Eukaryotic community composition in the sea surface microlayer across an east-west transect in the Mediterranean Sea

Birthe Zäncker[1,2], Michael Cunliffe[2,3], Anja Engel[1]

[1]GEOMAR, Helmholtz Centre for Ocean Research Kiel, 24105 Kiel, Germany
5 [2]Marine Biological Association of the UK, Plymouth, PL1 2PB, United Kingdom
[3]School of Biological and Marine Sciences, University of Plymouth, PL4 8AA, United Kingdom

Correspondence to: Anja Engel (aengel@geomar.de)

Abstract. The sea surface microlayer (SML) represents the boundary layer at the air-sea interface. Microbial eukaryotes in the SML potentially influence air-sea gas exchange directly by taking up and producing gases, and indirectly by excreting and

10 degrading organic matter, which may modify the viscoelastic properties of the SML. However, little is known about the controlling factors that influence microbial eukaryote community composition in the SML. We studied the composition of the microbial community, transparent exopolymer particles and polysaccharides in the SML during the PEACETIME cruise along a west-east transect in the Mediterranean Sea, covering the western basin, Tyrrhenian Sea and Ionian Sea. At the stations located in the Ionian Sea, fungi were found in high relative abundances determined by 18S sequencing efforts, making up a

15 significant proportion of the sequences recovered. At the same time, bacterial and phytoplankton counts were decreasing from west to east, while transparent exopolymer particle (TEP) abundance and total carbohydrate (TCHO) concentrations remained the same between Mediterranean basins. Thus, the presence of substrates for fungi, such as *Cladosporium* known to take up phytoplankton-derived polysaccharides, in combination with decreased substrate competition by bacteria suggests that fungi could be thriving in the neuston of the Ionian Sea and other low nutrient low chlorophyll (LNLC) regions.

1 Introduction

The sea surface microlayer (SML) constitutes the boundary layer between the ocean and the atmosphere (Liss and Duce, 2005; Zhang et al., 2003), and is around 1 to 1000 µm thick (Cunliffe and Murrell, 2009; Liss and Duce, 2005) with distinct physical and chemical properties compared to the underlying water (Cunliffe et al., 2013; Zhang et al., 2003). Due to the prominent position, the SML potentially has a substantial influence on air-sea exchange processes, such as gas transfer and sea spray

25 aerosol formation (Cunliffe et al., 2013; Engel et al., 2017).

The microbial food web plays a crucial role in ocean biogeochemistry and has been vastly studied. Despite the fact that microbes in the SML can directly and indirectly influence air-sea gas exchange, few studies have looked at the microbial community composition in the SML, particularly in terms of microbial eukaryotes. While phytoplankton throughout the water column play an important role in the ocean as primary producers, phytoneuston in the SML (Apts, 1989; Hardy and Apts,

30 1984; Naumann, 1917), might have an additional crucial role by impacting air-sea gas exchange (Ploug, 2008; Upstill-Goddard

et al., 2003). Early microscopic observations of the SML reported mostly diatoms, dinoflagellates and cyanobacteria (Hardy et al., 1988). More recent studies using 18S rRNA gene sequencing found a decreased protist diversity in the SML compared to underlying water with chrysophytes and diatoms enriched in the SML (Cunliffe and Murrell, 2010; Taylor and Cunliffe, 2014).

35 Not only phytoneuston, but also zooneuston, bacterioneuston and myconeuston might influence air-sea gas exchange processes by either parasitizing phytoneuston and thus impacting the primary productivity, or by degrading organic matter available in the SML and producing CO_2. While some studies have explored bacterioneuston diversity in the Mediterranean Sea (Agogué et al., 2005; Joux et al., 2006), fungi have not yet been characterized in the SML in this region. Fungi are however abundant in marine environments (Gladfelter et al., 2019; Grossart et al., 2019; Hassett et al., 2019), living a saprotrophic or parasitic

40 lifestyle and have been found in the Mediterranean Sea before (Garzoli et al., 2015; Gnavi et al., 2017), with the myconueston studied in other locations (Taylor and Cunliffe, 2014).

Phytoplankton and phytoneuston can release precursors such as carbohydrates which can aggregate and form gelatinous particles such as transparent exopolymer particles (TEP) (Chin et al., 1998; Engel et al., 2004; Verdugo et al., 2004). TEP contain mainly polysaccharides (Mopper et al., 1995; Passow, 2002) and occur ubiquitously in the ocean (Alldredge et al.,

45 1993; Passow, 2002). Due to their stickiness TEP can aggregate with other particles (Azetsu-Scott and Passow, 2004; Engel, 2000; Passow and Alldredge, 1995). When the aggregate becomes heavier due to the aggregation with additional particles, it eventually sinks out of the euphotic layer into the deep ocean and may thus play an important role in carbon export (Engel et al., 2004). However, the rate of TEP-related carbon export does not only depend on its production by phytoplankton, but also on microbial degradation.

50 Few studies have looked at spatial distribution of the microbial eukaryote communities in the SML and possible environmental drivers of community composition, especially in the open Mediterranean Sea, a characteristic low nutrient low chlorophyll (LNLC) region (Durrieu de Madron et al., 2011). The present study focuses on the organic matter (OM) and microbial eukaryotes distribution in relation to possible atmospheric inputs, focusing on the myconeuston community composition in the SML of the Mediterranean Sea using samples collected during the PEACETIME cruise in May and June 2017.

55 2 Material and methods

2.1 Sampling

Samples were collected during the PEACETIME cruise to the Mediterranean Sea onboard the RV *Pourquoi pas?* from the 10th May to the 11th June 2017. A total of 12 stations were sampled from 2.9°E to 19.8°E and 35.5°N to 42.0°N (Fig. 1) collecting water from the SML and the underlying water (ULW) at 20 cm below the SML. SML samples were collected from a zodiac

60 using a glass plate sampler (Cunliffe and Wurl, 2014; Harvey, 1966). The dimensions of the silicate glass plate (50 x 26 cm) resulted in an effective sampling surface area of 2,600 cm^2 considering both sides. To avoid contamination during sampling, the zodiac was located in front of the research vessel into the direction of the wind. The glass plate was immersed and

withdrawn perpendicular to the sea surface at a controlled rate of ~17 cm s^{-1}. With a Teflon wiper, SML samples were collected in acid cleaned and rinsed bottles (Cunliffe and Wurl, 2014). All sampling equipment was acid-cleaned (10 % HCl), rinsed

65 with Milli-Q and copiously rinsed with seawater from the respective depth once the sampling site was reached. The ULW samples were collected concurrently with two acid-cleaned and MilliQ rinsed glass bottles.

