Picocyanobacterial community structure and space-time dynamics in the subalpine Lake Maggiore (N. Italy)

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ABSTRACT

We investigated the community structure of freshwater picocyanobacteria (Pcy) in the oligotrophic subalpine Lake Maggiore (Northern Italy) using the least conserved sequences of the Internal Transcribed Spacer (ITS-1) fragment. The aim was to characterize the succession of Pcy Operational Taxonomic Units (OTUs) during a seasonal cycle at two environmental niches with distinctive light, temperature and mixing characteristics. ITS fragment length outlined a succession of OTUs from spring to summer, even in the absence of a pronounced spring peak. The vertical partitioning of Pcy OTUs down the water column, more evident in summer, indicates different community composition at the two depths. Redundancy Data Analysis (RDA) was carried out using temperature, pH and depth of mixing layer (Z_m), which passed the Monte Carlo test (p<0.05). A correlation between axis 1 and temperature, pH and Z_m of respectively 0.85, 0.64 and 0.61 was found. Our results indicate that lake thermal structure, defined by temperature and Z_m, has a significant influence on the succession of Pcy OTUs and on their dynamics in colonizing different ecological niches in the vertical gradient.

Key words: picocyanobacterial diversity, oligotrophic lake, Synechococcus*, ARISA, OTU.*

INTRODUCTION

Picocyanobacteria (Pcy, 0.2-2 μm) have been considered key organisms in ecological issues regarding phytoplankton diversity, competition models and niche partitioning (Stomp et al. 2004; Ahlgren, and Rocap 2006). The space-time dynamics of freshwater Pcy along environmental gradients have been extensively studied by means of microscopic and flow cytometric investigations (Callieri, and Piscia 2002; Crosbie et al. 2003a; Sarmento et al. 2008). In temperate lakes, the biomass maxima generally conform to a typical bimodal pattern, with a spring or early summer peak and a second peak in late summer or autumn (Stockner et al. 2000). This is the case in most of the subalpine deep lakes, such as Lake Maggiore (Callieri 2007), Lake Constance (Gaedke, and Weisse 1998), and Lake Stechlin (Padisák et al. 2003). Nevertheless, in Lake Maggiore during the process of re-oligotrophication (from 1992 on) the spring peak has been low or absent (Callieri 2010) as in the case of the ultraoligotrophic Lake Tahoe (Winder 2009).

The sharp fluctuations in Pcy population abundance are difficult to predict, as the pattern is caused by the interaction of various factors. Among the biotic factors, grazing (Callieri et al. 2002) and viral lysis (Personnic et al. 2009) strongly influence seasonality and prokaryote community structure. Abiotic forcing also plays a role in driving Pcy dynamics. Changing physical and chemical variables in temperate lakes seem to support the coexis-

tence of multiple Pcy genotypes (Postius, and Ernst 1999), resulting in a succession of Pcy subpopulations throughout the year. This pattern has been found in Lake Constance and suggests seasonal changes in Pcy community composition (Ernst et al. 2000). More recently, biomolecular tools (*e.g.*, fingerprinting, clone libraries, *Taq* nuclease assays) have permitted detailed tracking of *Synechococcus* in different lakes. In Lake Constance, the dynamics of single *Synechococcus* genotypes were followed over two years in a depth integrated sample: some strains dominated the population, while other isolates were only traced sporadically (Becker et al. 2002; 2007). In Lake Superior, two novel clusters of Pcy have been found that dominate pelagic waters (Ivanikova et al. 2007). Despite a number of studies tried to describe ideal habitat-related patterns of distribution of *Synechococcus* genotypes, many aspects of their possible niche differentiation along the vertical gradient remain unclear. In order to enlighten the potential structure and dynamics of persistent and differentiated subpopulations of Pcy we covered an annual cycle of distribution at two depths, which can be considered potential different niches for Pcy growth. The research took over in the oligotrophic Lake Maggiore (Northern Italy) where Pcy abundance was monitored since 1992. Moreover, the main parameters that are potential drivers for Pcy dynamics were analyzed. Pcy community structure was characterized through finger-printing approach (ARISA: Automated Ribosomal In-

tergenic Spacer Analyse, Fisher, and Triplett 1999) that is still used for non-quantitative analysis of microbial community structure (Ramette 2009) and of their dynamics (Newton et al. 2006).

