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

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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 degrading organic matter, which may modify the viscoelastic properties of the SML. However, little is known about the distribution of microbial eukaryotes 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 – likely of continental origin ~~via~~ and delivered by atmospheric deposition - were found in high relative abundances ~~determined by 18S sequencing efforts,~~ making up a significant proportion of the sequences recovered. ~~At the same time~~Concomitantly, bacterial and picophytoplankton counts ~~were~~ decrease~~ding~~ from west to east, while transparent exopolymer particle (TEP) abundance and total carbohydrate (TCHO) concentrations remained ~~the same between Mediterranean basins. Thus,~~constant in all basins. Our results suggest that 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~~might favour fungal dominance in the neuston of the Ionian Sea and other low nutrient low chlorophyll (LNLC) regions.

1 Introduction

The sea surface microlayer (SML) constitutes ~~the~~ a 1 to 100 µm thick boundary layer between the ocean and the atmosphere (Cunliffe and Murrell, 2010; 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, t~~The SML potentially has a substantial influence on air-sea exchange processes, such as gas transfer and sea spray aerosol formation (Cunliffe et al., 2013; Engel et al., 2017; Freney et al., 2020; Sellegri et al., in prep.).

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, and have mainly focussed~~ing~~ on bacteria (Agogué et al., 2005; Joux et al., 2006; Obernosterer et al., 2008)~~ and less on~~with little attention to microbial eukaryotes (Taylor and Cunliffe, 2014). 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, 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).

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~~ releasing 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 lifestyle and have been found in the Mediterranean Sea before (Garzoli et al., 2015; Gnavi et al., 2017)and ~~, within~~ the myconueston studied ~~in~~ at 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), occur ubiquitously in the ocean (Alldredge et al., 1993; Passow, 2002), and are an important structural component of the SML (Wurl and Holmes, 2008). Due to their stickiness TEP can aggregate with other denser 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 sink~~s~~ out of the euphotic layer into the deep ocean leading to ~~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~~ TEP production by phytoplankton, but also on their microbial degradation.

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 anti-estuarine circulations at the Strait of Gibraltar and the Straits of Sicily, transport low-nutrient surface waters into the basins, and deeper waters out of the basins, resulting in oligotrophic conditions ~~in the western~~ and ultra-oligotrophic conditions in the western and eastern Mediterranean basin, respectively (Krom et al., 2004; Mermex Group et al., 2011; Pujo-Pay et al., 2011; Tanhua et al., 2013). The present study focuses on TEP as important structural components of the SML and their precursors, carbohydrates, as well as microbial eukaryotes distribution, focusing on the myconeuston community composition in the SML ~~of the Mediterranean Sea~~ using samples collected during the PEACETIME cruise in the Mediterranean Sea during May and June 2017.

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~~ Water from the SML and the underlying water (ULW; 20 cm below the SML) was collected at 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 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~~positioned upwind and in front of the research vessel ~~into the direction of the wind~~. The glass plate was immersed and withdrawn perpendicular to the sea surface. With a Teflon wiper, SML samples were collected in acid cleaned and rinsed bottles (Cunliffe and Wurl, 2014). Approximately ~~total of app.~~ 1.5 L of SML sample was collected in the course of 1 h. Sampling times are listed in table 1. All sampling equipment was acid-cleaned (10 % HCl), rinsed with Milli-Q and copiously rinsed with seawater from the respective depth once the sampling site was reached. The ULW samples were collected ~~concurrently~~ simultaneously with two acid-cleaned and MilliQ rinsed glass bottles by immersing the closed bottles and opening them at app. 20 cm.

2.2 Gel particle determination

The abundance and area of TEP was measured microscopically (Engel, 2009). The sample volume (10-30 ml) was ~~determined onboard the ship~~chosen according to the prevailing ~~concentration of~~ TEP concentrations. Samples were filtered onto 0.4 µm Nucleopore membranes (Whatman) and stained with 1 ml Alcian Blue solution (0.2 g l^{-1} w/v) for 3 seconds. 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~~equipped with a Zeiss 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). A filter prepared with 10 ml MilliQ water served as a blank.

2.3 Bacterioplankton and bacterioneuston abundance

Bacterial cell numbers were determined from ~~a~~ 2 ml samples 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~~)~~ flow cytometer equipped with a 488 nm laser. Bacterial cells were detected by the~~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 standards.

2.4 Picophytoplankton and picophytoneuston abundance

Picophytoplankton and picophytoneuston cell numbers were determined from 2 ml samples fixed and stored as for bacterial abundances. ~~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~~(similar as in section 2.3). Enumeration of cells was conducted using a high 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). ~~This method is in accordance with previous method development~~The method used here (fixative addition + slow freezing) follows recommendations by Lepesteur et al. (~~Lepesteur et al.,~~ 1993).