2.2 Gel particle determination

The abundance and area of TEP and was measured microscopically (Engel, 2009). The sample volume (10-30 ml) was determined onboard the ship according to the prevailing concentration of TEP. Samples were filtered onto 0.4 µm Nucleopore

70 membranes (Whatman) and stained with 1 ml Alcian Blue solution (0.2 g l^{-1} w/v) for 3 s. Filters were mounted on Cytoclear® slides and stored at -20°C until analysis. Two filters per sample with 30 images each were analyzed using a Zeiss Axio Scope.A1 (Zeiss) and the AxioCam MRc (Zeiss). The pictures with a resolution of 1388 x 1040 pixels were saved using AxioVision LE64 Rel. 4.8 (Zeiss). All particles larger than 0.2 µm^2 were analyzed. ImageJ was subsequently used for image analysis (Schneider et al., 2012). 10 ml MilliQ water served as a blank.

75 ### 2.3 Bacterioplankton and bacterioneuston abundance

Bacterial cell numbers were determined from a 2 ml sample fixed with 100 µl glutaraldehyde (GDA, 1 % final concentration). Samples were stored at -20°C and stained with SYBR Green I (Molecular Probes) to determine abundance using a flow cytometer (Becton & Dickinson FACScalibur) with a 488 nm laser. A unique signature in a plot of side scatter (SSC) vs. green fluorescence (FL1) was used to detect bacterial cells. Yellow-green latex beads (Polysciences, 0.5 µm) were used as an internal

80 standard.

2.4 Phytoplankton and phytoneuston abundance

Phytoplankton and phytoneuston cell numbers were determined from a 2 ml sample fixed with 100 µl GDA (1 % final concentration) and stored at -20°C. Samples were filtered through a 50 µm filter and analyzed with a flow cytometer (Becton & Dickinson FACScalibur) using a 488 nm laser and a standard filter set-up. Enumeration of cells was conducted using a high

85 flow rate (app. 39-41 µl min^{-1}). The forward or right-angle light scatter (FALS, RALS) as well as the phycoerythrin and chl *a* related fluorescent signal was used to distinguish the cells. Cell counts were analyzed using the CellQuest Pro-Software (BD Biosciences).

2.5 Total combined carbohydrates

Samples (20 ml) for total hydrolysable carbohydrates (TCHO) > 1 kDa were filled into precombusted glass vials (8h, 500°C)

90 and stored at -20°C. High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was applied on a Dionex ICS 3000 ion chromatography system (Engel and Händel 2011) for TCHO analysis. Prior to analysis, samples were desalinated with membrane dialysis (1 kDa MWCO, Spectra Por) at 1°C for 5h. Samples were hydrolyzed for

20 h at 100°C with 0.8 M HCl final concentration with subsequent neutralization using acid evaporation (N$_2$, for 5 h at 50°C). Two replicates per TCHO sample were analyzed.

2.6 DNA extraction and eukaryote 18S rRNA gene sequencing

400 ml of sample was pre-filtered through a mesh with 100 µm pore size and subsequently filtered onto a Durapore membrane (Millipore, 47 mm, 0.2 µm) and immediately stored at -80°C. In order to improve cell accessibility for the DNA extraction, filters in cryogenic tubes were immersed in liquid nitrogen and the filter was crushed with a pestle. DNA was extracted according to a modified protocol from Zhou et al. (1996) by Wietz and colleagues (2015). The protocol included bead-beating, phenol-chloroform-isoamyl alcohol purification, isopropanol precipitation and ethanol washing. An additional protein-removal step by salting was used to avoid protein contamination.

Library preparation and sequencing was conducted at the Integrated Microbiome Resource at Dalhousie University, Halifax, Canada and is described in detail elsewhere (Comeau et al., 2017). Samples were PCR-amplified in two dilutions (1:1 and 1:10) using the 18S rRNA gene primers E572F and E1009R (Comeau et al., 2011). Prior to pooling, samples were cleaned up and normalized using the Invitrogen SequalPrep 96-well Plate kit (Thermo Fisher Scientific). Sequencing was conducted according to Comeau et al. (2017) on an Illumina MiSeq using 300+300 bp paired-end V3 chemistry.

Sequences were processed using the DADA2 pipeline (Callahan et al., 2016) and sequences shorter than 400 bp, longer than 444 bp, with more than 8 homopolymers or any ambiguous bases were discarded. Sequences were aligned with the 18S rRNA gene sequences of the SILVA 132 alignment (Quast et al., 2013). Subsequently, sequences that aligned outside of most of the dataset and chimeras were removed. Sequences were classified using the SILVA 132 database (Quast et al., 2013) and deposited at the European Nucleotide Archive (ENA accession number PRJEB23731). Sequences were not subsampled and sequence numbers per sample ranged from 1063 sequences (S8 SML) to 43,027 sequences (S5 SML), except for PCA, where all samples were subsampled to 1063 sequences.

2.7 Statistical analyses

Statistical analyses and maps were produced using R (R Core Team, 2014) and bathymetry information from NOAA (National Oceanic and Atmospheric Administration). The enrichment factor (EF) was used to compare the concentration of substance A in the SML to the concentration in the ULW and was calculated using the following Eq. (1):

$$EF = \frac{[A]_{SML}}{[A]_{ULW}} \qquad (1)$$

Where [A] is the concentration of a parameter in the SML or ULW (World Health Organization, 1995). An EF > 1 indicates enrichment, an EF < 1 indicates depletion and an EF = 1 indicates no change of a phytoplankton genus in the SML compared to the ULW. The significance of difference between the SML and ULW and between the basins of 18S eukaryote sequences and biogeochemical parameters were tested using the Kruskal-Wallis test and PERMANOVA. Correlations were calculated using Spearman's rank correlation.

2.8 Data obtained from the ship

125 Wind speed, salinity and seawater temperature at 5 m were obtained from the RV *Pourquoi pas?* software. Radiation measurements were obtained with the pyranometer Li-Cor Radiation Sensor (Li-200SZ) measuring wavelengths of 400 to 1100 nm. All parameters were measured every 5 min during the sampling on the zodiac outlined above and the average during the sampling period was taken for statistical analyses (Table 1).