MATERIALS AND METHODS

Study site, sampling strategy and environmental parameter measurements

The research was carried out in Lake Maggiore, a large deep subalpine lake, which is now in an oligotrophic state (Salmaso et al. 2007; Bertoni et al. 2010). A total of 12 samples were collected fortnightly at the deepest point (Ghiffa: 372 m) from March to September 2006 (for the dates see Tab. 1) at two fixed depths (3 m and 20 m) using Niskin bottles. The samples were kept in the dark and immediately processed (~1 hour) after sampling. The depth of 3 m was selected because Photosynthetically Active Radiation (PAR) is always higher than 10% of surface PAR and also because this is the typical depth of phytoplankton peak abundance in Lake Maggiore (Callieri, and Piscia 2002). The depth of 20 m was chosen because it is always deeper than the depth of mixing (Z_m) and has around 0.1% of surface PAR. Seven main parameters that affect growth and hence are potential drivers for Pcy dynamics were analyzed: temperature, mixing depth, PAR, *in vivo* chlorophyll, pH, Total Phosphorus (TP) and Soluble Reactive Phosphorus (SRP). The first five environmental variables were measured using the IDRONAUT OS316 multiparameter submersible probe.

Soluble Reactive Phosphorus (SRP) was analyzed after gentle filtration through GF/C (Whatman) filters, while Total Phosphorus (TP) was analyzed on unfiltered sample. The samples were digested with potassium persulphate at 125°C at 1.5 atm for 1 hour. Phosphorus concentrations were obtained through the ascorbate-reduced molybdenum method (A.P.H.A. 1992).

Picocyanobacteria enumeration

Samples (50 mL) were immediately fixed using formalin (1% final solution) cacodylate buffered and stored in the dark at 4°C for one day. Pcy were enumerated using an epifluorescence microscope (Zeiss Axioplan equipped with an HBO 100 W lamp, a Neofluar 100× objective, 1.25× additional magnification, and filter sets for blue and green light excitation, Zeiss filter set 09:BP450-490, FT510, LP520, Zeiss filter set 14: LP510-KP560, FT580, LP590) after filtration through 0.2 μm Nuclepore polycarbonate filters. Images for morphometric analysis and cell biovolume calculations were elaborated using an image analysis system (Image-Pro Plus software).

Community composition analysis (DNA extraction, Polymerase Chain Reaction and ARISA)

About 300 mL of lake water from each sample was concentrated on a 0.2 μm Supor® (Gelman Laboratory) and the filters kept at 20°C in 2 mL of lysis buffer (50 mM Tris, 40 mM EDTA, 400 mM NaCl, 0.75 M Sucrose) for no longer than 2 months. Nucleic acid extraction was performed directly from the filters using the UltraClean Bacterial Soil DNA Kit (Mobio Laboratories Inc., California) following the yield maximization protocol suggested by the manufacturer. DNA extractions were checked on 0.8% agarose-TBE (Tris-Borate-EDTA, Promega). Each extract was also quantified using a QuBit Fluorometer (Invitrogen) and stored at 20°C until further analysis.

PCR reactions were performed in a seminested procedure as described in Caravati et al. (2010). Briefly, 2 ng of total extracted DNA was used as a template for the first PCR using 16S-359F (5'-GGGGAATYTTC CG-CAATGGG-3') and 23S-125R (5'-GGGTTBCCC CATTCRG-3'). About 2 μL of each amplified sample were used as a template for the second PCR, performed with 16S-CSIF (5'-GYCACGCCCGAAGTCRTTAC-3', Janse et al. 2003) and 23S-125R (in this case TET labelled

Tab. 1. Environmental parameters measured during the campaign. Zeup: depth of the euphotic layer, Zm/Zeup: ratio of depth of mixing layer to euphotic layer, pH, T: temperature, Chl: *in situ* chlorophyll, TP: total phosphorus, SRP: soluble reactive phosphorus.

