J. Limnol., 66(2): 81-89, 2007

Photosynthetic characteristics and diversity of freshwater *Synechococcus* at two depths during different mixing conditions in a deep oligotrophic lake

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ABSTRACT

We studied the photosynthetic characteristics and genetic diversity of Synechococcus *assemblages at two different light and mixing conditions in a deep oligotrophic lake (Lake Maggiore, Northern Italy). Sampling was performed at the beginning of summer stratification, when the base of the photic zone was isolated by the presence of a shallower thermocline, and during late summer, when the mixing layer extended below the photic zone. Two depths were sampled (15 % and 1 % of surface PAR). To study the diversity of* Synechococcus, *we used a classical molecular fingerprinting technique (Denaturing Gradient Gel Electrophoresis, DGGE) with ambient samples and sequencing of the prominent bands. The ecotypes selected within the samples from different depths were different in photosynthetic characteristics and grew in a range of nutrient concentrations. The OTUs colonizing different niches in the water column did not show any significant clustering. However, the OTU richness was significantly different at the two depths in spring, reaching the highest values at 15 % of surface PAR. Cluster analysis of DGGE lanes provided evidence of different community compositions between spring and late summer. Sequencing of the most prominent bands showed one spring OTU affiliated to the* Synechococcus *subalpine cluster I, with 100% similarity to LM94, MW76B2 and MW15#2 (*sensu *Crosbie* et al. *2003a), not present in late summer. Another OTU present both in spring and late summer showed 100% similarity to MH301, a strain near to subalpine cluster II. Our results support the coexistence of ecotypes in the vertical gradients, rapidly acclimating and performing differently in the microhabitats.*

Key words: *freshwater picocyanobacteria,* Synechococcus *spp., photosynthetic characteristics, genetic diversity, Lake Maggiore*

1. INTRODUCTION

In freshwater ecosystems *Synechococcus*-like cyanobacteria dominate both the surface and deep layers (Stockner *et al*. 2000) and can be present as solitary single cells or in microcolonies (Passoni & Callieri 2001; Komárková 2002; Crosbie *et al*. 2003b), with more morphotypes than marine environments. Whether microenvironment peculiarities determine different genetic traits is in debate. From ecological studies, we know that *Synechococcus*, like many prokaryotes, is highly plastic and, in lakes, can acclimate to different environmental conditions and irradiance levels by adjusting their pigment composition (Hauschild *et al*. 1991; Callieri 1996; Vörös *et al*. 1998). By phylogenetic study of the 16S rDNA, the genus *Synechococcus* is known to be polyphyletic (Robertson *et al*. 2001), but the identification of species inside this taxon is still unclear. The large number of isolated strains suggests that closely related taxa have undergone widespread dispersal (Crosbie *et al*. 2003a) and contradicts the evidence that strain clusters developed similar ecotypes during independent adaptive radiations (Ernst *et al*. 2003). These molecular genetic studies claim that there is an urgent need for more data acquisition and the development of different approaches. A general consensus on the importance of the parallel study of genetic and ecological characteristics of this taxon is necessary for a comprehensive evaluation of the different ecotypes.

To get insight into the freshwater *Synechococcus* ecotypes/genotypes in a deep oligotrophic subalpine lake we compared its photosynthetic characteristics and diversity (1) at two depths under different irradiances, and (2) at spring and late summer peaks of abundance during different mixing conditions.

To study the genetic diversity of *Synechococcus* we used an application of DGGE (Denaturing Gradient Gel Electrophoresis) (Muyzer *et al*. 1993), one of the classical molecular fingerprinting techniques. DGGE gives information on OTU diversity and richness and, through sequencing of the gel bands, we can compare the sample to sequences published in genomic databases in order to infer phylogenetic affiliation. In the marine environment, researchers have found compartmentalization of *Prochlorococcus* and *Synechococcus* clades in the horizontal and vertical "space" at contrasting environmental conditions (Fuller *et al*. 2006). However, in freshwater, as far as we know, no data exist on the depth-dependent genetic diversity of *Synechococcus,* or its possible correlation with physiological adaptations. Similarly, we set out to compare the diversity of *Synechococcus* to the photosynthetic performance of subpopulations (*sensu* Ernst *et*

al. 2000). We addressed the difficult question of whether, in lakes, the genus *Synechococcus* consists of multiple coexisting ecotypes that are genetically closely related but physiologically distinct. For bacteria it is known that competition and fluctuating environments increase phenotypic diversity (Corno & Jürgens 2006). However, in the specific case of *Synechococcus* it is still unknown, for example, if adaptation to low light (in order to survive at the limit of the photic zone) can be a force selecting an appropriate strain or if the adaptability of the genus itself is sufficient to let them survive in extreme conditions.