3 Results

130 ### 3.1 Microbial eukaryote community composition in the SML and ULW

The eukaryotic communities in the SML and the ULW were similar (ANOSIM, p=0.039, R=0.1002). The cruise track allowed for sampling in three basins of the Mediterranean Sea: the western basin (Provencal + Algerian basin), the Tyrrhenian Sea and the Ionian Sea. Looking at the three different basins sampled (Fig. 1), differences were detected in their eukaryotic community composition (Fig. 2). ANOSIM showed that the differences in the eukaryotic community composition were slightly larger

135 across basins than between SML and ULW (p=0.0025, R=0.2263). However, the overall diversity and evenness (based on shannon and pielou indices) were not significantly different between basins (Fig. S1).

16 orders were found in relative abundances over 5 % of the total eukaryotic community in one or more of all 12 stations (Fig. 3). The communities in the SML and ULW at most stations were similar, with Dinophyceae and Syndiniales (Dinoflagellata), and an unidentified Eukaryote class dominating the eukaryotic community. Zooneuston were found in most of the SML

140 samples, but rarely (n=2) in the ULW samples. Zooneuston were comprised of Ploimida (Rotifer), Maxillopoda (Cyclopoida and Calanoida) and Scyphozoa (Semaeostomeae).

Myconeuston and mycoplankton were found in high relative abundances in three ULW samples and in the corresponding SML samples (S8, ION_2, S7) of the Ionian Sea. At station S7 ULW, fungi made up more than half (54 %) of the total number of retrieved sequences. The vast majority of fungal ASVs (64 out of 69) belonged to Ascomycota and Mucoromycota with the

145 remaining five belonging to the Chytridiomycota (n=3), Basidiomycota and Neocallimastigomycota. Figure 4 displays all fungal ASVs that were recovered throughout the cruise and their relative abundance. It becomes apparent that while fungal ASVs make up a significant amount of sequences in the Ionian Sea (stations to the right of fig. 4), they were barely detectable at the other stations (p=0.014 for differences on fungal ASV level between basins tested with PERMANOVA).

3.2 Concentrations and SML enrichments of microorganisms and organic matter

150 Bacterial numbers did not show any significant differences between depths. In the SML, bacterial abundances ranged from 2.0 x 10^5 to 1.0 x 10^6 cells ml^{-1} with an average of 5.2 x 10^5 ± 2.3 x 10^5 cells ml^{-1}. In the ULW, bacterial numbers were on average 4.6 x 10^5 ± 1.5 x 10^5 cells ml^{-1} (range of 2.2 x 10^5 – 6.9 x 10^5 cells ml^{-1}).

Phytoneuston abundance was on average $3.3 \times 10^3 \pm 1.9 \times 10^3$ cells ml^{-1} and phytoplankton in the ULW $2.3 \times 10^3 \pm 1.7 \times 10^3$ cells ml^{-1} ($1.4 \times 10^3 - 8.5 \times 10^3$ cells ml^{-1} in the SML, $9.5 \times 10^2 - 7.1 \times 10^3$ cells ml^{-1} in the ULW). Overall, cell counts

155   determined by flow cytometry were significantly higher in the SML than in the ULW (p=0.002, n=12).

TEP concentration was on average $1.4 \times 10^7 \pm 9.7 \times 10^6$ particles l^{-1} ($3.6 \times 10^6 - 3.7 \times 10^7$ TEP l^{-1}) in the SML. In the ULW, the average TEP concentrations were $3.6 \times 10^6 \pm 2.1 \times 10^6$ particles l^{-1} ($6.8 \times 10^5 - 7.5 \times 10^6$ TEP l^{-1}) in the ULW. TEP area in the SML was on average $9.7 \times 10^7 \pm 1.2 \times 10^8$ mm^2 l^{-1} (1.5×10^7 and 4.5×10^8 mm^2 l^{-1}). TEP area was lower in the ULW with an average of $2.3 \times 10^7 \pm 1.1 \times 10^7$ ($2.9 \times 10^6 - 3.9 \times 10^7$ mm^2 l^{-1}). Both TEP abundance and area were significantly enriched in

160   the SML (Fig. 2) with values of p=0.01 and p=0.007, respectively. While irradiation, water temperature and salinity did not correlate with TEP abundance or area, wind speed did have a significant negative correlation with TEP abundance in the SML ($R^2 = -0.73$) and TEP area in the SML ($R^2 = -0.75$) and the enrichment factor of TEP area ($R^2 = -0.63$).

TCHO concentrations were similar between SML and ULW (Fig. 2), with no significant differences between depths (778 ± 294 nM ($562 - 1684$ nM) in the SML and 605 ± 97 nM ($525 - 885$ nM) in the ULW.

165   ## 4 Discussion

4.1 Eukaryotic diversity in the surface of the Mediterranean Sea

The eukaryotic community composition between the SML and the ULW only differed slightly, with larger spatial heterogeneity and significant differences between the communities of the Western, Tyrrhenian and Ionian basins. The shannon diversity did not differ significantly between depths or basins, however there was a slight decrease of species richness from

170   west to east (Fig. S1), possibly due to the transition to more oligotrophic conditions from west to east, as water exchange with the Atlantic is most pronounced in the western basin (Reddaway and Bigg, 1996) and organisms have to adapt to a more oligotrophic environment the further east they come.

Looking at the phytoplankton community (Fig. 3), it becomes apparent that no diatoms were present at high concentrations. In seasonal studies, diatoms have been important during blooms in March and April in the Mediterranean Sea, but later in the

175   year when a stratified water column was established, their importance decreased (Marty et al., 2002). Even though diatoms most likely were not dominant in the samples, finding no diatom orders over 1 % in at least one of the samples might also indicate a bias of the primers used. Another point that becomes apparent from Figure 3 is the dominance of dinoflagellate genera. Several studies have shown that dinoflagellates have a large number of 18S rRNA gene copies in comparison to other phytoplankton groups, and therefore the abundance of dinoflagellates in 18S rRNA gene sequencing is often overestimated

180   (Godhe et al., 2008; Guo et al., 2016).

Previous studies suggested various factors that potentially drive the phytoplankton community composition. Radiation, especially in the SML, where often high levels of UV-radiation occur, could potentially cause damage by photoinhibition. Dinoflagellates, one of the dominating phytoplankton groups, can however produce photoprotective compounds, including mycosporine-like amino acids (MAAs) (Carreto et al., 1990; Häder et al., 2007). Even though dinoflagellates can produce

185 MAAs, they can still be inhibited by high UV radiation (Ekelund, 1991). However, looking at the current study, no inhibition by UV radiation can be inferred from the data because phytoplankton were enriched despite high radiation values (e.g. stations S4 and 7) (Table 1).

