Spatial-temporal study of cluster 5 picocyanobacteria and exopolymeric microgels in Lake Maggiore

Cristiana Callieri*, Raffaella Sabatino, Andrea Di Cesare, Roberto Bertoni

CNR–IRSA Water Research Institute, Molecular Ecology Group, Verbania, Italy

ABSTRACT

In the oligotrophic Lake Maggiore, the majority of picocyanobacteria are phycoerythrin-bearing *Synechococcus*-type belonging to cluster 5 (Pcy-5). Their distribution was followed in a seven-year study along a depth gradient from the surface down to 350 m in relation to Transparent Exopolymer Particles (TEP), Total Organic Carbon (TOC), Chlorophyll-*a* (Chl-*a*) and water temperature. Pcy-5 abundances exhibit pronounced inter-annual variability, showing years of high numerical abundances as well as years with low numbers. In the upper 20 m, Pcy-5 peaks at around 10-15 m and then progressively decreases. Here, the Pcy-5 presence has been outlined for the first time in the deep layers of a deep lake, thus opening an interesting discussion on these organisms' survival mechanisms in the absence of the light needed to perform photosynthesis. The relation of Pcy-5 with extracellular microgels was significant in autumn, when peaks of both variables were observed. In the other seasons, TEP was correlated with temperature and Chl-*a*, indicating the autochthonous origin of this fraction.

INTRODUCTION

Picocyanobacteria are small prokaryotes (size range 0.2-2 μm) of the order Chroococcales ubiquitous in freshwater and marine ecosystems (Zwirglmaier *et al*., 2008; Callieri, 2008). Picocyanobacteria are mainly constituted by the polyphyletic genus *Synechococcus*, which is one of the most abundant autotrophic prokaryotes living in oceans and lakes (Flombaum *et al*., 2013; Callieri *et al*., 2012). In freshwaters, the genus *Synechococcus* comprises many unicellular strains and isolates that fall into cluster 5 (Castenholz *et al*., 2001; Cabello-Yeves *et al*., 2018). Moreover, their relative abundance on the total autotrophic biomass in freshwater and marine ecosystems increases with a decrease in the trophic status (Bell and Kalff, 2001; Callieri *et al*., 2007), indicating global ecological importance in most aquatic systems. In Lake Maggiore, picocyanobacteria are likewise mainly composed of the genus *Synechococcus* according to morphological classification (Callieri and Pinolini, 1995), and the isolated strains fall in the subalpine cluster I (Callieri *et al*., 2013), now subcluster 5.2 (*Cyanobium*, *sensu* Dorè *et al*., 2020).

These tiny Gram-negative cyanobacteria have a particular cell membrane composition, with the outer peptidoglycan layer considerably thicker than that of most gram-negative bacteria (Hoiczyk and Hansel, 2000). Moreover, *Synechococcus* spp. can form an external S-layer of glycoproteins on the cell surface (Ernst *et al*., 1996), important for cell adhesion and protection (Šmarda *et al*., 2002), that confers to the cell a suitable site for microgel particle formation starting from certain precursors. These sticky microgel particles are predominantly composed of acidic polysaccharides stainable with Alcian blue (Alldredge *et al*., 1993; Passow *et al*., 2001), and are known to be the main constituent of Transparent Exopolymer Particles (TEP). Such particles are present in oceans and inland waters and have an important role in the food webs, increasing the size of single cells by aggregating autotrophic and heterotrophic cells (Burd and Jackson, 2009; Deng *et al*., 2015). Algae of different sizes and groups (pico and nano phytoplankton) can release TEP-precursors, generally in periods of senescence or decline of the population, such as after a bloom (Passow, 2002a; Grossart *et al*., 2006; Berman-Frank *et al*., 2007). Nevertheless, the production of TEP in *Synechococcus* cultures has been recently demonstrated not only under chemical and physical stress conditions but also during cell growth (Callieri *et al*., 2019a). The process of TEP formation involves abiotic processes like coagulation or gelation from polysaccharide fibrils present on the cell surface (Meng *et al*., 2013). TEP contain polysaccharides, proteins, lipids, and amino acids (Passow, 2002b), giving it a composition similar to that of Extracellular Polymeric Substances (EPS). However, unlike EPS, TEP are present in the aquatic environment as discrete particles and not as cell-surface attached or dissolved molecules (Meng *et al*., 2013).

TEP may constitute an important fraction of the Total Organic Carbon (TOC) present in aquatic environments, but its seasonal and spatial dynamics are still poorly understood (Callieri *et al*., 2017). This is the case in Lake Maggiore, where TEP make up to 54% of the TOC (Callieri *et al*., 2021). In a recent study on the Borromeo Basin (Lake Maggiore, Italy), TEP showed a clear vertical heterogeneity from surface to bottom, which is related to the distribution of the autotrophic microorganisms that represent the main source of these particles (Callieri *et al*., 2021). In that annual study, TEP was correlated with diatom chlorophyll in spring, and with the number of picocyanobacteria in autumn.

TOC is an important indicator of the trophic state of aquatic environments because it summarizes in a single variable the result of the activities of microbial communities. The study of TOC, moreover, is important due to the conspicuous role of lakes as an active component in the global carbon cycle (Armstrong, 2010). It follows that the study of TEP distribution, as an important fraction of TOC, can also be central to the study of lake ecosystems.

The study of the multi-year spatial-temporal evolution of TOC and its fractions from the 1980s to the present date documents the evolution of the trophic state of Lake Maggiore, highlighting its progressive, albeit discontinuous, transformation, and its return to its original conditions of oligotrophy (Rogora *et al*., 2021). Although this trend is evident, in recent years the appearance of anomalous blooms of cyanobacteria and green algae has been connected to raises in the concentration of TOC and TEP (Callieri *et al*., 2021).

Here we present the results of a seven-year study of TEP and Pcy-5 dynamics in the subalpine Lake Maggiore, also considering the deep hypolimnetic layer. The main aim of this work was to verify the presence of Pcy-5 also in the deep zone of the lake and to ascertain if Pcy-5 relation to TEP, already found in *Synechococcus* cultures (Callieri *et al*., 2019a), was also evident in the environment. Therefore, we present distribution maps of TEP, TOC, total Chlorophyll-*a* (Chl-*a*), and temperature, as well as an analysis of their correlations with Pcy-5 numbers, along the entire water column from surface to 350 m from 2015 to 2021.