Sampling	Z_{eup}(m)	Z_m/Z_{eup}	pH	T (°C)	Chl (μg L⁻¹)	TP (μg L⁻¹)	SRP (μg L⁻¹)
			3 m – 20 m	3 m – 20 m	3 m – 20 m	3 m – 20 m	3 m – 20 m
22 March	26.0	3.85	7.5-7.3	6.70-6.22	1.16-0.47	11.5-10.5	2.2-6.7
06 April	12.5	4.00	7.8-7.6	8.20-7.32	1.12-2.23	7.4-6.3	0.3-02
20 April	12.5	2.80	8.5-7.7	9.80-7.19	1.68-3.33	14.0-9.2	1.3-1.4
10 May	8.50	0.88	8.9-7.6	13.46-8.72	3.77-2.15	13.4-8.6	0.2-0.8
24 May	12.5	0.52	8.4-7.2	16.70-8.49	1.55-0.65	10.8-6.2	1.3-1.3
07 June	12.0	0.71	8.4-7.7	14.96-8.86	0.97-1.14	11.1-7.8	1.3-1.1
21 June	16.0	0.22	-	22.08-9.76	0.79-1.26	-	-
20 July	12.5	1.24	8.9-7.5	25.28-10.87	1.17-1.63	10.9-9.7	1.1-1.2
09 August	12.5	0.92	8.9-7.4	22.87-9.87	0.68-0.55	9.4-6.8	0.7-1.6
23 August	13.0	0.77	8.8-7.4	21.60-10.86	0.55-0.31	7.1-7.9	0.8-1.8
05 September	10.0	1.50	8.9-8.0	21.43-12.24	0.94-0.44	3.0-7.0	-
19 September	16.0	0.78	8.6-7.4	20.19-12.78	0.65-0.34	10.1-8.5	0.9-1.8

in 5'). The PCRs were executed in a volume of 50 μL containing forward and reverse primers (each at 0.3 μM) and 20 μL of PCR Master Mix (Promega). Thirty PCR cycles were used for the consecutive amplifications step, consisting of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, preceded by 3 min of denaturation at 94°C and followed by a final extension of 10 min at 72°C. The final amplifications were purified using a PCR Purification Kit (Qiagen) and quantified using a QuBit Fluorometer (Invitrogen), and stored at 20°C.

Pcy community structure was analyzed using the ARISA fingerprinting technique, which amplifies the Internal Transcribed Spacer (ITS) region in the rRNA operon plus a small part of the 16S rRNA and 23S rRNA genes (Fisher, and Triplett 1999). The purified TET-labelled PCR products were denatured (3 μL of PCR product, 2.5 μL of deionised formamide and 0.5 μL of internal size standard; ROX 2500, Applied Biosystems) at 94°C for 2 min, then chilled on ice and carried for the analysis. Electrophoresis was carried out at 2800 V for 7 h in a denaturing 5% acrylamide gel with an ABI Prism 377 automated sequencer. The ARISA Fragments Length was determined by comparison with the ROX 2500 internal size standard (Applied Biosystems) by using the Local Southern size-calling method in GeneScan 3.7 (Applied Biosystems). Each ARISA peak corresponds to one genetic variant and was used as proxy for a single taxon or OTU. The profiles of different runs were aligned using the procedure described in Caravati et al. (2010). We binned the peaks in different fixed windows depending on fragment length (300/700-3bp, 700/1000-5bp, >1000-10bp). The peak heights of all ARISA profiles collected from the samples were then standardized (Dunbar et al. 2001). Peaks smaller than 350 bp and longer than 1300 bp in size were not considered because the amplified fragments are composed of the ITS region plus 300-350 bp belonging to the adjacent genes. All the "shoulder peaks" were eliminated. The detection threshold (0.24% of total fluorescence) was calculated according to Luna and coworkers (2006).

Calculations, data treatment and statistical analysis

The depth of mixing layer (Z_m) can be defined as the depth at which the temperature gradient is greatest and was calculated by analyzing the vertical thermal profile. Finding two different gradients, we selected the one with the highest gradient (Hocking, and Straškraba 1999). The maximum depth of the light zone is designated as the euphotic depth (Z_{eup}), and is estimated as the depth where light energy is reduced to 1% of the intensity immediately below the surface. The ratio Z_m/Z_{eup} is an indirect estimate of light availability in the mixing layer.

Richness, Shannon-Weaver diversity index and evenness were calculated according to Pielou (Pielou 1984) based on ARISA OTU relative fluorescence. The similarity index (Bray-Curtis) within pairs of samples was also calculated using the PopTools free software and clusters were built using the Unweighted Paired Group Mean Average (UPGMA).

Statistical differences were tested *via* a paired *t*-test and Two Way ANOVA. Normality and homoscedasticity were checked and transformations were applied when necessary to fulfil these conditions. All statistics were performed using SigmaStat 3.1.

The relationships among species (OTUs) and environmental variables were explored *via* Redundancy Data Analysis (RDA, CANOCO 4.5). The environmental variables used in the multivariate data analysis were temperature, mixing depth, PAR, *in vivo* chlorophyll, TP, SRP and pH. All the variables were included in the model, following Manual Forward Selection (ter Braak, and Šmilauer 2002). In this process, each variable is included in the model manually in consecutive steps. Every new variable was tested and permuted (Montecarlo Test, 1000 permutations) and only the environmental variables that had $p<0.05$ were definitely included in the RDA. Each individual OTU was square root transformed to avoid causing imbalances in the analyses due to the most abundant OTUs.

