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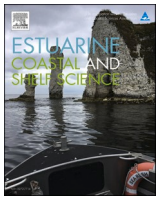

Hypervariable DOM properties in coastal NW Mediterranean sea -evidences of strong human influences and potential consequences for the heterotrophic base of planktonic food webs

Clara Dignan [a,*], Véronique Lenoble [a], Nicole Garcia [b], Benjamin Oursel [b], Ana-Marija Cindrić [c], Benjamin Misson [a]

[a] Université de Toulon, Aix Marseille Univ., CNRS, IRD, MIO, Toulon, France
[b] Aix Marseille Univ, Université de Toulon, CNRS, IRD, MIO, Marseille, France
[c] Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, 10000, Zagreb, Croatia

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ABSTRACT

Marine dissolved organic matter (DOM) plays a key role in the carbon cycle. There is growing interest for its chemical and ecological properties but its variability in the very heterogeneous coastal environments is poorly documented. In this study, we assess the spatial and temporal variations in marine coastal DOM chemical properties and its potential to fuel the growth of the heterotrophs in the planktonic food-web. We sampled two northwestern Mediterranean coastal stations under contrasting terrestrial and human influences. From January to July 2022, dissolved organic carbon (DOC) concentration progressively increased, although highly variable spatially. DOM composition varied temporally rather than spatially. These variations appeared related to a combination of direct freshwater inputs (tracked by salinity variations), direct human contaminations (tracked by concentrations in copper and lead), and primary production (tracked by concentrations in chlorophyll *a*). An incubation experiment was used at each sampling date to evaluate the growth potential of heterotrophic prokaryotes and compare it to variations in DOM properties. Significantly higher growth was observed with DOM from a site under higher terrestrial and human influences. Water temperature exerted a higher control on growth than DOM properties. Correlation analysis with DOM sources suggested the uncoupling of phytoplanktonic production and growth of heterotrophic prokaryotes, which appeared better supported by human contaminations and, to a lesser extent, freshwater inputs. Sediment resuspension in harbors and antifouling paints could represent two important sources of bioavailable resources, favoring fast heterotrophic growth and higher net production, respectively. This work suggests that human activities and constructions in harbors have the potential to strengthen the heterotrophic basis of the planktonic food web.

1. Introduction

Marine dissolved organic matter (DOM) is one of the largest carbon pools on the planet (Hedges et al., 1992; Hansell et al., 2009) and an important and influential component of global carbon cycle models (Farrington, 1992). In the last decades, its spatial and temporal variability across ocean basins and its importance in the biological carbon pump have been described (e.g. Hansell et al., 2009; Santinelli, 2015). Its dynamics at various time scales is far from being fully understood. An ecological perspective linking production and consumption processes is emerging, although the role of key environmental drivers is debated (Shen and Benner, 2020, 2022; Dittmar et al., 2021; Lennartz and Dittmar, 2022). Through to the major contribution of DOM to global ocean primary production (Gattuso et al., 1998), highly-dynamic marine coastal areas area as hot spot of DOM production and processing. Along the coast, DOM originates from different sources. An important part of coastal marine DOM is autochthonous, mainly resulting from phytoplanktonic activity. It varies along the year because of ecological successions (Sommer et al., 2012; Romagnan et al., 2015; Yucel, 2018). Unlike offshore environments, coastal areas also receive significant DOM inputs from allochthonous sources. Terrestrial DOM from biological production and decomposition feed coastal waters through rivers

* Corresponding author. Université de Toulon, Avenue de l'Université, 83130, La Garde, France.
E-mail address: clara.dignan@univ-tln.fr (C. Dignan).

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and water run-off from the watershed (Jonsson et al., 2017; Retelletti Brogi et al., 2021). Due to increasing demographic pressure, terrestrial and marine human activities threaten marine coastal ecosystems (Halpern et al., 2008). This situation is particularly relevant around the Mediterranean Sea, given the ever-increasing human population growth and footprint on its shoreline (UNEP/MAP and Plan Bleu, 2020). So, there is a third source of DOM distinct from natural origins – anthropogenic DOM. Anthropogenic DOM is a complex mixture of organic compouds generated by human activities, is transported to coastal waters via rivers, urban runoff, atmospheric deposition, and direct discharge (Vila-Costa et al., 2020). With this diversity of DOM sources, the high spatial and temporal heterogeneity of coastal environments is now challenging our ability to precisely depict consequences of human influences from natural ones on these ecosystems. Increasing scientific observations focused on coastal DOM appears as a first step in the long way necessary to identify and then manage the most influent human activities.

Once produced or brought to the ocean, DOM fate is under the control of various factors among which heterotrophic prokaryotes (HP) are key players. Being the main DOM consumers, able to transform labile compounds into more recalcitrant ones, HP control the microbial carbon pump (Jiao et al., 2010) and reinforce the basis of planktonic food webs through heterotrophic assimilation. This heterotrophic base sustains higher trophic levels through the microbial loop (Azam et al., 1983). HP contribution to DOM fate is a part of a close relationship with DOM properties and environmental conditions, both influencing HP metabolic activity and growth. Indeed, DOM intrinsic properties influence its processing by HP communities (Amon and Benner, 2001; Shen and Benner, 2020). Abiotic parameters, for example temperature (Pomeroy and Wiebe, 2001) or inorganic nutrients availability (Thingstad et al., 1997), also influence DOM processing by HP. There are three main biotic influences. First, HP development is under strong top-down pressure by grazers and viruses in the marine environment, limiting DOM processing rates (e.g. Liu et al., 2014; Silva et al., 2019). Secondly, competition with phytoplankton for inorganic nutrients can reduce the ability of HP to efficiently use DOM (Thingstad et al., 2005). Last but not least, DOM reworking by HP communities themselves tends to lower DOM lability and furthers contributes to limit DOM processing by HP (Mentges et al., 2019, 2020). Exposed to a diversity of processes, these numerous controls of DOM fate can result in major changes in biogeochemical functioning. In the coastal environment, terrestrial discharge is for example known for its ability to shift the base of planktonic food webs from a mainly autotrophic to a mainly heterotrophic metabolism (Wikner and Andersson, 2012; Jonsson et al., 2017, Barrera-Alba et al., 2019; Navarro et al., 2023). Numerous human activities on the coasts, especially concentrated in large, urbanized bays, could also influence biogeochemical fluxes trough imbalances in DOM properties (Vila-Costa et al., 2020; Martinot et al., 2023). Here again, an important observational effort is now needed understand and model coastal DOM dynamics in this highly variable context for a more sustainable management of human activities.