MATERIALS AND METHODS

Study site and sampling

Lake Maggiore is a large, deep, subalpine lake (212 km^2, Z_{max} 372 m) in Northern Italy, recovered from cultural eutrophication and now in oligo-mesotrophic conditions, with annual average total phosphorus, measured on the whole water column, around 12 µg L^{-1} (Rogora *et al*., 2018). Lake Maggiore is one of the most studied lakes in Europe and its long-term chemical, physical and biological data are published in a variety of journals (Bertoni *et al*., 2010; Salmaso and Mosello, 2010; Bertoni *et al*., 2016; Salmaso *et al*., 2020). Sampling was performed in Lake Maggiore at the station of maximum depth (372 m), using a 5 L Van Dorn type bottle, at 10 depths along the water column (0, 5, 10, 15, 20, 50, 100, 200, 300, 350 m).

In the seven years of the study (2015-2021), monthly sampling dates were selected to obtain a detailed picture of the seasonal cycle of TEP, TOC and Chl-*a* concentrations together with Pcy-5 cell number. In parallel, other limnological parameters (for most of the years: water temperature, conductivity, pH, dissolved oxygen) were measured *in situ* with a multiparametric probe (CTD316, Idronaut, Brugherio, Italy) and presented in the report of the International Commission for the Protection of Italian-Swiss Waters (CIPAIS, available at: www. cipais.org).

Pcy-5 cell counting and Chlorophyll-*a* measurements

Samples were immediately fixed with filtered formaldehyde (0.2 µm pore size membrane, 1% final concentration) and stored at 4°C in the dark. A flow cytometer Accuri C6 (Becton Dickinson, Oxford, UK), equipped with a 20 mW 488 nm Solid State Blue Laser and a 14.7 mW 640 nm Diode Red Laser, was used to quantify picocyanobacteria.

Pcy-5 were quantified using light scattering signals (forward and side light scatter named FSC-H and SSC-H, respectively), orange fluorescence (FL2-H channel = 585/40 nm) and red fluorescence (FL3-H channel >670 nm and FL4-H channel 675/25). The density plots of FL2-H vs FL3-H allowed for the optimal gating design and the quantification of the phycoerythrin-rich (PE) and phycocyanin-rich (PC) Pcy-5 cells (Callieri *et al*., 2016).

All data were acquired at a pre-set flow rate of 35 µL min^{-1}, in order to keep the number of total events below 1000 per second. The BD Accuri C6 resident software (v.1.0.264.21) was used for cytogram gating and data processing.

Chl-*a* concentration was measured by Pulse-Amplitude-Modulation Phytoplankton Analyzer (PhytoPAM, Heinz Walz, GmbH, Effeltrich, Germany). PhytoPAM was equipped with the Optical Unit ED 101US/MP, the phyto ML (25 measuring LED in the 4 wavelenghs and 12 actinic LED 655nm), and the phyto AL (37 actinic LED 655nm) (Schreiber *et al*., 1986). The Chl-*a* concentration (µg l^{-1}) was obtained using specific Chl calibration as reference and reference spectra using pure culture (performed by Walz H.).

Total Organic Carbon (TOC) and Transparent Exopolymer Particles (TEP)

TOC concentration was measured using a total organic carbon analyzer (TOC-L High-Sensitivity Model, Shimadzu, Kyoto, Japan) equipped with ASI-L autosampler. The CO$_2$ derived from high-temperature oxidation is measured with a Non-Dispersive Infrared Detector (NDIR). Two replicates for each sample were analyzed. Running in TOC mode, the Shimadzu analyzer removes the inorganic carbon from the sample by acid addition and bubbling. For each analysis 250 µL of the sample are injected in the oxidation furnace and, for each sample, the TOC concentration is computed by averaging five analyses. The concentration of TEP was measured spectrophotometrically (787 nm) following the protocol of Alldredge *et al*., (1993). In short, the samples were filtered onto polycarbonate membranes (0.4 µm pore size) under low vacuum (<10 mbar, 150mm Hg), stained with 500 µL of 0.22 µm pre-filtered 0.02% Alcian Blue (8GX, Fluka; Sigma-Aldrich, St. Louis, USA) aqueous solution, in 0.06% glacial acetic acid (pH 2.5) for 30 second (Alldredge *et al*., 1993). Subsequently, the membranes were left immersed for 2 hours in 6 mL of 80% (v/v) sulphuric acid and removed before reading the resulting liquid fraction at the spectrophotometer.

The concentration of TEP was expressed as Gum Xanthan (GX) equivalent (µg GX eq L^{-1}) following Passow and Alldredge (1995) and converted in µg C L^{-1} using the conversion factor of 0.63 (Engel, 2004; Berman-Frank *et al*., 2016).

Statistical analyses

Statistical analyses were carried out to: 1) assess which biotic (Pcy-5 abundance, Chl-*a* concentration) and abiotic (TOC concentration, depth, temperature, and season) variables can affect TEP concentration during the sampling years (2015-2021); 2) deepen our understanding of the role of Pcy-5 in the production of TEP in a natural environment.

We applied a Linear Mixed Effect Model (LMEM) and extrapolated partial R2 values, which represent the extent of the variance explained by a variable in the model, and p values, which express the significance of the variable. All statistical analyses were conducted in the R environment v3.6.0 (R Core Team, 2019; R Foundation for Statistical Computing, Vienna, Austria), using the package *lme4* v1.1-31 (*lmer*) to perform the LMEM and *car* v3.1-1 (*Anova*) and *partR2* v0.9.1 (*partR2*) to obtain the partial R2 and p values, respectively.

RESULTS

Pcy-5 number, Chlorophyll-*a* concentration and temperature

In Lake Maggiore the study of Pcy-5 distribution began in 2000, but only recently have these microorganisms been counted at different depths of the water column thanks to the acquisition of a flow cytometer, which allows fast and accurate counting. Examples of cytograms are presented in Figure 1, which show the clouds of PE and PC cells in 2016 at different depths, including the 200 and 350 m depths (upper graphs), and the typical presence of eukaryotic cells in May and of microcolonies and filamentous forms in September (lower graphs), as recurrently observed in late summer during all the years of study.