RESULTS

Physico-chemical characteristics

Lake Maggiore is a temperate holo-oligomictic lake. The isopleth map (not shown) shows the distribution of the thermocline from May to early October. Thermal stability of water masses (indicated by Z_m) extends from 3.5 m (June 21) to over 30 m (early spring) (Fig. 1). The depth of the euphotic zone Z_{eup} (1% of surface PAR) reached its
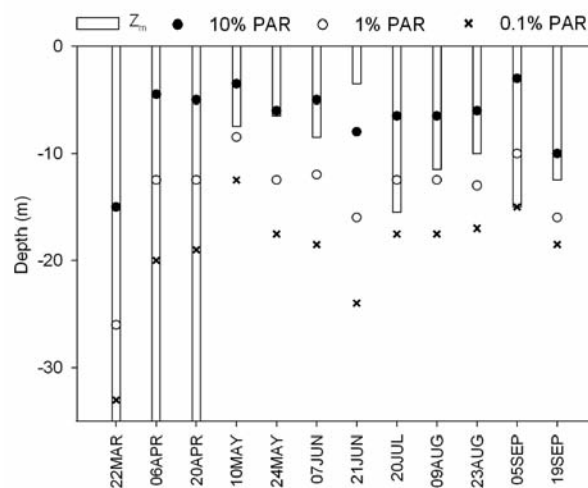

Fig. 1. Dynamics of mixing layer depth (Z_m=hystograms), at 10%, 1% and 0.1% of surface PAR (respectively closed circles, open circles and crosses). The horizontal lines mark sampling depths.

maximum in March (26 m) and its minimum in May (8.5 m) (Tab. 1).

The depth of 10% of PAR was deeper than 3 m and that of 0.1% PAR was often around 20 m (Fig. 1). During stratification, the two selected depths were separated by the thermocline, so that the depth of 3 m was always in the mixing zone and had more than 10% of surface PAR and the depth of 20 m was in the hypolimnion with limited light. The two depths therefore represent different light niches for Pcy.

The ratio Z_m/Z_{eup} progressively decreases from March to June and remains around 1 in summer and autumn (Tab. 1). This ratio is an estimate of light availability in the mixing layer therefore the highest values in March and April indicate a possible light limitation at the beginning of stratification. The lowest value was reached on June 21 with clear water and shallow termocline.

TP reached its peak on April 20 at 3 m (14 µg L^{-1}) and its minimum on September 5 at 3 m (3 µg L^{-1}) (Tab. 1). SRP was <2 µg L^{-1} at both depths with the exception of the 6.7 µg L^{-1} reached at 20 m at the beginning of stratification. There was no significant difference (paired *t*-test) between TP and SRP at 3 m and at 20 m. On the other hand there was a significant inverse correlation between TP and Pcy numbers at 3 m (r Spearman=0.655, p <0.05). *in vivo* chlorophyll a (Tab. 1) showed a spring peak, in April at 20 m and in May at 3 m.

In summer, chlorophyll a was around 1 µg L^{-1} but frequently fell below this value at both depths, indicating the actual oligotrophic state of the lake. The pH showed significant difference between the two depths (Mann Whitney Rank Sum Test, p <0.001), with higher values at 3 m, particularly in summer.

Picocyanobacterial community structure
(dynamics, morphology and diversity)

In September, Pcy reached 350×10^3 cells mL^{-1} at 3 m (Fig. 2). At 20 m, Pcy abundance was never higher than 50×10^3 cells mL^{-1}. On average over the period, Pcy cell biovolume was 0.63 µm^3 cell^{-1} and showed its high-est value in March (1.44 µm^3 cell^{-1}). The lowest biovolumes were recorded in May (0.12 µm^3 cell^{-1}).

At 20 m, diversity (Shannon Index) was higher in summer and lower in early spring. Conversely, the highest richness and diversity at 3 m was found from March to July. There was a significant difference (*t*-test, p=0.04) between the Shannon Index at 3 m and at 20 m, with the highest values found at 20 m. The diversity was only inversely correlated with the number of Pcy at 3 m (*r* Spearman=0.6, p=0.046).