In this context, this study aims at documenting spatiotemporal variability of DOM properties in a Mediterranean coastal area, to compare contributions of phytoplanktonic, terrestrial and human influences and to further provide pieces of evidence of ecological consequences. With those aims in mind, we combined (i) in situ sampling for the characterization of DOM chemical properties and tracers of autochthonous, terrestrial and anthropogenic sources and (ii) experimental assessment of DOM potential to support HPgrowth under low top-down pressure. We observed a very large variability in DOM chemical properties with unexpected short-term and spatial changes that override seasonal trends. DOM ability to support the heterotrophic base of the food web appears largely controlled by terrestrial and human influences, and strongly modulated by water temperature.

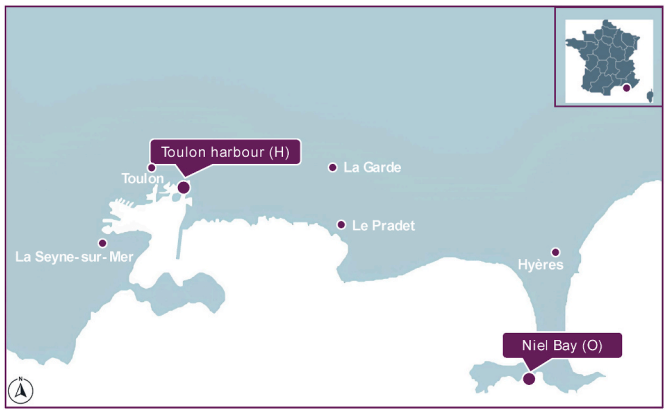

Fig. 1. Map of the study area and sampling stations.

2. Material and methods

2.1. Study area and sample collection

The first coastal sampling station was located in Niel Bay on the Giens peninsula ("O", 43.035325; 6.127527), within Port-Cros National park, located along the French coasts in the northwestern Mediterranean, in an area open to the sea and regularly mixed by the currents. It is exposed to very limited human pressure and its location in a rocky peninsula strongly reduces any terrestrial influence. A second sampling station was chosen in Toulon Bay, a site exposed to high levels of anthropogenic activities (1st French naval base, ferry transport, civil harbours, industry, sewage and aquaculture). Human activities in this area have generated multiple chemical contaminations in the water as well as in the sediment (Tessier et al., 2011; Coclet et al., 2018; Layglon et al., 2020) and influence biological functioning (Coclet et al., 2018, 2019, 2020; Paix et al., 2021). This bay is also subjected to freshwater discharge, mainly through 2 river outlets (Las and Eygoutier river; Durrieu et al., 2023; Nicolau et al., 2012). In this wide bay, the sampling station was located in the civil harbour of Toulon ("H", 43.118460; 5.934092), representative of civil harbours on the French Mediterranean coast (i.e. in the centre of a city, landlocked, with very limited tides, hosting leisure boats as well as artisanal fishing activity). Both sites being located within a restricted geographic area (~18 km), they were exposed to similar climatic conditions (Fig. 1).

These two stations were sampled every two to three weeks, from January 2022 to July 2022. Water temperature (°C), salinity and chlorophyll *a* ($\mu g.L^{-1}$) were measured using a multiparameter probe (Hydrolab® DS5X). For the different chemical analyses, surface seawater (1 m depth) was sampled at each site with previously washed and conditioned material (see Supplementary Material for details). One liter of seawater was sampled using a Van-Dorn bottle and transferred in an acid-cleaned fluorinated ethylene propylene (FEP) bottle for chemical characterization. Similarly, 15L of surface seawater were collected at each site and transferred in a 20L acid-cleaned low density polyethylene (LDPE) bottle for incubation experiments. The samples were brought back in the lab within 2 h and processed immediately to avoid any major changes in the DOM properties and the prokaryotic communities. For dissolved organic carbon (DOC) concentration measurement, aliquots of 24 mL taken from the 1L FEP bottle were filtered through 0.2 µm polyesthersulfone (PES) syringe filters, collected in preconditioned glass tubes, acidified (10% v/v HCl, analytical grade, Fisher Scientific) and stored at 4 °C until analysis. Following the same filtration protocol, 24 mL of filtered water were stored in preconditioned glass tubes at 4 °C for further fluorescent DOM (fDOM) evaluation and 50 mL were stored at −20 °C in centrifuge tubes (Falcon) for nitrate, nitrite, dissolved organic nitrogen (DON), phosphate and dissolved organic phosphorus (DOP) concentration measurements.

Ten mL of filtered water were also collected in Trace Metal Grade centrifugation tubes (Falcon®), acidified (0.2% v/v HNO_3 sp, analytical grade, Fisher Scientific) and stored at 25 °C until analysis for Cu and Pb concentration measurement by HR-ICP-MS.

2.2. Experimental design

To set an experimental approach representative of in situ conditions, we incubated natural microbial communities in natural dissolved substances pools. For that purpose, at each sampling date, heterotrophic microbial communities and dissolved substances from each site were isolated by filtration with previously washed and conditioned material (see Supplementary Material for details). Seven hundred millilitres of seawater were filtered through a pre-conditioned PES filter (Whatman, 0.2 μm, 47 mm) to recover pools of dissolved substances. In parallel, 100 mL of seawater were filtered through a pre-conditioned GF/F (Whatman, 0.7 μm, 47 mm) to isolate the free heteretrophic prokaryotic (HP) community from each sample.

Laboratory experiments consisted in incubating each pool of dissolved substances to the HP community of the same sampling site. In addition, each dissolved pool was also incubated with the HPcommunity of the other site to evaluate the consistency of DOM potential to support HPgrowth. The experimental conditions were named “HinH” for the harbour community in its own dissolved substances, “OinO” for the open area community in its own dissolved substances, “HinO” for the harbour community in open area dissolved susbtances and “OinH” for the open area community in harbour dissolved substances This experimental approach developed within a previous work (Dignan et al., 2023) was hereby adapted to take into account the influence of in situ temperature and potentially delayed microbial growth by winter temperature. Thus, incubations lasted 72 h and were performed in the dark at the *in-situ* temperature recorded during the sampling (or at the average temperature in case of difference between the two sampling stations). Incubations resulted in 4 experimental conditions at each sampling date. For each replicate, 54 mL of sterile seawater (<0.2 μm) containing the dissolved substances was inoculated with 6 mL of seawater containing the HP community (<0.7 μm) in a preconditioned 60 mL FEP bottle.

Subsamples (1 mL) were taken after 24, 48 and 72 h of incubation, fixed with glutaraldehyde (0.25% final concentration) and stored at −80 °C prior to HP enumeration by flow cytometry. Differences in net growth and in the maximum heterotrophic prokaryote abundance (mHPA) reached during incubation were used as proxies to compare DOM potential to support HPgrowth.