The abundance map for the seven-year period 2015-2021 (Figure 2) showed a decrease in their numbers as the depth increases, but it also confirmed the presence of these cyanobacteria even at high depths. In the layer around 75 m, particularly in 2017 and 2019, there is a clear increase in cell number, also visible, even with lower numbers, around 250 m. The highest numbers of Pcy-5 were measured in August 2019 at 15 m (6.75×10^5 cells mL^{-1}) and in May 2020 at 10 m (5.42×10^5 cells mL^{-1}). If we compare the map of Pcy-5 with that of Chl-*a* in the upper 100 m (Figure 3), we notice that in the 20-40 m water layer they are very scarce and other fractions of phytoplankton contribute more significantly to Chl-*a* concentration: likely the eukaryotic fraction. During the years 2017 and 2021 we observed particularly low numbers of Pcy-5, even though at high depths they were always present. In the 100-350 m layer, generally considered not suitable for the growth of Pcy-5, we calculated an average number of 1333 cells mL^{-1} in the seven years of the study, with a significant trend toward a decrease from 2015 to 2021 (Tau=-0.3539, $p<0.001$) (Figure 1S).

Chl-*a* concentrations peaked in April during 2015 with 12.6 µg L^{-1}, mainly due to diatoms (pers. comm. G. Morabito). The mean value in the 0-20 m zone in the seven years was 2.25 µg L^{-1}, while at 100 m was 0.35 µg

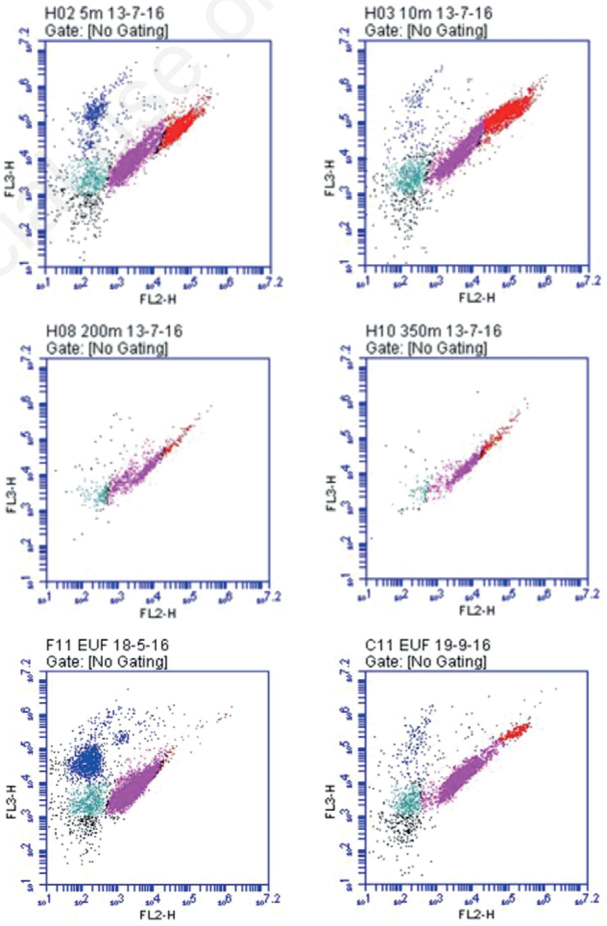

Figure 1. Examples of cytograms (FL2 phycoerythrin vs FL3 chlorophyll) obtained by flow cytometer analysis and showing the fingerprints of autofluorescent picocyanobacteria in Lake Maggiore at 5, 10, 200 and 350 m in July 2016 (upper 4 graphs) and in the euphotic zone in May and September (lower 2 graphs). In violet the more numerous fraction of PE Pcy-5, in green of PC Pcy-5, in red the microcolonies, and in blue the picoeukaryotes.

L^{-1}. It is interesting to note a significant trend toward a decrease in concentrations in the deep layers from 2015 to 2017 followed by an increase in 2018 and a drop to values around 0.40 µg L^{-1} that remained constant till 2021 (Tau=-0.0678, p=0.4280) (Figure 1S).

Temperature showed the usual pattern of a deep subalpine lake with a maximum value of 25.6°C at the surface on July 2019 and a minimum of 6.5°C at 350 m in January 2015 (Figure 4). Complete overturn has not been observed since the seventies due to the increase in winter air temperatures and the decrease in the wind (Dresti, 2022). Over the seven years, a significant trend towards an increase in values was observed in the layer 100-350 m (Tau=0.9260, p<0.001) (Figure 1S).

Total Organic Carbon (TOC) and Transparent Exopolymer Particles (TEP)

The TOC dynamics in Lake Maggiore in the seven-year period studied are well evident in the concentration map (Figure 5). The first observation is that no recurring and repeatable annual trend could be found; instead, we found a clear interannual variability, that was not so marked in previous decades (Bertoni *et al*. 2016). The maximum concentration measured during the period was 1953 µg C L^{-1} and the minimum 600 µg C L^{-1}. The second observation is a pronounced decrease in concentration with depth: the mean value in the 0-20 m layer was 1161 µg C L^{-1}, while in the 100-350 m layer it was of 853 µg C L^{-1}. In 2021 the concentrations were particularly low in the deep layers.

The TEP concentration map shows a more recurrent pattern in contrast with TOC, with two years of very low concentration (2017 and 2021) and the other years (2018-19-20) with the highest concentrations (up to 3000 µg C L^{-1}) reached in September (Figure 6). In particular, in 2019 high concentrations were also measured in the deep layers, where usually TEP concentrations are very low. One characteristic of TEP is that during winter they are very low and only begin to appear from June/July; however, in some years, these particles reach very high concentrations in the first 10/15 m in September, in coincidence with the appearance of picocyanobacteria bloom and foam events (Callieri *et al*., 2022). The scatterplots on a log:log scale between TEP:Chl-*a* and TEP:Pcy-5 showed the relations among these parameters and how the highest values are prevalent in summer and autumn (Figure 7).

Statistical analyses

Before analyzing the data, we performed a correlation test in order to reduce the number of variables to use subsequently, retaining only one variable of each pair in case of correlation coefficients ≥0.65. Temperature and TOC showed a correlation (Figure 2S), therefore we decided to discard the latter from the following analysis. Then, we applied a LMEM to explain TEP variations, using Pcy-5, Chl-*a*, depth, temperature, and season as fixed effect variables, and the year as a random effect variable to minimize annual fluctuations. The results showed that temperature is the variable that explains most of the variance of TEP (p=0.0010). Also Chl-*a* was significantly correlated to TEP (p=0.0209) (Table 1). Moreover, we further investigated the effect of Pcy-5 on TEP variations, introducing in the model, as explanatory factors, the interaction between these microorganisms and each of the other variables (Table 1).