2. MATERIAL AND METHODS

2.1. Study site and in situ measurements

Lake Maggiore is a large (212 km^2), deep (372 m), subalpine lake, which has been the site of limnological studies since the first half of the past century (Baldi *et al*. 1953; de Bernardi *et al*. 1988; Bertoni & Callieri 1989; Callieri & Piscia 2002). The lake recovered from the eutrophic state reached in the late 1970s (maximum in-lake TP concentration at winter mixing 30 $\mu g\ l^{-1}$) and is now oligotrophic (TP around 10 $\mu g\ l^{-1}$) (Calderoni *et al*. 2006).

Sampling was performed at the station of the maximum depth, which has been chosen as the reference station for a long-term study on the lake (http://www.ise.cnr.it/lter). Discrete samples were taken with a 5 litre Van Dorn bottle. Profiles of temperature and Photosynthetic Active Radiation (PAR) were obtained using a multiparameter probe (IDRONAUT, mod. OS316).

2.2. Sampling

We took samples in two periods of the year corresponding to the spring and late summer picocyanobacteria peaks, typical of deep, temperate oligotrophic lakes (Callieri & Pinolini 1995; Padisák *et al*. 1997). In each period, sampling was performed once a week for three consecutive weeks (16-6, 22-6, 30-6 and 31-8, 8-9, 14-9) in order to follow the short-term evolution of picocyanobacteria assemblages. Two depths were chosen, namely 1 % (8-15 m) and 15 % of surface PAR (3 m).

2.3. Counting

Picocyanobacteria were immediately preserved in formalin (2 % final solution) cacodilate buffered, stored in darkness at 4°C and processed within two weeks. Counting was performed on polycarbonate filters (Nuclepore, 0.2 μm pore size) by autofluorescence of phycoerythrin (Zeiss Axioplan microscope equipped with an HBO 100 W lamp, a Neofluar 100 x objective,1.25 x 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). Cells were measured with an image analysis system (Image ProPlus sofware and CoolSNAP pro-cf videocamera; Media Cybernetics, Silver Spring, MD, USA). Cell volumes were transformed to carbon using a conversion factor of 200 fg C μm^{-3} (Weisse 1993).

2.4. Chemical analyses

Total Phosphorus (TP) and Total Nitrogen (TN) were determined according to A.P.H.A. (1985) after mineralization of the samples with persulfate-boric acid (Valderrama 1981). Soluble Reactive Phosphorus (SRP) was determined according to A.P.H.A. (1985) after samples filtration through GF/C (Whatman) filters.

The Total Inorganic Carbon (TIC) availability was determined from pH and alkalinity measurements. In order to determine the Chlorophyll *a* (Chl *a*) content of picophytoplankton, up to 250 ml of sampled water were filtered through a 2.0 μm pore size polycarbonate filter (Osmonics™) and the filtrate was subsequently filtered through a 0.2 μm pore size polycarbonate filter (Osmonics™). The Chlorophyll *a* concentration was determined fluorometrically by Perkin-Elmer LS-2 Filter Fluorometer (Stainton *et al*. 1974) after pigment extraction with methanol (Talling & Driver 1961; Holm-Hansen & Riemann 1978).

2.5. Primary production

For each date, primary production (PP) was measured using the ^{14}C technique (Steeman-Nielsen 1951, 1952). Dark bottle measurements were substituted by the "time 0" organic ^{14}C measurement by adding the isotope to the dark bottle and immediately filtering and analyzing (Fahnenstiel *et al*. 1994). Lake water was sampled before starting the incubations and 4 replicate vials (25 ml each) were immediately filled with lake water. We added 1.48 kBq $NaH^{14}CO_3\ ml^{-1}$ (Amersham) to each vial, and then incubated the samples *in situ* for 4 hours around noon. Incubations were carried out with the vials suspended at the two sampling depths in the euphotic zone. After incubation, 500 μl aliquots were taken to check total activity. In order to estimate size fractionated primary productivity (>2 μm and <2 μm) the samples were filtered using plastic disposable syringes and plastic 25 mm filter holders. Polycarbonate filters (Osmonics™) of 2 μm were used for the autotrophic fraction (>2 μm). The filtrate was concentrated on 0.22 μm nitrocellulose membranes (Millipore™). Filters were acidified with 200 μl 1N HCl for 60 minutes in 20 ml- scintillation vials. After adding 10 ml of scintillation liquid the vials were counted in a Beckman LS3801 scintillation Counter. Photosynthetic carbon assimilation was calculated based on the proportion between ^{14}C uptake and TIC availability (Steeman-Nielsen 1951, 1952).

Tab. 1. Sequences of primers used and relative target regions.