2.3. Chemical analyses for field samples

DOC concentrations were determined by high temperature catalytic oxidation using a Shimadzu TOC-VCSH carbon analyzer with an accuracy of 0.1 $mgC.L^{-1}$. DOC analyses were validated by comparison with the environmental matrix reference material (SUPER-05, Canada) (Louis et al., 2009). Nutrients (nitrate NO_3^- and phosphate PO_4^{3-}) were measured by colorimetric methods using an automated Technicon Autoanalyser III (Treguer and Le Corre, 1975) and total matter concentrations (total nitrogen TN and total phosphorus TP) were determined using the wet-oxidation technique (Raimbault et al., 1999). Dissolved organic nitrogen (DON) and phosphate (DOP) concentrations were deduced by subtracting nitrate concentration from total nitrogen concentration and phosphate concentration from total phosphorus concentration, respectively.

DOM quality was estimated using the composition of DOM fluorescent fraction. Three-dimensional excitation-emission matrices (EEM) of each sub-sample were recorded using a Fluoromax + spectrofluorimeter (Horiba) using a 1 × 1 cm quartz cell. EEMs are used to distinguish fluorescence signals due to various groups of chromophores. The excitation wavelength varied between 220 and 450 nm in 5 nm increments, while the emission was measured between 220 and 600 nm. By using the drEEM toolbox (Murphy et al., 2013), the EEMs were corrected from the Milli-Q water EEM measured under the same conditions. EEMs were elaborated to remove and interpolate the Rayleigh and Raman scatter peaks. EEMs were normalized to the Raman signal of water, dividing the fluorescence by the integrated Raman band of Milli-Q water (λex = 350 nm, λem = 371–428 nm), measured on the same day of analysis. Thus, fluorescence intensity is expressed in water-equivalent Raman units (R. U.) (Lawaetz and Stedmon, 2009). Parallel factor analysis (PARAFAC) was performed on all EEMs collected during time tracking in MATLAB R2018a (Mathworks, Natick, MA). PARAFAC analysis was performed using two to seven component models with non-negativity constraints. The final four-component model was chosen based on residual analysis, split-half analysis, and visual inspection (Stedmon and Bro, 2008). The table referencing peak locations for EEM components of marine organic matter (Table S.I 1) and the OpenFluor database, a database of environmental fluorescence spectra, were used to characterize the four components. OpenFluor compares excitation and emission spectra of the validated components with all the components present in the database and allows to compare the spectra by using the Tucker congruence coefficient (TCC; Murphy et al., 2014).

Two trace metals, copper (Cu) and lead (Pb) were considered as proxies of different human influences in the highly contaminated Toulon Bay (Tessier et al., 2011). Cu contamination is linked to the use of antifouling paints (Lagerström et al., 2020). The long residence time of the waters within the bay coupled to the absence of tides of the Mediterranean Sea leads to an important contamination of the waters, fluctuating with the seasons and the hydrodynamics of the area (Mazoyer et al., 2020). Consequently, Cu appears strongly enriched in harbours of the bay (Coclet et al., 2018). Hotspots of sediments Pb contamination were highlighted near Toulon, resulting from Second World War boats scuttling (Dang et al., 2015). Through waves, boat traffic or dredging, sediments are remobilised in the water, leading to some metals desorption, especially Pb (Dang et al., 2020; Layglon et al., 2020). The concentrations of dissolved Cu and Pb were measured by High Resolution Inductively Coupled Plasma Mass Spectrometer (Element 2, HR ICP-MS, Thermo), with a 10-fold dilution for seawater samples to reduce salt-matrix effect. All samples were spiked with an Internal Standard (Indium). A certified reference material (CASS-5, Nearshore seawater reference material for metals, National Research Council Canada) was used as a quality control of HR ICP-MS measurements. Two separate CASS-5 control samples were measured after every 10–15 samples. Matrix matching calibration (in 10-fold diluted CASS-5 sample) was used for concentration quantification. Determined concentrations of metals in CASS-5 sample were within 10% of the certified reference.

2.4. Heterotrophic prokaryotes abundances (HPA) during incubations

After thawing, samples were stained with SYBR Green (1X final concentration) for 15 min in the dark and HP were counted with an Accuri C6 flow cytometer (BD Biosciences). Fifty microliters were run at a flow rate of 35 $\mu L\ min^{-1}$. Non-fluorescent polystyrene microspheres (Flow Cytometry Size Calibration Kit, Thermo Fisher Scientific) were used as a size standard. Particles considered as HP were smaller than 2.0 μm, exhibited low complexity (low SSC), emitted green fluorescence and no red fluorescence (Grégori et al., 2001). Data were acquired using BD Accuri CFlow Plus software and HPA expressed as cells per milliliter ($cell.mL^{-1}$). Maximal HP abundances (mHPA), always recorded after 72h, were used for interpretations. Additionally, net growths at three distinct time intervals were calculated to segment the growth curves and better understand the development dynamics of the microbial communities under the experimental conditions. Rapid growth (EG) corresponds to the net growth between 0 and 24 h. Intermediate growth represents net growth between 24 and 48 h while late growth (LG) represents net growth between 48 and 72 h.