2.5 Total combined carbohydrates

Samples (20 ml) for total hydrolysable carbohydrates (TCHO) > 1 kDa were filled into precombusted glass vials (8h, 500°C) and stored at -20°C. In the home lab, TCHO analysis was carried out using 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~~ by membrane dialysis (1 kDa MWCO, Spectra Por) at 1°C for 5h and~~. Samples were~~ hydrolyzed for 20 h at 100°C in HCl (~~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 analy~~z~~ed for each sample.

2.6 DNA extraction and eukaryote 18S rRNA gene sequencing

~~400 ml of sample was pre-filtered~~Water samples for sequencing (400 ml each) were passed through a ~~mesh with~~ 100 µm pore size mesh in order to ~~avoid~~ remove meta-zooplankton that could dominate~~being captured on the filters and dominating~~ the ~~retrieved~~ 18S sequences 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 et al.~~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 database (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). However, for principal component analysis (PCA), except for PCA, where all samples were subsampled down 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 parameter A in the SML ($[A]_{SML}$) to the concentration in the ULW ($[A]_{ULW}$) and was calculated as follows using the following (Eq. (1; World Health Organization, 1995)):

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

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 ULWdifference between the SML and the ULW. The significance of differences 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

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

3 Results

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),However, differences were detected in their eukaryotic community composition (Fig. 2) of the basins sampled (western Mediterranean, Tyrrhenian Sea and Ionian Sea). ANOSIM

showed that the differences in the eukaryotic community composition were slightly larger 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 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 (Stations 7, 8~~,~~ and ION_2~~, S7~~) ~~o~~in~~f~~ the Ionian Sea. In the ULW of station 7~~At station S7 ULW~~, fungi made up more than half (54 %) of the total number of retrieved sequences. The vast majority of fungal amplicon sequence variants (ASVs) (64 out of 69) belonged to Ascomycota and Mucoromycota with the remaining five belonging to the Chytridiomycota (n=3), Basidiomycota and Neocallimastigomycota. ~~Figure 4 displays a~~All fungal ASVs that were recovered throughout the cruise and their relative abundance are shown in Figure 4. ~~It becomes apparent that w~~While fungal ASVs ma~~k~~de 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 i~~o~~n fungal ASVs ~~level~~ between basins tested with PERMANOVA).

3.2 Concentrations and SML enrichments of microorganisms and organic matter

Bacterial numbers did not show any significant differences between ~~depths~~layers (Fig. 5A). 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 ~~–~~to 6.9 x 10^5 cells ml^{-1}) (Fig. 5).

Picophytoneuston (0.2 – 20 µm size range) abundance was on average 3.3 x 10^3 ± 1.9 x 10^3 cells ml^{-1} in the SML and picophytoplankton abundance ~~in the ULW~~ was on average 2.3 x 10^3 ± 1.7 x 10^3 cells ml^{-1} in the ULW (range of 1.4 x 10^3 ~~–~~to 8.5 x 10^3 cells ml^{-1} in the SML, 9.5 x 10^2 to~~–~~ 7.1 x 10^3 cells ml^{-1} in the ULW). Overall, cell counts determined by flow cytometry were significantly higher in the SML than in the ULW (p=0.002, n=12; Fig. 5B).

TEP concentration averaged~~was on average~~ 1.4 x 10^7 ± 9.7 x10^6 particles l^{-1} (ranging between 3.6 x 10^6 and~~–~~ 3.7 x 10^7 TEP l^{-1}) in the SML. In the ULW, the average TEP concentration~~s were~~was 3.6 x 10^6 ± 2.1 x10^6 particles l^{-1} (ranging between 6.8 x 10^5 and~~–~~ 7.5 x 10^6 TEP l^{-1}) in the ULW. TEP area in the SML was on average 9.7 x 10^7 ± 1.2 x 10^8 mm^2 l^{-1} (1.5 x 10^7 ~~and~~ to 4.5 x 10^8 mm^2 l^{-1}). TEP area was lower in the ULW with an average of 2.3 x 10^7 ± 1.1 x 10^7 (2.9 x 10^6 ~~–~~to 3.9 x 10^7 mm^2 l^{-1}). Both TEP abundance and area were significantly enriched in the SML (Fig. 5~~;) 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~~ for TEP area (R^2 = -0.63).

TCHO concentrations were similar between the SML and ULW (Fig. 5E), with no significant differences between depths (778 ± 294 nM (range 562 –to 1684 nM) in the SML and 605 ± 97 nM (range 525 –to 885 nM) in the ULW).