4.2 Fungi in the Ionian Sea

Figures 3 and 4 show the relative prevalence of fungi in the Ionian Sea and their scarce reduced distribution in the western
190 basin and the Tyrrhenian Sea. Most of the ASVs present in the Ionian Sea belonged to Ascomycota and Mucoromycota. With fungi making up a significant part of the apparent eukaryotic community in the Ionian Sea (more than half of the sequences retrieved at one station), the question arises as to what drives the higher fungal numbers in this region of the Mediterranean Sea.

Not only fungal abundances increased in the Ionian Sea, but also ASVs identified as Solanales (Nicotiana) had quite high
195 abundances in the easternmost stations. Since Solanales are land plants, presence of their DNA could suggest a possible strong terrestrial influence on the Ionian Sea, either deposited by dust or by rain previous to this research campaign in the Ionian Sea or in other areas closeby. Station FAST_2 in the western basin was highly influenced by dust input in the area (Guieu et al., 2020; Tovar-Sánchez et al., 2020). This resulted not only in a high increase in TEP abundance in the SML, but also in a distinct increase in the abundance of unidentified dinoflagellates in the SML (Fig. 3). The details of the dust input on the organic matter
200 and microbial community composition in the SML and the ULW are discussed elsewhere (Engel et al., *in prep*). However, figure 4 shows that no fungi were found at station FAST_2 neither in the SML nor in the ULW, showing that dust input does not necessarily deposit fungi to the surface ocean, which potentially also holds true for the Ionian Sea. In addition, the highest amount of fungi was found in the ULW and not the SML, making a direct atmospheric influence unlikely. In addition to atmospheric inputs, riverine inputs can also influence the Mediterranean Sea (Martin et al., 1989). However, the Ionian Sea
205 itself does not experience vast riverine input and riverine influence is even less pronounced in the open sea, making riverine sources of mycophyta unlikely. Ascomycota and Mucoromycota have been recovered from a variety of marine environments (Bovio, 2019; Grossart et al., 2019; Hassett et al., 2019), thus implying that they also might be thriving in the SML of the Mediterranean Sea instead of being the result of terrestrial input.

Overall, the most abundant fungal ASV in the Ionian Sea, ASV 8, was identified as belonging to genus *Cladosporium* which
210 has been found in marine environments before (Cunliffe et al., 2017). Another explanation for the high abundance of fungi in the Ionian Sea might be that they are more adapted to dealing with the low nutrient conditions found in the more eastern basin of the Mediterranean Sea.

Bacterial and microalgal numbers determined by flow cytometry decreased significantly from west to east, with bacteria showing the greatest decline. At the same time, TCHO and TEP were still abundant in the Ionian Sea. TEP are often enriched
215 in the SML of various oceans (Engel and Galgani, 2016; Jennings et al., 2017; Wurl et al., 2009; Wurl and Holmes, 2008). In previous studies, TEP enrichment was highest over oligotrophic regions (Jennings et al., 2017; Zäncker et al., 2017). This is in good accordance with the present study in the veryoligotrophic eastern Mediterranean Sea (Durrieu de Madron et al., 2011;

Fogg, 1995; Wikner and Hagstrom, 1988) where low phytoplankton abundances, but high TEP enrichments of 1.1-17.3 were found. Wind speed correlated negatively with TEP abundance and area in the SML, showing that wind can negatively affect

220 TEP concentrations at the air-sea interface as has been previously suggested (Sun et al., 2018).

Since exchange of water with the Atlantic is mostly pronounced in the western basin, nutrient limitation increases going eastwards in the Mediterranean Sea. TEP production has been shown to be independent of stoichiometric ratios in the surrounding water before (Corzo et al., 2000). Since especially in the SML, light limitation rarely occurs and TEP might serve as light protection (Elasri and Miller, 1999; Ortega-Retuerta et al., 2009), phytoplankton might still photosynthesize and

225 excrete carbohydrates that assemble to TEP. This would not only explain the lack of difference of TEP abundance between basins, but also TCHO concentrations. However, TCHO could also be produced by cell lysis (due to nutrient depletion) and subsequent release of intracellular compounds into the surrounding water.

TCHO and TEP could therefore provide available substrate and microhabitats for marine fungi with reduced competition by bacteria in the Ionian Sea. *Malassezia* and *Cladosporium* have been shown to assimilate carbon derived from TEP-associated

230 algal polysaccharides in the English Channel (Cunliffe et al., 2017), which highlights that *Cladosporium* and other fungi might be able to make use of the substrate under decreased bacterial competition in the Ionian Sea.

5 Conclusions

The present study shows that even though flow cytometry counts suggest that bacteria and phytoplankton numbers are reducing from west to east of the Mediterranean Sea, organic matter such as microgels and TCHO are still prevalent in surface waters.

235 Our findings from the Ionian Sea suggest that accumulation of organic substrates in the surface under oligotrophic conditions may favour certain taxa such as fungi which can benefit from decreased competition by bacteria. In LNLC regions, where phytoplankton and bacterial counts are typically low, but TEP enrichment is high in the SML might be a specific ecosystem where fungi are able to thrive and to control organic matter degradation.

Data availability

All biogeochemical data will be made available at the French INSU/CNRS LEFE CYBER database (data manager, webmaster:
250   Catherine Schmechtig). All sequence data is available at the European Nucleotide Archive (ENA accession number
PRJEB23731).

Author contributions

BZ, MC and AE wrote the paper and contributed to the data analysis. BZ participated in the sample treatment.

Competing interests

255   The authors declare that they have no conflict of interest

References

Agogué, H., Casamayor, E. O., Bourrain, M., Obernosterer, I., Joux, F., Herndl, G. J. and Lebaron, P.: A survey on bacteria
inhabiting the sea surface microlayer of coastal ecosystems, FEMS Microbiol. Ecol., 54(2), 269–280,
doi:10.1016/j.femsec.2005.04.002, 2005.

260   Alldredge, A. L., Passow, U. and Logan, B. E.: The abundance and significance of a class of large , transparent organic particles
in the ocean, Deep. Res. I, 40(6), 1131–1140, 1993.

Apts, J. T. H. C. W.: Photosynthetic carbon reduction: high rates in the sea-surface microlayer, Mar. Biol., 101(3), 411–417,
doi:10.1007/bf00428138, 1989.

Azetsu-Scott, K. and Passow, U.: Ascending marine particles: Significance of transparent exopolymer particles (TEP) in the
265   upper ocean, Limnol. Oceanogr., 49(3), 741–748, doi:10.4319/lo.2004.49.3.0741, 2004.

Bovio, E.: Marine fungi from sponges: biodiversity, chemodiversity and biotechnological applications., 2019.

Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. and Holmes, S. P.: DADA2: High resolution sample
inference from Illumina amplicon data, Nat. Methods, 13(7), 581–583, doi:10.1038/nmeth.3869.DADA2, 2016.

Carreto, J. I., Carignan, M. O., Daleo, G. and DeMarco, S. G.: Ocurrence of mycosporine-like amino acids in the red-tide
270   dinoflagellate Alexandrium excavatum:uv-photoprotective compounds?., J. Plankton Res., 12(5), 909–921 [online] Available
from: http://193.144.41.80:8086/2431.pdf, 1990.

Chin, W.-C., Orellana, M. V. and Verdugo, P.: Spontaneous assembly of marine dissolved organic matter into polymer gels,
Nature, 394(July), 1998.

Comeau, A. M., Li, W. K. W., Tremblay, J. É., Carmack, E. C. and Lovejoy, C.: Arctic ocean microbial community structure
275   before and after the 2007 record sea ice minimum, PLoS One, 6(11), doi:10.1371/journal.pone.0027492, 2011.

Comeau, A. M., Douglas, G. M. and Langille, M. G. I.: Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research, mSystems, 2(1), e00127-16, doi:10.1128/mSystems.00127-16, 2017.

Corzo, A., Morillo, J. A. and Rodriquez, S.: Production of transparent exopolymer particles (TEP) in cultures of Chaetoceros calcitrans under nitrogen limitation, Aquat. Mar. Ecol., 23, 63–72, 2000.

280   Cunliffe, M. and Murrell, J. C.: The sea-surface microlayer is a gelatinous biofilm., ISME J., 3(9), 1001–1003, doi:10.1038/ismej.2009.69, 2009.

Cunliffe, M. and Murrell, J. C.: Eukarya 18S rRNA gene diversity in the sea surface microlayer: implications for the structure of the neustonic microbial loop., ISME J., 4(3), 455–458, doi:10.1038/ismej.2009.133, 2010.

Cunliffe, M. and Wurl, O.: Guide to best practices to study the ocean's surface, Occas. Publ. Mar. Biol. Assoc. United Kingdom

285   [online] Available from: http://www.solas-int.org/files/solas-int/content/downloads/Activities/SCOR WG/SCOR_GuideSeaSurface_2014.pdf, 2014.

Cunliffe, M., Engel, A., Frka, S., Gašparović, B., Guitart, C., Murrell, J. C., Salter, M., Stolle, C., Upstill-Goddard, R. and Wurl, O.: Sea surface microlayers: A unified physicochemical and biological perspective of the air-ocean interface, Prog. Oceanogr., 109, 104–116, doi:10.1016/j.pocean.2012.08.004, 2013.

290   Cunliffe, M., Hollingsworth, A., Bain, C., Sharma, V. and Taylor, J. D.: Algal polysaccharide utilisation by saprotrophic planktonic marine fungi, Fungal Ecol., 30, 135–138, doi:10.1016/j.funeco.2017.08.009, 2017.

Durrieu de Madron, X., Guieu, C., Sempéré, R., Conan, P., Cossa, D., D'Ortenzio, F., Estournel, C., Gazeau, F., Rabouille, C., Stemmann, L., Bonnet, S., Diaz, F., Koubbi, P., Radakovitch, O., Babin, M., Baklouti, M., Bancon-Montigny, C., Belviso, S., Bensoussan, N., Bonsang, B., Bouloubassi, I., Brunet, C., Cadiou, J. F., Carlotti, F., Chami, M., Charmasson, S., Charrière,

295   B., Dachs, J., Doxaran, D., Dutay, J. C., Elbaz-Poulichet, F., Eléaume, M., Eyrolles, F., Fernandez, C., Fowler, S., Francour, P., Gaertner, J. C., Galzin, R., Gasparini, S., Ghiglione, J. F., Gonzalez, J. L., Goyet, C., Guidi, L., Guizien, K., Heimbürger, L. E., Jacquet, S. H. M., Jeffrey, W. H., Joux, F., Le Hir, P., Leblanc, K., Lefèvre, D., Lejeusne, C., Lemé, R., Loÿe-Pilot, M. D., Mallet, M., Méjanelle, L., Mélin, F., Mellon, C., Mérigot, B., Merle, P. L., Migon, C., Miller, W. L., Mortier, L., Mostajir, B., Mousseau, L., Moutin, T., Para, J., Pérez, T., Petrenko, A., Poggiale, J. C., Prieur, L., Pujo-Pay, M., Pulido-Villena,

300   Raimbault, P., Rees, A. P., Ridame, C., Rontani, J. F., Ruiz Pino, D., Sicre, M. A., Taillandier, V., Tamburini, C., Tanaka, T., Taupier-Letage, I., Tedetti, M., Testor, P., Thébault, H., Thouvenin, B., Touratier, F., Tronczynski, J., Ulses, C., Van Wambeke, F., Vantrepotte, V., Vaz, S. and Verney, R.: Marine ecosystems' responses to climatic and anthropogenic forcings in the Mediterranean, Prog. Oceanogr., 91(2), 97–166, doi:10.1016/j.pocean.2011.02.003, 2011.

Ekelund, N. G. A.: The Effects of UV-B Radiation on Dinoflagellates, J. Plant Physiol., 138(3), 274–278, 1991.

305   Elasri, M. O. and Miller, R. V: Study of the Response of a Biofilm Bacterial Community to UV Radiation Study of the Response of a Biofilm Bacterial Community to UV Radiation, Appl. Environ. Microbiol., 65(5), 2025–2031, doi:0099-2240, 1999.

Engel, A.: The role of transparent exopolymer particles (TEP) in the increase in apparent particle stickiness (alpha) during the decline of a diatom bloom, J. Plankton Res., 22(3), 485–497, doi:10.1093/plankt/22.3.485, 2000.

310   Engel, A.: Determination of marine gel particles, in Practical Guidelines for the Analysis of Seawater, edited by O. Wurl, pp.
        125–142, CRC Press Taylor & Francis Group, Boca Raton, FL., 2009.

Engel, A. and Galgani, L.: The organic sea-surface microlayer in the upwelling region off the coast of Peru and potential
implications for air-sea exchange processes, Biogeosciences, 13(4), 989–1007, doi:10.5194/bg-13-989-2016, 2016.

Engel, A., Thoms, S., Riebesell, U., Rochelle-Newall, E. and Zondervan, I.: Polysaccharide aggregation as a potential sink of
315   marine dissolved organic carbon, Nature, 428(April), 929–932, doi:10.1038/nature02506.1., 2004.

