) using r-package “phytclass” (v.1.0.0) (Hayward  
158 et al., 2023) that is based on CHEMTAX program (Mackey et al., 1996).

159 Table 1. List of the pigments used in this study and their taxonomic significance  
160 (Roy et al., 2011).

<b>Chlorophylls</b>	<b>Taxonomic or biochemical significance</b>
Alloxanthin	Cryptophytes
Bacteriochlorophyll-a	Photoheterotrophic bacteria
Chlorophyll-b	Chlorophytes, Prasinophytes
19-Butanoyloxyfucoxanthin	Haptophytes, Pelagophytes

Fucoxanthin	Diatoms, Haptophytes, some Dinoflagellates, Pelagophytes
19-Hexanoyloxyfucoxanthin	Haptophytes
Lutein	Chlorophytes, Prasinophytes,
Neoxanthin	Prasinophytes, Chlorophytes
Peridinin	Dinoflagellates
Prasinoxanthin	Prasinophytes
Violaxanthin	Chlorophytes, Prasinophytes
Zeaxanthin	Cyanobacteria, Chlorophytes, Prasinophytes, Flavobacteria

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161

## 162 **2.4 Bacterioplankton Community Analysis**

163 Samples for genomic DNA were collected at 5 and 500 m. Around 5 L of seawater  
164 was filtered through 3.0- $\mu\text{m}$  and then 0.22- $\mu\text{m}$  filters (Nucleopore, USA) using a  
165 peristaltic pump and stored frozen (-80°C). The genomic DNA was lysed with  
166 lysozyme (20 mg mL<sup>-1</sup>, 45 min at 37°C, Sigma-Aldrich, USA), proteinase K (20 mg  
167 mL<sup>-1</sup>, 60 min at 55°C, Sigma-Aldrich, USA), and extracted using Zymobionics  
168 DNATM Miniprep Kit (Cat. No.: D6005, Zymo Research, USA). PCR-amplification  
169 using the universal 16S rRNA 515F and 926R primer pair covering the v4-v5  
170 hypervariable region (Parada et al., 2016) and sequenced on a MiSeq platform  
171 (Illumina, USA) at LGC Genomics GmbH (Berlin, Germany).

172 Amplicon reads were processed in RStudio (v2023.09.1+). FastQ files were  
173 processed into amplicon sequence variants (ASVs) using the R-package ‘dada2’  
174 (v1.16.1) (Callahan et al., 2016). Filtering settings were truncLen=c(190,200),  
175 maxN=0, minQ=2, maxEE=c(3,3) and truncQ=0, followed by merging using

176 minOverlap=10 and chimera removal. After singleton removal, we obtained an  
177 average of 53,387 reads per sample. ASVs were taxonomically classified using the  
178 SILVA SSU Reference dataset (release v138.1) (McLaren and Callahan, 2021).  
179 ASVs with less than three counts in less than 3% of samples were excluded. ASVs  
180 were further analyzed using R-packages ‘phyloseq’ (v1.42.0) (McMurdie and  
181 Holmes, 2013), ‘ampvis2’ (v2.8.3) (Andersen et al., 2018), and ‘microeco’ (v1.4.0)  
182 (Liu et al., 2021).

183

## 184 ***2.5 Statistical Analyses***

185 Data processing and statistical analyses were conducted in RStudio (v2023.09.1+).  
186 Statistical analyses focused on changes within a Julian year, since the sampling  
187 gaps over the course of the three-year time series made it inapt to determine  
188 monthly averages or inter-annual variation within the discrete variables.

189 For all environmental parameters, arithmetic means and standard  
190 deviations were calculated using the ‘base’ package (v4.2.1) and Spearman  
191 correlations were corrected using a false discovery rate (FDR) adjusted p-value  
192 (also called q-value) of 0.05. Degradation indices of DHAA were calculated based  
193 on Kaiser and Benner (2009) by summarizing L- and D-enantiomer  
194 concentrations. Integrations of the upper 500 m were performed using trapezoidal  
195 integration.

196 For the amplicon data, alpha-diversity was analyzed with ‘iNEXT’ (v3.0.0)  
197 (Hsieh et al., 2016) and permutational multivariate analysis of variance  
198 (PERMANOVA) were calculated using vegan (‘adonis2’ and ‘vegdist’ functions,  
199 v2.6.4) (Oksanen et al., 2022). Log<sub>2</sub>fold-change (log<sub>2</sub>FC) had an adjusted p-value

200 of 0.05 using the ‘DESeq2’ (v1.38.3) (Love et al., 2014) to investigate the differences  
201 in bacterioplankton community between the free-living (FL) and particle-attached  
202 (PA) size fractions. Distance-based redundancy analysis (dbRDA) was fit with  
203 significant environmental vectors using vegan (‘envfit’ function) to investigate  
204 seasonal environmental drivers of FL bacterioplankton community at 5 m  
205 (Buttigieg and Ramette, 2014). Similarly, Pearson correlations with an FDR-  
206 adjusted p-value was performed between ASVs and the environmental data using  
207 ‘microeco’ (function ‘cal\_cor’, v1.4.0) to investigate drivers of taxa (Liu et al., 2021).

208 Visualizations were performed in MATLAB with M\_Map (Pawlowicz, 2020)  
209 and using R-package ggplot2 (v3.4.3) (Wickham, 2016). All scripts are publicly  
210 available via <https://github.com/anabelvonjackowski/>.

211

## 212 ***2.6 Remote Sensing and Simulation Approaches***

213 Remote sensing data products were used to fill in the gaps between discrete  
214 sampling events of temperature, salinity, and chl-*a* at MOLA. For sea surface  
215 temperature (SST), L4 satellite data was downloaded from the Copernicus Marine  
216 Environment Monitoring Service (CMEMS), resulting in a high-resolution dataset  
217 (VHR, 1/40°, ~1.4 km) of daily SST averages (Buongiorno Nardelli et al., 2013).

218 For salinity, we used two remote sensing data products to fill in the gaps  
219 between discrete sampling events. Sea surface salinity (SSS) lacks a high-  
220 resolution satellite-derived data for the Mediterranean (SSS, > 30 km with the  
221 SMOS satellite) (Olmedo et al., 2018) and artifacts (>40) in global ocean datasets.  
222 Consequently, we chose to apply two simulations with assimilations of  
223 observations: a reanalysis for the January 2019 to June 2021 period and an

224 operational model from August to December 2021 to finalize the time series. The  
225 high resolution ( $1/24^\circ$ ,  $\sim 4$  km) of the daily reanalysis (Escudier et al., 2021), the  
226 daily CMEMS analysis, and the forecast model (Clementi et al., 2019) allowed the  
227 assimilation of *in situ* temperature and salinity vertical profiles and satellite sea  
228 level anomaly along the track data through an OceanVar assimilation scheme.

229 For sea surface chl- $\alpha$ , mass concentration data was downloaded from the L4  
230 CMEMS satellite-derived product at 1km resolution (Berthon and Zibordi, 2004;  
231 Volpe et al., 2018, 2019). The data was reconstructed using satellite-merged  
232 (SeaWiFS, MODI, MERIS, VIIRS-SNPP & JPSS1) bio-optical multi-sensors that  
233 use empirical and semi-analytical algorithms to calculate the remotely sensed chl-  
234  $\alpha$ . Daily outputs were reprocessed with climatological data for the smoothing  
235 procedure of the sensor merging, in addition with the DINEOF (Data  
236 INterpolating Empirical Orthogonal Functions) algorithm to fill in missing data  
237 due to cloud cover. Since oligotrophic waters of the Mediterranean are greener and  
238 less blue than other ocean basins, the algorithm parameterized at a regional level  
239 (an updated version of MedOC4) provides a more accurate estimate compared to  
240 the overestimated chlorophyll by the global algorithm. Furthermore, algorithm  
241 assessment and validation concluded that the product is in good agreement with  
242 historical *in situ* chlorophyll data.

243

### 244 **3 Results**

245 Discrete sampling and remote-sensing approaches showed well-defined seasonal  
246 patterns in temperature and salinity. The *in situ* surface (5 m) temperatures  
247 ranged from 13 to 24°C ( $n=20$ ), while satellite-derived sea surface temperatures

248 (SST) ranged between 13 to 25°C ( $n=1096$ ) with rapid increases from ~15°C in  
249 May to >18°C in June 2019 and 2021 compared to ~15°C in April to >18°C in June  
250 in 2020. The *in situ* surface salinity ranged from 37.7 to 38.2 PSU ( $n=20$ ), while  
251 satellite-derived sea surface salinity (SSS) ranged between 37.5 and 38.4 PSU  
252 ( $n=1057$ ), both suggesting the occurrence of LSW (PSU < 37.9, >3 days) events but  
253 with inter-annual variability between 2019 to 2021. LSWs likely ranged from 17  
254 June to 29 July 2019, 09 June to 21 July 2020, and 28 May to 28 June 2021 (Fig.  
255 1c). At 500 m, temperatures remained ~14°C and salinity ~39 PSU ( $n=5$ ) year-  
256 round, but data are only available for 2021.

257         The remote-sensing approach confirmed that spring bloom in the NWMED  
258 occurred between February and May. Satellite-derived sea surface chl-*a* ranged  
259 from 0.05 to 0.60 µg L<sup>-1</sup> ( $n=1096$ ), which was slightly lower than the *in situ* surface  
260 chl-*a* that ranged from 0.07 to 1.0 µg L<sup>-1</sup> ( $n=21$ ; Fig. 1c). Both satellite-derived and  
261 *in situ* chl-*a* show inter-annual variability in bloom dynamics between 2019 to  
262 2021. Discrete measurements of chl-*a* increased by 85% from February to March  
263 before declining 37% from March to April until the beginning of the new productive  
264 season in November (Fig. 1c). Phytoplankton taxonomy estimated from pigment  
265 biomarkers suggests that the spring bloom was dominated by cryptophytes,  
266 haptophytes, and prasinophytes at the beginning of March and diatoms in early  
267 February as well as the end of April (Fig. 2a). Accordingly, chl-*a* showed significant  
268 positive correlations with cryptophytes ( $r=0.83$ ,  $p<0.001$ ), haptophytes ( $r=0.84$ ,  
269  $p<0.001$ ), prasinophytes ( $r=0.88$ ,  $p<0.001$ ), dinoflagellates ( $r=0.86$ ,  $p<0.001$ ), and  
270 diatoms ( $r=0.63$ ,  $p=0.01$ , Supplementary Fig. 1). Chlorophytes were only observed

271 in spring 2021, while *Synechococcus* appeared to grow in spring, summer, and fall  
272 (Fig. 2a and 3).

273 Maximal inorganic nutrient concentrations were observed in winter, which  
274 gradually decreased during the productive season until late fall. At 5 m, nitrate  
275 ( $\text{NO}_3^-$ ) ranged from below the detection limit to  $2.3 \mu\text{mol L}^{-1}$  ( $n=21$ ), nitrite ( $\text{NO}_2^-$ )  
276 ranged from  $0.010$  to  $0.25 \mu\text{mol L}^{-1}$  ( $n=21$ ), and silicate ( $\text{SiO}_4$ ) ranged from  $0.25$  to  
277  $2.0 \mu\text{mol L}^{-1}$  ( $n=21$ ). Ammonium ( $\text{NH}_4^+$ ) ranged from below the detection limit to  
278  $0.068 \mu\text{mol L}^{-1}$  ( $n=21$ ), and phosphate ( $\text{PO}_4^{3-}$ ) ranged from  $0.010$  to  $0.80 \mu\text{mol L}^{-1}$   
279 ( $n=21$ ). At 500 m,  $\text{NO}_3^-$  ranged from  $7.7$  to  $9.1 \mu\text{mol L}^{-1}$  ( $n=18$ ),  $\text{NO}_2^-$  ranged from  
280  $0.010$  to  $0.31 \mu\text{mol L}^{-1}$  ( $n=18$ ),  $\text{PO}_4^{3-}$  ranged from  $0.31$  to  $0.41 \mu\text{mol L}^{-1}$  ( $n=18$ ), and  
281  $\text{SiO}_4$  ranged from  $5.1$  to  $7.6 \mu\text{mol L}^{-1}$  ( $n=18$ ).