The space-time dynamics (Fig. 3) of the standardized relative fluorescence signal of each individual OTU explored by ITS analysis showed a very complex distribution with a succession of different OTUs. OTU 1139,
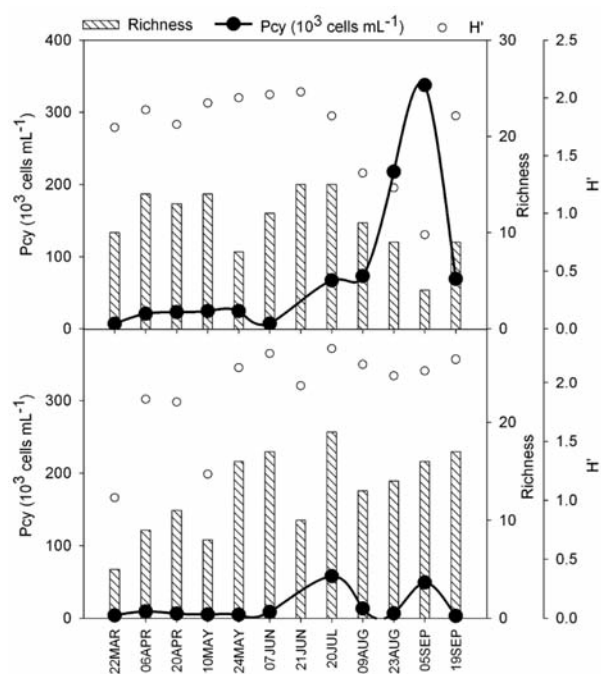

Fig. 2. Dynamics of Pcy abundances (line with closed circles), OTU richness (hystogram) and Shannon diversity index (H'= open circles) at 3 m (upper panel) and 20 m (lower panel), in Lake Maggiore.

typical of spring months at both depths, was replaced by OTU 1073 in summer and contributed up to 70% of the total fluorescence signal at 3 m in September. OTU 588 was characteristic of deeper samples in summer and OTU 526 constituted an important fraction (up to 33%) in summer samples at both depths.

The similarity between pairs of samples (Fig. 4) calculated using the Bray-Curtis index revealed (distance linkage=0.35) two different subclusters which characterize spring and late summer Pcy communities. Moreover, in the summer two distinct clusters of Pcy (0.28 distance) were formed at 3 m and at 20 m. This separation between the two depths was not visible in spring samples.

RDA multivariate analysis (Fig. 5) was performed using only three environmental variables which passed the Monte Carlo test (*p* <0.05): temperature (T, *p* <0.001), pH (pH, *p*=0.01) and mixing depth (Z_m, *p*=0.037). The eigenvalues of the first two ordination axes were respectively 0.653 and 0.214. We found correlations between axis 1 and temperature, pH and Z_m of respectively 0.85, 0.64, 0.61. OTU distributions were controlled by variations in physico-chemical parameters. In particular, the co-occurrence of OTUs 1073, 539 and 987 was positively correlated with temperature, while the occurrence of OTUs 588, 754 and 732 was inversely correlated with temperature. Some OTUs (685, 376, 480 and others) were not correlated with any of the environmental variables. In
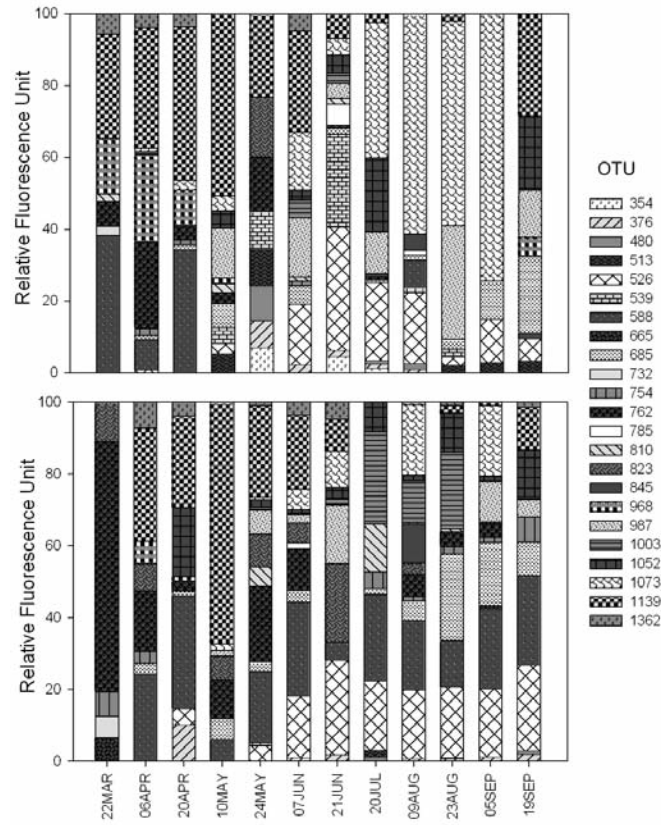
Fig. 3. Space-time dynamics (upper panel: 3 m and lower panel: 20 m) of the percentage of operational taxonomic units (OTU, >5%) obtained using ARISA.
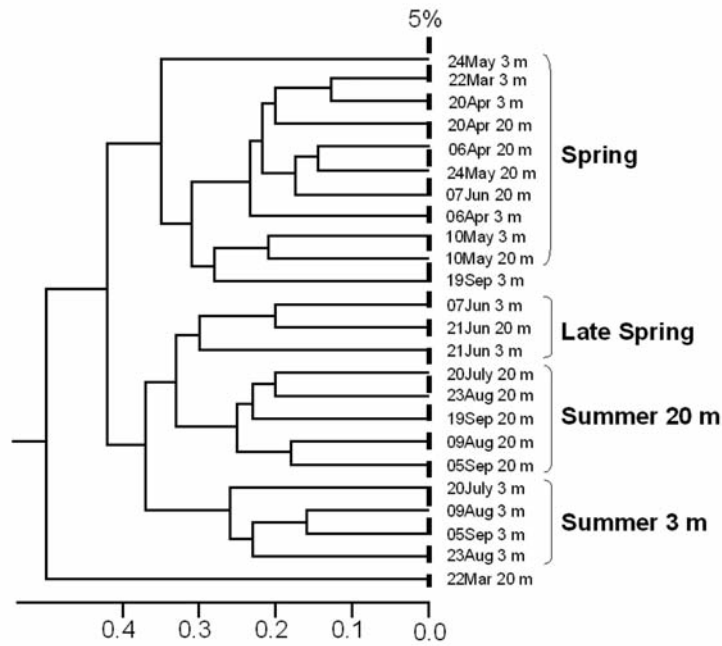