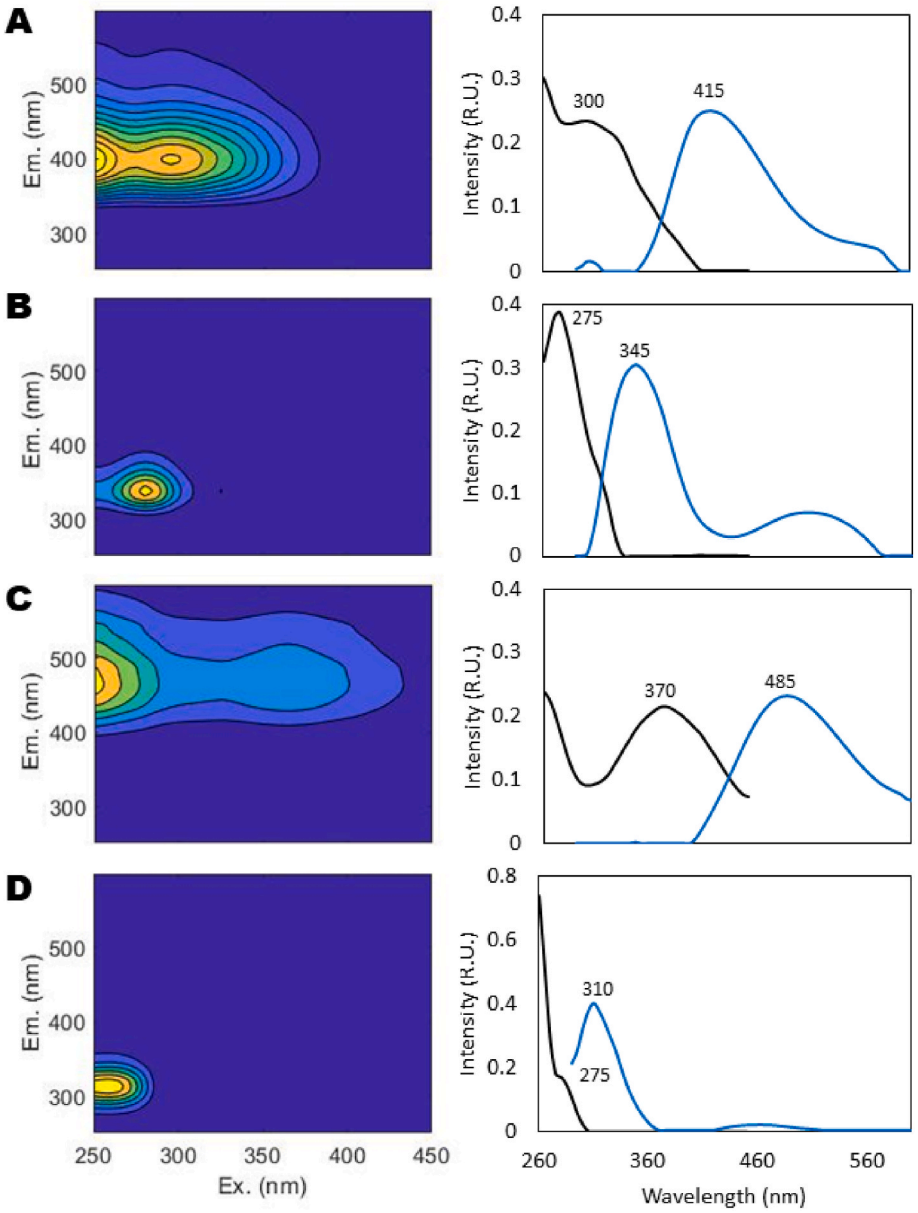

Fig. 2. Fluorescence signatures of the PARAFAC components identified in the dataset. Four components were identified: (**A**) C1 marine humic-like, (**B**) C2 tryptophan-like, (**C**) C3 terrestrial fulvic-like, (**D**) C4 Tyrosine-like. Contour plots of components C1 - C4 are ordered by decreasing percent explained, with emission wavelength on the y-axis, excitation on the x-axis, and shading representing the relative intensity of emission. Corresponding line plots to the right of each contour plot represent the excitation (black curve) and emission (blue curve) spectra of each component. The x-axis in the line plots is excitation or emission wavelength, with relative intensity on the y-axis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.5. Statistical analyses

All statistical analyses were performed with RStudio 2022.7.0.548 software (RStudio Team, 2022) under R 4.2.0 environment. The variability of DOM properties according to sampling stations was represented through PCA with *stats* and *ggplot2* packages. Significant contribution of the represented variables was verified with the *envfit* function of the *vegan* package.

After checking the normal distribution and homoscedasticity of the variances with Shapiro and Levene tests, respectively, analyses of variance (ANOVA) were used to compare the mHPA obtained under each experimental condition at every sampling date using rstatix package. If the ANOVA detected a significant difference ($p < 0,05$), a pairwise T test, with the Bonferroni method for the p-value correction, was then used to distinguish which samples were significantly different from the others ($p < 0,05$). Wilcoxon tests were used to compare the average values of environmental variables between the two sites over the entire monitoring period.

To investigate the relationships between DOM properties and potential sources of variation, Spearman rank pairwise correlation tests were performed using the "vegan" package in R. Corresponding heatmaps were built using ggplot2. For specific correlation analysis between environmental variables and HP growth, we considered growth kinetics in addition to mHPA. For that, daily net growth was calculated by subtracting HPA recorded at the beginning of the 24h time frame to HPA recorded at the end. Spearman rank pairwise correlation tests were then performed and are represented through heatmaps, as described above.

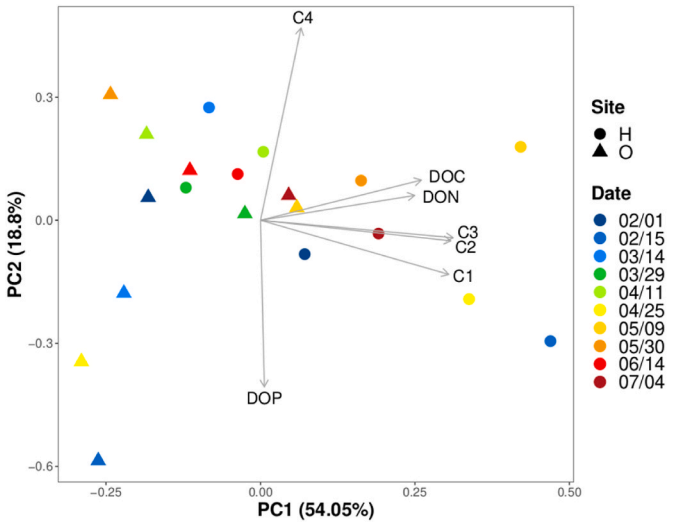

Fig. 3. Principal component analysis of the variation of DOM properties through time and space. H: harbour; O: open area. DOC: dissolved organic carbon, DON: dissolved organic nitrogen, DOP: dissolved organic phosphorus. C1 to C4 are representing the fluorescent components identified with PARAFAC: marine humic-like (C1), tryptophan-like (C2), terrestrial fulvic-like (C3) and tyrosine-like (C4). Indicated sampling dates correspond to year 2022.

3. Results and discussion

3.1. Spatial and temporal variability in DOM chemical properties and potential sources

During the monitoring, DOC showed significant variations at both stations (from ~60 to ~85 μM at station O, and from ~70 to 105 μM at station H; Figure S.I. 1). Such values are in the range of the reported values for this long-time studied area (Dang et al., 2018; Coclet et al., 2019; Layglon et al., 2022; Durrieu et al., 2023). The average DOC concentrations increased throughout the temporal sequence. While average DOC concentration appeared significantly higher at H ($p < 0.05$), temporal variations within a given station were of similar extent to inter-stations variations (~15 μM). DON and DOP concentrations varied between ~4 and 15 μM and ~0.1 and 0.3 μM, respectively. These ranges are in line with previous observations in another northwestern Mediterranean coastal site located close to an urbanized bay (Céa et al., 2015). No clear temporal trend could be observed (Figure S.I. 1), and the temporal punctual variations within one station were more important than inter-stations variations. Differences in average concentrations in DON and DOP between H and O were not statistically significant ($p > 0.05$).

