Quantitative dominance of seasonally persistent filamentous cyanobacteria (Planktothrix rubescens) in the microbial assemblages of a temperate lake

Silke Van den Wyngaert,1 Michaela M. Salcher, Jakob Pernthaler, Michael Zeder, and Thomas Posch*

Department of Limnology, Institute of Plant Biology, University of Zürich, Kilchberg, Switzerland

Abstract

The spatiotemporal changes in abundance and biomass of heterotrophic bacteria, of three major bacterial phylogenetic groups, and of picocyanobacteria in the upper 20 m of a deep prealpine lake (Lake Zurich, Switzerland) were monitored during a seasonally persistent bloom of the toxigenic filamentous cyanobacterium Planktothrix rubescens. In addition, bacterial 16S ribosomal deoxyribonucleic acid (rDNA) sequences were collected at one instance from the bloom layer and from waters above and below this zone. P. rubescens comprised up to 70% of particulate organic carbon during summer stratification and autumnal mixis and thus by far exceeded the total biomass of both other phytoplankton and of prokaryotes. A strong negative correlation was found between the estimated basin-wide biomass of P. rubescens and of heterotrophic bacteria, and there was different spatial niche preference of filamentous vs. picocyanobacteria. Only members of the Cytophaga–Flavobacterium lineage of Bacteroidetes showed an increasing tendency of association with the P. rubescens population, in particular at the onset of autumnal mixing. Although the filamentous cyanobacterium was the dominant primary producer throughout the year, it did not seem to be a carbon source for heterotrophic bacteria at all. We conclude that P. rubescens represents a powerful competitor of autotrophic and heterotrophic prokaryotes, likely due to both its specific physiological (phototrophic) properties and its protection against zooplankton grazing. This competitiveness might be regarded as another reason for its mass occurrence in numerous lakes of the Northern hemisphere.

Autotrophic and heterotrophic microorganisms in the plankton of lakes and oceans are connected by multifarious relationships. Heterotrophic bacteria profit from organic substrates fixed by the phytoplankton and released during nutrient limited growth (Børsheim et al. 2005) or upon disintegration of algal cells. At the same time, the two groups compete for growth-limiting nutrients, e.g., for phosphorus in freshwater systems (Lövdal et al. 2007), with heterotrophic bacteria being more effective than algae at low nutrient concentrations (Mindl et al. 2005).

The interactions between heterotrophic bacteria and primary producers in freshwaters have mainly been investigated in the context of artificially induced (Riemann and Søndergaard 1986) or natural (Zeder et al. 2009) short-lived blooms of prokaryotic and eukaryotic phytoplankton species. The typically observed patterns, e.g., during phytoplankton spring blooms, are an increase of dissolved organic carbon and of bacterial cell numbers and activity (Simon et al. 1998) during or after the maximum concentrations of chlorophyll a (Chl a) and a subsequent formation of macroscopic organic aggregates (due to the agglutination of senescent algae) colonized by bacteria that differ from single-celled planktonic taxa (Grossart et al. 1997; Schweitzer et al. 2001). Such transient phytoplankton blooms are often accompanied by pronounced changes in microbial community composition, e.g., a disproportionate rise of bacteria affiliated with Flavobacteria (Zeder et al. 2009), Betaproteobacteria (Salcher et al. 2008), or Actinobacteria (Salcher et al. 2010). By contrast, considerably less is known about the coexistence of bacteria with phytoplankton groups that are seasonally more persistent (Šimek et al. 2008).