Engel, A., Bange, H. W., Cunliffe, M., Burrows, S. M., Friedrichs, G., Galgani, L., Herrmann, H., Hertkorn, N., Johnson, M.,
Liss, P. S., Quinn, P. K., Schartau, M., Soloviev, A., Stolle, C., Upstill-Goddard, R. C., van Pinxteren, M. and Zäncker, B.:
The Ocean's Vital Skin: Toward an Integrated Understanding of the Sea Surface Microlayer, Front. Mar. Sci., 4(May), 165,
doi:10.3389/fmars.2017.00165, 2017.

320   Fogg, G. E.: Some comments on picoplankton and its importance in the pelagic ecosystem, Aquat. Microb. Ecol., 9(1), 33–
39, doi:10.3354/ame009033, 1995.

Garzoli, L., Gnavi, G., Tamma, F., Tosi, S., Varese, G. C. and Picco, A. M.: Sink or swim: Updated knowledge on marine
fungi associated with wood substrates in the Mediterranean Sea and hints about their potential to remediate hydrocarbons,
Prog. Oceanogr., 137, 140–148, doi:10.1016/j.pocean.2015.05.028, 2015.

325   Gladfelter, A. S., James, T. Y. and Amend, A. S.: Marine fungi, Curr. Biol., 29, 2019.

Gnavi, G., Garzoli, L., Poli, A., Prigione, V., Burgaud, G. and Varese, G. C.: The culturable mycobiota of Flabellia petiolata:
First survey of marine fungi associated to a Mediterranean green alga, PLoS One, 12(4), 1–20,
doi:10.1371/journal.pone.0175941, 2017.

Godhe, A., Asplund, M. E., Härnström, K., Saravanan, V., Tyagi, A. and Karunasagar, I.: Quantification of diatom and
330   dinoflagellate biomasses in coastal marine seawater samples by real-time PCR, Appl. Environ. Microbiol., 74(23), 7174–7182,
doi:10.1128/AEM.01298-08, 2008.

Grossart, H.-P., Van den Wyngaert, S., Kagami, M., Wurzbacher, C., Cunliffe, M. and Rojas-Jimenez, K.: Fungi in aquatic
ecosystems, Nat. Rev. Microbiol., 17(6), 339–354, 2019.

Guieu, C., D'Ortenzio, F., Dulac, F., Taillandier, V., Doglioli, A., Petrenko, A., Barrillon, S., Mallet, M., Pierre, N. and
335   Desboeufs, K.: Process studies at the air-sea interface after atmospheric deposition in the Mediterranean Sea : objectives and
strategy of the PEACETIME oceanographic campaign (May – June 2017), 2020.

Guo, L., Sui, Z. and Liu, Y.: Quantitative analysis of dinoflagellates and diatoms community via Miseq sequencing of actin
gene and v9 region of 18S rDNA, Sci. Rep., 6(May), 1–9, doi:10.1038/srep34709, 2016.

Häder, D.-P., Kumar, H. D., Smith, R. C. and Worrest, R. C.: Effects of solar UV radiation on aquatic ecosystems and
340   interactions with climate change, Photochem. Photobiol. Sci., 6(3), 267–285, doi:10.1039/B700020K, 2007.

Hardy, J. T. and Apts, C. W.: The sea-surface microlayer: phytoneuston productivity and effects of atmospheric particulate
matter, Mar. Biol., 82, 293–300, 1984.

Hardy, J. T., Coley, J. A., Antrim, L. D. and Kiesser, S. L.: A hydrophobic large-volume sampler for collecting aquatic surface

11

microlayers: characterization and comparison with the glass plate method, Can. J. Fish. Aquat. Sci., 45(5), 822–826, 1988.

345 Harvey, G.: Microlayer collection from the sea surface: a new method and intial results, Limnol Ocean., 11(4), 608–613, 1966.

Hassett, B. T., Borrego, E. T., Vonnahme, T. R., Rämä, T., Kolomiets, M. V. and Gradinger, R.: Arctic marine fungi: Biomass, functional genes, and putative ecological roles, ISME Journal2, 13(6), 1484–1496, 2019.

Jennings, M. K., Passow, U., Wozniak, A. S. and Hansell, D. A.: Distribution of transparent exopolymer particles (TEP) across an organic carbon gradient in the western North Atlantic Ocean, Mar. Chem., 190, 1–12, doi:10.1016/j.marchem.2017.01.002, 350 2017.

Joux, F., Agogue, H., Obernosterer, I., Dupuy, C., Reinthaler, T., Herndl, G. J. and Lebaron, P.: Microbial community structure in the sea surface microlayer at two contrasting sites in the northwestern Mediterranean Sea., Aquat. Microb. Ecol., 42, 91–104, doi:10.3354/ame042091, 2006.

Liss, P. S. and Duce, R. A.: The sea surface and global change, Cambridge University Press, Cambridge., 2005.

355 Martin, J. M., Elbaz-Poulichet, F., Guieu, C., Loÿe-Pilot, M. D. and Han, G.: River versus atmospheric input of material to the mediterranean sea: an overview, Mar. Chem., 28(1–3), 159–182, doi:10.1016/0304-4203(89)90193-X, 1989.

Marty, J. C., Chiavérini, J., Pizay, M. D. and Avril, B.: Seasonal and interannual dynamics of nutrients and phytoplankton pigments in the western Mediterranean Sea at the DYFAMED time-series station (1991-1999), Deep. Res. Part II Top. Stud. Oceanogr., 49(11), 1965–1985, doi:10.1016/S0967-0645(02)00022-X, 2002.

360 Mopper, K., Zhou, J., Ramana, K. S., Passow, U., Dam, H. G. and Drapeau, D. T.: The role of surface active carbohydratesin the flocculation of a diatom bloom in a mesocosm, Deep Sea Res. Part I Oceanogr. Res. Pap., 42(1), 43–73, 1995.

Naumann, E.: Über das Neuston des Süsswassers, Biol. Cent., 37(2), 98–106, 1917.

Ortega-Retuerta, E., Passow, U., Duarte, C. and Reche, I.: Effects of ultraviolet B radiation on (not so) transparent exopolymer particles, Biogeosciences, 6, 3071–3080, doi:10.5194/bgd-6-7599-2009, 2009.

365 Passow, U.: Transparent Exopolymer Particles in Aquatic Environments, Prog. Oceanogr., 55(3–4), 287–333, doi:10.1016/S0079-6611(02)00138-6, 2002.

Passow, U. and Alldredge, A. L.: Aggregation of a diatom bloom in a mesocosm: The role of transparent exopolymer particles (TEP), Deep. Res. Part II, 42(1), 99–109, doi:10.1016/0967-0645(95)00006-C, 1995.