The PARAFAC analysis performed on the EEMs allowed to identify four fluorescent components, meaning that four main groups of fluorophores characterize the fDOM pool (Fig. 2). Component 1 showed spectroscopic characteristics similar to Coble's peak M (Table S.I. 1) described as marine humic-like organic matter ($C1_{MarHum\text{-}like}$) (Coble, 2007). OpenFluor database found 129 matches (TCC >0.95) with its excitation and emission spectrum. Marine humic-like is associated to complex and degraded DOM linked to autochthonous biological activity in the water column (Romera-Castillo et al., 2010). It is now recognized that marine humic-like substances can be directly released by phytoplankton (Bachi et al., 2023). Component 2 showed spectroscopic characteristics similar on par with Coble's peak T (Table S.I. 1) described as tryptophan-like ($C2_{Trp\text{-}like}$). OpenFluor database found 43 matches (TCC >0.95) with its excitation and emission spectrum. Tryptophan-like are protein compounds and are considered a biodegradable fraction of DOM (Bachi et al., 2023). Component 3 showed spectroscopic characteristics similar on par with Coble's peaks C (Table S.I. 1) described as terrestrial fulvic-like ($C3_{TerFul\text{-}like}$). OpenFluor database found 42 matches (TCC >0.95) with its excitation and emission spectrum. Terrestrial humic-like often associated with decomposed plant material and soil of terrigenous origin (McKnight and Aiken, 1998). Component 4 showed spectroscopic characteristics similar on par with Coble's peaks B (Table S.I. 1) described as tyrosine-like ($C4_{Tyr\text{-}like}$) and are like tryptophan-like protein compounds. OpenFluor database found 5 matches (TCC >0.95) with its excitation and emission spectrum (Coble, 1007).

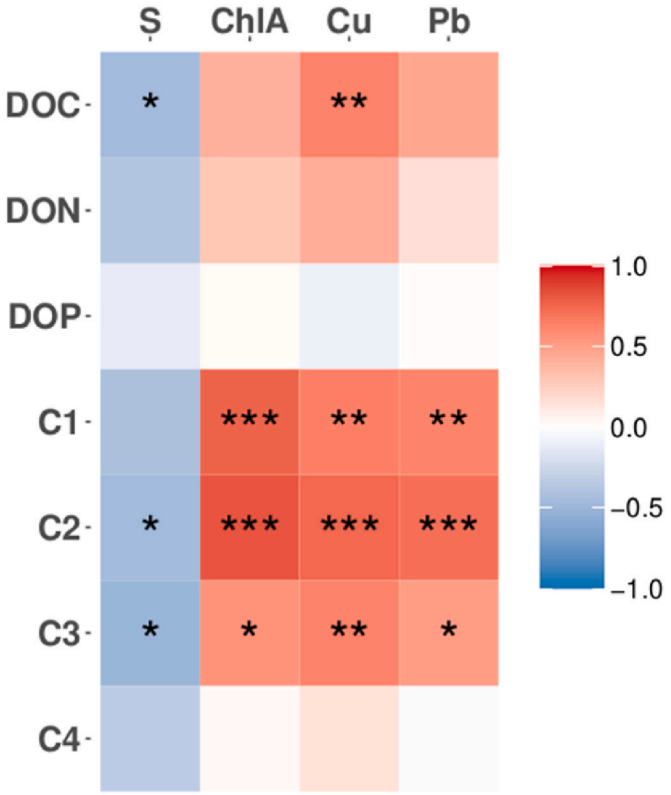

Fig. 4. Correlation analysis between variations in DOM chemical properties and tracers of potential sources. DOC: dissolved organic carbon, DON: dissolved organic nitrogen, DOP: dissolved organic phosphorus. C1 to C4 are representing the fluorescent components identified with PARAFAC: marine humic-like (C1), tryptophan-like (C2), terrestrial fulvic-like (C3) and tyrosine-like (C4). Potential sources were tracked with salinity (S), chlorophyll *a* (Chl a), dissolved copper concentration (Cu), dissolved lead concentration (Pb). The color scale corresponds to Spearman's correlation coefficients. 1, 2 and 3 stars represent a $p < 0.05$, <0.01 and < 0.001, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

At station O (Figure S.I. 1), the fluorescence intensity of the different compounds showed very limited variation. At station H, they were significantly enriched when compared to station O ($p < 0.01$).

Overall variability in DOM properties (DOC, DON, DOP and fluorescent components) at the two studied stations throughout the temporal sequence were visualized by a Principal Component Analysis (PCA) (Fig. 3). The two-dimension representation covered ~70% of the observed variations. This representation highlights significant spatial variability between the two sites along the PC1 axis and considerable heterogeneity at station H along the same axis. This variability seems primarily related to DOC, DON concentrations and $C1_{MarHum\text{-}like}$, $C2_{Trp\text{-}like}$ and $C3_{TerFul\text{-}like}$ fluorescent components, which are generally higher but particularly variable at station H. Furthermore, the PCA also reveals temporal heterogeneity at site O along the PC2 axis, mainly linked to DOP concentration and $C4_{Tyr\text{-}like}$ fluorescent compounds. There is no seasonal trend, whatever the considered station, pointing to occasional