282 Maximal POC concentrations and minimum DON concentrations were  
283 observed in winter, while DOC and DOP concentrations decreased to a minimum  
284 spring and accumulated during the productive season. At 5 m, concentrations of  
285 POC ranged from  $4.1$  to  $19 \mu\text{mol L}^{-1}$  ( $n=21$ ) and generally decreased from winter  
286 and throughout the productive season until fall (Supplementary Fig. 2). DOC  
287 ranged from  $58$  to  $102 \mu\text{mol L}^{-1}$  ( $n=21$ ), DON concentrations ranged from  $4.5$  to  $7.6$   
288  $\mu\text{mol L}^{-1}$  ( $n=19$ ), and DOP concentrations ranged from  $0.038$  to  $0.13 \mu\text{mol L}^{-1}$  ( $n=19$ ;  
289 Supplementary Fig. 2). DOC:DON:DOP ratios increased from 13:92:1 in spring,  
290 14:96:1 in summer, 15:68:1 in fall, and decreased to 15:77:1 in winter. DOC  
291 significantly negatively correlated with chl- $\alpha$  ( $r=-0.59$ ,  $p<0.001$ ). At 500 m, POC  
292 concentrations ranged from  $1.8$  to  $16 \mu\text{mol L}^{-1}$  ( $n=20$ ) or  $1529$  to  $4733 \text{ mmol m}^{-2}$   
293 and peaked in winter and late summer ( $n=20$ ; Supplementary Fig. 3). DOC  
294 concentrations ranged from  $45$  to  $94 \mu\text{mol L}^{-1}$  ( $n=18$ ), DON ranged from  $2.8$  to  $5.9$



295  $\mu\text{mol L}^{-1}$  ( $n=18$ ), and DOP ranged from below the detection limit to  $0.13 \mu\text{mol L}^{-1}$   
296 ( $n=18$ ; Supplementary Fig. 3).

297 DHAA concentrations showed seasonal variability with an accumulation of  
298 L-DHAA in March and July compared to a gradual accumulation of D-DHAA until  
299 late summer. At 5 m, DHAA ranged from  $0.65$  to  $1.17 \mu\text{mol L}^{-1}$  ( $n=13$ ) that  
300 negatively correlated with salinity ( $r=-0.62$ ,  $p<0.05$ ) and diatoms ( $r=-0.82$ ,  $p<0.01$ ).  
301 Calculating degradation indices (DI) showed positive DIs (less degraded) in spring  
302 and during the LSW events in June compared to negative DIs (more degraded) in  
303 winter (Fig. 2b). Further investigating the enantiomer composition showed that  
304 L-DHAA ranged from  $0.38$  to  $0.67 \mu\text{mol L}^{-1}$  ( $n=13$ ; Fig. 2c) and significantly  
305 negatively correlated to diatoms ( $r=-0.77$ ,  $p<0.01$ ; Supplementary Fig. 1). In  
306 contrast, D-DHAA ranged from  $40$  to  $68 \text{ nmol L}^{-1}$  ( $n=15$ ; Fig. 2c) and significantly  
307 increased with the Julian Day (JD) ( $r=0.72$ ,  $p=0.0025$ ), while significantly  
308 negatively correlating with chl- $a$  ( $r=-0.69$ ,  $p<0.01$ ) and phytoplankton groups that  
309 bloomed in March (Supplementary Fig. 1). Specifically, individual monomers  
310 showed that all L-DHAA increased more than two-fold from February to March  
311 (Fig. 2d), while several L-DHAA (Histidine, Lysine, Serine, Tyrosine, and Valine)  
312 decreased more than 1.3-fold in April (Fig. 2e). Similar to their L-DHAA  
313 counterparts, B-Alanine, D-Alanine, and D-Glx also increased in April, whereas  
314 D-Serine and D-Valine decreased in April (Fig. 2f). Generally, B-Alanine and D-  
315 DHAA increased throughout the year (Fig. 2f). At 500, DHAA ranged from  $0.42$  to  
316  $4.6 \mu\text{mol L}^{-1}$  with L-DHAA ranging from  $0.17$  to  $1.7 \mu\text{mol L}^{-1}$  ( $n=15$ ) peaking in  
317 spring and D-DHAA ranging from  $27$  to  $142 \text{ nmol L}^{-1}$  ( $n=15$ ) increasing towards  
318 fall (Supplementary Fig. 3).

319 Seasonal changes were observed in the bacterioplankton abundance and  
320 community composition at 5 m. Bacterioplankton abundances ranged from 3.8 to  
321  $13 \times 10^5$  cells  $\text{mL}^{-1}$  ( $n=20$ ) with a notable shift in high (HNA) and low nucleic acid  
322 (LNA) subgroups. HNA cells ranged from 1.8 to  $5.2 \times 10^5$  cells  $\text{mL}^{-1}$  ( $n=20$ ) and  
323 peaked in late February to May and July to September. LNA cells ranged from 1.6  
324 to  $8.9 \times 10^5$  cells  $\text{mL}^{-1}$  ( $n=20$ ) and peaked in late February to early March and July  
325 to August (Supplementary Fig. 2). Assessing the amplicon data showed that the  
326 sequencing depth sufficiently covered the bacterioplankton diversity free-living  
327 (FL) fraction and particle-attached (PA)  $\sim 99.7\%$  (Supplementary Fig. 4). At 5 m,  
328 the FL community significantly changed with the JD (PERMANOVA,  $p < 0.01$ )  
329 along with the PA community that also significantly changed with the JD  
330 (PERMANOVA,  $p < 0.05$ ; Supplementary Table 3). Furthermore, alpha-diversity  
331 indices of the FL showed a significant increase in richness ( $r=0.69$ ,  $p=0.0041$ ) and  
332 Shannon diversity ( $r=0.52$ ,  $p=0.045$ ) with the JD (Supplementary Fig. 5, outlier  
333 JD 27). At the class level, FL and PA were represented by Alphaproteobacteria,  
334 Gammaproteobacteria, Bacteroidetes, and Cyanobacteria up to 80%. At the family  
335 level, FL community was dominated by SAR11 clade I  $\sim 18.8\%$ , SAR11 clade II  
336  $\sim 6.2\%$ , and Flavobacteriaceae  $\sim 12.8\%$  year-round, while Cyanobiaceae showed  
337 monthly fluctuations. The PA community was dominated by Cyanobiaceae  $\sim 21.7\%$   
338 and families in Bacteroidetes  $\sim 19.8\%$  but also reoccurring Pirellulaceae  $\sim 2.8\%$ ,  
339 Puniceococcaceae 2.6%, and Vibrionaceae  $\sim 3.5\%$  (Fig. 3). Testing the seasonal  
340 enrichment showed significant changes in SAR11 clades, SAR86 clade, and  
341 AEGEAN-169 marine group during spring, summer, or winter in FL ( $\log_2\text{FC}$

342  $q < 0.001$ ) compared to Planctomycetes in winter in PA ( $\log_2FC$   $q < 0.001$ ,  
343 Supplementary Fig. 6).

344 Seasonal changes were less pronounced in the bacterioplankton abundance  
345 and community composition at 500 m. Bacterial abundances ranged from 0.97 to  
346  $9.6 \times 10^5$  cells  $mL^{-1}$  ( $n=19$ ) with HNA ranging from 0.48 to  $5.1 \times 10^5$  cells  $mL^{-1}$  ( $n=19$ )  
347 and LNA ranged from 0.47 to  $4.5 \times 10^5$  cells  $mL^{-1}$  ( $n=19$ ). Assessing the amplicon  
348 data showed that the sequencing depth sufficiently covered the bacterioplankton  
349 diversity FL fraction and PA  $\sim 99.3\%$  (Supplementary Fig. 4). The alpha-diversity  
350 indices gradually decreased by JD in the FL, while alpha-diversity peaked in  
351 summer within the PA (Supplementary Fig. 5). At the class level, the FL was  
352 dominated by Nitrososphaeria, whereas the PA community had representatives of  
353 Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes up to 50% (Fig. 3). At  
354 family level, FL community was dominated by Nitrosopumilaceae  $\sim 32.3\%$  along  
355 with SAR11 clades I  $\sim 8.5\%$  and clade II  $\sim 4.7\%$ . PA community was almost year-  
356 round, represented by Nitrosopumilaceae, Pirellulaceae, Crocinitomicaceae, and  
357 Phycisphaeraceae  $\sim 4\%$ .

358 Combining environmental parameters with the FL bacterioplankton  
359 community suggests DOM-microbe interactions. A distance-based redundancy  
360 analysis (dbRDA) was used to assess the influence of environmental explanatory  
361 variables (arrows) on the ASV abundance data. At 5m, the dbRDA showed that  
362 salinity, dinoflagellates, and pelagophytes significantly constrained the ordination  
363 space of the response variables (points) corresponding to the winter and spring  
364 samples ( $p < 0.05$ , Fig 4a). In contrast, temperature, D-Asx, and DON significantly  
365 constrained the ordination space corresponding to summer and fall ( $p < 0.05$ , Fig.

366 4a). Attempting direct links between environmental parameters and the FL  
367 bacterioplankton community showed that *Candidatus* Nitrosopumilus and  
368 common bloom-associated genera within the Roseobacter lineage and  
369 Flavobacteriiaeae positively correlated with phytoplankton groups, while  
370 negatively correlating with temperature ( $p\text{-adj}<0.05$ ; Fig. 4b). In contrast, genera  
371 such as *Candidatus* Aquiluna, *DS001*, and other Roseobacter group members  
372 positively correlated to several L-DHAA ( $p\text{-adj}<0.05$ , Fig. 4b). At 500 m, data were  
373 insufficient to derive the significant environmental variables, but L-DHAA  
374 constrained the ordination space corresponding to winter samples, while DON,  
375 DOP, and D-DHAA constrained the ordination space of summer and fall samples  
376 (Supplementary Fig. 7). Direct links were limited in the mesopelagic depth with  
377 few genera showing significant links to L-DHAA, B-Alanine, and D-Serine  
378 (Supplementary Fig. 7).