Primer	Sequence (5' to 3')	Target region
CYA359F	GGG GAA TY[b]T TCC GCA ATG GG	359-378
CYA781R(a)	GAC TAC TGG GGT ATC TAA TCC CAT T	781-805
CYA781R(b)	GAC TAC AGG GGT ATC TAA TCC CTT T	781-805

numbering refers to position on 16S rRNA of *E.coli*
[b] Y= C/T

2.6. Nucleic acid extraction and PCR amplification

We concentrated about 300 ml of lake water from each environmental sample on 0.2 μm sterile hydrophilic polyethersulfone Supor® (Gelman Laboratory) and kept at -20°C in 2 ml of lysis buffer (50 mM Tris, 40 mM EDTA, 400 mM NaCl, 0,75 M Sucrose; Giovannoni *et al*. 1990) for no longer than 2 months.

DNA used for DGGE protocols was extracted from the filters using the UltraClean Soil DNA Isolation Kit (MoBio) and cast through 1 % (w/v) agarose in TBE buffer for visual estimation under UV transillumination (Gel DOC XR System (Bio-Rad) with Quantity One® (Bio-Rad), after staining with ethidium bromide (EB).

For amplification of the 16S rDNA, 20 nM of primers CYA359F and equimolar mix of CYA781R(a) and CYA781R (b) (Tab. 1) (Nübel *et al*. 1997) were included in the reaction with approximately 50 ng of DNA extracts and 25 μl Taq PCR Master Mix Kit (QIAGEN), for a volume of 50 μl of 0.2 μm sterile filtered water. The amplification profile consisted of an initial denaturation at 96°C for 5 min followed by 10 touchdown cycles of 94°C for 45s, 65°C (-1°C each cycle) for 45s and 72°C for 2 min, then 20 cyles of 94°C for 45s, 55°C for 45s and 72°C for 2 min (Don *et al*. 1991; Muyzer *et al*. 1993). A final elongation step of 72°C for 5 min was performed. PCR reactant concentrations were as follows: 20 nM each primer, 50 ng DNA template and 25 μl Taq PCR Master Mix Kit (QIAGEN), for a volume of 50 μl of 0.2 μm sterile filtered water.

2.7. DGGE analysis and sequencing

The PCR product was electrophoresed (for 16 h at 150V) through a polyacrylamide gel (1 mm) with 37 to 70 % denaturing gradient, in which 100 % is defined as 7 M urea and 40 % (vol/vol) formamide in a Bio-Rad D-Code. The gel was then incubated for 20 min in 4°C in SYBR® Green I Nucleic Acid Gel Stain (Cambrex) dissolved in MilliQ water. Denatured DNA bands were detected under UV illumination with Gel DOC XR System (Bio-Rad) and elaborated by Quantity One® (Analyse Software, Bio-Rad).

A density profile through each lane was assessed, the bands detected (regarded as operational taxonomic units, OTUs) and the relative contribution of each band to the total band signal was calculated in the lane after applying a rolling disk as background subtraction. Bands with a relative intensity of < 0.5% of the total intensity of the lane were disregarded. A distance matrix of the lanes was calculated using the BrayCurtis Coefficient and a cluster (UPGMA, unweighted-pair-group mean average) was inferred using the program MEGA3 (Molecular Evolutionary Genetic Analysis).

In order to sequence the DGGE bands, we excised the most prominent bands of the 12 lanes of SYBR® Green-stained DGGE gel using a sterile scalpel and DNA eluted overnight at 4°C in 500 μl of TE buffer (10 mM Tris-HCl, EDTA 1 mM) (Nübel *et al*. 1997). One microliter of each solution containing excised bands was re-amplified using the same primers and the same PCR conditions, as described above. Next, we sequenced the PCR products using the forward primer under the following conditions: 2.5 μl primer CYA359F (1 μM), 2 μl of BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), 4 μl Tris buffer (2.5x), 3 μl pre-amplified DNA, and 8.5 μl of MilliQ water to a final volume of 20 μl (termocycling conditions: 96°C for 1 min, 30 cycles at 96°C for 10s, 50°C for 5 s and 60°C for 4 min). The final PCR products were purified using the QIAquick PCR purification kit (QIAGEN). Sequencing was performed in a ABI PRISM 310 (Applied Biosystems) sequencer.

3. RESULTS

3.1. In situ measurements and ecological parameters

Temperature and light profiles (Fig. 1) at the six sampling dates indicate two distinct hydrologic situations: in spring, the thermocline depth was shallower than the depth of 1 % of surface PAR (limit of the photic zone), while in late summer the thermocline depth extended deeper than the photic zone. The exception was on September 8, when the euphotic zone extended to 18 m, much deeper than the thermocline depth. In late summer the thermocline was much more pronounced than in spring. In general, during spring samplings the *Synechococcus* assemblages living at 1 % of surface PAR were confined to the bottom of the photic zone whereas in late summer the assemblages living in the photic zone stayed in the mixing layer, thus having the possibility to be transported into the near surface zone.