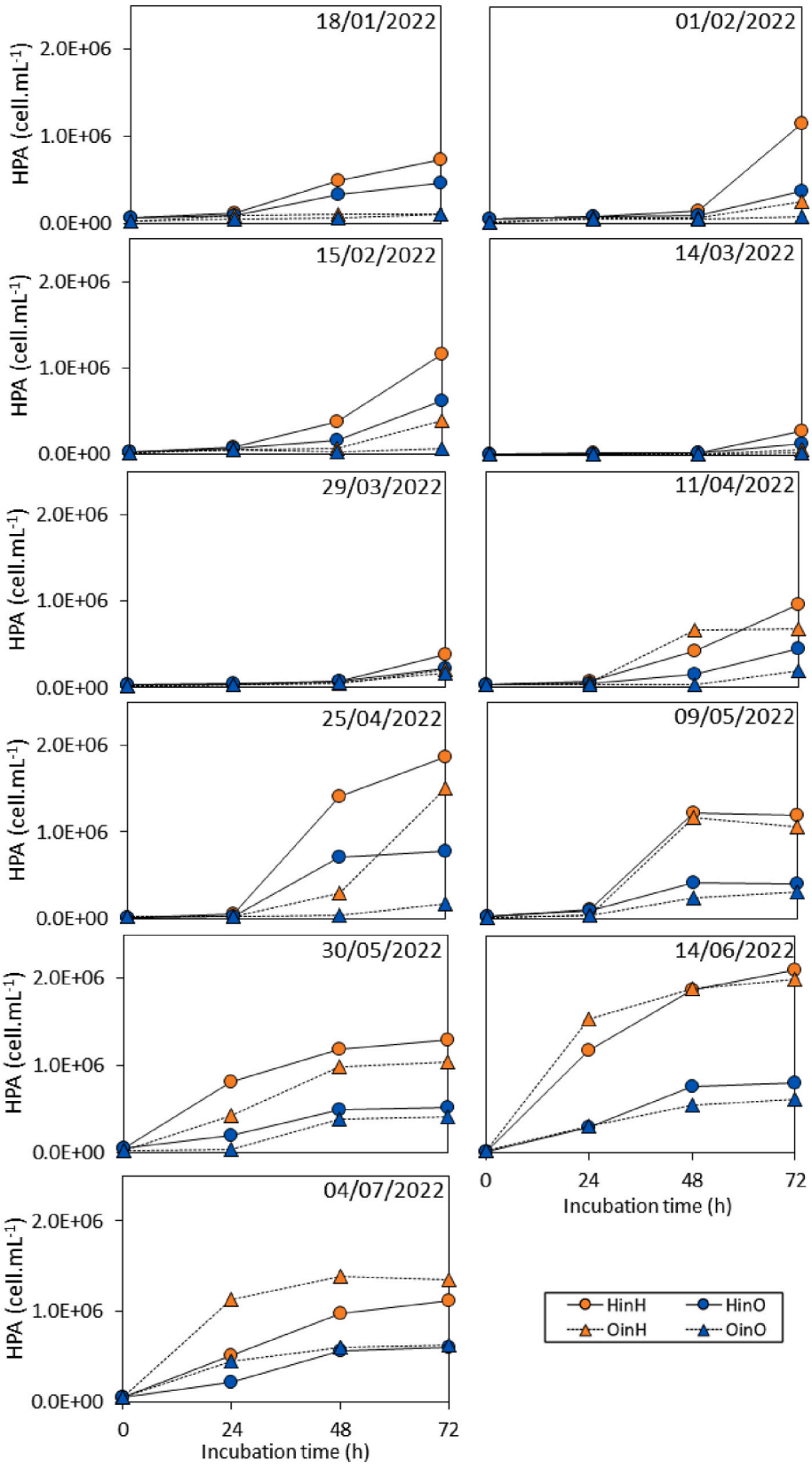

Fig. 5. Heterotrophic prokaryote abundances (HPA) as a function of DOM and community origins for each campaign. HP origin is specified by point shapes: circles for Harbour (H), triangles for Open area (O). DOM origin is specified by point colours: orange for Harbour (inH), blue for Open area (inO). Error bars represent the standard deviation between experimental triplicates. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

variations.

These numerous spatial and temporal variations in DOM chemical properties could reflect different processes and sources. First, significantly higher concentrations in chlorophyll *a* were observed at H ($p < 0.001$), which agrees with previous observation of phytoplanktonic diversity suggesting higher production in the most enclosed parts of Toulon Bay (Coclet et al., 2018; Delpy et al., 2018). Then, other sources could explain these higher concentrations, like the input of freshwaters or the concomitant input of organic matter and contaminants related to human activities. Salinity was significantly lower, and trace metallic concentration was significantly higher at H ($p < 0.05$). Most of the salinity variations can be related to the difference in land influence between the two sampling stations, illustrated by the presence of two rivers (Las and Eygoutier) in Toulon Bay. They are usually characterized by small discharge, except during the typical Mediterranean violent but short storms (Durrieu et al., 2023; Nicolau et al., 2012). Spatial differences in trace metallic contamination are in good agreement with previous observations in the area (Coclet et al., 2018, 2019; Layglon et al., 2020). Concerning temporal variability, salinity, Chl a, and trace metals did not fluctuate much at station O when strong variations were recorded at station H (Figure S.I. 2). Station O is prone to very limited terrestrial and human influences in contrast to station H which is also located in an area showing high variability in hydrodynamics (Dufresne et al., 2014; Mazoyer et al., 2020).

As all these processes and sources could influence DOM chemical properties, a correlation analysis was performed (Fig. 4). Looking at DOM quantitative properties, DON and DOP did not present any significant correlation with any of the studied source tracers. Salinity was negatively correlated with DOC, suggesting DOC-rich freshwater inputs. Terrestrial discharges through freshwater often represent an important source of DOC to the coastal sea, as observed elsewhere in the oligotrophic Mediterranean Sea (e.g. Navarro et al., 2023). The positive relationship between Cu and DOC suggests a common source, probably the antifouling paints (Valkirs et al., 2003; Hobbs et al., 2022). The new generation of antifouling paints are designed to be self-polishing (Watermann and Eklund, 2019), which means that they are progressively degrading through hydrolysis, releasing a whole set of compounds in the water, including some organic compounds from the polymeric matrix (Yebra et al., 2005). Surprisingly, DOC concentration was not correlated to chlorophyll *a* concentration, an observation that sets the difference with other Mediterranean contexts (Avril, 2002; Santinelli, 2015; 2020).

The composition of DOM, expressed through fluorescent components $C1_{MarHum\text{-}like}$ and $C2_{Trp\text{-}like}$, was highly linked with chlorophyll *a* (Fig. 4) suggesting the influence of fresh phytoplanktonic production of DOM and heterotrophic reworking on its chemical quality. Indeed, protein-like fluorescent component (such as $C2_{Trp\text{-}like}$ in this study) is known to be produced and exudated during marine phytoplankton exponential growth (Bachi et al., 2023). Moreover, marine humic-like fluorescence ($C1_{MarHum\text{-}like}$) is often associated with autochthonous fluorescent DOM and its presence has historically been linked to elevated biological activity in the water column (Nieto-Cid et al., 2005; Stedmon and Markager, 2005; Romera-Castillo et al., 2010). Recently, it has also been recognized that marine humic-like substances can be directly released by phytoplankton (Bachi et al., 2023). In addition, negative correlations between fluorescence intensities and salinity suggest an indirect contribution of freshwater inputs that could stimulate phytoplanktonic production. The correlation between salinity and fluorescent component $C3_{TerFul\text{-}like}$ supports the hypothesis of influent terrestrial inputs of DOM. Lastly, DOM composition appeared related to the studied metals. The observed positive correlations indicate a negligible toxic influence and suggest a shared origin. These findings strongly support the hypothesis that antifouling paints and sediment remobilization significantly impact DOM chemical properties. Thus, all of these correlations imply a complex coupling between terrigenous, sedimentary, anthropogenic inputs and microbial responses in the balances of production and consumption of the DOM in the harbours.

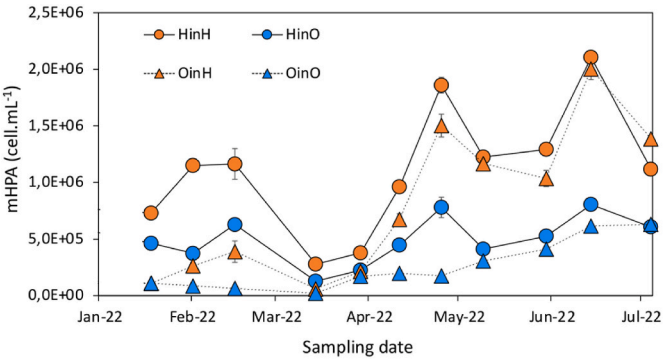

Fig. 6. Temporal variability of maximum heterotrophic prokaryotes abundance (mHPA) as a function of community and DOM origin. Community origin is specified by point shapes: circles for Harbour (H), triangles for Open area (O). DOM origin is specified by point colours orange for Harbour (inH), blue for Open area (inO). Error bars represent the standard deviation between experimental triplicates. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In the studied area, quantity and quality of DOM is presenting a large variability. These spatial and temporal variations are deeply linked with covarying potential terrestrial and anthropogenic sources of direct and indirect influences.