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 only slightly, with larger spatialhigher horizontal 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 west to east could be observed (Fig. S1), possibly due related to the transition from oligotrophic to ultra oligotrophic conditions from west to east, asgiven the more water exchange with the Atlantic is most pronounced water exchange with the Atlantic 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 nNo diatoms were present at high relative abundances in our samples. In seasonal studies in the Mediterranean Sea, diatom contribution can be significants have been important during blooms in March and April in the Mediterranean Sea, but later in the year, as the water column stratifies, year when a stratified water column was established, their importance contribution decreaseds (Marty et al., 2002). Even though diatoms most likely were not dominant in the samples, the extremely low abundance (< 1%) of diatoms in finding no diatom orders over 1 % in at least one of the samples might also indicate a bias of the primers used or removal of larger cells and aggregates duringof the pre-filtration removing larger cells and aggregates step. Another potential biaspoint that becomes apparent from Figure 3 is the dominance of dinoflagellate genera (Fig. 3). Several studies have shown that dDinoflagellates have a large number of 18S rRNA gene copies in comparison to other phytoplankton groups, and therefore the abundance of dinoflagellates their abundance in 18S rRNA gene sequencing is often overestimated (Godhe et al., 2008; Guo et al., 2016).

Previous studies suggested various factors that potentially drive the phytoplankton community composition. In addition to buoyancy of cells, radiation, especially in the SML, where often high levels of UV-radiation occur, could potentially cause damage by photoinhibition. While dDinoflagellates, 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 MAAs, they can still be inhibited by high UV radiation (Ekelund, 1991). However, looking at the currentIn the present study, no inhibition by UV radiation can be inferred from is not indicated in the data because since phytoplankton were was enriched in the SML despite high radiation values (e.g. stations S4 and 7;) (Table 1). At the same time, TEP were significantly enriched during the this sampling campaign while the phytoplankton community did not show significant differences. Previous studies suggested that TEP can protect phytoplankton and bacteria from UV radiation (Elasri and Miller, 1999; Ortega-Retuerta et al., 2009). Further Process oriented studies would be needed to determine whether TEP

production was higher in the SML due to ~~phytoneuston~~ UV protection of phytoneuston or whether TEP formation rates were higher in the SML due to wind and wave shear at the surface (Carlson, 1993; Cunliffe et al., 2013).

4.2 Fungi in the Ionian Sea

~~Figures 3 and 4 show the relative prevalence of f~~Fungi were prevalent in the Ionian Sea ~~and their scarce reduced distribution~~ (more than half of the sequences retrieved at one station), while scarce in the western basin and the Tyrrhenian Sea (Fig. 3 and 4). Most of the fungal 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), t~~The question arises as to what drives the higher fungal relative abundances in this region of the Mediterranean Sea. While fungi, like dinoflagellates and other eukaryotic groups, can have varying amounts of 18S rDNA gene copy numbers, the patchy distribution of fungi found in this study makes a consistent bias unlikely. Marine fungi can live a saprotrophic lifestyle, degrading and recycling high molecular weight organic matter (Chrismas and Cunliffe, 2020; Cunliffe et al., 2017) and potentially competing with functionally similar bacteria. Some marine fungi are also phytoplankton parasites, potentially altering phytoplankton community composition through selective parasitism (Amend et al., 2019; Grossart et al., 2019).. At present, we have a very limited understanding of diversity and functional role of fungi in the SML (myconeuston). One previous study of the coastal myconeuston in the Western English Channel off Plymouth (UK) showed that the SML was dominated by both Ascomycota and Basidiomycota (Taylor and Cunliffe, 2014), while our results support the dominance of Ascomycota in the SML~~compared to Ascomycota dominating in this study~~.

So far, not many studies have looked at fungi in LNLC regions. A global comparison of fungal distribution (Hassett et al., 2020) has found that fungal diversity determined by amplicon sequencing varies between different oceanic regions with *Exophiala*, belonging to Ascomycota, dominating the Ligurian Sea ~~samples~~ and an unclassified Ascomycota being the most abundant taxon in other regions~~, similar to our study being dominated by Ascomycota~~.

~~Not only fungal relative abundances increased in the Ionian Sea, but also~~ ASVs identified as Solanales (Nicotiana) had quite high relative 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, linked to wet or dry deposition that occurred before and/or during our sampling period in this basin~~at ION~~. This is also corroborated by air mass trajectory backtracking using the HYSPLIT model (Fig. S3) which showed that aerosols ~~likely~~ were most likely of continental origin (Fu et al., in prep). This is ~~,~~ also confirmed by atmospheric measurements indicating that chemical composition of dry and wet depositions were influenced by Eastern European air masses (Desboeufs et al., in prep.). 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 coincided not only with a ~~high~~ strong increase in TEP abundance in the SML, but also with a distinct increase in the relative abundance of unidentified dinoflagellates in the SML (Fig. 3). The details of the impact of dust input on the organic matter 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 (Fig. 4), showing that dust input does not necessarily deposit fungi to the surface ocean, ~~which~~ this