Fig. 4. UPGMA clustering of Bray-Curtis similarity indexes for the picocyanobacterial community of Lake Maggiore, obtained through ARISA.
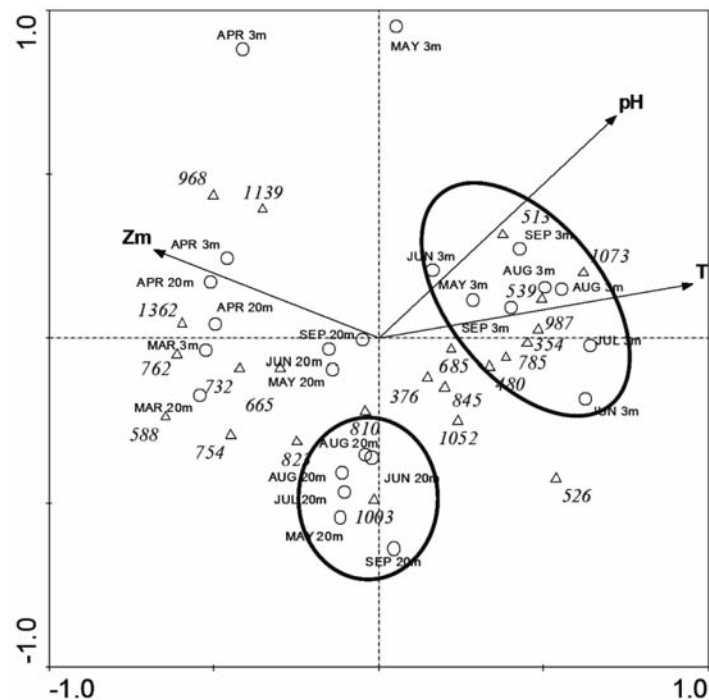
Fig. 5. Triplots (RDA) along the two main axes of variation for the dominant OTU (triangles), significant environmental variables (p <0.05) and sampling dates (circles). The two clusters of 3 m and 20 m are marked with ellipses. T = temperature, Zm = mixing depth.

this ordination plot, the sampling data in summer were distributed differently for the two depths: 3 m data were controlled by the first axis, while 20 m data were controlled by the second axis.