3.2. Stimulation potential of the heterotrophic basis of the planktonic foodweb

During incubation experiments, HPA increased in all conditions. An exponential growth phase was observed before growth slowed down when the community reached the carrying capacity of the system (Fig. 5). However, from January 18th to March 29th no plateau was observed, suggesting that the carrying capacity might not have been reached after three days of incubation, the communities being still in the exponential growth phase. In comparison of the potential dissolved substances to sustain heterotrophic growth, the maximum Heterotrophic Procaryotic Abundance (mHPA) reached in each incubation was used. It was systematically reached after 72h of incubation.

The use of dissolved substances from H resulted in a high temporal variability (HinH: $1.38 \pm 0.88 \times 10^6$ cell.mL^{-1}, OinH: $1.09 \pm 0.90 \times 10^6$ cell.mL^{-1}) and promoted the highest mHPA (Fig. 6). With dissolved substances from O, variations were smaller though still important (HinO: $5.49 \pm 0.25 \times 10^5$ cell.mL^{-1}, OinO: $3.14 \pm 0.22 \times 10^5$ cell.mL^{-1}). No clear temporal trend could be depicted, and several punctual important variations were observed. Average mHPA appeared significantly lower with dissolved substances from O than with those from H ($p < 10^{-4}$) which agrees with previous observations in the same geographic area (Dignan et al., 2023). Differences in growth stimulation between the two pools of dissolved substances were well supported whatever HPorigin from April to July. However, the influence of HP origin was predominant from January to March and our experimental approach did only reveal a minor influence of the origin of the dissolved substances. Indeed, the growth of heterotrophic prokaryotes increased faster with the port community, regardless of the dissolved substances used, suggesting a higher reactivity of HP from H. More work is now needed to explore this trend associated to winter and rather cold waters. Longer incubations experiments to reach the carrying capacities would be helpful to better contextualize and validate this observation.

Utilization of marine DOM by HP for biomass and energy production is known to be determined by DOM quantitative and qualitative properties (Del Giorgio and Cole, 1998; Amon and Benner, 2001; Shen and Benner, 2020), but inorganic nutrients supply can also play a key role when DOM composition does not meet HP stoichiometric requirements

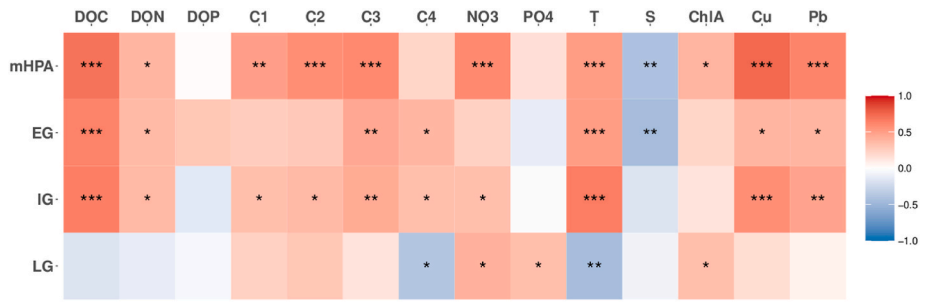

Fig. 7. Correlation analysis between heterotrophic prokaryotes growth and environmental characteristics. Final net growth and growth kinetics were considered for all experimental conditions taken together. mHPA: maximal heterotrophic prokaryotes abundance, EG: early growth (net growth between 0 and 24h), IG: intermediate growth (net growth between 24 and 48h), LG: late growth (net growth between 48 and 72h). DOC: dissolved organic carbon, DON: dissolved organic nitrogen, DOP: dissolved organic phosphorus, NO3: nitrate, PO4: phosphate. C1 to C4 are representing the fluorescent components identified with PARAFAC: marine humic-like (C1), tryptophan-like (C2), terrestrial fulvic-like (C3) and tyrosine-like (C4). T: temperature, S: salinity, ChlA: chlorophyll *a*, Cu: dissolved copper concentration, Pb: dissolved lead concentration. The color scale corresponds to Spearman correlation coefficients. 1, 2 and 3 stars represent a $p < 0.05$, <0.01 and < 0.001, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Thingstad et al., 1997 AME). Moreover, through its influence on microbial metabolism, water temperature is known to regulate HP growth rates (Pomeroy and Wiebe, 2001), especially when nutrient supplies are not limiting (Thingstad and Aksnes, 2019). To better evidence the abiotic variability that could explain the observed variations in HP growth according to time and space, we also considered water temperature, as well as nitrate and phosphate concentrations in addition to environmental variables presented above (Figure S.I.3). Seawater temperature gradually increased over the studied period, rising from ~13 °C in January to ~25 °C in July. It presented similar temporal variations at both stations, H being slightly colder in winter and warmer in summer. Phosphate concentrations measured at H (0.06 ± 0.11 μM) could be compared to previous measurements in another northwestern Mediterranean site (Céa et al., 2015). They were significantly lower at O ($p < 0.01$) and very low compared to previous observations (0.01 ± 0.01 M; μCéa et al., 2015). While it appeared highly stable at O, important punctual increases were observed at H, reaching concentrations up to 20 times higher than at O (Figure S.I.3). During the studied period, nitrate concentrations remained low and stable at O (0.29 ± 0.28 μM), while they were significantly higher ($p < 10^{-4}$), highly variable (6.73 ± 8.10 μM, Figure S.I.3) and elevated at H when compared to another French Mediterranean coastal station under the influence of an urbanized bay (Céa et al., 2015).

Correlation analyses between HP growth metrics and environmental variables in the whole dataset highlighted numerous highly significant correlations (Fig. 7). The correlation with water temperature is in good agreement with its well-known effect on microbial metabolism and growth (Pomeroy and Wiebe, 2001). Considering DOM properties and inorganic N and P supplies, DOC concentration appeared as the most influent factor for growth kinetics and total net growth (Fig. 7), growth being faster and more important when DOC concentration was higher. This observation is supporting the debated role of DOCconcentration on microbial behaviours (e.g. Shen and Benner, 2020, 2022; Lennartz and Dittmar, 2022). Qualitative properties of DOM also appeared influent, especially stimulating mHPA, when $C1_{MarHum\text{-}like}$, $C2_{Trp\text{-}like}$ and $C3_{Ter\text{-}Ful\text{-}like}$ fluorescence and DON concentrations were higher (Fig. 7). The influence of nitrate concentration appeared like these DOM properties. These observations are in line with the influence of DOM qualitative properties on HP growth (Del Giorgio and Cole, 1998; Amon and Benner, 2001; Shen and Benner, 2020).