potentially also holds true for the Ionian Sea. ~~In addition, the highest relative abundance of fungi was found in the ULW and not the SML, making a simple atmospheric influence without any subsequent thriving of certain fungal taxa unlikely~~. In addition to atmospheric inputs, riverine inputs can also influence the Mediterranean Sea (Martin et al., 1989). However, the Ionian Sea 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 rather than originating solely from terrestrial sources ~~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 has been found in marine environments before (Cunliffe et al., 2017). Another explanation for the high relative 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~~ largest decline (Tovar-Sánchez et al., 2020). While ~~overall~~ total microalgal abundances determined by flow cytometry were ~~rather~~ low in the SML and in the ULW, they were comparable to other studies on~~looking at the~~ phytoplankton abundance in the SML of the Mediterranean Sea (Joux et al., 2006). The microalgal numbers ~~from 5 200 m~~between 5 and 200 m water depth (data not shown) were higher than at the air-sea interface. Even though overall bacterial numbers decrease, further molecular analyses would be needed to determine if the bacterial community is changing from west to east and if certain bacterial taxa can benefit from the ultra oligotrophic conditions. ~~At the same time~~In contrast to bacteria and phytoplankton, spatial trends in TCHO and TEP ~~were still abundant in the Ionian Sea~~, as well as DOC in the SML and DOC and POC in the ULW ~~which~~ did not show ~~changes~~ significant differences between the Ionian Sea and the other basins (Freney et al., 2020; Trueblood et al., 2020). TEP are often enriched in the SML ~~of various oceans~~ (Engel and Galgani, 2016; Jennings et al., 2017; Wurl et al., 2009; Wurl and Holmes, 2008), in particular~~. In previous studies, TEP enrichment was highest~~ over oligotrophic regions (Jennings et al., 2017; Zäncker et al., 2017). This is in good accordance with trends observed in the present study ~~in the ultra oligotrophic eastern Mediterranean Sea~~ (Durrieu de Madron et al., 2011; Fogg, 1995; Wikner and Hagstrom, 1988) ~~where low picophytoplankton abundances, but high TEP enrichments of 1.1 17.3 were found in the present study~~. Wind speed correlated negatively with TEP abundance and area in the SML, showing that wind can negatively affect TEP concentrations at the air-sea interface as has been previously suggested (Sun et al., 2018).

Since exchange of water with the Atlantic is most~~ly~~ pronounced in the western basin and an anti-estuarine circulation prevails in the Mediterranean Sea, 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~~ in a previous study (Corzo et al., 2000). Since ~~especially~~ in the SML, light limitation rarely occurs and TEP can potentially protect phytoplankton from ~~might serve as~~ light damage ~~protection~~ (Elasri and Miller, 1999; Ortega-Retuerta et al., 2009), phytoplankton might still photosynthesize and excrete carbohydrates that assemble to TEP. This would not only explain the lack of difference ~~of~~ in TEP abundances between

basins, but also TCHO concentrations. ~~However,~~ TCHO ~~could~~ can 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~~ also 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 algal polysaccharides in the English Channel (Cunliffe et al., 2017), highlighting that~~which highlights that~~ *Cladosporium* and other fungi might be able to profit from the~~make use of the substrate under~~ decreased bacterial competition in the Ionian Sea. Further~~In addition~~, previous studies have shown that the Eastern Mediterranean Sea ~~shows~~ contains higher concentrations of organic pollutants (Berrojalbiz et al., 2011a, 2011b) ~~and~~ while a *Cladosporium* strain has the capacity~~been observed~~ to degrade polycyclic aromatic hydrocarbons (Birolli et al., 2018), highlighting another potential substrate for the fungi detected in the Ionian Sea.

5 Conclusions

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

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Data availability

All biogeochemical data will be made available at the French INSU/CNRS LEFE CYBER database (data manager, webmaster: 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