379

## 380 **4 Discussion**

### 381 *4.1 The spring bloom in the NWMED*

382 The haptophyte- and diatom-dominated blooms caused L-DHAAs and the  
383 degradation index to peak at 5 m during spring (Fig. 2). The phytoplankton spring  
384 bloom composition at MOLA was similar to nearby coastal time series *Service*  
385 *Observation Laboratory Arago* (SOLA), *Blanes Bay* near Barcelona, Spain, and  
386 *DYFAMED* near Villefranche-sur-Mer, France (Marty et al., 2002; Latasa et al.,  
387 2010; Gutiérrez-Rodríguez et al., 2011; Nunes et al., 2018). However, relative  
388 abundances of *Synechococcus* were higher at MOLA compared to the Adriatic Sea  
389 (Trano et al., 2022). The increase of phytoplankton-derived L-DHAA could be

390 similar across the NWMED with a release of hydroxyl-amino acids (L-Serine and  
391 L-Threonine) and glycine from diatom cell walls post-bloom in March (Hecky et  
392 al., 1973; Dauwe and Middelburg, 1998). In June, the peak of aromatic-DHAA (L-  
393 Phenylalanine and L-Tyrosine) and L-Asx might have been released by cell  
394 exudation or lysis of diatoms (Hecky et al., 1973). Furthermore, the different  
395 pattern of L-Glx, which was low during the first diatom bloom but increased to 135  
396 nmol L<sup>-1</sup> during the second diatom bloom in April, might suggest a succession of  
397 diatom populations. *Chaetoceros* sp. and *Rhizosolenia* sp. tend to bloom in early  
398 spring, while *Pseudo-nitzschia* sp. has been observed to bloom at SOLA between  
399 March and May (Charles et al., 2005; Quiroga, 2006). Overall, chl-*a* and L-DHAA  
400 concentrations were over 2-fold higher in surface waters at MOLA compared to  
401 more oligotrophic sites during spring and summer, such as Bermuda Ocean Time  
402 Series Station (BATS) and Hawaii Ocean Time Series (HOT) (Lomas et al., 2013;  
403 Wirtz and Smith, 2020; Ianiri et al., 2022). Studying phytoplankton bloom  
404 dynamics is essential to understand biogeochemical and microbial interactions.  
405 We strongly encourage time series to analyze larger phytoplankton via microscopy  
406 or automated imaging systems, e.g., imaging flow cytobot (IFCB) (Olson and Sosik,  
407 2007; Sosik and Olson, 2007), and additional sampling efforts for smaller  
408 phytoplankton, e.g., flow cytometry, to better understand the mechanisms of  
409 bloom formation and links to biochemistry at offshore time series.

410         DOM bioavailability positively correlated with bacterial abundances and  
411 coincided with copiotroph ecological strategies. Heterotrophs can be generally  
412 distinguished as either HNA or LNA, where HNA are considered to be a more  
413 substrate-driven and metabolically active subpopulation than LNA (Robertson

414 and Button, 1989; Sherr et al., 2006; Bouvier et al., 2007). Furthermore,  
415 heterotrophs can be distinguished as either copiotrophs that compete under high  
416 DOM concentrations or oligotrophs adapted to low concentrations (Koch, 2001;  
417 Giovannoni et al., 2014). In terms of composition, Bacteroidetes (Flavobacteriaceae)  
418 and members of the *Roseobacter* group are non-seasonal and known copiotroph  
419 bacteria since they are more competitive under bioavailable DOM levels in the  
420 NWMED (Lambert et al., 2019; Auladell et al., 2022). The positive correlations  
421 with phytoplankton groups are indicative of labile DOM and diatom-derived DOM  
422 consumption (Sarmiento et al., 2013; Landa et al., 2018). Alongside the  
423 copiotrophs, we find oligotrophs such as SAR11 (Fig. 3). Within SAR11, the  
424 ecotype-like clades exhibit pronounced seasonal cycles and confirm previous  
425 observations that SAR11 clade *Ib* is associated with replete nutrient  
426 concentrations and phytoplankton biomass, while clade *Ia* are adapted to thrive  
427 under basal DOM levels (Salter et al., 2015). SAR11 ecotypes appear to be  
428 differentially involved in the labile and humic substrate processing during the  
429 spring bloom and can not be classified into these over-simplified ecological  
430 strategies (Malmstrom et al., 2004; Alonso-Sáez and Gasol, 2007; Laghdass et al.,  
431 2012). Still, a bacterioplankton community with fairly balanced proportions of  
432 copiotrophs and oligotrophs can simultaneously process the phytoplankton-  
433 derived protein-like substances that contribute to the labile DOC pool and humic-  
434 like fluorescent organic matter that contribute to the recalcitrant DOC pool  
435 released during the spring bloom (Yamashita and Tanoue, 2003; Romera-Castillo  
436 et al., 2010; Sánchez-Pérez et al., 2020). Furthermore, experiments have shown  
437 that picocyanobacteria and bacterioplankton produce humic-like fluorescent

438 organic matter under phosphorus-replete conditions during the spring bloom  
439 (Romano et al., 2014; Zhao et al., 2017; Bouchachi et al., 2023). The humic-like  
440 fluorescent organic matter would be less bioavailable than phytoplankton-derived  
441 compounds and possibly serve oligotrophs. Overall, the copiotrophs appear to be  
442 processing labile DOM, like *Roseobacter*, while oligotrophs might be processing  
443 relatively refractory DOM, like SAR11 clade *Ia*, and supports the notion that  
444 trophic strategies follow a gradient from copiotrophy to oligotrophy at the end of  
445 spring (Lemonnier et al., 2020).

446

#### 447 *4.2 Microbial loop dynamics from summer until winter*

448 LSWs are diluted mesoscale structures that detach from the Rhône River plume  
449 to the open Mediterranean Sea and impact biogeochemical processes around June  
450 to July each year (Diaz et al., 2008). Our study confirms that LSWs are low in chl-  
451 *a* and dissolved inorganic nitrogen (DIN) but contain bioavailable DOM that  
452 sustains bacterial activity (Laghdass et al., 2010; Gonzalez et al., 2019). In 2020,  
453 we observed a strong increase in several L-DHAA and DI, therefore  
454 bioavailability, during the LSW (JD 175) compared to after the LSW (JD 203; Fig.  
455 2). Enrichments of phenylalanine and valine could have been released from  
456 *Chaetoceros* sp. (Poulet and Martin-Jézéquel, 1983; Sarmiento et al., 2013).  
457 Isoleucine and leucine are known to contribute to the highly labile fraction of DOM  
458 (Carlson and Ducklow, 1995; Cherrier et al., 1996), promoting bacterioplankton  
459 growth in summer.

460 Bacterioplankton were increasingly exposed to D-DHAA towards fall (Fig.  
461 2). DHAA accumulated on the surface waters during summer, which appeared as

462 relatively short-term fluctuations in individual L-DHAA but a steady increase of  
463 D-DHAA concentrations until fall (Fig. 2). Since D-DHAA amino acids are proxies  
464 of bacterial-derived DOM, their presence indicates more degraded DOM at the end  
465 of the accumulation period. To further understand the accumulation in the D-  
466 DHAA pool, “canonical” DHAA (D-Alanine, D-Asx, D-Glx) showed relatively  
467 higher concentrations and greater seasonal variability than “non-canonical”  
468 DHAA (D-Leucine and D-Valine; Fig. 2), which is similar to previous observations  
469 (Broek et al., 2019; Ianiri et al., 2022). Canonical DHAA are more abundant in the  
470 HMW material and non-canonical DHAA are more abundant in the LMW material  
471 (Ianiri et al., 2022), which could suggest a preference of bacterioplankton for  
472 canonical D-DHAA. For example, D-Alanine is a key component of autotrophic and  
473 heterotrophic bacterial peptidoglycan (Schleifer and Kandler, 1972; Kaiser and  
474 Benner, 2008; Cava et al., 2011), which would be relatively rapidly remineralized  
475 compared to recalcitrant DHAA that accumulates as part of the LMW DON  
476 towards fall (Ianiri et al., 2022). Nonetheless, D-DHAA can be used as a nitrogen  
477 source for bacterial growth, to modulate cell wall biogenesis or act as a chemotactic  
478 warning signal, but many of the related functions and metabolic pathways remain  
479 unknown (Zhang et al., 2016; Yu et al., 2020; Irazoki et al., 2023). The seasonal  
480 changes in the DHAA pool amid few correlations to the bacterioplankton  
481 community (Fig. 4) highlight the complex interactions among the abiotic and biotic  
482 factors in the microbial loop. More research with longer sampling frequencies of  
483 time series-based studies and higher resolution of ‘omic approaches (e.g., meta-  
484 and transcriptomics) are needed to better unravel the interactions between the



485 composition of the amino acid pool and microbial communities in the NW  
486 Mediterranean.

487

#### 488 ***4.3 Mesopelagic DOM-Bacterioplankton interactions***

489 Organic matter seasonal variations in euphotic and mesopelagic zones will affect  
490 the deep ocean bacterioplankton community and their metabolism. In MOLA,  
491 mesopelagic POC and L-DHAA concentrations peaked during the spring and  
492 summer, while D-DHAA concentrations remained fairly constant at depth  
493 (Supplementary Fig. 3). In comparison, individual and total L-DHAA  
494 concentrations were over 2-fold higher at MOLA than previously reported from  
495 more oligotrophic sites during spring and summer, such as BATS and HOT (Kaiser  
496 and Benner, 2008; Ianiri et al., 2022). The enrichment of L-DHAA at MOLA could  
497 be due to a reduced DHAA degradation since bacterial production, which is around  
498 2-3 times lower at the bottom of the euphotic zone relative to BATS (Gonzalez et  
499 al., 2019; Liu et al., 2022). Alternatively, the elevated chl-*a* at the surface relative  
500 to previous observations (section 4.1) could have enhanced organic matter export  
501 at MOLA through various pathways (phytoplankton, aggregates zooplankton  
502 migration or mixing processes (Siegel et al., 2016; Le Moigne, 2019). Surface  
503 productivity combined with strong continental (westerly/northerly) winds that  
504 cause dense shelf water cascading events favor POC export in the southwestern  
505 Gulf of Lyon, near MOLA (Puig et al., 2008; Sanchez-Vidal et al., 2008; Many et  
506 al., 2021). Furthermore, assuming that sinking or ascending particles contributed  
507 to the POC pool at MOLA, these sinking particles release amino acids into the  
508 surrounding water (Smith et al., 1992; Grossart and Ploug, 2000; Baumas et al.,

509 2023). Finally, our 500m samples are taken near the seafloor, where sediment  
510 resuspensions into the nepheloid layer could have also been a source of L-DHAA.  
511 The diverse pool of available organic substrates shapes the diverse community  
512 across the different fractions in mesopelagic and relative to the euphotic zone  
513 (Ghiglione et al., 2012; Wilson et al., 2017; Baumas et al., 2021; Sebastián et al.,  
514 2021). We encourage future studies and time series that measure microbial  
515 variables to observe organic matter degradation, aggregation, and sinking in the  
516 meso- and bathypelagic zones since anthropogenic climate change affects the  
517 entire water column (Buttigieg et al., 2018; González-Dávila and Santana-  
518 Casiano, 2023; Baumas and Bizic, 2024).

519

## 520 **5 Conclusion**

521 Our assessment of the DOM pool and links to the phyto- and bacterioplankton  
522 communities revealed a seasonality in the surface waters and mesopelagic zone.  
523 In the surface, springtime phytoplankton blooms released L-DHAA, which  
524 increased HNA bacteria abundances and lowered alpha-diversity, thereby  
525 promoting bacterioplankton with copiotroph ecological strategies. A notable input  
526 of bioavailable DOC occurred during LSW events during summer. During  
527 summer, the accumulation of DOC and D-DHAA, the presence of LNA bacteria,  
528 and declining richness suggest that a broader community of less active  
529 bacterioplankton target more refractory substrates into fall. Our study highlights  
530 the seasonal variation in L- and D- enantiomers of DHAA and possible  
531 associations with the microbial community. We strongly encourage microbial time  
532 series observatories to incorporate or expand the range of discrete parameters to

533 incorporate biochemical measurements, such as DHAA, as tracers of bacterial  
534 molecules in the DON pool.