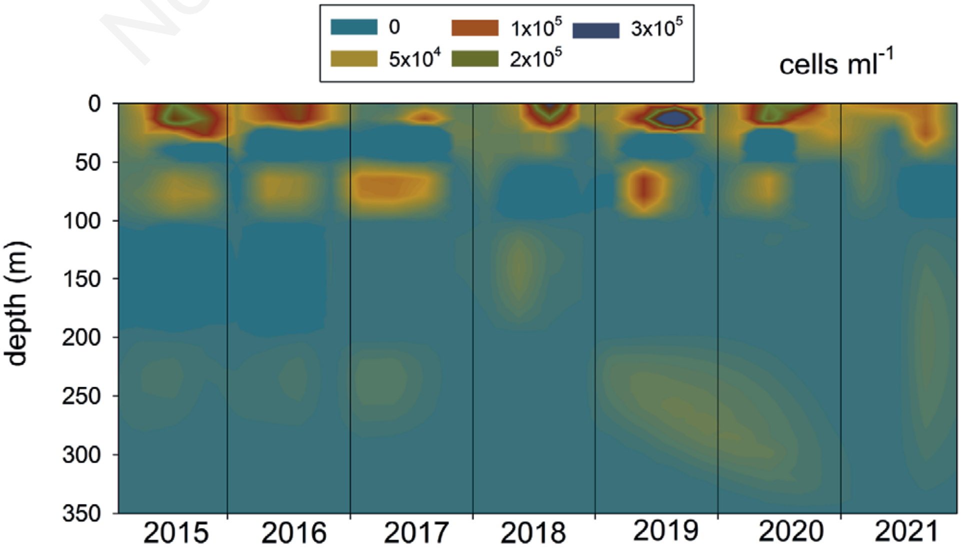

Figure 2. Total Pcy-5 cell number at Ghiffa station in Lake Maggiore.

There was a significant correlation between Pcy-5 and TEP only if season and temperature were introduced as explanatory factors (Table 1). The results showed that TEP and Pcy-5 were correlated on a seasonal basis (p=0.00002): in autumn, when Pcy-5 abundances reached their peak, also high TEP concentrations were found (Figure 7B).

DISCUSSION

This is the first detailed study of Pcy-5 spatial and temporal distribution with maps of their abundance from the surface to the bottom (350 m) over the medium-term (seven years).

Table 1. Statistical results of the Linear Mixed Effect Model (LMEM) assessing the influence of the biotic and abiotic variables on TEP concentration over the years (2015-2021). For Pcy-5 a significant p-value on TEP was obtained by isolating the temperature and the season effect.

	p-value	R^2
MODEL		0.3216
Pcy-5	0.1467	0.0043
Chl-*a*	0.0209*	0.0099
Depth	0.6267	0.0021
Season	0.0753	0.0123
Temperature	0.0010**	0.0255
Pcy-5 : Chl-*a*	0.8619	
Pcy-5 : Depth	0.3412	
Pcy-5 : Season	0.00002***	0.0421
Pcy-5 : Temperature	0.00003***	0.0351

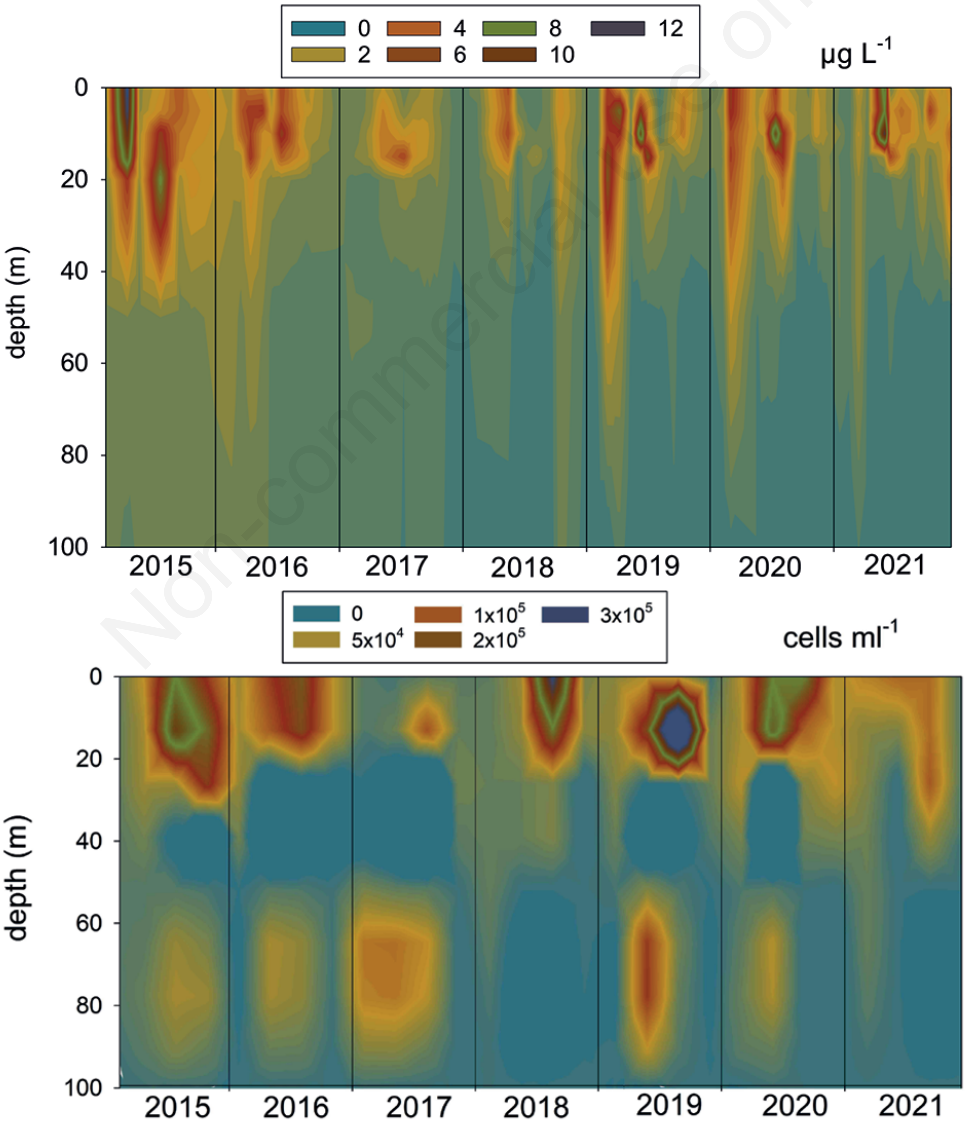

Figure 3. Chlorophyll-*a* concentration map (upper panel) compared with the Pcy-5 number (up to 100 m, lower panel) at Ghiffa station in Lake Maggiore.