The authors declare that they have no conflict of interest

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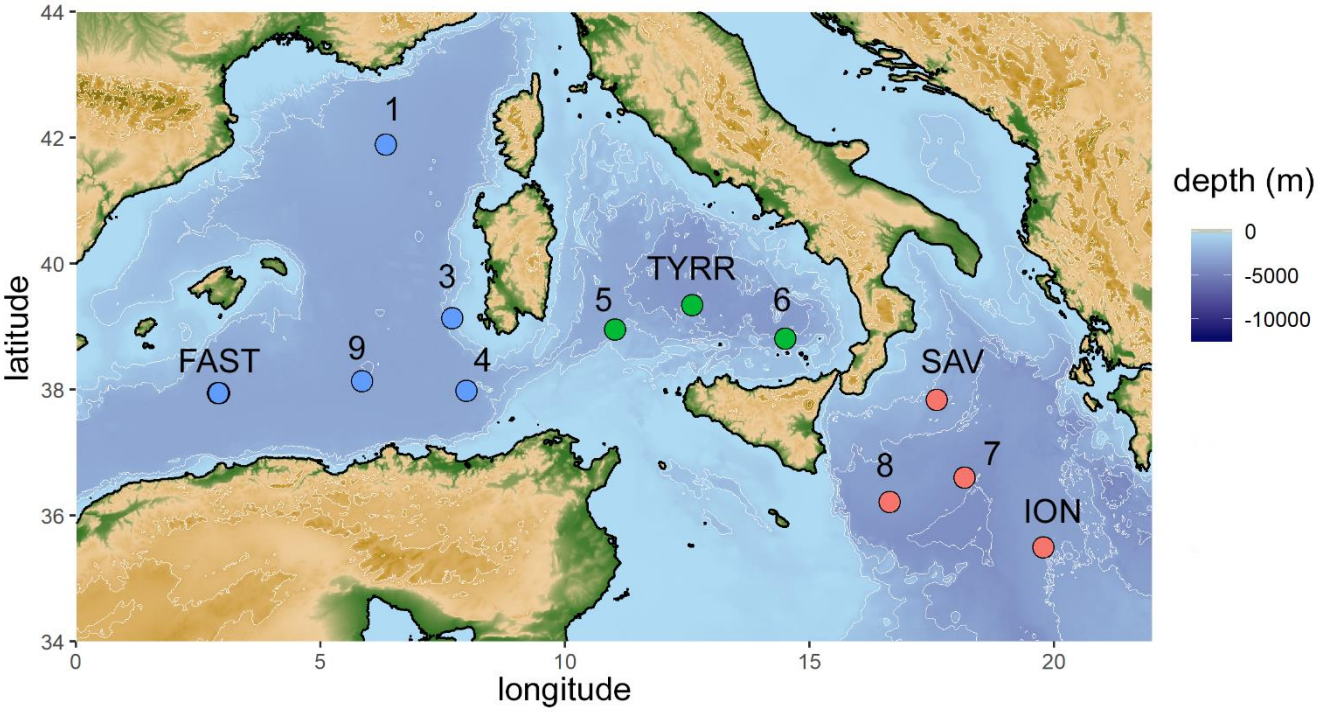

Figure 1: Map of the stations sampled during the PEACETIME cruise in the Mediterranean Sea in May/June 2017. Stations FAST and TYRR were sampled twice. Colours represent sampled basins (blue: western basin, green: Tyrrhenian Sea, red: Ionian Sea).