535  
536 **CRedit authorship contribution statement.** Anabel von Jackowski: Data  
537 curation, Formal analysis, Visualization, Writing – original draft, Writing –  
538 review & editing. Nawal Bouchachi; Conceptualization, Investigation, and Data  
539 Curation. Quentin-Boris Barral; Methodology, Software, and Visualization. Paul  
540 Labatut: Investigation. Barbara Marie: Investigation and Formal analysis. Olivier  
541 Crispi: Investigation and Formal analysis. Karine Escoubeyrou: Investigation and  
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567 **Data Availability, Products, and Datasets.** Environmental data for DOM,  
568 DHAA enantiomers, and pigments are uploaded to PANGAEA under the accession  
569 number 967436 (von Jackowski et al., 2024). Raw fastq files have been deposited  
570 at ENA under accession number PRJEB75023.

- 571
- 572 • Mediterranean Sea - High Resolution And Ultra High Resolution Sea  
573 Surface Temperature Analysis, E.U. Copernicus Marine Service  
574 Informa-tion product [Data set]. Available at:  
575 • [https://resources.marine.copernicus.eu/?option=com\\_csw&view=details&pr  
576 oduct\\_id=SST\\_MED\\_SST\\_L4\\_NRT\\_OBSERVATIONS\\_010\\_004](https://resources.marine.copernicus.eu/?option=com_csw&view=details&product_id=SST_MED_SST_L4_NRT_OBSERVATIONS_010_004) (Accessed:  
577 September 2023)

- 577 • Mediterranean Sea, Bio-Geo-Chemical, L4, monthly means, daily gapfree  
578 and climatology Satellite Observations (1997-ongoing), E.U. Copernicus  
579 Marine Service Informa-tion product [Data set]. Available at:
- 580 • [https://data.marine.copernicus.eu/product/OCEANCOLOUR\\_MED\\_BGC\\_](https://data.marine.copernicus.eu/product/OCEANCOLOUR_MED_BGC_L4_MY_009_144/)  
581 [L4\\_MY\\_009\\_144/](https://data.marine.copernicus.eu/product/OCEANCOLOUR_MED_BGC_L4_MY_009_144/) (Accessed: September 2023)
- 582 • Mediterranean Sea Physics Reanalysis, E.U. Copernicus Marine Service  
583 Informa-tion product [Data set]. Available at:
- 584 • [https://data.marine.copernicus.eu/product/MEDSEA\\_MULTIYEAR\\_PHY\\_](https://data.marine.copernicus.eu/product/MEDSEA_MULTIYEAR_PHY_006_004/)  
585 [006\\_004](https://data.marine.copernicus.eu/product/MEDSEA_MULTIYEAR_PHY_006_004/) (Accessed: September 2023)
- 586 • Mediterranean Sea Physics Analysis and Forecast, E.U. Copernicus  
587 Marine Service Informa-tion product [Data set]. Available at:
- 588 • [https://data.marine.copernicus.eu/product/MEDSEA\\_ANALYSISFORECA](https://data.marine.copernicus.eu/product/MEDSEA_ANALYSISFORECAST_PHY_006_013/)  
589 [ST\\_PHY\\_006\\_013](https://data.marine.copernicus.eu/product/MEDSEA_ANALYSISFORECAST_PHY_006_013/) (Accessed: September 2023)

#### 590 Documentations:

- 591 • Buongiorno Nardelli B., C.Tronconi, A. Pisano, R.Santoleri, 2013: High  
592 and Ultra-High resolution processing of satellite Sea Surface Temperature  
593 data over Southern European Seas in the framework of MyOcean project,  
594 *Rem. Sens. Env.*, 129, 1-16, <https://doi.org/10.48670/moi-00172>
- 595 • Berthon, J.-F., Zibordi, G.: Bio-optical relationships for the northern  
596 Adriatic Sea. *Int. J. Remote Sens.*, 25, 1527-1532, 2004,  
597 <https://doi.org/10.1080/01431160310001592544>
- 598 • Volpe, G., Buongiorno Nardelli, B., Colella, S., Pisano, A. and Santoleri, R.  
599 (2018). An Operational Interpolated Ocean Colour Product in the  
600 Mediterranean Sea, in *New Frontiers in Operational Oceanography*,  
601 edited by E. P. Chassignet, A. Pascual, J. Tintorè, and J. Verron, pp. 227–  
602 244, <https://doi.org/10.17125/gov2018.ch09>
- 603 • Volpe, G., Colella, S., Brando, V. E., Forneris, V., Padula, F. L., Cicco, A.  
604 D., ... & Santoleri, R. (2019). Mediterranean ocean colour Level 3  
605 operational multi-sensor processing. *Ocean Science*, 15(1), 127-146,  
606 <https://doi.org/10.5194/os-15-127-2019>
- 607 • Escudier, R., Clementi, E., Cipollone, A., Pistoia, J., Drudi, M., Grandi, A.,  
608 Lyubartsev, V., Lecci, R., Aydogdu, A., Delrosso, D., Omar, M., Masina, S.,  
609 Coppini G., Pinardi, N. (2021). A High Resolution Reanalysis for the  
610 Mediterranean Sea. *Frontiers in Earth Science*, 9, 1060,  
611 <https://www.frontiersin.org/article/10.3389/feart.2021.702285>
- 612 • Clementi, E., Aydogdu, A., Goglio, A. C., Pistoia, J., Escudier, R., Drudi,  
613 M., Grandi, A., Mariani, A., Lyubartsev, V., Lecci, R., Cretí, S., Coppini,  
614 G., Masina, S., & Pinardi, N. (2021). Mediterranean Sea Physical Analysis  
615 and Forecast (CMEMS MED-Currents, EAS6 system) (Version 1) [Data  
616 set]. Copernicus Monitoring Environment Marine Service (CMEMS),  
617 [https://doi.org/10.25423/CMCC/MEDSEA\\_ANALYSISFORECAST\\_PHY\\_0](https://doi.org/10.25423/CMCC/MEDSEA_ANALYSISFORECAST_PHY_006_013_EAS8)  
618 [06\\_013\\_EAS8](https://doi.org/10.25423/CMCC/MEDSEA_ANALYSISFORECAST_PHY_006_013_EAS8)

#### 620 **References**

- 621 Alderkamp, A., Sintes, E., Herndl, G., 2006. Abundance and activity of major groups of  
622 prokaryotic plankton in the coastal North Sea during spring and summer. *Aquatic*  
623 *Microbial Ecology* 45, 237–246.

624 Alonso-Saez, L., Balagué, V., Sã, E.L., Sanchez, O., Gonzalez, J.M., Pinhassi, J., Massana, R.,  
625 Pernthaler, J., Pedros-Alio, C., Gasol, J.M., 2007. Seasonality in bacterial diversity in  
626 north-west Mediterranean coastal waters: assessment through clone libraries,  
627 fingerprinting and FISH: Seasonality in marine bacterial diversity. *FEMS Microbiology*  
628 *Ecology* 60, 98–112.

629 Alonso-Sáez, L., Gasol, J.M., 2007. Seasonal Variations in the Contributions of Different  
630 Bacterial Groups to the Uptake of Low-Molecular-Weight Compounds in  
631 Northwestern Mediterranean Coastal Waters. *Applied and Environmental*  
632 *Microbiology* 73, 3528–3535.

633 Andersen, K.S., Kirkegaard, R.H., Karst, S.M., Albertsen, M., 2018. ampvis2: an R package to  
634 analyse and visualise 16S rRNA amplicon data. doi:10.1101/299537

635 Auladell, A., Barberán, A., Logares, R., Garcés, E., Gasol, J.M., Ferrera, I., 2022. Seasonal  
636 niche differentiation among closely related marine bacteria. *The ISME Journal* 16,  
637 178–189.

638 Azúa, I., Goiriena, I., Baña, Z., Iriberry, J., Unanue, M., 2014. Release and Consumption of d-  
639 Amino Acids During Growth of Marine Prokaryotes. *Microbial Ecology* 67, 1–12.

640 Baumas, C., Bizic, M., 2024. A focus on different types of organic matter particles and their  
641 significance in the open ocean carbon cycle. *Progress in Oceanography* 224, 103233.

642 Baumas, C., Fuchs, R., Garel, M., Poggiale, J.-C., Memery, L., Le Moigne, F.A.C., Tamburini, C.,  
643 2023. Reconstructing the ocean’s mesopelagic zone carbon budget: sensitivity and  
644 estimation of parameters associated with prokaryotic remineralization.  
645 *Biogeosciences* 20, 4165–4182.

646 Baumas, C.M.J., Le Moigne, F.A.C., Garel, M., Bhairy, N., Guasco, S., Riou, V., Armougom, F.,  
647 Grossart, H.-P., Tamburini, C., 2021. Mesopelagic microbial carbon production  
648 correlates with diversity across different marine particle fractions. *The ISME Journal*  
649 15, 1695–1708.

650 Berthon, J.-F., Zibordi, G., 2004. Bio-optical relationships for the northern Adriatic Sea.  
651 *International Journal of Remote Sensing* 25, 1527–1532.

652 Bouchachi, N., Obernosterer, I., Marie, B., Crispi, O., Ortega-Retuerta, E., 2023. Phosphorus  
653 limitation determines the quality of dissolved organic matter released by marine  
654 heterotrophic prokaryotes. *Limnology and Oceanography Letters* 8, 330–339.

655 Bouvier, T., Del Giorgio, P.A., Gasol, J.M., 2007. A comparative study of the cytometric  
656 characteristics of High and Low nucleic-acid bacterioplankton cells from different  
657 aquatic ecosystems. *Environmental Microbiology* 9, 2050–2066.

658 Broek, T.A.B., Bour, A.L., Ianiri, H.L., Guilderson, T.P., McCarthy, M.D., 2019. Amino acid  
659 enantiomers in old and young dissolved organic matter: Implications for a microbial  
660 nitrogen pump. *Geochimica et Cosmochimica Acta* 247, 207–219.

661 Bunse, C., Pinhassi, J., 2017. Marine Bacterioplankton Seasonal Succession Dynamics. *Trends*  
662 *in Microbiology* 25, 494–505.

663 Buongiorno Nardelli, B., Tronconi, C., Pisano, A., Santoleri, R., 2013. High and Ultra-High  
664 resolution processing of satellite Sea Surface Temperature data over Southern  
665 European Seas in the framework of MyOcean project. *Remote Sensing of*  
666 *Environment* 129, 1–16.

667 Buttigieg, P.L., Fadeev, E., Bienhold, C., Hehemann, L., Offre, P., Boetius, A., 2018. Marine  
668 microbes in 4D — using time series observation to assess the dynamics of the ocean  
669 microbiome and its links to ocean health. *Current Opinion in Microbiology* 43, 169–  
670 185.

671 Buttigieg, P.L., Ramette, A., 2014. A guide to statistical analysis in microbial ecology: a  
672 community-focused, living review of multivariate data analyses. *FEMS Microbiology*  
673 *Ecology* 90, 543–550.

674 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016.  
675 DADA2: High-resolution sample inference from Illumina amplicon data. *Nature*  
676 *Methods* 13, 581–583.