The first observation we can make is that Pcy-5 abundances exhibit pronounced inter-annual variability, showing years of high cell numbers (2015, 2019) as well as years of very low numbers (2017, 2021) (Figure 2). In the first 20 m, Pcy-5 peak around 10-15 m and then progressively decrease. However, in the deeper, lightless water mass, below 50 m, we observe the presence of Pcy-5 in well-defined niches between 50 and 100 m and around 250 m. The presence of Pcy-5 in deep waters without the light necessary to perform photosynthesis has already been observed, but mainly in marine systems (Sohorin *et al*., 2011; Miller *et al*., 2017; Callieri *et al*., 2019b). Pcy-5 have been found in the subtropical NW Pacific and in the Gulf of Aqaba in meso- and bathypelagic waters, transported from the epipelagic zone down the water column by convective mixing (Sohorin *et al*., 2011; Miller *et al*., 2017), as well as in the deep anoxic water of the Black Sea (Callieri *et al*., 2019b; Di Cesare *et al*., 2020). However, to our knowledge, the presence of Pcy-5 in deep lakes has not yet been reported. This is most likely due to the fact that the study of autotrophic communities is normally restricted to the euphotic zone, leaving out the deep aphotic zone. The cytograms of samples from different depths (Figure 1) clearly show that the autotrophic communities at 350 m are pre-

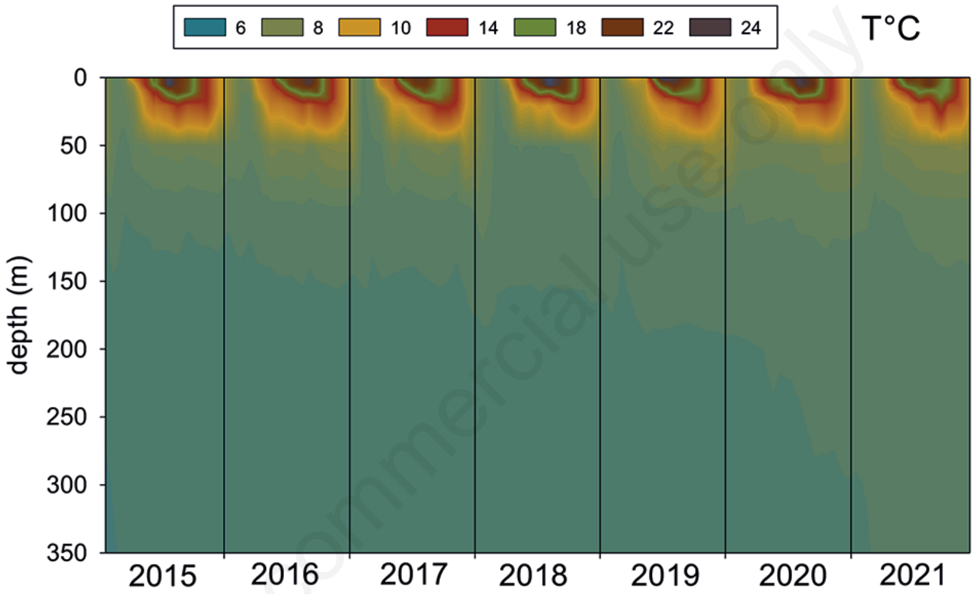

Figure 4. Temperature (°C) map at Ghiffa station in Lake Maggiore.

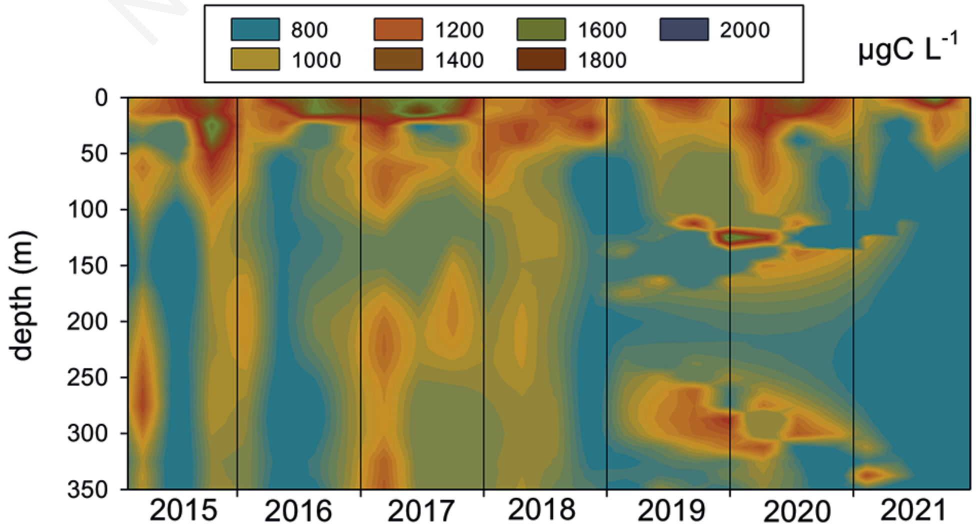

Figure 5. Total Organic Carbon (TOC) map at Ghiffa station in Lake Maggiore.

dominantly composed of picocyanobacteria, while picoeukaryotes or larger cells are generally absent. In marine systems, the presence of *Prochlorococcus* and *Synechococcus* in the deep layer was explained by assuming that they could adapt and tolerate repeated periods of light energy deprivation due to periodic deep mixing that transports cells from the euphotic layer to deeper waters (Coe *et al*., 2021). Small subpopulations of dark-tolerant cells could be transported again to the surface and contribute to the dispersal of new genotypes dark-tolerant (Coe *et al*., 2021). In the case of the meromictic Black Sea the genome of the *Synechococcus* strains (BS55D and BS56D) isolated from the 750 m, contained genes encoding various compounds that enable them to exploit heterotrophic or fermentation pathways (Callieri *et al*., 2019b). In general, we can hypothesize that adaptation mechanisms similar to those found in marine en-