Tab. 2. Main chemical parameters measured in Lake Maggiore at 15 % (upper panel) and at 1 % of surface PAR (lower panel). TN =Total Nitrogen, SRP = Soluble Reactive Phosphorus, TP = Total Phosphorus, TIC = Total Inorganic Carbon, PAR = Photosynthetic Active Radiation.

15 % **PAR**	TN (mg l^{-1})	s.d.	SRP (μg l^{-1})	s.d.	TP (μg l^{-1})	s.d.	TIC (mg l^{-1})	PAR (μmol m^{-2} s^{-1})
16-06-04	0.98	0.08	1.04	1.13	9.70	1.35	10.01	154
22-06-04	0.97	0.07	2.11	0.72	12.03	0.97	9.79	52
30/06/04	0.84	0.05	2.84	1.65	12.52	2.17	9.89	872
31/08/04	0.60	0.03	1.89	0.72	9.94	0.37	8.85	82
08/09/04	0.79	0.01	1.74	-	6.64	1.09	9.35	399
14/09/04	0.75	0.04	1.02	-	10.77	3.56	9.62	395

1 % **PAR**	TN (mg l^{-1})	s.d.	SRP (μg l^{-1})	s.d.	TP (μg l^{-1})	s.d.	TIC (mg l^{-1})	PAR (μmol m^{-2} s^{-1})
16-06-04	0.98	0.02	0.46	1.03	9.65	1.11	9.69	15
22-06-04	0.95	0.02	2.03	-	12.42	0.30	9.65	1
30/06/04	0.93	0.07	3.27	0.41	10.41	0.51	10.02	25
31/08/04	0.81	0.06	0.01	-	10.96	0.34	9.17	3
08/09/04	0.89	0.03	1.09	0.10	8.29	2.90	8.60	32
14/09/04	0.81	0.03	0.01	-	11.64	1.93	9.40	75

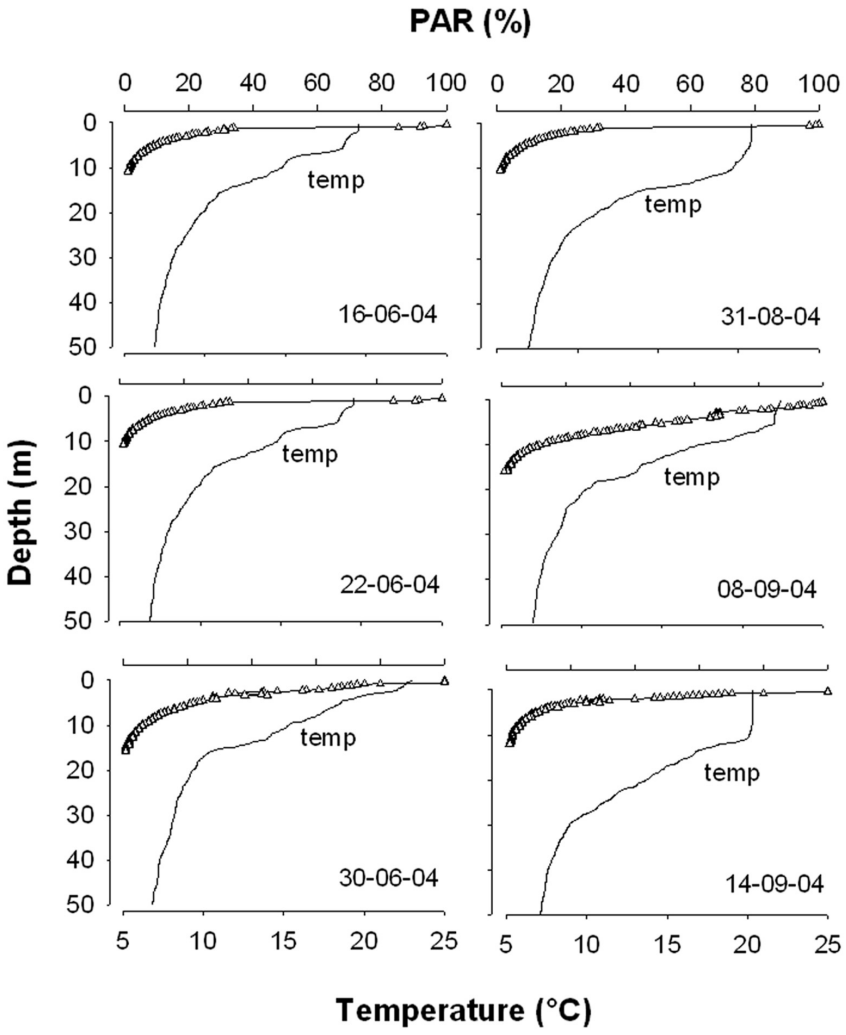

Fig. 1. Profiles of temperature (line) and percentage of surface PAR (triangles) in the two study periods, in Lake Maggiore 2004.