677 Carlson, C.A., Ducklow, H.W., 1995. Dissolved organic carbon in the upper ocean of the  
678 central equatorial Pacific Ocean, 1992: Daily and finescale vertical variations. *Deep*  
679 *Sea Research Part II: Topical Studies in Oceanography* 42, 639–656.

680 Carlson, C.A., Ducklow, H.W., Michaels, A.F., 1994. Annual flux of dissolved organic carbon  
681 from the euphotic zone in the northwestern Sargasso Sea. *Nature* 371, 405–408.

682 Cava, F., Lam, H., De Pedro, M.A., Waldor, M.K., 2011. Emerging knowledge of regulatory  
683 roles of d-amino acids in bacteria. *Cellular and Molecular Life Sciences* 68, 817–831.

684 Charles, F., Lantoine, F., Brugel, S., Chrétiennot-Dinet, M.-J., Quiroga, I., Rivière, B., 2005.  
685 Seasonal survey of the phytoplankton biomass, composition and production in a  
686 littoral NW Mediterranean site, with special emphasis on the picoplanktonic  
687 contribution. *Estuarine, Coastal and Shelf Science* 65, 199–212.

688 Cherrier, J., Bauer, J., Druffel, E., 1996. Utilization and turnover of labile dissolved organic  
689 matter by bacterial heterotrophs in eastern North Pacific surface waters. *Marine*  
690 *Ecology Progress Series* 139, 267–279.

691 Clementi, E., Pistoia, J., Escudier, R., Delrosso, D., Drudi, M., Grandi, A., Lecci, R., Cretí, S.,  
692 Ciliberti, S., Coppini, G., Masina, S., Pinardi, N., 2019. Mediterranean Sea Physical  
693 Analysis and Forecast (CMEMS MED-Currents 2016-2019):  
694 MEDSEA\_ANALYSIS\_FORECAST\_PHY\_006\_013.  
695 doi:10.25423/CMCC/MEDSEA\_ANALYSIS\_FORECAST\_PHY\_006\_013\_EAS4

696 Copin-Montégut, G., Avril, B., 1993. Vertical distribution and temporal variation of dissolved  
697 organic carbon in the North-Western Mediterranean Sea. *Deep Sea Research Part I:*  
698 *Oceanographic Research Papers* 40, 1963–1972.

699 Dauwe, B., Middelburg, J.J., 1998. Amino acids and hexosamines as indicators of organic  
700 matter degradation state in North Sea sediments. *Limnology and Oceanography* 43,  
701 782–798.

702 Davis, J., Benner, R., 2007. Quantitative estimates of labile and semi-labile dissolved organic  
703 carbon in the western Arctic Ocean: A molecular approach. *Limnology and*  
704 *Oceanography* 52, 2434–2444.

705 Diaz, F., Naudin, J.-J., Courties, C., Rimmelin, P., Oriol, L., 2008. Biogeochemical and  
706 ecological functioning of the low-salinity water lenses in the region of the Rhone  
707 River freshwater influence, NW Mediterranean Sea. *Continental Shelf Research* 28,  
708 1511–1526.

709 Escoubeyrou, K., Tremblay, L., 2014. Quantification of free, dissolved combined, particulate,  
710 and total amino acid enantiomers using simple sample preparation and more robust  
711 chromatographic procedures. *Limnology and Oceanography: Methods* 12, 421–431.

712 Escudier, R., Clementi, E., Cipollone, A., Pistoia, J., Drudi, M., Grandi, A., Lyubartsev, V., Lecci,  
713 R., Aydogdu, A., Delrosso, D., Omar, M., Masina, S., Coppini, G., Pinardi, N., 2021. A  
714 High Resolution Reanalysis for the Mediterranean Sea. *Frontiers in Earth Science* 9,  
715 702285.

716 Evans, C.A., O'Reilly, J.E., Thomas, J.P., 1987. A handbook for the measurement of chlorophyll  
717 a and primary production, BIOMASS scientific series. Texas A&M University with  
718 support from SCAR, College Station, Texas.

719 Galand, P.E., Pereira, O., Hochart, C., Auguet, J.C., Debroas, D., 2018. A strong link between  
720 marine microbial community composition and function challenges the idea of  
721 functional redundancy. *The ISME Journal* 12, 2470–2478.

722 Ghiglione, J.-F., Galand, P.E., Pommier, T., Pedrós-Alió, C., Maas, E.W., Bakker, K., Bertilson, S.,  
723 Kirchman, D.L., Lovejoy, C., Yager, P.L., Murray, A.E., 2012. Pole-to-pole biogeography  
724 of surface and deep marine bacterial communities. *Proceedings of the National  
725 Academy of Sciences* 109, 17633–17638.

726 Giovannoni, S.J., Cameron Thrash, J., Temperton, B., 2014. Implications of streamlining  
727 theory for microbial ecology. *The ISME Journal* 8, 1553–1565.

728 Gonzalez, M.-L., Blain, S., Obernosterer, I., 2019. Seasonal freshening of NW Mediterranean  
729 surface water impacts microbial heterotrophic activity and dissolved organic matter.  
730 *Estuarine, Coastal and Shelf Science* 230, 106448.

731 González-Dávila, M., Santana-Casiano, J.M., 2023. Long-term trends of pH and inorganic  
732 carbon in the Eastern North Atlantic: the ESTOC site. *Frontiers in Marine Science* 10,  
733 1236214.

734 Grossart, H.-P., Ploug, H., 2000. Bacterial production and growth efficiencies: Direct  
735 measurements on riverine aggregates. *Limnology and Oceanography* 45, 436–445.

736 Gutiérrez-Rodríguez, A., Latasa, M., Scharek, R., Massana, R., Vila, G., Gasol, J.M., 2011.  
737 Growth and grazing rate dynamics of major phytoplankton groups in an oligotrophic  
738 coastal site. *Estuarine, Coastal and Shelf Science* 95, 77–87.

739 Hancock, R., 1960. The amino acid composition of the protein and cell wall of  
740 *Staphylococcus aureus*. *Biochimica et Biophysica Acta* 37, 42–46.

741 Hansell, D.A., 2013. Recalcitrant Dissolved Organic Carbon Fractions. *Annual Review of  
742 Marine Science* 5, 421–445.

743 Hayward, A., Pinkerton, M.H., Gutierrez-Rodriguez, A., 2023. PHYTOCLASS: A pigment-based  
744 chemotaxonomic method to determine the biomass of phytoplankton classes.  
745 *Limnology and Oceanography: Methods* 21, 220–241.

746 Hecky, R.E., Mopper, K., Kilham, P., Degens, E.T., 1973. The amino acid and sugar composition  
747 of diatom cell-walls. *Marine Biology* 19, 323–331.

748 Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of  
749 species diversity ( Hill numbers). *Methods in Ecology and Evolution* 7, 1451–1456.

750 Ianiri, H.L., Shen, Y., Broek, T.A.B., McCarthy, M.D., 2022. Bacterial sources and cycling  
751 dynamics of amino acids in high and low molecular weight dissolved organic nitrogen  
752 in the ocean. *Marine Chemistry* 241, 104104.

753 Irazoki, O., Ter Beek, J., Alvarez, L., Mateus, A., Colin, R., Typas, A., Savitski, M.M., Sourjik, V.,  
754 Berntsson, R.P.-A., Cava, F., 2023. d-amino acids signal a stress-dependent run-away  
755 response in *Vibrio cholerae*. *Nature Microbiology* 8, 1549–1560.

756 Jones, V., Meador, T.B., Gogou, A., Migon, C., Penkman, K.E.H., Collins, M.J., Repeta, D.J.,  
757 2013. Characterisation and dynamics of dissolved organic matter in the Northwestern  
758 Mediterranean Sea. *Progress in Oceanography* 119, 78–89.

759 Jørgensen, N., Tranvik, L., Berg, G., 1999. Occurrence and bacterial cycling of dissolved  
760 nitrogen in the Gulf of Riga, the Baltic Sea. *Marine Ecology Progress Series* 191, 1–18.

761 Kaiser, K., Benner, R., 2008. Major bacterial contribution to the ocean reservoir of detrital  
762 organic carbon and nitrogen. *Limnology and Oceanography* 53, 99–112.

763 Kaiser, K., Benner, R., 2009. Biochemical composition and size distribution of organic matter  
764 at the Pacific and Atlantic time-series stations. *Marine Chemistry* 113, 63–77.

765 Koch, A.L., 2001. Oligotrophs versus copiotrophs. *BioEssays* 23, 657–661.

766 Laghdass, M., Catala, P., Caparros, J., Oriol, L., Lebaron, P., Obernosterer, I., 2012. High  
767 Contribution of SAR11 to Microbial Activity in the North West Mediterranean Sea.  
768 *Microbial Ecology* 63, 324–333.

769 Laghdass, M., West, N.J., Batailler, N., Caparros, J., Catala, P., Lantoine, F., Oriol, L., Lebaron,  
770 P., Obernosterer, I., 2010. Impact of lower salinity waters on bacterial heterotrophic  
771 production and community structure in the offshore NW Mediterranean Sea.  
772 *Environmental Microbiology Reports* 2, 761–769.

773 Lam, H., Oh, D.-C., Cava, F., Takacs, C.N., Clardy, J., De Pedro, M.A., Waldor, M.K., 2009. D-  
774 Amino Acids Govern Stationary Phase Cell Wall Remodeling in Bacteria. *Science* 325,  
775 1552–1555.

776 Lambert, S., Tragin, M., Lozano, J.-C., Ghiglione, J.-F., Vaultot, D., Bouget, F.-Y., Galand, P.E.,  
777 2019. Rhythmicity of coastal marine picoeukaryotes, bacteria and archaea despite  
778 irregular environmental perturbations. *The ISME Journal* 13, 388–401.

779 Landa, M., Blain, S., Harmand, J., Monchy, S., Rapaport, A., Obernosterer, I., 2018. Major  
780 changes in the composition of a Southern Ocean bacterial community in response to  
781 diatom-derived dissolved organic matter. *FEMS Microbiology Ecology* 94.  
782 doi:10.1093/femsec/fiy034

783 Latasa, M., Scharek, R., Vidal, M., Vila-Reixach, G., Gutiérrez-Rodríguez, A., Emelianov, M.,  
784 Gasol, J., 2010. Preferences of phytoplankton groups for waters of different trophic  
785 status in the northwestern Mediterranean Sea. *Marine Ecology Progress Series* 407,  
786 27–42.

787 Le Moigne, F.A.C., 2019. Pathways of Organic Carbon Downward Transport by the Oceanic  
788 Biological Carbon Pump. *Frontiers in Marine Science* 6, 634.

789 Lemonnier, C., Perennou, M., Eveillard, D., Fernandez-Guerra, A., Leynaert, A., Marié, L.,  
790 Morrison, H.G., Memery, L., Paillard, C., Maignien, L., 2020. Linking Spatial and  
791 Temporal Dynamic of Bacterioplankton Communities With Ecological Strategies  
792 Across a Coastal Frontal Area. *Frontiers in Marine Science* 7, 376.

793 Liu, C., Cui, Y., Li, X., Yao, M., 2021. *microeco* : an R package for data mining in microbial  
794 community ecology. *FEMS Microbiology Ecology* 97, fiae255.

795 Liu, S., Baetge, N., Comstock, J., Opalk, K., Parsons, R., Halewood, E., English, C.J.,  
796 Giovannoni, S., Bolaños, L.M., Nelson, C.E., Vergin, K., Carlson, C.A., 2020. Stable  
797 Isotope Probing Identifies Bacterioplankton Lineages Capable of Utilizing Dissolved  
798 Organic Matter Across a Range of Bioavailability. *Frontiers in Microbiology* 11,  
799 580397.