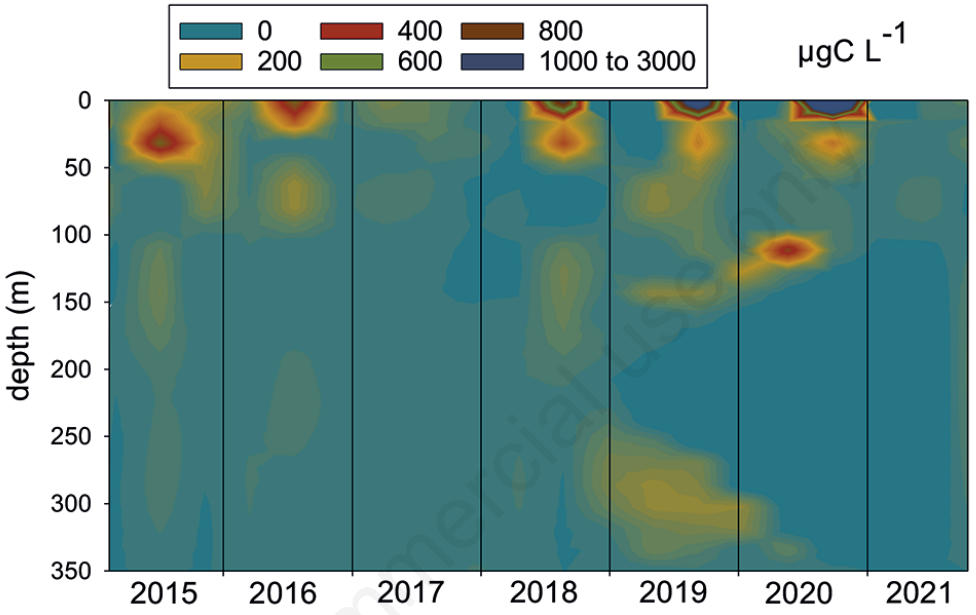

Figure 6. Transparent Exopolymer Particles (TEP) map at Ghiffa station in Lake Maggiore.

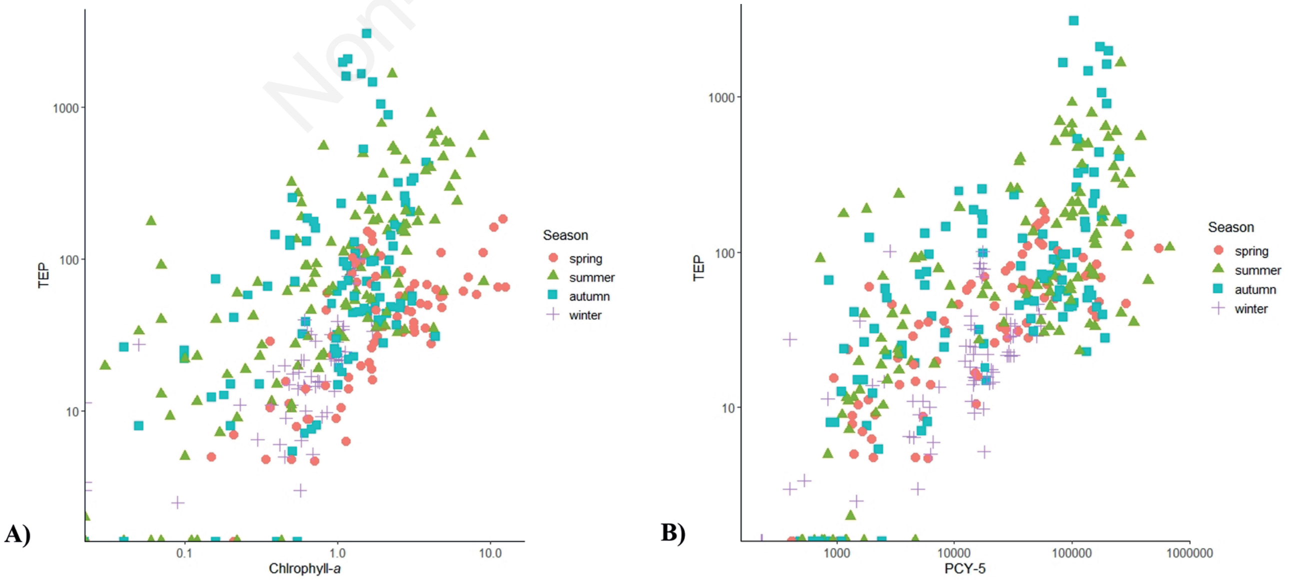

Figure 7. Scatterplots on a log:log scale between A) TEP:Chl-a and B) TEP:Pcy-5 data of the whole water column of Lake Maggiore, during the period 2015-2021. The different symbols refer to the seasons.

vironments can also be conceivable for Pcy-5 in a deep lake.

The comparison of Pcy-5 abundances with TEP concentration maps indicates a substantially similar distribution. During 2017 and 2021, TEP and Pcy-5 were very low, whereas both peaked in 2019 and 2020. This picture is confirmed by the model used in the statistical analyses, from which Pcy-5 resulted to be significantly correlated to TEP if the season effect is removed (Table 1) and it is particularly evident in autumn. This correlation, however, is not present when all seasons are considered. On the other hand, Pcy-5's ability to produce extracellular substances has been demonstrated in laboratory experiments: under both stress and normal growth conditions, marine and freshwater Pcy-5 were able to form TEP and EPS (Thornton and Chen, 2017; Deng *et al*., 2016; Callieri *et al*., 2019a). In Lake Maggiore, on a smaller dataset, TEP variability was explained mainly by Chl-*a*, a proxy for phytoplankton biomass, and to a lesser extent by picocyanobacteria (Callieri *et al*., 2017). With our large seven-year dataset, we showed that in nature a correlation between TEP and Pcy-5 is visible in autumn, when this component of phytoplankton is dominant over other groups. In a littoral to pelagic gradient in Lake Maggiore similar results were obtained (Callieri *et al*., 2021). September was shown to be the month in which numerous colonial forms like *Microcystis aeruginosa*, *Aphanothece* spp. and *Aphanocapsa* spp. and even single cells of *Synechococcus* spp. appear in the lake (Callieri *et al*., 2021). We can therefore assume an essential role in TEP production of the colonial forms of Pcy-5 that are often immersed in mucilaginous organic matrices.