On two occasions, we found multiple thermoclines (on 29 June: 2, 9, 14 m; on 8 September: 9, 18 m), which is a condition often encountered in oligomictic Lake Maggiore (Barbanti & Ambrosetti 1989).

The chemical analyses performed in the two periods (Tab. 2) showed similar characteristics of lake water at the two depths and a more pronounced difference of the SRP between June and September. A well-defined period of P limitation was evident in September with SRP concentrations below detection limits. Nitrogen was never limiting, and ranged between 600-980 μg l^{-1}. Inorganic carbon was around 9-10 mg l^{-1}, indicating favourable conditions for primary production.

Synechococcus abundances (Fig. 2A) were not statistically different at the two depths (*t*-test for dependent variables, df 5, P = 0.44 and P = 0.30 respectively). The marked variability observed during the study periods indicates a weekly variation of cell number. The peak of cell number was reached in September at 3 m (160 x 10^3 cell ml^{-1}).

The mean cell volume was statistically different (*t*-

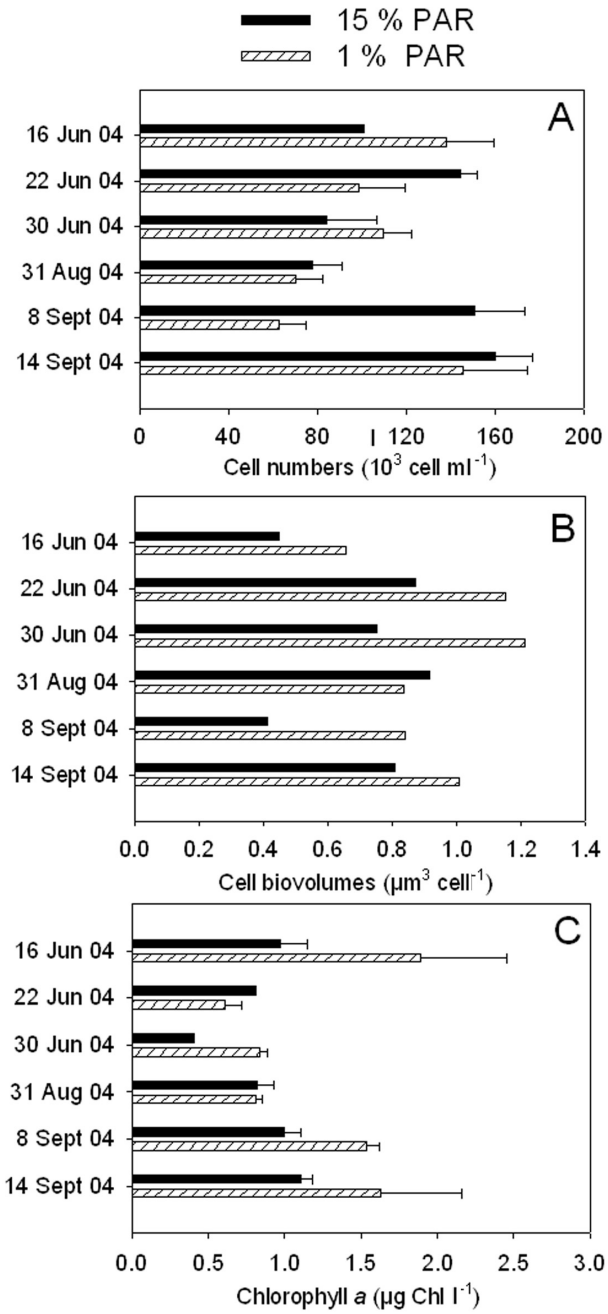

Fig. 2. Numbers of picocyanobacteria (10^3 cells ml^{-1}) (A), cell volume (μm^3 cell^{-1}) (B), Chlorophyll *a* concentrations (μg l^{-1}) (C), at the two depths during the two study periods, in Lake Maggiore 2004.

test for dependent variables, df 5, P = 0.02) at the two depths, with the larger cells at 1% PAR (0.70 and 0.95 μm^3 $cell^{-1}$ at 3 and 10 m respectively) (Fig. 2B)