800 Liu, S., Longnecker, K., Kujawinski, E.B., Vergin, K., Bolaños, L.M., Giovannoni, S.J., Parsons,  
801 R., Opalk, K., Halewood, E., Hansell, D.A., Johnson, R., Curry, R., Carlson, C.A., 2022.  
802 Linkages Among Dissolved Organic Matter Export, Dissolved Metabolites, and  
803 Associated Microbial Community Structure Response in the Northwestern Sargasso  
804 Sea on a Seasonal Scale. *Frontiers in Microbiology* 13, 833252.

805 Lomas, M.W., Bates, N.R., Johnson, R.J., Knap, A.H., Steinberg, D.K., Carlson, C.A., 2013. Two  
806 decades and counting: 24-years of sustained open ocean biogeochemical  
807 measurements in the Sargasso Sea. *Deep Sea Research Part II: Topical Studies in*  
808 *Oceanography* 93, 16–32.



809 Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion  
810 for RNA-seq data with DESeq2. *Genome Biology* 15, 550.

811 Mackey, M., Mackey, D., Higgins, H., Wright, S., 1996. CHEMTAX - a program for estimating  
812 class abundances from chemical markers: application to HPLC measurements of  
813 phytoplankton. *Marine Ecology Progress Series* 144, 265–283.

814 Malmstrom, R.R., Kiene, R.P., Cottrell, M.T., Kirchman, D.L., 2004. Contribution of SAR11  
815 Bacteria to Dissolved Dimethylsulfoniopropionate and Amino Acid Uptake in the  
816 North Atlantic Ocean. *Applied and Environmental Microbiology* 70, 4129–4135.

817 Many, G., Ulses, C., Estournel, C., Marsaleix, P., 2021. Particulate organic carbon dynamics in  
818 the Gulf of Lion shelf (NW Mediterranean) using a coupled hydrodynamic–  
819 biogeochemical model. *Biogeosciences* 18, 5513–5538.

820 Marty, J.-C., Chiavérini, J., Pizay, M.-D., Avril, B., 2002. Seasonal and interannual dynamics of  
821 nutrients and phytoplankton pigments in the western Mediterranean Sea at the  
822 DYFAMED time-series station (1991–1999). *Deep Sea Research Part II: Topical Studies*  
823 *in Oceanography* 49, 1965–1985.

824 McLaren, M.R., Callahan, B.J., 2021. Silva 138.1 prokaryotic SSU taxonomic training data  
825 formatted for DADA2.

826 McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive  
827 Analysis and Graphics of Microbiome Census Data. *PLoS ONE* 8, e61217.

828 Nunes, S., Latasa, M., Gasol, J., Estrada, M., 2018. Seasonal and interannual variability of  
829 phytoplankton community structure in a Mediterranean coastal site. *Marine Ecology*  
830 *Progress Series* 592, 57–75.

831 Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P.,  
832 O’Hara, R., Simpson, G., Solymos, P., Stevens, M., 2022. vegan: Community Ecology  
833 Package. R package version 2.5-7. Preprint at 3--1.

834 Olmedo, E., Taupier-Letage, I., Turiel, A., Alvera-Azcárate, A., 2018. Improving SMOS Sea  
835 Surface Salinity in the Western Mediterranean Sea through Multivariate and  
836 Multifractal Analysis. *Remote Sensing* 10, 485.

837 Olson, R.J., Sosik, H.M., 2007. A submersible imaging-in-flow instrument to analyze nano-  
838 and microplankton: Imaging FlowCytobot. *Limnology and Oceanography: Methods* 5,  
839 195–203.

840 Ortega-Retuerta, E., Marrasé, C., Muñoz-Fernández, A., Sala, M.M., Simó, R., Gasol, J.M.,  
841 2018. Seasonal dynamics of transparent exopolymer particles (TEP) and their drivers  
842 in the coastal NW Mediterranean Sea. *Science of The Total Environment* 631–632,  
843 180–190.

844 Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small  
845 subunit rRNA primers for marine microbiomes with mock communities, time series  
846 and global field samples: Primers for marine microbiome studies. *Environmental*  
847 *Microbiology* 18, 1403–1414.

848 Park, J.T., Strominger, J.L., 1957. Mode of Action of Penicillin: Biochemical Basis for the  
849 Mechanism of Action of Penicillin and for Its Selective Toxicity. *Science* 125, 99–101.

850 Pawlowicz, R., 2020. M\_Map: A mapping package for MATLAB.

851 Pinhassi, J., Hagström, Å., 2000. Seasonal succession in marine bacterioplankton. *Aquatic*  
852 *Microbial Ecology* 21, 245–256.

853 Piontek, J., Handel, N., De Bodt, C., Harlay, J., Chou, L., Engel, A., 2011. The utilization of  
854 polysaccharides by heterotrophic bacterioplankton in the Bay of Biscay (North  
855 Atlantic Ocean). *Journal of Plankton Research* 33, 1719–1735.

856 Poulet, S.A., Martin-Jézéquel, V., 1983. Relationships between dissolved free amino acids,  
857 chemical composition and growth of the marine diatom *Chaetoceros debile*. *Marine*  
858 *Biology* 77, 93–100.

859 Puig, P., Palanques, A., Orange, D.L., Lastras, G., Canals, M., 2008. Dense shelf water  
860 cascades and sedimentary furrow formation in the Cap de Creus Canyon,  
861 northwestern Mediterranean Sea. *Continental Shelf Research* 28, 2017–2030.

862 Pujo-Pay, M., Raimbault, P., 1994. Improvement of the wet-oxidation procedure for  
863 simultaneous determination of particulate organic nitrogen and phosphorus  
864 collected on filters. *Marine Ecology Progress Series* 105, 203–207.

865 Qian, J., Mopper, K., 1996. Automated High-Performance, High-Temperature Combustion  
866 Total Organic Carbon Analyzer. *Analytical Chemistry* 68, 3090–3097.

867 Quiroga, I., 2006. Pseudo-Nitzschia Blooms in the Bay of Banyuls-sur-Mer, Northwestern  
868 Mediterranean Sea. *Diatom Research* 21, 91–104.

869 Ras, J., Claustre, H., Uitz, J., 2008. Spatial variability of phytoplankton pigment distributions  
870 in the Subtropical South Pacific Ocean: comparison between in situ and predicted  
871 data. *Biogeosciences* 5, 353–369.

872 Riedel, T., Dittmar, T., 2014. A Method Detection Limit for the Analysis of Natural Organic  
873 Matter via Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Analytical*  
874 *Chemistry* 86, 8376–8382.

875 Robertson, B.R., Button, D.K., 1989. Characterizing aquatic bacteria according to population,  
876 cell size, and apparent DNA content by flow cytometry. *Cytometry* 10, 70–76.

877 Romano, S., Dittmar, T., Bondarev, V., Weber, R.J.M., Viant, M.R., Schulz-Vogt, H.N., 2014.  
878 Exo-Metabolome of *Pseudovibrio* sp. FO-BEG1 Analyzed by Ultra-High Resolution  
879 Mass Spectrometry and the Effect of Phosphate Limitation. *PLoS ONE* 9, e96038.

880 Romera-Castillo, C., Sarmiento, H., Álvarez-Salgado, X.A., Gasol, J.M., Marraséa, C., 2010.  
881 Production of chromophoric dissolved organic matter by marine phytoplankton.  
882 *Limnology and Oceanography* 55, 446–454.

883 Roy, S., Llewellyn, C.A., Egeland, E.S., Johnsen, G. (Eds.), 2011. *Phytoplankton Pigments:*  
884 *Characterization, Chemotaxonomy and Applications in Oceanography*, 1st ed.  
885 Cambridge University Press.

886 Salter, I., Galand, P.E., Fagervold, S.K., Lebaron, P., Obernosterer, I., Oliver, M.J., Suzuki, M.T.,  
887 Tricoire, C., 2015. Seasonal dynamics of active SAR11 ecotypes in the oligotrophic  
888 Northwest Mediterranean Sea. *The ISME Journal* 9, 347–360.

889 Sánchez-Pérez, E.D., Pujo-Pay, M., Ortega-Retuerta, E., Conan, P., Peters, F., Marrasé, C.,  
890 2020. Mismatched dynamics of dissolved organic carbon and chromophoric dissolved  
891 organic matter in the coastal NW Mediterranean Sea. *Science of The Total*  
892 *Environment* 746, 141190.

893 Sanchez-Vidal, A., Pasqual, C., Kerhervé, P., Calafat, A., Heussner, S., Palanques, A., Durrieu  
894 De Madron, X., Canals, M., Puig, P., 2008. Impact of dense shelf water cascading on  
895 the transfer of organic matter to the deep western Mediterranean basin. *Geophysical*  
896 *Research Letters* 35, 2007GL032825.

897 Sarmiento, H., Romera-Castillo, C., Lindh, M., Pinhassi, J., Sala, M.M., Gasol, J.M., Marrase, C.,  
898 Taylor, G.T., 2013. Phytoplankton species-specific release of dissolved free amino  
899 acids and their selective consumption by bacteria. *Limnology and Oceanography* 58,  
900 1123–1135.

901 Schleifer, K.H., Kandler, O., 1972. Peptidoglycan types of bacterial cell walls and their  
902 taxonomic implications. *Bacteriological Reviews* 36, 407–477.

903 Sebastián, M., Ortega-Retuerta, E., Gómez-Consarnau, L., Zamanillo, M., Álvarez, M.,  
904 Arístegui, J., Gasol, J.M., 2021. Environmental gradients and physical barriers drive  
905 the basin-wide spatial structuring of Mediterranean Sea and adjacent eastern  
906 Atlantic Ocean prokaryotic communities. *Limnology and Oceanography* 66, 4077–  
907 4095.

908 Sherr, E.B., Sherr, B.F., Longnecker, K., 2006. Distribution of bacterial abundance and cell-  
909 specific nucleic acid content in the Northeast Pacific Ocean. *Deep Sea Research Part*  
910 *I: Oceanographic Research Papers* 53, 713–725.

911 Siegel, D.A., Buesseler, K.O., Behrenfeld, M.J., Benitez-Nelson, C.R., Boss, E., Brzezinski, M.A.,  
912 Burd, A., Carlson, C.A., D’Asaro, E.A., Doney, S.C., Perry, M.J., Stanley, R.H.R.,  
913 Steinberg, D.K., 2016. Prediction of the Export and Fate of Global Ocean Net Primary  
914 Production: The EXPORTS Science Plan. *Frontiers in Marine Science* 3.  
915 doi:10.3389/fmars.2016.00022

916 Smith, D.C., Simon, M., Alldredge, A.L., Azam, F., 1992. Intense hydrolytic enzyme activity on  
917 marine aggregates and implications for rapid particle dissolution. *Nature* 359, 139–  
918 142.

919 Sosik, H.M., Olson, R.J., 2007. Automated taxonomic classification of phytoplankton sampled  
920 with imaging-in-flow cytometry. *Limnology and Oceanography: Methods* 5, 204–216.

921 Sugimura, Y., Suzuki, Y., 1988. A high-temperature catalytic oxidation method for the  
922 determination of non-volatile dissolved organic carbon in seawater by direct injection  
923 of a liquid sample. *Marine Chemistry* 24, 105–131.