In our study, the growth of heterotrophic prokaryotes (HP) was not correlated with the fluorescent compound $C4_{tyrosine\text{-}like}$. This lack of correlation may be surprising as these proteic compounds are generally considered relatively labile and biodegradable for microorganisms (Kawasaki and Benner, 2006). However, the lability can vary depending on specific conditions of the studied ecosystem. For instance, the chemical structure of these compounds plays a crucial role in their lability: simple tyrosine derivatives are more easily degraded than complex molecules or polymers containing tyrosine-like structures (Stedmon and Markager, 2005). The availability of specific enzymes necessary for the degradation of these compounds is also critical (Madigan et al., 1997). Furthermore, the presence of competitive microorganisms and interactions with other DOM compounds can influence the biodegradability of tyrosine-like compounds. Phosphorus content of DOM did not present any correlation with HP growth and only a weak correlation between inorganic phosphorus and HP growth was observed (Fig. 7). Despite the well-documented phosphorus limitation on HPgrowth in the Mediterranean Sea (e.g. Céa et al., 2015; van Wambeke et al., 2002), the proximity of both study sites to the shoreline ensured sufficient phosphorus inputs to support observed growth rates. Finally, correlations between HP growth metrics and nitrate, DON and fDOM were weaker and no longer significant when considering a single sampling station (data not shown). Thus, these substances would be more responsible of spatial variations in the potential of dissolved pools to sustain heterotrophic growth than the temporal one. On the other hand, temperature and DOC concentration were significantly correlated at both the stations, suggesting their combined influence on temporal variabilities in HP growth sustainment.

Correlations with tracers of potential sources highlighted interesting patterns. Indeed, chlorophyll a appeared very poorly correlated to HP growth metrics whereas salinity was significantly and negatively correlated to early and total net growth (Fig. 7). This observation suggests that HP growth was more stimulated by freshwater inputs than by variations in phytoplanktonic production, even though salinity variations were small. Similarly, Cu and Pb showed far stronger and more significant correlations with early and total net growth than chlorophyll *a* (Fig. 7). Positive correlations demonstrate the absence of toxicity of these metals in our study and suggest that the heterotrophic base of the planktonic food web in the considered marine area could largely be sustained by human activities (faster growth and higher carrying capacity of the system). While it is generally considered that phytoplanktonic production is the main source of organic substrates for HP growth in oligotrophic marine environments, shifts in the autotrophic/heterotrophic balance have been observed in environments under strong allochthonous inputs such as estuaries (Barrera-Alba et al., 2019; Jonsson et al., 2017; Wikner and Andersson, 2012). Our results strongly support the idea of such shifts in the studied area, even in the absence of major riverine inputs, and point out the strong influence of human activities on biogeochemical functioning at the base of the planktonic foodweb. Such impact could be linked to direct inputs of dissolved substances but also to increased water residence time because of human constructions, and subsequent accumulation favouring eutrophication. Indeed, in the same area, an important increase of water residence time has been demonstrated at rather large scale under the influence of the

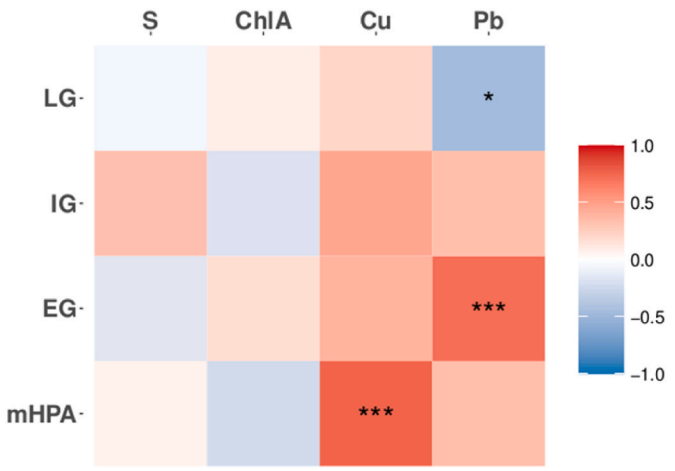

Fig. 8. Correlation analysis between heterotrophic prokaryotes growth in water from the harbour and tracers of potential sources of dissolved resources. Calculations are based on data from HinH and OinH experimental conditions. mHPA: maximal heterotrophic prokaryotes abundance, EG: early growth (net growth between 0 and 24h), IG: intermediate growth (net growth between 24 and 48h), LG: late growth (net growth between 48 and 72h), S: salinity, ChlA: chlorophyll *a*, Cu: dissolved copper concentration, Pb: dissolved lead concentration. The color scale corresponds to Spearman correlation coefficients. 1, 2 and 3 stars represent a $p < 0.05$, <0.01 and < 0.001, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

main seawall of Toulon Bay (Dufresne et al., 2014), an influence that is probably even more important in enclosed docks such as Toulon civil harbour.

To get more insights into human influences, another correlation analysis was performed focusing on the results obtained with water from H (Fig. 8). Correlation analysis revealed no significant relationships between the studied parameters and chlorophyll *a* or salinity, suggesting that the observed correlations primarily reflect spatial differences among sampling stations. Interestingly, Cu and Pb demonstrated different significant and positive correlations with growth metrics, higher concentrations in Cu being associated to higher total net growth and higher Pb concentrations being associated to higher early growth. In Toulon Bay, the highest concentrations in dissolved Cu concentrations were observed in civil harbours (Coclet et al., 2018, 2019), suggesting antifouling paints as a major source for this contamination. Our study thus hypothesizes that the concentration of boats in civil harbours release organic compounds that have positive consequences for biological functioning, such as an increased carrying capacity of the system without increasing growth rates. Concerning Pb, it was demonstrated both in laboratory and fieldwork experiments that sediment resuspension was a major source of dissolved Pb (Dang et al., 2015, 2020; Layglon et al., 2020). Our work suggests that such resuspension influences dissolved nutritive substances in the water column and affect biogeochemical functioning at the base of the planktonic foodweb by favouring fast uptake and assimilation of organic resources without changing the carrying capacity of the system. Such intriguing observations now deserve more in depth analyses of DOM composition to test these hypotheses.

4. Conclusion

This study evidences a strong variability of DOM chemical properties in a coastal NW Mediterranean area. Important temporal changes were observed, with a seasonal pattern for DOC concentration and punctual changes in DOM qualitative properties. Such changes were amplified in a nutrient-rich harbour when compared to an oligotrophic open coastal site. Consequently, DOM ability to support heterotrophic growth was far higher when originating from the harbour, and more linked to quantitative DOM properties than to qualitative ones under our experimental conditions. Terrestrial and anthropogenic sources appeared to be the main support of heterotrophic growth, which was additionally regulated by water temperature.

CRediT authorship contribution statement

Clara Dignan: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Véronique Lenoble:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Nicole Garcia:** Writing – review & editing, Validation, Formal analysis. **Benjamin Oursel:** Writing – review & editing, Validation, Formal analysis. **Ana-Marija Cindrić:** Writing – review & editing, Validation, Formal analysis. **Benjamin Misson:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2024.108925.

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