Although TEP is they are a fraction of TOC, the concentration of mucilaginous substances seems to exceed that of the TOC during lake foam production (Callieri *et al.*, 2022), probably because the analytical protocol for measuring TEP implies concentration trough filtration, whilst the total TOC analysis via high-temperature oxidation utilizes microliters amount of sample. This could lead to an over-accumulation of TEP above the filters in one case and a loss of mucilaginous TEP particles in the other.

TOC is the variable that best illustrates, alongside nutrients, the trophic evolution of Lake Maggiore. In fact, the current TOC concentration in the lake is the result of the input of organic matter of autochthonous and allochthonous origin, net of TOC decomposed by bacteria and sequestered at the bottom by sedimentation. It is currently difficult to determine the different weight of meteoclimatic and anthropogenic forces in determining the interannual variability observed, because of the absence of macroscopic pollution phenomena that would suggest a causal relationship with the increase in TOC. However, it is clear that the trophic state of Lake Maggiore is in a precarious balance between oligotrophy and mesotrophy, and therefore the utmost attention must be paid to the management of this ecosystem to avoid a shift towards worse conditions.

CONCLUSIONS

The results of our multi-year study on the spatial and temporal dynamics of Pcy-5 in Lake Maggiore and its relationships with TEP, TOC, Chl-*a*, and temperature suggest the existence of more robust causal relationships between these variables than what can be assumed on the basis of annual observations. This prompts further efforts to move from a speculative to an empirical view of the relationship between TEP and picoplanktonic populations.

Corresponding author: Cristiana Callieri, CNR–IRSA Water Research Institute, Microbial Ecology Group, Verbania, Italy.
E-mail: cristiana.callieri@irsa.cnr.it

Authors' contributions: All the authors made a substantive intellectual contribution, performed part of the experiments. All the authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: CIPAIS - International Commission for the Protection of Italian-Swiss Waters.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Key words: Picocyanobacteria cluster 5, *Synechococcus spp.*, TEP, TOC, Lake Maggiore, deep picocyanobacterial.

Acknowledgements: We thank Filippo Bertoni for assistance with editing the text, and Gabriele Tartari for providing temperature data.

Received: 29 November 2022.
Accepted: 15 December 2022.

Advances in Oceanography and Limnology, 2022; 13:11043
DOI: 10.4081/aiol.2022.11043

REFERENCES

Alldredge AL, Passow U, Logan BE, 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. Deep Sea Res. Part I Oceanogr. Res. Pap. 40:1131–40.

Armstrong A, 2010. Lake carbon. Nature Geosci. 3:151.

Bell T, Kalff L, 2001. The contribution of picophytoplankton in marine and freshwater systems of different trophic status and depth. Limnol. Oceanogr. 46:1243-8.

Berman-Frank I, Rosenberg G, Levitan O, Haramaty L, Mari X,

2007. Coupling between autocatalytic cell death and transparent exopolymeric particle production in the marine cyanobacterium *Trichodesmium*. Environ. Microbiol. 9:1415–22.

Berman-Frank I, Spungin D, Rahav E, Wambeke FV, Turk-Kubo K, Moutin T, 2016. Dynamics of transparent exopolymer particles (TEP) during the VAHINE mesocosm experiment in the New Caledonian lagoon. Biogeosciences. 13:3793-805.

Bertoni R, Bertoni M, Morabito G, Rogora M, Callieri C, 2016. A non-deterministic approach to forecasting the trophic evolution of lakes. J. Limnol. 75:42-252.

Bertoni R, Callieri C, Corno G, Rasconi S, Caravati E, Contesini M, 2010. Long-term trends of epilimnetic and hypolimnetic bacteria and organic carbon in a deep holo-oligomictic lake. Hydrobiologia. 644:279–87.

Burd AB, Jackson GA, 2009. Particle aggregation. Annu. Rev. Mar. Sci. 1:65–90.

Cabello-Yeves PJ, Picazo A, Camacho A, Callieri C, Rosselli R, Roda-Garcia JJ, et al., 2018. Ecological and genomic features of two widespread freshwater picocyanobacteria. Environ. Microbiol. 20:3757–71.

Callieri C, 2008. Picophytoplankton in freshwater ecosystems: the importance of small sized phototrophs. Freshwater Rev. 1:1-28.

Callieri C, Amalfitano S, Corno G, Bertoni R, 2016. Grazing-induced *Synechococcus* microcolony formation: experimental insights from two freshwater phylotypes. FEMS Microbiol. Ecol. 92:fiw154.

Callieri C, Coci M, Corno G, Macek M, Modenutti B, Balseiro E, Bertoni R, 2013. Phylogenetic diversity of nonmarine picocyanobacteria. FEMS Microbiol. Ecol. 85:293-301.

Callieri C, Corno G, Contesini M, Fontaneto D, Bertoni R, 2017. Transparent exopolymer particles (TEP) are driven by chlorophyll *a* and mainly confined to the euphotic zone in a deep subalpine lake. Inland Waters. 7:118-27.

Callieri C, Cronberg G, Stockner J, 2012. Freshwater Picocyanobacteria: Single Cells, Microcolonies and Colonial Forms. In: Whitton B (ed). Ecology of Cyanobacteria II: Their Diversity in Time and Space. Second edition. p. 229-269. Springer Netherlands, Heidelberg, Germany.

Callieri C, Hernandez-Aviles JS, Eckert EM, Rogora M, Tartari G, Sforzi T, et al., 2021. Transparent exopolymeric particles (TEP), phytoplankton and picocyanobacteria along a littoral-to-pelagic depth-gradient in a large subalpine lake. J. Limnol. 80:2021.

Callieri C, Modenutti B, Queimaliños C, Bertoni R, Balseiro E, 2007. Production and biomass of picophytoplankton and larger autotrophs in Andean ultraoligotrophic lakes: differences in light harvesting efficiency in deep layers. Aquat. Ecol. 80:345-62.

Callieri C, Pinolini ML, 1995. Picoplankton in Lake Maggiore, Italy. Int.Rev.Ges. Hydrobiol. 80:491-550.

Callieri C, Sabatino R, Di Cesar A, Bertoni R, Crippa E, Eckert EM, 2022. Carbonio organico totale (TOC), sua componente mucillaginosa (TEP) clorofilla e picocianobatteri. In: CNR IRSA. Sede di Verbania. Ricerche sull'evoluzione del Lago Maggiore. Aspetti limnologici. Programma triennale 2019-2021. Campagna 2021 e Rapporto triennale 2019-2021. Commissione Internazionale per la protezione delle acque italo-svizzere (Ed.):170-180.