Chl *a* concentration (Fig. 2C) results were highly variable with a generalized concentration increase at 1 % of surface PAR. Nevertheless, the differences between the two depths were not significant (*t*-test for dependent variables, df 14, P = 0.158). The picocyanobacteria chlorophyll-specific production (Fig. 3, upper panel) showed a significant difference among depths (*t*-test, df 23, P = 0.0001). At 15 % of surface PAR the values were always higher and ranged from 0.7 to 5.4 mg C (mg Chl)$^{-1}$ h^{-1}, as compared to a variation between 0.17 and 0.81 mg C (mg Chl)$^{-1}$ h^{-1} at 1 % of surface PAR. Picocyanobacteria efficiency, i.e. the production per unit Chl and unit mole of photons was significantly higher at 1 % of PAR (mean: 13.4 mg C (mg Chl)$^{-1}$ mol^{-1} m^{-3}) than at 15 % of PAR (mean: 6.5 mg C (mg Chl)$^{-1}$ mol^{-1} m^{-3}) (Fig. 3, lower panel). No significant difference was found between spring and late summer samples.

3.2. Genotypic classification

DGGE profiles showed a relatively low number of bands through both seasons, never exceeding 10 different OTUs in a single sample with an average value of OTU richness of 6.25 OTUs per lane. Communities at 1% PAR showed relatively constant richness levels, ranging between 5 and 8 OTUs, while communities at 15% PAR exhibited more unstable values, ranging from 3 to 10 OTUs per sample (Fig. 4). Some very clear and pronounced bands were common to all samples while others were only present in spring samples. We found a rather constant number of taxonomic units (DNA bands) in the 3 consecutive samples from each period, at the two depths, suggesting slight differences between depths in the same period.

From the density profile of the DGGE lanes, we used the relative contribution of each band to the total band signal to calculate the distance matrix and produce a cluster analysis (Fig. 4). The cluster displayed two groups at 0.21 distance. The sample from August 31 (3 m) was an outgroup, because its analysis was very poor due to problems with the PCR amplification of the extracted DNA. The two clusters demonstrated the difference between the spring and late summer fingerprinting image of the picocyanobacteria community in Lake Maggiore. On the other hand, in spring, the relative OTU richness (Fig. 4) was significantly higher (Paired t-test, P = 0.02) at the depth of 15 % surface PAR as compared to the 1 %. In autumn, the difference between the depths was not significant (Paired t-test, P = 0.07).

When excised and sequenced, the more pronounced spring bands showed 100 % similarity to *Synechococcus* LM94, MW76B2 and MW15-2 which are phycoerythrin-rich strains of subalpine cluster I, Group B (Crosbie *et al*. 2003a). One band common both to late summer and spring was 100 % similar to *Synechococcus* MH301, near subalpine cluster II.

4. DISCUSSION

Picocyanobacteria in Lake Maggiore have been studied since 1992 (Callieri & Pinolini 1995) and represent 85-90 % of total picophytoplankton number (Callieri & Piscia 2002). They are *Synechococcus*-type single celled in spring and tend to form small microcolonies in late summer (Passoni & Callieri 2001). During this study picocyanobacteria cell volumes were different at the two depths examined, and the cells at 15 % of surface PAR (3 m) were significantly smaller than those at 1% of surface PAR. This morphological difference along the photic zone could indicate either a different metabolic state of the cells in the vertical gradient (cells with low divi-