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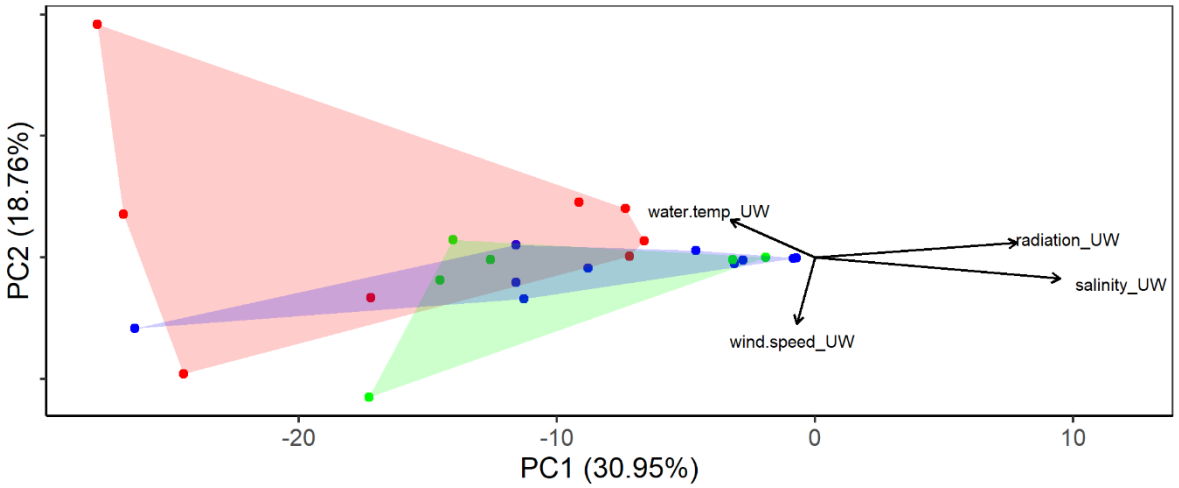