Ploug, H.: Cyanobacterial surface blooms formed by Aphanizomenon sp. and Nodularia spumigena in the Baltic Sea: Small-370 scale fluxes, pH, and oxygen microenvironments, Limnol. Oceanogr., 53(3), 914–921, doi:10.4319/lo.2008.53.3.0914, 2008.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. and Glöckner, F. O.: The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools, Nucleic Acids Res., 41(D1), 590–596, doi:10.1093/nar/gks1219, 2013.

R Core Team: R: A language and environment for statistical computing. Vienne, Austria: R Foundation for Statistical 375 Computing, Vienna., 2014.

Reddaway, J. M. and Bigg, G. R.: Climatic change over the Mediterranean Sea and links to the more general atmospheric circulation, Int. J. Clim. Chang., 16, 651–661, 1996.

Schneider, C. a, Rasband, W. S. and Eliceiri, K. W.: NIH Image to ImageJ: 25 years of image analysis, Nat. Methods, 9(7), 671–675, doi:10.1038/nmeth.2089, 2012.

380 Sun, C., Sperling, M. and Engel, A.: Effect of wind speed on the size distribution of biogenic gel particles in the sea surface microlayer : Insights from a wind wave channel experiment, , (October), 1–39, doi:10.5194/bg-15-3577-2018, 2018.

Taylor, J. D. and Cunliffe, M.: High-throughput sequencing reveals neustonic and planktonic microbial eukaryote diversity in coastal waters, J. Phycol., 50(5), 960–965, doi:10.1111/jpy.12228, 2014.

Tovar-Sánchez, A., Rodríguez-Romero, A., Engel, A., Zäncker, B., Fu, F., Marañón, E., Pérez-Lorenzo, M., Bressac, M.,
385 Wagener, T., Triquet, S., Siour, G., Desboeufs, K. and Guieu, C.: Characterizing the surface microlayer in the Mediterranean Sea: Trace metal concentrations and microbial plankton abundance, Biogeosciences, 17(8), 2349–2364, doi:10.5194/bg-17-2349-2020, 2020.

Upstill-Goddard, R. C., Frost, T., Henry, G. R., Franklin, M., Murrell, J. C. and Owens, N. J. P.: Bacterioneuston control of air-water methane exchange determined with a laboratory gas exchange tank, Global Biogeochem. Cycles, 17(4), 1108,
390 doi:10.1029/2003GB002043, 2003.

Verdugo, P., Alldredge, A. L., Azam, F., Kirchman, D. L., Passow, U. and Santschi, P. H.: The oceanic gel phase: A bridge in the DOM-POM continuum, Mar. Chem., 92(1-4 SPEC. ISS.), 67–85, doi:10.1029/2002GL016046, 2004.

Wietz, M., Wemheuer, B., Simon, H., Giebel, H. A., Seibt, M. A., Daniel, R., Brinkhoff, T. and Simon, M.: Bacterial community dynamics during polysaccharide degradation at contrasting sites in the Southern and Atlantic Oceans, Environ.
395 Microbiol., 17(10), 3822–3831, doi:10.1111/1462-2920.12842, 2015.

Wikner, J. and Hagstrom, A.: Evidence for a tightly coupled nanoplanktonic predator-prey link regulating the bacterivores in the marine environment, Mar. Ecol. Prog. Ser., 50, 137–145, doi:10.3354/meps050137, 1988.

World Health Organization: The sea-surface microlayer and its role in global change, Geneva., 1995.

Wurl, O. and Holmes, M.: The gelatinous nature of the sea-surface microlayer, Mar. Chem., 110(1–2), 89–97,
400 doi:10.1016/j.marchem.2008.02.009, 2008.

Wurl, O., Miller, L., Röttgers, R. and Vagle, S.: The distribution and fate of surface-active substances in the sea-surface microlayer and water column, Mar. Chem., 115(1–2), 1–9, doi:10.1016/j.marchem.2009.04.007, 2009.

Zäncker, B., Bracher, A., Röttgers, R. and Engel, A.: Variations of the Organic Matter Composition in the Sea Surface Microlayer: A Comparison between Open Ocean, Coastal, and Upwelling Sites Off the Peruvian Coast, Front. Microbiol.,
405 8(December), 1–17, doi:10.3389/fmicb.2017.02369, 2017.

Zhang, Z., Liu, L., Liu, C. and Cai, W.: Studies on the sea surface microlayer: II. The layer of sudden change of physical and chemical properties, J. Colloid Interface Sci., 264(1), 148–159, doi:10.1016/S0021-9797(03)00390-4, 2003.

Zhou, J., Bruns, M. A. and Tiedje, J. M.: DNA recovery from soils of diverse composition, Appl. Environ. Microbiol., 62(2), 316–322, doi:D - NLM: PMC167800 EDAT- 1996/02/01 MHDA- 1996/02/01 00:01 CRDT- 1996/02/01 00:00 PST - ppublish,
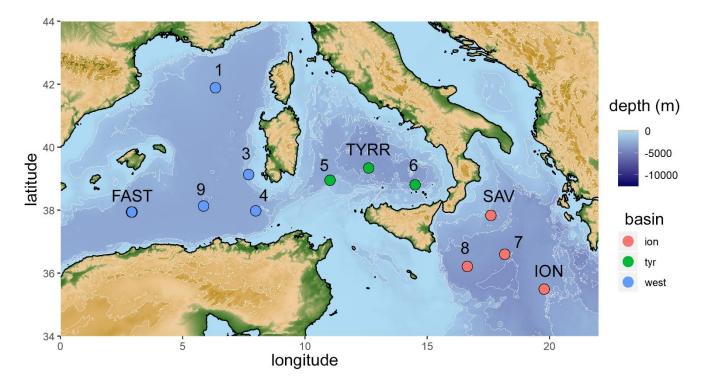410 1996.