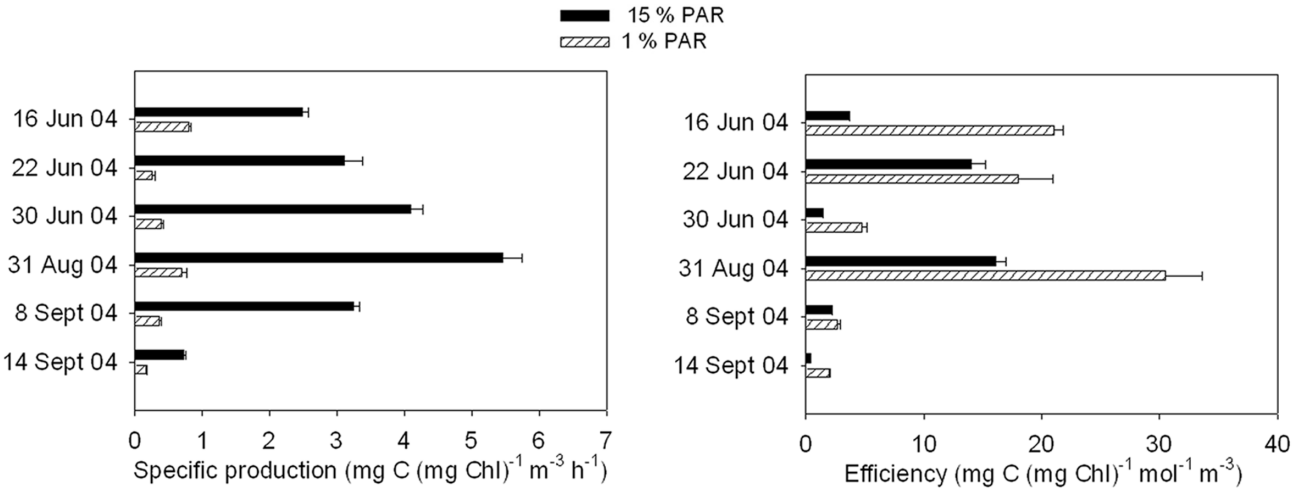

Fig. 3. Chlorophyll specific production (mg C (mg Chl)$^{-1}$ m^{-3} h^{-1}, left) and photosynthetic efficiency (mg C (mg Chl)$^{-1}$ mol^{-1} m^3, right), measured in the pico fraction at the two depths on six dates, in Lake Maggiore 2004.

sion rate which slowly enlarge their volume but do not divide), or even the presence of different strains. Picocyanobacteria assemblages of the two depths in Lake Maggiore also showed differences in chlorophyll *a* concentrations, as well as in photosynthetic characteristics. The surface communities were more productive but less photosynthetically efficient. No difference was found in morphology and in photosynthetic characteristics of *Synechococcus* cells between the spring and late summer samplings. Therefore, working from the measures of functional activity of picocyanobacteria (Chl *a* content, cell volume, cell production and efficiency) we expected to find a pronounced difference in OTU's diversity at different niches along the water column, rather than between the two different seasons.

However, the genotypic analyses showed opposite signals. The DGGE fingerprints proved two different clusters that markedly differentiate the relative OTU diversity of the spring and late summer picocyanobacteria. The results of the sequenced bands yielded evidence of the presence of one OTU that was phylogenetically similar to group B of the subalpine cluster I, which was only present in spring (see Crosbie *et al*. 2003a). Another OTU sequenced was common to all the spring and late summer samples. In contrast, the OTUs colonizing different niches in the water column vertical profile did not show any significant clustering. In other words, different picocyanobacteria ecotypes (i.e. with distinct photosynthetic characteristics), met along the photic zone and did not form clustered groups. Instead, the OTU richness was significantly different at the two depths in spring, reaching the highest values (10) at 15 % of surface PAR. This result is partly explained by the hydrologic situation of the spring period, when the shallow thermocline created a density barrier between the two depths. Actually, in spring, notwithstanding the lower cell abundances in the shallower layer, the picocyanobacteria community was characterized by a higher richness and was composed of smaller, more photosynthetically active cells. The absence of a true mixing layer in spring actually influences the number of OTUs present at the shallower layer, enabling the increase of species coexistence. This situation was not observed in late summer when the mixing layer extended deeper than the photic zone and allowed cells to be transported and mixed through the entire photic zone. We know that temperature gradient and water column stability influence picocyanobacterial community dynamics in both

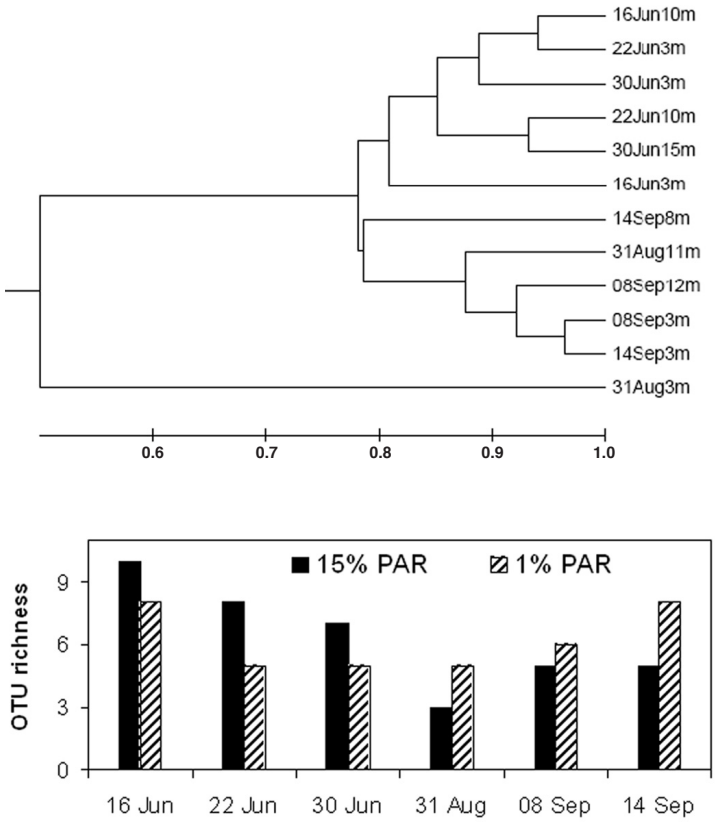

Fig. 4. Cluster analysis of picocyanobacteria communities based upon significant (> 0.5 % of total lane brightness) DGGE bands. Similarities are expressed as the Bray-Curtis Coefficient, which compares the relative contribution of single OTUs to the total amplified DNA of each band, when making pairwise comparisons between samples. Clustering was performed with UPGMA. Richness in bands of each sample is reported by grouping 15 % PAR (3 m) samples and 1 % PAR (between 8 and 15 m depth).