924 Teeling, H., Fuchs, B.M., Bennke, C.M., Krüger, K., Chafee, M., Kappelmann, L., Reintjes, G.,  
925 Waldmann, J., Quast, C., Glöckner, F.O., Lucas, J., Wichels, A., Gerdt, G., Wiltshire,  
926 K.H., Amann, R.L., 2016. Recurring patterns in bacterioplankton dynamics during  
927 coastal spring algae blooms. *eLife* 5, e11888.

928 Thingstad, T.F., Hagström, Å., Rassoulzadegan, F., 1997. Accumulation of degradable DOC in  
929 surface waters: Is it caused by a malfunctioning microbial loop? *Limnology and*  
930 *Oceanography* 42, 398–404.

931 Trano, A.C., Piredda, R., Balestra, C., Bastianini, M., Gasol, J.M., Casotti, R., 2022. Diversity of  
932 Free-Living and Particle-Attached Prokaryotes in a River-Influenced Coastal Area of  
933 the Northern Adriatic Sea. *Frontiers in Marine Science* 9, 912528.

934 Vila-Reixach, G., Gasol, J., Cardelús, C., Vidal, M., 2012. Seasonal dynamics and net  
935 production of dissolved organic carbon in an oligotrophic coastal environment.  
936 *Marine Ecology Progress Series* 456, 7–19.

937 Volpe, G., Buongiorno Nardelli, B., Colella, S., Pisano, A., Santoleri, R., 2018. An Operational  
938 Interpolated Ocean Colour Product in the Mediterranean Sea, in: Chassignet, E.P.,  
939 Pascual, A., Tintoré, J., Verron, J. (Eds.), *New Frontiers in Operational Oceanography*.  
940 GODAE OceanView. doi:10.17125/gov2018.ch09

941 Volpe, G., Colella, S., Brando, V.E., Forneris, V., La Padula, F., Di Cicco, A., Sammartino, M.,  
942 Bracaglia, M., Artuso, F., Santoleri, R., 2019. Mediterranean ocean colour Level 3  
943 operational multi-sensor processing. *Ocean Science* 15, 127–146.

944 von Jackowski, A., Becker, K.W., Wietz, M., Bienhold, C., Zäncker, B., Nöthig, E., Engel, A.,  
945 2022. Variations of microbial communities and substrate regimes in the eastern Fram  
946 Strait between summer and fall. *Environmental Microbiology* 24, 4124–4136.

947 von Jackowski, A., Bouchachi, N., Labatut, P., Marie, Barbara, B., Crispi, O., Escoubeyrou, K.,  
948 Paulin, C.-H., Dimier, C., Ras, Ortega-Retuerta, E., 2024. Phytoplankton and DOM at  
949 MOLA 2019-2021. doi:10.1594/PANGAEA.967436

950 von Jackowski, A., Grosse, J., Nöthig, E.-M., Engel, A., 2020. Dynamics of organic matter and  
951 bacterial activity in the Fram Strait during summer and autumn. *Philosophical*  
952 *Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*  
953 378, 20190366.

954 Wagner, S., Schubotz, F., Kaiser, K., Hallmann, C., Waska, H., Rossel, P.E., Hansman, R., Elvert,  
955 M., Middelburg, J.J., Engel, A., Blattmann, T.M., Catalá, T.S., Lennartz, S.T., Gomez-  
956 Saez, G.V., Pantoja-Gutiérrez, S., Bao, R., Galy, V., 2020. Soothsaying DOM: A Current  
957 Perspective on the Future of Oceanic Dissolved Organic Carbon. *Frontiers in Marine*  
958 *Science* 7, 341.

959 Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

960 Wilson, B., Müller, O., Nordmann, E.-L., Seuthe, L., Bratbak, G., Øvreås, L., 2017. Changes in  
961 Marine Prokaryote Composition with Season and Depth Over an Arctic Polar Year.  
962 *Frontiers in Marine Science* 4. doi:10.3389/fmars.2017.00095

963 Winkler, L.W., 1888. Die Bestimmung des im Wasser gelösten Sauerstoffes. *Berichte der*  
964 *deutschen chemischen Gesellschaft* 21, 2843–2854.

965 Wirtz, K., Smith, S.L., 2020. Vertical migration by bulk phytoplankton sustains biodiversity  
966 and nutrient input to the surface ocean. *Scientific Reports* 10, 1142.

967 Yamashita, Y., Tanoue, E., 2003. Chemical characterization of protein-like fluorophores in  
968 DOM in relation to aromatic amino acids. *Marine Chemistry* 82, 255–271.

969 Yu, Y., Yang, J., Zheng, L.-Y., Sheng, Q., Li, C.-Y., Wang, M., Zhang, X.-Y., McMinn, A., Zhang, Y.-  
970 Z., Song, X.-Y., Chen, X.-L., 2020. Diversity of D-Amino Acid Utilizing Bacteria From  
971 Kongsfjorden, Arctic and the Metabolic Pathways for Seven D-Amino Acids. *Frontiers*  
972 *in Microbiology* 10, 2983.

973 Zhang, G., Sun, H.J., 2014. Racemization in Reverse: Evidence that D-Amino Acid Toxicity on  
974 Earth Is Controlled by Bacteria with Racemases. *PLoS ONE* 9, e92101.

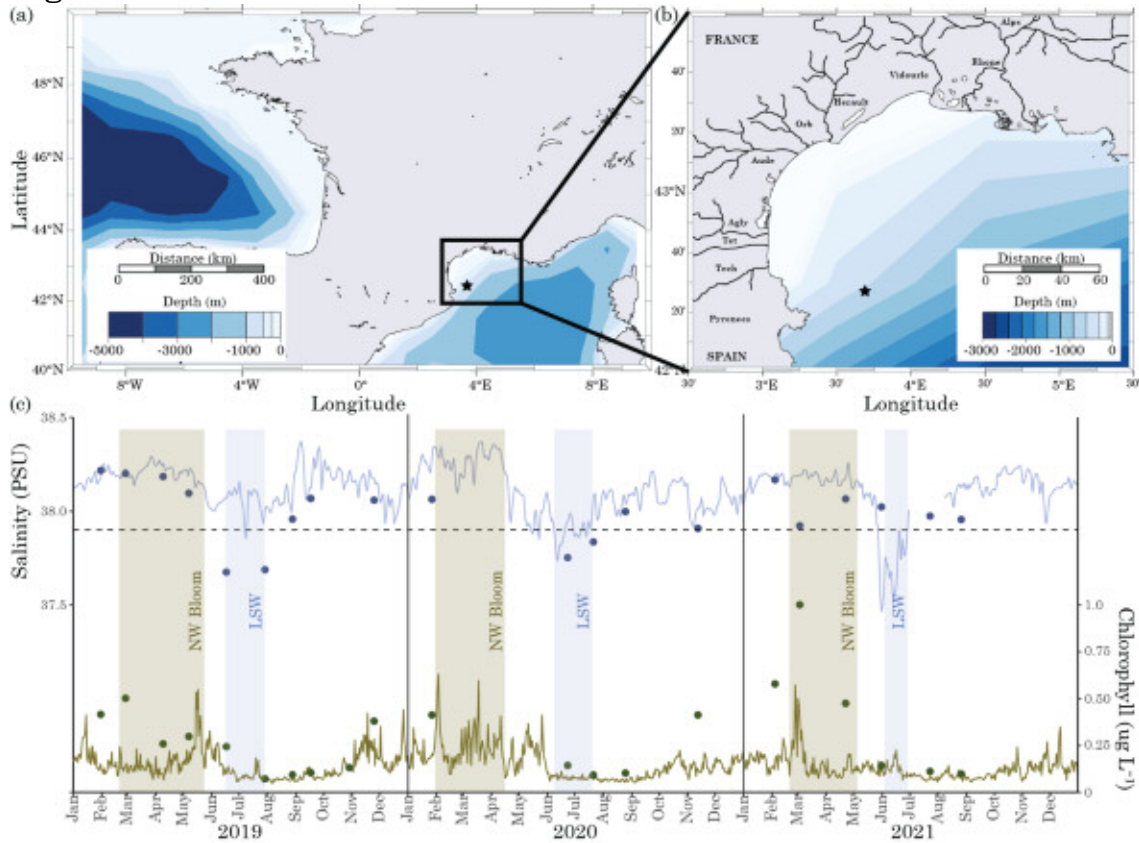
975 Zhang, Z., Zheng, Q., Jiao, N., 2016. Microbial D-amino acids and marine carbon storage.  
976 *Science China Earth Sciences* 59, 17–24.

977 Zhao, Z., Gonsior, M., Luek, J., Timko, S., Ianiri, H., Hertkorn, N., Schmitt-Kopplin, P., Fang, X.,  
978 Zeng, Q., Jiao, N., Chen, F., 2017. Picocyanobacteria and deep-ocean fluorescent  
979 dissolved organic matter share similar optical properties. *Nature Communications* 8,  
980 15284.