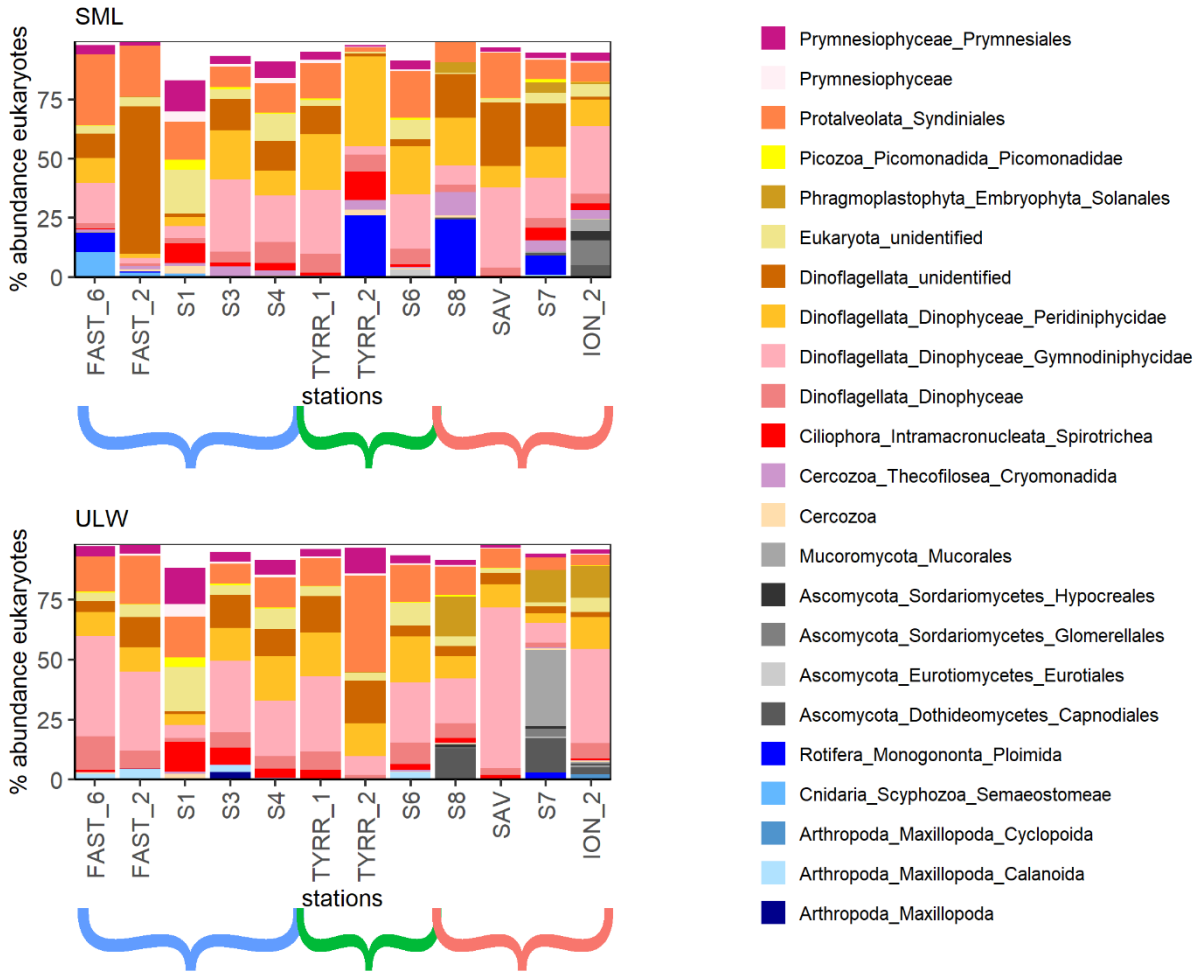
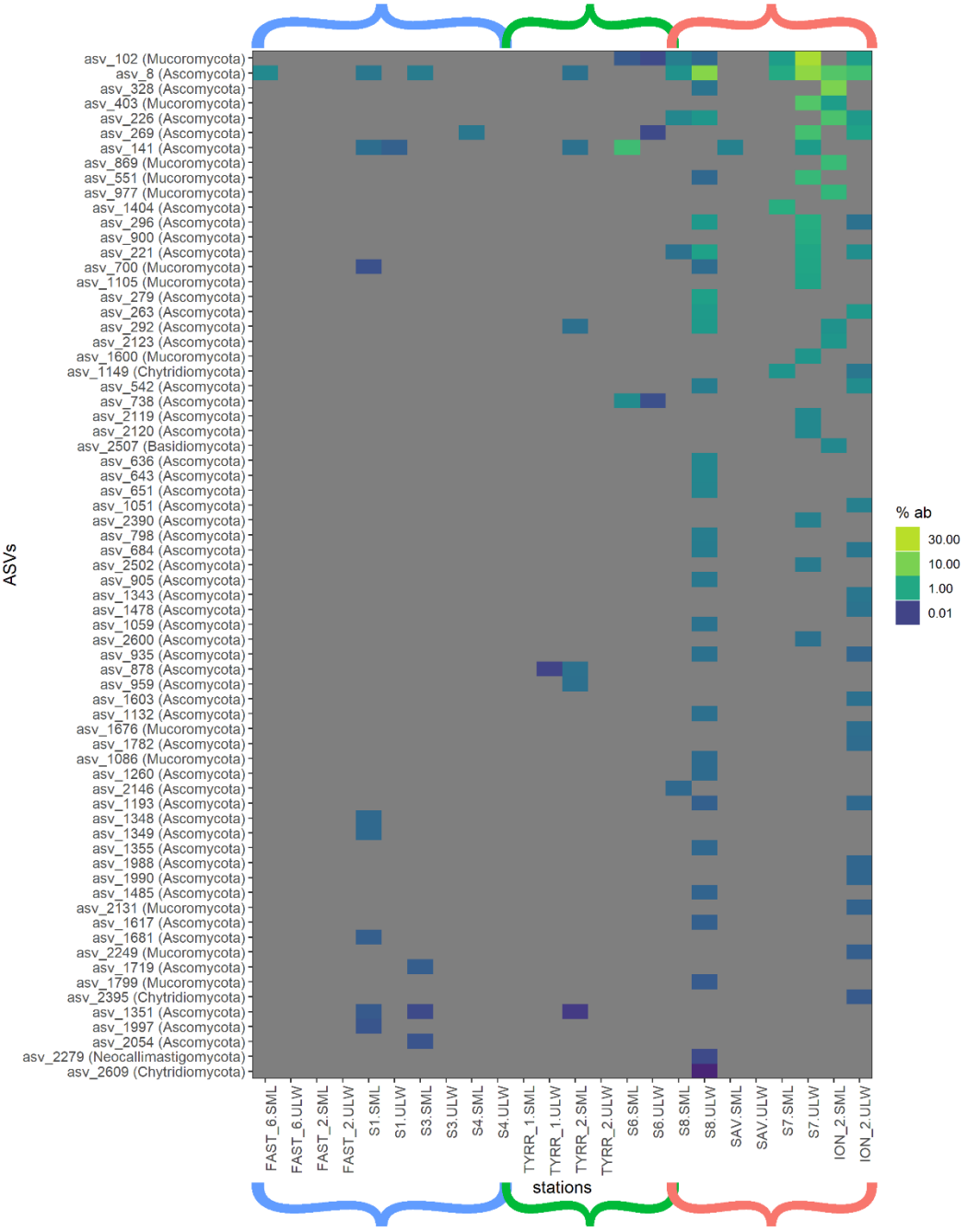