marine and freshwater environments (Camacho *et al*. 2003; Fuller *et al*. 2005).

In Lake Constance, seasonal dynamics of population and subpopulations of *Synechococcus*-type picocyanobacteria were demonstrated (Becker *et al*. 2002). In laboratory experiments using *Synechococcus* strains isolated from Lake Constance in spring and late summer (Postius *et al*. 1998) it has been found that changing the environmental conditions may cause dominance of the better adapted organisms. Nevertheless, to date, the partitioning of specific *Synechococcus* clades or ecotypes into specific niches *in situ* has not yet been demonstrated in freshwater.

Using dot blot hybridization technique, the predominance of genotypes from a single clade of *Synechococcus* population was demonstrated in the Red Sea (Fuller *et al*. 2003). The other cultures isolated, lacking PE, represented only a minor component in the natural environment. This community composition, which was rather homogeneous in vertical space, turned out to be composed of a few strains, dominant over the others, as a result of niche differentiation.

Recently was found that the *Prochlorococcus* HL (high light) ecotype (*sensu* Moore *et al*. 1998) was dominant over the season, while the LL (low light) ecotype was negligible and only appeared in late summer below 50 m (Fuller *et al*. 2005). This indicates that different ecotypes can coexist, but not necessarily dominate, at the same time at different depths; they appear to exploit a particular refuge niche in different seasonal periods. This hypothesis is also based on the evidence that picocyanobacteria are the winning competitors for phosphorus but at the cost of a selection towards the more adapted ecotypes.

Our data showed a difference in SRP concentrations in spring and late summer, with strong P-limitation conditions in September. In that month, total phytoplankton biomass was lower than in spring (Morabito *et al*. 2005) and picocyanobacteria represented more than 50 % of total autotrophic biomass (S. Galafassi, Thesis). The ecological conditions in late summer appeared distinct from those in spring and potentially able to select different genotypes, in accordance with our results of OTU's diversity. Conversely, the photosynthetic characteristics and the physiological traits of spring and late summer picocyanobacteria assemblages were highly variable inside the two periods and showed no significant difference between seasons.

The initial hypothesis of a genetically distinct *Synechococcus* population located at different depths along the water column according to their most favourable temperature and light conditions appears improbable. Instead, it is likely that picocyanobacterial strains coexist in the vertical gradient, rapidly acclimate (without an actual genetic adaptation) in the mixing layer and perform differently in the microhabitats. In particular, the OTU richness of spring samples was higher in the upper layer, above the thermocline. This study also revealed the presence of a spring picocyanobacterial strain affiliated to the subalpine cluster I, which disappeared in late summer, as well as a ubiquitous strain affiliated to subalpine cluster II.

Our results should be confirmed by further studies performed over an entire year. The importance of long-term molecular studies has been recently emphasized (Fuller *et al*. 2005) as critical to the better interpretation of the succession of subpopulations of picocyanobacteria.

5. CONCLUSIONS

In conclusion, our study on the photosynthetic and genetic characteristics of *Synechococcus* has shown that picocyanobacteria near the surface and at the deeper edge of the photic zone photosynthesize with different efficiency. Nevertheless, fingerprinting analyses of these samples did not reveal any relevant difference in OTU diversity. In spring, with a shallow thermocline, OTU richness was different at the two depths. In particular, richness was higher in the shallower layer, where the cells were more photosynthetically active and smaller, than in the deeper one.

The sequences obtained from DGGE excised bands revealed the presence of one spring OTU phylogenetically similar to the group B of the subalpine cluster I and of one OTU common to spring and late summer samples. This confirms that in Lake Maggiore, like in other subalpine lakes, different *Synechococcus* strains may occur in spring and in late summer.

ACKNOWLEDGEMENTS

We thank Mario Contesini for help with sampling, and Paola Giacomotti for analyses of the inorganic carbon. We are also grateful to the laboratories of the ICM (Istituto Cantonale di Microbiologia), Bellinzona (CH), where part of the genetic analyses were performed.

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Received: October 2007
Accepted: December 2007