DISCUSSION

Picocyanobacteria seasonal succession

Numerous studies of Pcy dynamics and activity in Lake Maggiore have demonstrated the importance of this autotrophic size-fraction as percent biomass contribution to total autotrophic biomass (Callieri 2007). As for most lakes in temperate regions, maximum Pcy abundance generally conforms to a typical bimodal pattern, with a spring or early summer peak and a second peak in summer or autumn (Stockner et al. 2000).

However, if we look at the long-term series of Pcy abundance, not only in Lake Maggiore (Callieri, and Piscia 2002) but also in Lake Constance (Gaedke, and Weisse 1998) and Lake Stechlin (Padisák et al. 2003), not all years are clearly bimodal. The interannual variability of Pcy dynamics, with the absence of a spring peak, is likely due to weak spring stratification and relatively deep vertical mixing (Weisse 1993). Our study in Lake Maggiore showed the absence of a spring peak in Pcy and of a DCM (Deep Chlorophyll Maxima) at 20 m during 2006. Nevertheless, the ITS-1 fragment length clearly outlined a succession of OTUs from spring to summer at both 3 m and 20 m. This fact demonstrates that, even if there was

no bimodal pattern of cell numbers, bimodal community composition succession was evident. The information given by ITS fragment length varies among taxa so that Pcy microdiversity can be studied with ARISA to identify different ecotypes, even if the data obtained are not quantitative (Brown et al. 2005). A rapid succession of individual Pcy clades, illustrating the patchy structure of the Pcy community, was described over a quite small spatial/temporal scale for lakes in the English Lake District using quantitative PCR (Sánchez-Baracaldo et al. 2008). Although we did not perform quantitative PCR, we still obtained a fairly detailed picture of OTU succession in Lake Maggiore. Similar results have been obtained for other microbial communities (Hewson, and Fuhrman 2004; Danovaro et al. 2006) and, in the case of Pcy, may result in the succession of *Synechococcus* ecotypes already hypothe-sized by Postius, and Ernst (1999). Such a variety of genotypic diversity among Pcy communities also reflects the different morphotype composition observed in spring and summer assemblages, with a dominant presence of single cells in spring and an increase in microcolonies in late summer (Callieri 2010). Previous studies using different fingerprinting techniques on 16S DNA provided evidence of different community composition in spring and late summer in Lake Maggiore (Callieri et al. 2007). Sequencing of the most prominent bands showed one spring OTU affiliated with *Synechococcus* subalpine cluster I, with 100% similarity to LM94, MW76B2 and MW15#2 (*sensu* Crosbie et al. 2003b),

which was not present in late summer. Another OTU, present both in spring and in late summer, showed 100% similarity to MH301, a strain similar to subalpine cluster II (Callieri et al. 2007). In the present study, we used the least-conserved ITS region and extended the measurements to a seasonal scale, thus strengthening previous results. We also observed the constant presence of two OTUs throughout the year. This finding was in accordance with the more accurate quantification of the genotype *Synechococcus* BO 8807 isolated in the pelagic zone of Lake Constance (Becker et al. 2007).

Niche partitioning along the vertical gradient

Light is considered a major factor in niche differentiation in various aquatic ecosystems. While the adaptation of Pcy ecotypes to high and low light has been assessed in the oceans (Johnson et al. 2006; Scanlan et al. 2002), in freshwaters niche adaptation mechanisms of Pcy (mainly *Synechococcus* and *Cyanobium*) with respect to light are not so clear (Kettler et al. 2007). Temperature, as well as light, is another important factor in niche differentiation. In marine habitats, temperatures under the threshold of 14°C have been identified as being directly related to mean annual Pcy abundance (Li 1998). In freshwaters maximal concentrations of Pcy were observed at an optimum temperature of between 18°C and 20°C and at the depth of the thermocline (Callieri, and Piscia 2002). Thermal conditions are important not only for the ambient water temperature per se, but for the maintenance of a density gradient resisting settlement (Callieri 2007).

In this study, we selected two niches: one at a depth of 3 m that receives from 100 to 300 µmol photons m^{-2} s^{-1} and has a mean temperature of 17°C and another at 20 m with 1 to 10 µmol photons m^{-2} s^{-1} and a temperature of 9°C (Caravati 2003). The range of photon flux and the different temperatures can be optimal for distinguishing differently adapted Pcy, particularly in summer when stratification occurs and separates the two environmental niches.

The data showed vertical partitioning of Pcy OTUs down the water column, more evident in summer (Fig. 4), when two Pcy clusters, composed of different OTUs, clearly separate at 3 m and 20 m. This indicates differences in community composition between the two depths. Our results support the coexistence of ecotypes in the vertical gradient, rapidly acclimating and performing differently in the various microhabitats.