Callieri C, Sathicq MB, Cabello-Yeves PJ, Eckert EM, Hernández-Avilés JS, 2019a. TEP production under oxidative stress of the picocyanobacterium *Synechococcus*. J. Limnol. 78:271-83.

Callieri C, Slabakova V, Dzhembekova N, Slabakova N, Peneva E, Cabello-Yeves PJ, et al., 2019b. The mesopelagic anoxic Black Sea as an unexpected habitat for *Synechococcus* challenges our understanding of global "deep red fluorescence". ISME J. 13:1676-87.

Castenholz RW, Wilmotte A, Herdman M, Rippka R, Waterbury JB, Iteman I, Hoffmann L, 2001. Phylum BX. Cyanobacteria. In: Bergey's Manual Syst. Bacteriol. Springer, New York, USA. p. 473–599.

Coe A, Biller SJ, Thomas E, Boulias K, Bliem C, Arellano A, et al., 2021 Coping with darkness: the adaptive response of marine picocyanobacteria to repeated light energy deprivation. Limnol. Oceanogr. 66:3300-12.

Deng W, Cruz BN, Neuer S, 2016. Effects of nutrient limitation on cell growth, TEP production and aggregate formation of marine *Synechococcus*. Aquat. Microb. Ecol. 78:39-49.

Deng W, Monks L, Neuer S, 2015. Effects of clay minerals on the aggregation and subsequent settling of marine *Synechococcus*. Limnol. Oceanogr. 60:805−16.

Di Cesare A, Dzhembekov N, Cabello-Yeves PJ, Eckert EM, Slabakova V, Slabakova N, et al., 2020. Genomic comparison and spatial distribution of different *Synechococcus* phylotypes in the Black Sea. Front. Microbiol. 11:1979.

Doré H, Farrant GK, Guyet U, Haguait J, Humily F, Ratin M, et al., 2020. Evolutionary mechanisms of long-term genome diversification associated with niche partitioning in marine picocyanobacteria. Front. Microbiol. 11:2129.

Dresti C, 2022. Evoluzione del regime di mescolamento e della dinamica dei nutrienti attraverso l'utilizzo di un modello numerico accoppiato ecologico-idrodinamico del lago Maggiore. In: CNR IRSA. Sede di Verbania. Ricerche sull'evoluzione del Lago Maggiore. Aspetti limnologici. Programma triennale 2019-2021. Campagna 2021 e Rapporto triennale 2019-2021. Commissione Internazionale per la protezione delle acque italo-svizzere (Ed.):114-122.

Engel A, 2004. Distribution of transparent exopolymer particles (TEP) in the northeast Atlantic Ocean and their potential significance for aggregation processes. Deep Sea Res. Part I: Oceanogr. Res. Pap. 51:83-92.

Ernst AC. Postius C, Böger P, 1996. Glycosylated surface proteins reflect genetic diversity among *Synechococcus* species of Lake Constance. Arch. Hydrobiol. Spec. Issues Advanc. Limnol. 48:1-6.

Flombaum P, Gallegos J, Gordillo R, Rincón J, Zabala L, Jiao N, et al., 2013. Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. Proc. Natl. Acad. Sci. U.S.A. 110:9824-29.

Grossart HP, Kiørboe T, Tang KW, Allgaier M, Yam EM, Ploug H, 2006. Interactions between marine snow and heterotrophic bacteria: aggregate formation and microbial dynamics. Aquat. Microb. Ecol. 42:19-26.

Hoiczyk E, Hansel A, 2000. Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J. Bacteriol. 182:1191-9.

Meng S, Rzechowicz M, Winters H, Fane AG, Liu Y, 2013.

Transparent exopolymeric particles (TEP) and their potential effect on membrane biofouling Appl. Microbiol. Biotechnol. 97:5705-10.

Miller D, Pfreundt U, Hou S, Lott SC, Hess WR, Berman-Frank I, 2017. Winter mixing impacts gene expression in marine microbial populations in the Gulf of Aqaba. Aquat. Microb. Ecol. 80:223-42.

Passow U, 2002a. Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. Mar. Ecol. Prog. Ser. 236:1-12.

Passow U, 2002b. Transparent exopolymer particles (TEP) in aquatic environments. Prog. Oceanogr. 55:287-333.

Passow U, Alldredge AL, 1995. A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). Limnol. Oceanogr. 40:1326-35.

Passow U, Shipe RF, Murray A, PaK DK, Brzezinski MA, Alldredge AL, 2001. Origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter. Continental Shelf Res. 21:3-346.

R Core Team, 2019. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available at: http://www.R-project.org/.

Rogora M, Austoni M, Caroni R, Giacomotti P, Kamburska L, Marchetto A, et al., 2021. Temporal changes in nutrients in a deep oligomictic lake: the role of external loads versus climate change. J. Limnol. 80:2051.

Rogora M, Buzzi F, Dresti C, Leoni B, Lepori F, Mosello R, et al, 2018. Climatic effects on vertical mixing and deep-water oxygen content in the subalpine lakes in Italy. Hydrobiologia 824:33-50.

Salmaso N, Buzzi F, Capelli C, Cerasino L, Leoni B, Lepori F, Rogora M, 2020. Responses to local and global stressors in the large southern perialpine lakes: Present status and challenges for research and management. J. Great Lakes Res. 46:752-66.

Salmaso N, Mosello R, 2010. Limnological research in the deep southern subalpine lakes: synthesis, directions and perspectives. Adv. Oceanogr. Limnol. 1:29-66.

Schreiber U, Schliwa U, Bilger W, 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. 10:51-62.

Šmarda J, Šmajs D, Komrska J, Krzyzanek V, 2002. S-layers on cell walls of cyanobacteria. Micron 33:257-77.

Sohrin R, Isaji M, Obara Y, Agostini S, Suzuki Y, Hiroe Y, et al., 2011. Distribution of *Synechococcus* in the dark ocean. Aquat. Microb. Ecol. 64:1-14.

Thornton DC, Chen J, 2017. Exopolymer production as a function of cell permeability and death in a diatom (*Thalassiosira weissflogii*) and a cyanobacterium (*Synechococcus elongatus*). J. Phycol. 53:245-60.

Zwirglmaier K, Jardillier L, Ostrowski M, Mazard S, Garczarek L, Vaulot D, et al., 2008. Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environ. Microbiol. 10:147-61.