Figure 2: Principal Component Analyses (PCA) using the eukaryotic community composition at the~~in~~ ASV level (see text for a detailed description) with environmental factors plotted. Colours distinguish the three different basins sampled (blue: western basin, green: Tyrrhenian Sea, red: Ionian Sea).



545

Figure 3: Eukaryotic community composition at the~~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 the western Mediterranean (blue)~~Mediterranean Sea basins (blue: western basin, green:~~, the Tyrrhenian Sea (green) and the ~~, red:~~ Ionian Sea (red).



550

Figure 4: Heatmap of fungal relative ASV abundances ~~on ASV level throughout the cruise within~~ all sequences samples. Colour brackets indicate the different basins as in Fig. 3 and Fig. 2 ~~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~~corresponds to the absence of the ASV in the respective sample.

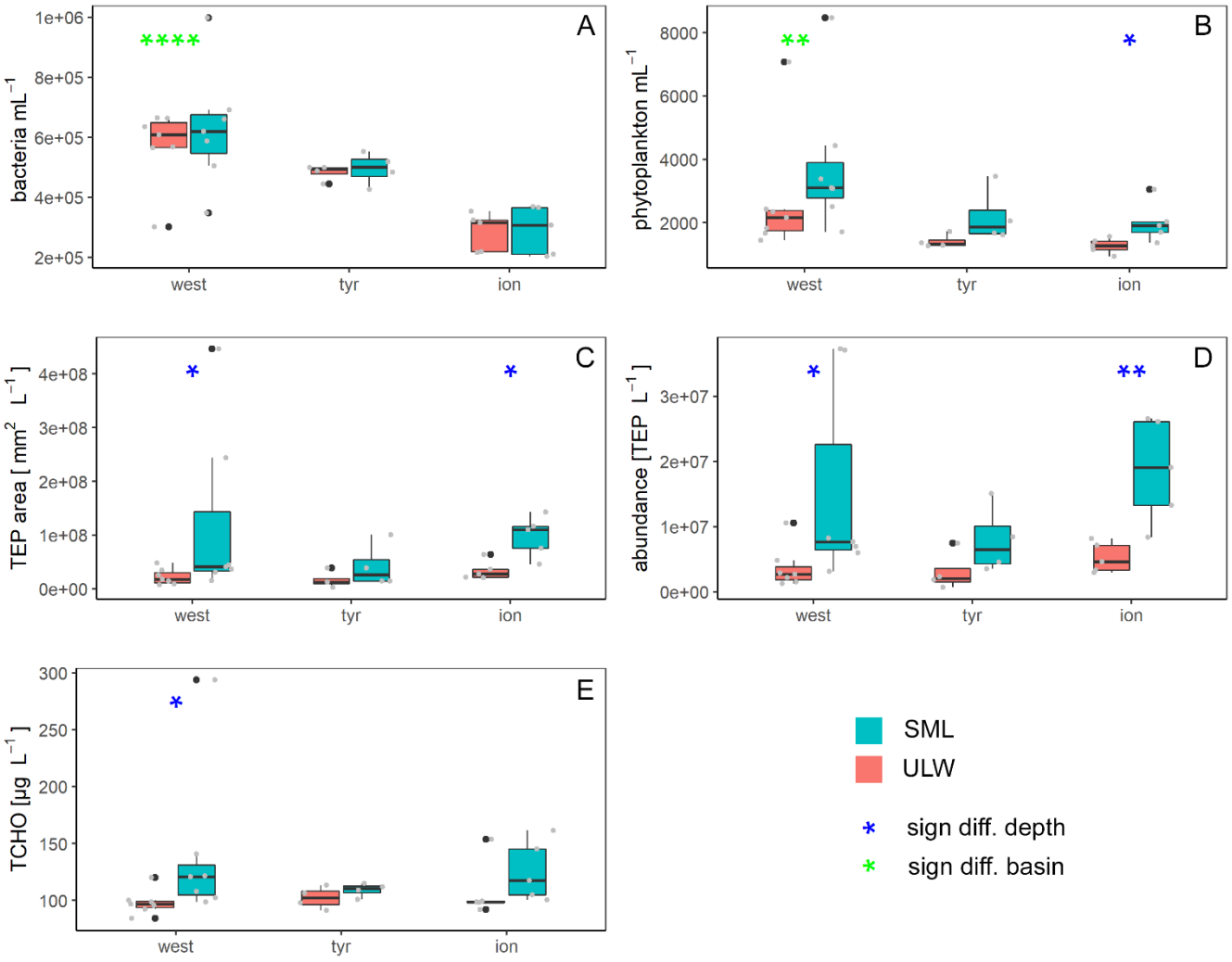


Figure 5: ~~Abundance~~ Boxplots of bacteria (A) and phytoplankton (B) abundance as well as area (C) and concentrations (D) of Transparent Exopolymer Particles (TEP) and total carbohydrates (TCHO) (E) ~~across sampled~~for each basin~~s~~ in the Mediterranean Sea: western basin (west), Tyrrhenian Sea (tyr) and Ionian Sea (ion). Blue stars mark significant SML 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. Black dots ~~mark~~ correspond to outliers ~~of the boxplots~~, grey dots to the~~mark the~~ measured values and concentrations.

Table 1: Name, position and eEnvironmental influences conditions at the stations sampled throughout the cruise. Temperature and salinity were collected at 5 m water depth.

station	Latitudelat	lonLongitude	Local sampling time	wind speed [m s^{-1}]	water temperature [°C] in 5m	salinity [PSU] in 5m	irradiation [W m^{-2}]
S1	41.8918	6.3333	15:45	9.7	16.4	38.2	1297.8
S3	39.1333	7.6835	10:00	2.9	18.7	37.2	2343.2
S4	37.9832	7.9768	10:30	3.5	19.8	37.1	2270.2
TYRR_1	39.34	12.5928	11:00	3.4	20.3	37.8	2253.1
TYRR_2	39.3398	12.5928	12:30	2.5	21.1	37.7	2311.1
S6	38.8077	14.4997	9:00	5.2	20.4	37.4	2215.5
SAV	37.8401	18.1658	12:00	1.5	20.1	38.5	
S7	36.6035	18.1658	7:00	2.5	20.8	38.5	16.8
ION_2	35.4892	19.7765	9:45	6.4	21.1	38.8	1235.3
S8	36.2103	16.631	7:45	1.9	21.2	37.9	2144.0
FAST_2	37.946	2.9102	8:30	3.1	21.7	36.7	627.4
FAST_6	37.0466	2.9168	8:30	5.1	21.9	36.6	1787.1