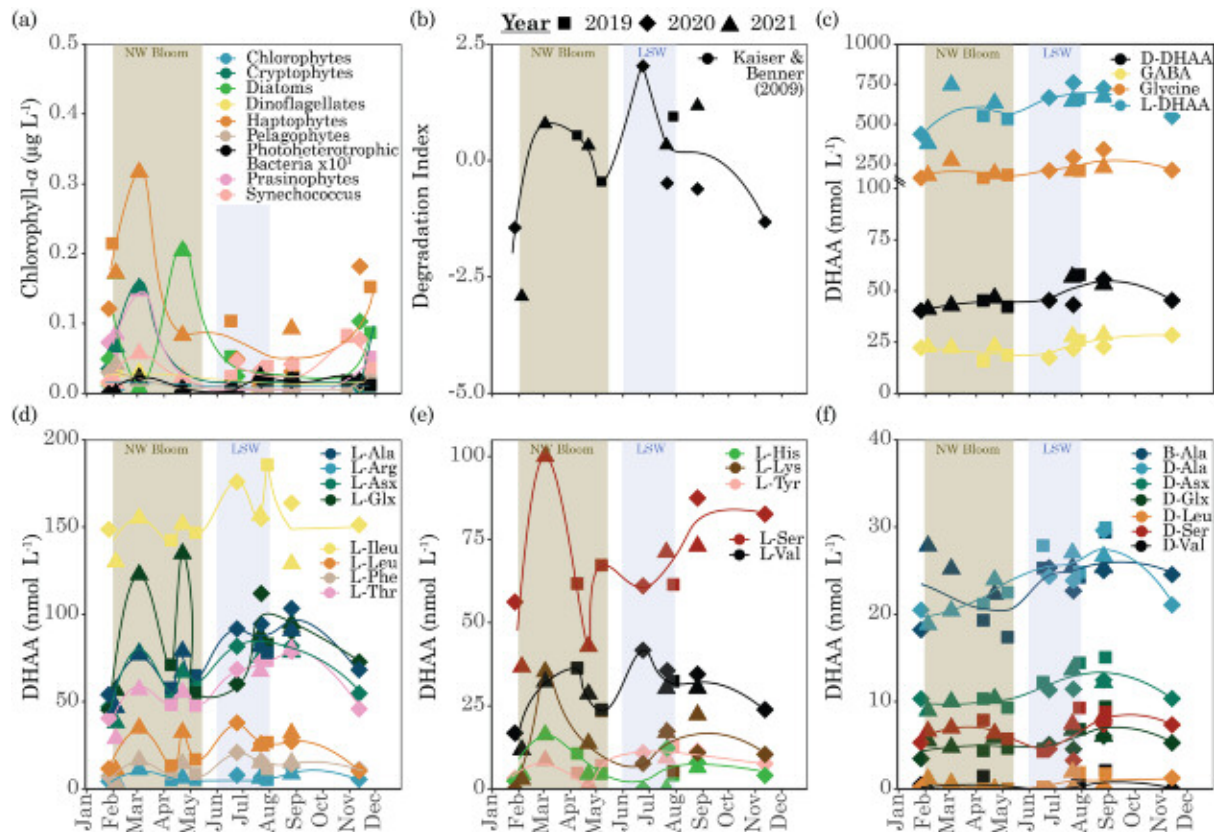
981

982

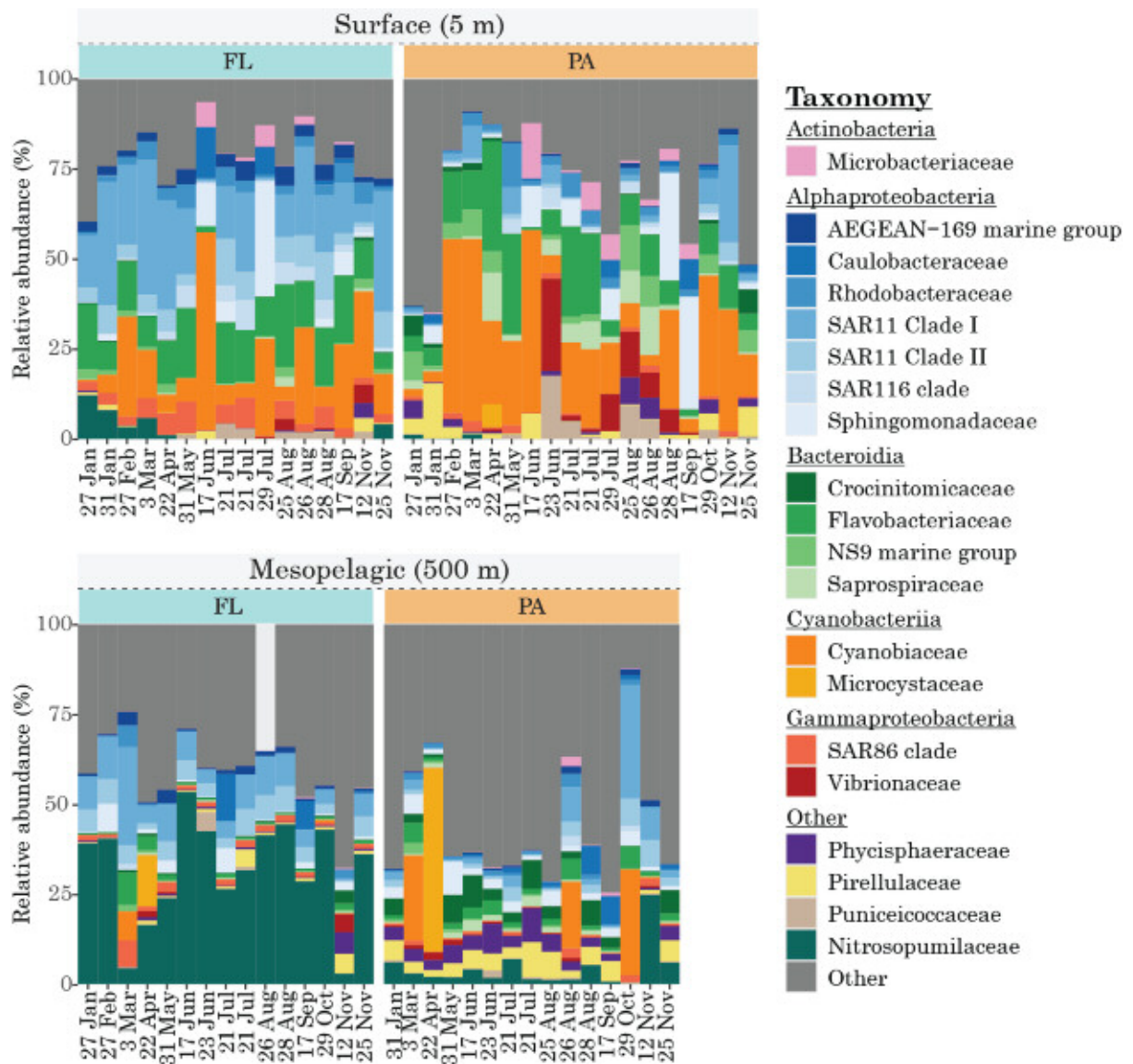
983 **Figures**



984  
 985 **Figure 1. Bathymetry and temporal conditions at Microbial Observatory**  
 986 **Laboratory Arago (MOLA) in the northwestern Mediterranean Sea**  
 987 **between 2019 and 2021.** The MOLA time series station (star) is located off (a)  
 988 northern Catalonia in the (b) drainage basin of the Agly, Aude, Hérault, Orb,  
 989 Rhône, Tech, Têt, and Vidourle into the Gulf of Lyon. The (c) satellite-derived (line)  
 990 and subsurface (5 m, circles) of salinity (blue) and chlorophyll-a (green) at MOLA.  
 991 Remote sensing allowed for a differentiation between coastal and offshore  
 992 northwestern blooms. Low salinity water (LSW) events are below 37.9 PSU  
 993 (dashed line) for more than three consecutive days.  
 994

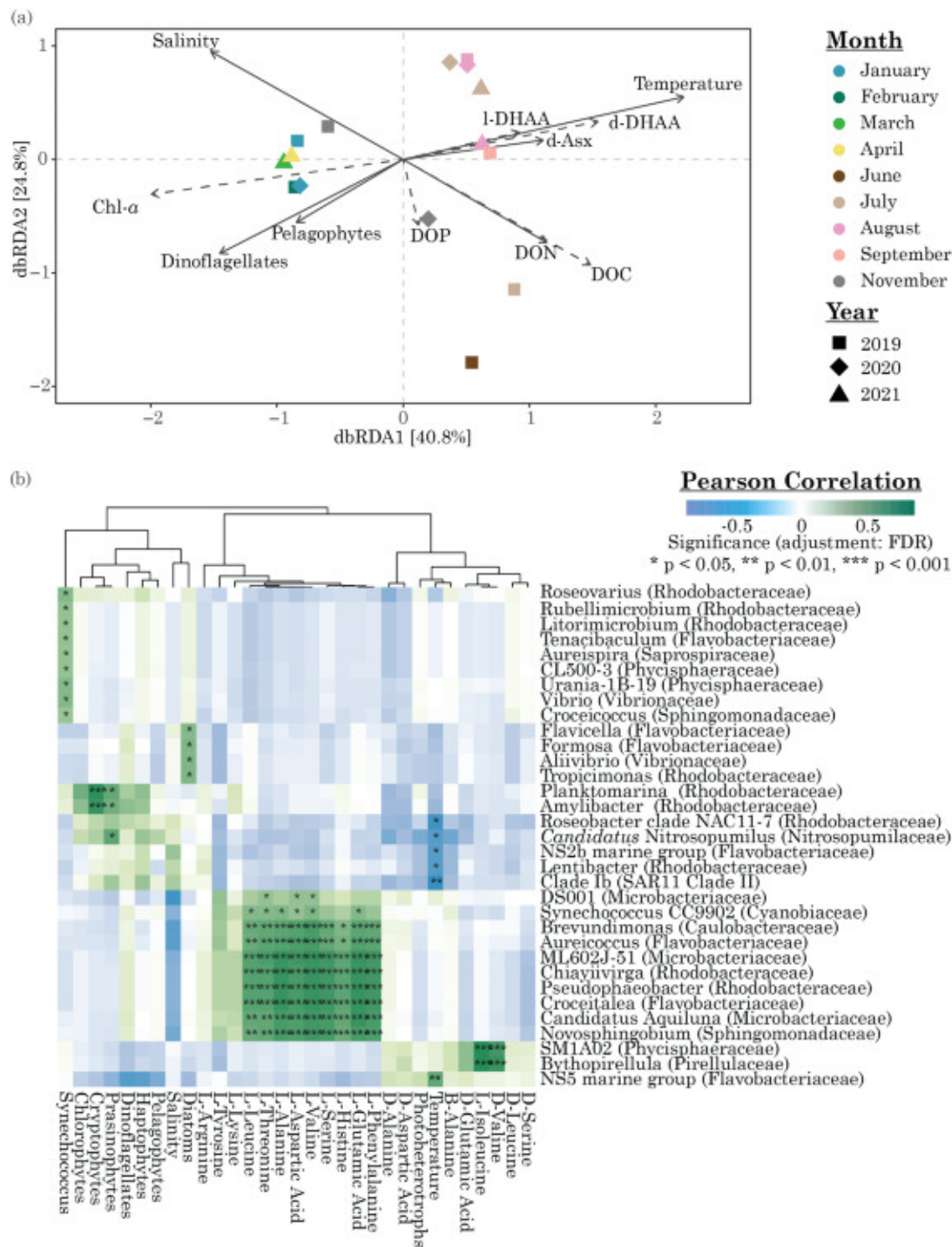


995  
 996 **Figure 2. Seasonal dynamics of phytoplankton biomass and dissolved**  
 997 **hydrolyzable amino acids (DHAA) at 5 meters of the Microbial**  
 998 **Observatory Laboratory Arago.** The (a) phytoplankton biomass estimated  
 999 from pigment biomarkers and bacteriochlorophyll-a for photoheterotrophic  
 1000 bacteria, (b) DHAA degradation index, (c) total concentrations of L- and D-DHAA  
 1001 and non-isomers gamma-aminobutyric acid (GABA) and glycine, (d) L-DHAA that  
 1002 peak twice during the NWMED bloom, (e) L-DHAA that only peak once during the  
 1003 NWMED bloom, (f) B- and D-DHAA. The shape corresponds to the year and  
 1004 abbreviations refer to: Ala, Alanine; Asx, Aspartic acid and Asparagine; Arg,  
 1005 Arginine; Glx, Glutamic acid and Glutamine; His, Histidine; Ileu, Isoleucine; Leu,  
 1006 Leucine; Lys, Lysine; Phe, Phenylalanine; Ser, Serine; Thr, Threonine; Tyr,  
 1007 Tyrosine; Val, Valine.  
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**Figure 3. Seasonal dynamics of the top 20 bacterioplankton families at the Microbial Observatory Laboratory Arago. Dominant orders in the free-living (FL) and particle-attached (PA) community at 5 m and 500 m depth.**



1014  
 1015 **Figure 4. Links between DOM and the free-living (FL) bacterioplankton**  
 1016 **community at 5 meters of the Microbial Observatory Laboratory Arago.**  
 1017 The redundancy analysis shows the significant environmental parameters and  
 1018 ordination of the samples. A (b) pearson correlation between environmental  
 1019 parameters and the bacterioplankton community at genus level within the top 20  
 1020 families and false discovery rate (FDR) adjusted p-values (\* p<0.05, \*\* p<0.01, \*\*\*  
 1021 p<0.001). Abbreviations refer to: Asx, Aspartic acid and Asparagine; Chl-a,  
 1022 Chlorophyll-a; dissolved hydrolyzable amino acid, DHAA; dissolved organic  
 1023 carbon, DOC; dissolved organic nitrogen, DON; dissolved organic phosphorus,  
 1024 DOP.