Figure 1: Map of the stations sampled during the PEACETIME cruise in the Mediterranean Sea in May/June 2017. Stations FAST and TYRR were sampled twice. Colours represent sampled basins (blue: western basin, green: Tyrrhenian Sea, red: Ionian Sea).
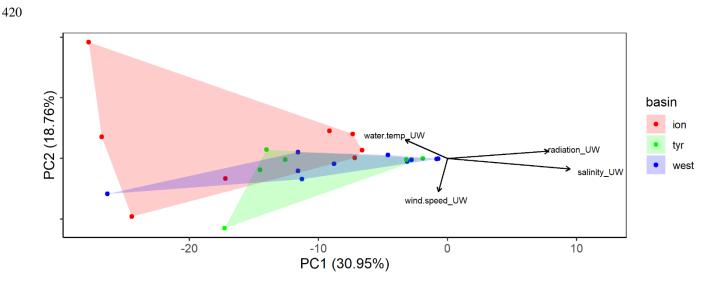
420

Figure 2: Principal Component Analyses (PCA) using the eukaryotic community composition on ASV level with environmental factors plotted. Colours distinguish the three different basins sampled (blue: western basin, green: Tyrrhenian Sea, red: Ionian Sea).
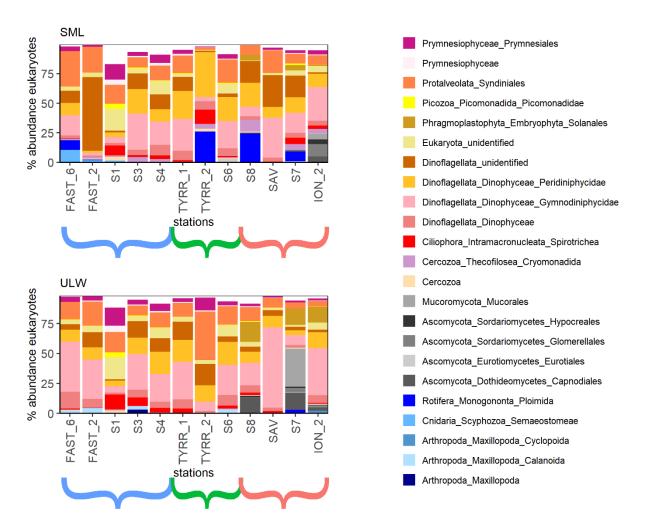
425

Figure 3: Eukaryotic community composition on order level (all taxa over 5 percent in at least one of the samples are displayed). Stations ordered from west to east with brackets indicating Mediterranean Sea basins (blue: western basin, green: Tyrrhenian Sea, red: Ionian Sea).
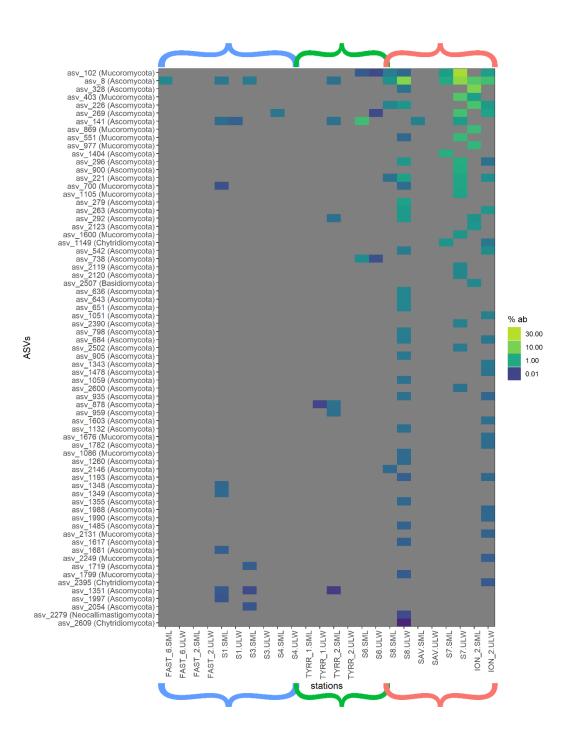
430

Figure 4: Heatmap of fungal relative abundances on ASV level throughout the cruise with brackets indicating the Mediterranean Sea basins (blue: western basin, green: Tyrrhenian Sea, red: Ionian Sea). Grey indicates that the ASV was not found in the respective sample.
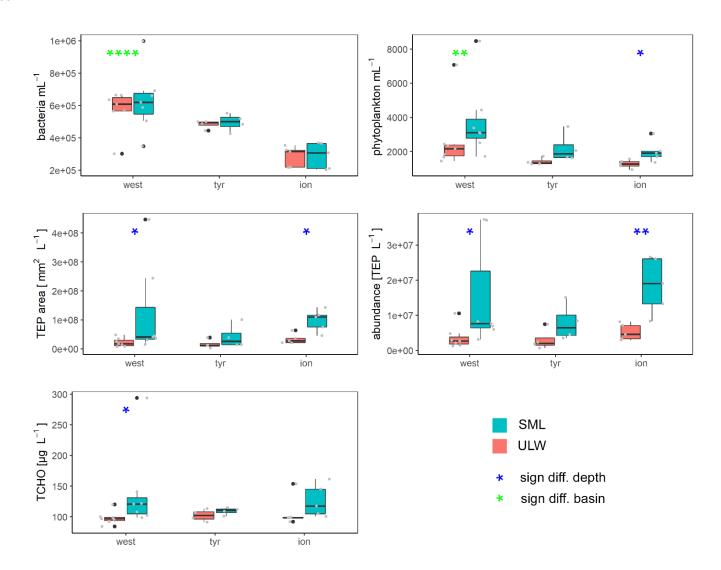
435

Figure 5: Abundance of phytoplankton and bacteria as well as area and concentrations of Transparent Exopolymer Particles (TEP) and total carbohydrates (TCHO) across sampled basins in the Mediterranean Sea. Blue stars mark significant SML
440 **enrichment/depletion, green stars mark significant differences between the three basins (Kruskall-Wallis tests used for significance levels). Signficance levels: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001.**

19

Table 1: Environmental influences at the stations sampled throughout the cruise.

station	lat	lon	wind speed [m s⁻¹]	water temp [°C] in 5m	salinity [PSU] in 5m	irradiation [W m⁻²]
S1	41.8918	6.3333	9.7	16.4	38.2	1297.8
S3	39.1333	7.6835	2.9	18.7	37.2	2343.2
S4	37.9832	7.9768	3.5	19.8	37.1	2270.2
TYRR_1	39.34	12.5928	3.4	20.3	37.8	2253.1
TYRR_2	39.3398	12.5928	2.5	21.1	37.7	2311.1
S6	38.8077	14.4997	5.2	20.4	37.4	2215.5
SAV	37.8401	18.1658	1.5	20.1	38.5	
S7	36.6035	18.1658	2.5	20.8	38.5	16.8
ION_2	35.4892	19.7765	6.4	21.1	38.8	1235.3
S8	36.2103	16.631	1.9	21.2	37.9	2144.0
FAST_2	37.946	2.9102	3.1	21.7	36.7	627.4
FAST_6	37.0466	2.9168	5.1	21.9	36.6	1787.1

445