In a previous study, we observed that Pcy has different photosynthetic characteristics at different depths in Lake Maggiore, but were unable to show differences in OTU composition in the vertical profile of the water column using DGGE and the 16S DNA gene (Callieri et al. 2007). In the present study, we documented the presence or absence of OTUs at two different depths over an entire sea-

sonal cycle. In this way, we clarified that in absence of any stable stratification, the community composition in terms of OTUs were similar at 3 m and 20 m up to the end of April. The appearance of stratification in May creates different niches colonized by specific OTUs. This is the case for OTU 588, which remains in the 20 m layer but almost disappears in the upper layer. In marine systems, distinct Pcy lineages have also been shown to occur in waters having different environmental characteristics; this feature is evident over large spatial scales (Fuller et al. 2006; Zwirglmaier et al. 2007, 2008). The depthrelated distribution pattern of Pcy OTUs we observed is a first clear indication of niche partitioning in a large deep oligotrophic lake where *Synechococcus* is found at different depths (Callieri et al. 2007).

Effects of environmental variables on Pcy dynamics

Of the 7 parameters examined, only three were found to be significant in RDA analysis: temperature ($p<0.001$), Z_m ($p=0.037$) and pH ($p<0.01$). The analysis was highly significant: 86.7% of the variance was explained by two axes. pH resulted to be significant in explaining OTU dynamics, but the effect of this parameter could be indirect. Temperate lakes typically show a summer increase in pH parallel to the increase in phytoplanktonic production, which decreases free CO_2. This fact could also induce changes in the Pcy community structure. Temperature is highly positively correlated with axis 1 (0.85). Lake thermal structure, defined by temperature (T) and depth of mixing layer (Z_m), influences Pcy abundance and dynamics through the effect of temperature per se and mass water movements in response to density gradients. Higher temperatures generally enhance the growth rate of phytoplankton and increase the reaction rate of RuBisCo (Beardall, and Raven 2004). However, it is difficult to separate the influence of temperature on the abundance of Pcy in lakes from the influence of other seasonal dependent variables. Weisse (1993) suggested that temperature is an important factor in triggering the onset of Pcy growth in marine and freshwater environments, but not in regulating their population dynamics. No data exist on the effects of temperature on Pcy diversity in natural systems. Our results indicate that some OTUs are typical of periods with higher temperature (summer) and others are observed when lake water is colder (Fig. 5). In Lake Maggiore, the maximum Pcy concentration occurs near the thermocline and at temperatures between 18°C and 20°C (Callieri, and Piscia 2002). However, the significant relation we found between temperatures and Pcy OTUs should be interpreted more as side-effect of seasonal changes.

In other freshwater and marine systems, the temperature gradient and stability of the water column appears to affect Pcy abundance and dynamics (Camacho et al. 2003;

Fuller et al. 2005). Here we would like to add a piece of information we found concerning the relationship between Z_m and Pcy diversity. In May and June, we observed the lowest values of Z_m in conjunction with the highest OTU diversity at 3 m. In July, the increase in Z_m corresponded to high richness at both depths. The ratio Z_m/Z_{eup} (significantly correlated to Z_m: r=0.89, p=0.0001) indicates the proportion between the light and dark zones in the mixing layer, related to algal light-adaptive response (Reynolds 1984). A low value of this ratio indicates that the mixing zone is well illuminated, while a high value shows that light is limited in the mixing zone. It is known that Pcy outcompete other autotrophs in low light conditions (Callieri 2007) and this could be the reason for the increase in diversity at 20 m. In other words, the high Z_m/Z_{eup} ratio and the higher diversity observed in July suggest that Pcy communities can stimulate their adaptive traits in conditions of light limitation and consequently increase their diversity. Although light was not a significant factor in the RDA analysis, we think its importance is expressed through the Z_m/Z_{eup} ratio. In this sense, light is an important factor but only if considered as an intrinsic property of the water mass, with its own thermal structure.

In conclusion the Pcy community structure in Lake Maggiore showed a remarkable succession of the OTUs defined by the least conserved ITS-1 fragment length. The thermal structure, defined by temperature (T) and depth of mixing layer (Z_m), influences OTU dynamics. There are "summer" OTUs typical of warmer waters in superficial layers and "early spring" OTUs typical of colder waters. In summer, microdiversity was clearly visible in the vertical gradient, with an increase of diversity at 20 m.

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