The filamentous cyanobacterium Planktothrix rubescens is the dominant primary producer in the mesotrophic, prealpine Lake Zurich, Switzerland ( Micheletti et al. 1998). A mass occurrence of this species has also been observed in numerous other lakes of the Northern Hemisphere (Ernst et al. 2009). Typically, the population is established in the metalimnion during early summer, where it forms an increasingly concentrated layer. Upon the onset of autumnal mixing it is dispersed throughout the epilimnetic water body and subsequently transported through the deepening mixed zone (Micheletti et al. 1998). P. rubescens can account for half of the total phytoplankton biomass in Lake Zurich in summer and for even more during the autumn and winter months. Several ecophysiological traits enhance the competitive abilities of this cyanobacterium in stratified lakes compared with other prokaryotic and eukaryotic phytoplankton species, such as adaptation to low irradiance and temperatures (Walsby and Schanz 2002), harmful secondary metabolites (Blom et al. 2006), or gas vesicles that allow filaments to regulate their vertical position within the stratified water column (Walsby et al. 1998). Planktothrix spp. are among the most important producers of hepatotoxic microcystins in freshwaters (Kurmayr and Gumpenberger 2006), which effectively provides a population-level protection against zooplankton grazing (Kurmayr and Jüttner 1999; Blom et al. 2006). In contrast to other cyanobacterial species, the filaments of P.
rubescens are typically void of attached bacterial cells (Feuillade et al. 1988), suggesting that they may actively resist microbial colonization.

Since *P. rubescens* is capable of photoheterotrophy (Feuillade et al. 1988; Zotina et al. 2003), it might be in direct competition with both other phototrophic and heterotrophic prokaryotic microorganisms. It is, therefore, conceivable that there is a niche segregation between these cyanobacteria and the autotrophic picoplankton. Moreover, a possible relationship with the heterotrophic bacterioplankton at a seasonal scale might be reflected in contrasting patterns of biomass distributions, as well as in the presence (or abundance) of particular bacterial groups or genotypes in the layer of highest *P. rubescens* densities. We therefore assessed the abundances and/or biomasses of heterotrophic bacteria, coccoi and filamentous cyanobacteria in the epilimnion and metalimnion of Lake Zurich at weekly intervals for a period of 6 months. Sampling started with the increasing growth of metalimnetic *P. rubescens* at the beginning of stable summer stratification and ended after the onset of autumnal mixis, i.e., after the first entrainment of cyanobacterial filaments in the upper water body. Three phyla of heterotrophic bacteria were distinguished that typically respond to phytoplankton bloom situations (Zeder et al. 2009; Salcher et al. 2010). In addition, the fine-scale taxonomic differences in the bacterial assemblages within, above, and below the zone of maximal *P. rubescens* densities were analyzed in detail on one occasion. We expected a negative correlation of the total heterotrophic bacterial assemblage with *P. rubescens* parameters and supposed a biomass dominance of the cyanobacterium compared with heterotrophic prokaryotes.

Methods

Lake Zurich and *P. rubescens*—Lake Zurich is a large (66.8 km²), deep (136 m, mean depth = 49.9 m) prealpine lake in a densely populated region. The theoretical renewal time of the entire water volume (3.34 km³) is 1.2 yr. Besides its recreational uses, it serves as the major source of drinking water for the city of Zurich. Partial mixis of the upper water body (down to 40 m) occurs in autumn; holomixis takes place in early spring (March or April). Lake Zurich is characterized by a strong metalimnetic oxygen depletion lasting from summer stratification until autumnal mixis. The harmful cyanobacterium *P. rubescens* shows recurrent mass developments (blooms) between late autumn and winter, first described for the year 1897 (earlier undocumented blooms are likely).

Sampling—Weekly sampling started on 03 July with summer stratification (development of the metalimnetic *P. rubescens* population) and ended with autumnal mixis on 12 November 2007. The sampling site was near the deepest point of the lake (47°18′25.24″N, 8°34′37.8″E). Samples were collected with a water sampler (Uwitec) in 2.5-m intervals from surface to 20 m depth (eight samples per profile). Forty milliliters of sample were fixed with 0.2-μm pore size prefilteter formaldehyde (2% final concentration) for determination of abundance and biovolume of microorganisms. Another 40 mL were fixed with buffered paraformaldehyde (pH 7.4, 2% final concentration) for bacterial identification by fluorescence in situ hybridization followed by catalyzed reporter deposition (CARD-FISH). The following limnological parameters were measured in situ: Profiles of photosynthetically active radiation were determined with a spherical quantum sensor (LI-COR) from surface in 1-m intervals to the depth with an irradiance of lower than 0.05 μmol m⁻² s⁻¹. Temperature and oxygen were also measured in 1-m intervals from surface to 20-m depth (Wissenschaftlich-Technische Werkstätten GmbH). Chlorophyll *a* (Chl *a*) was determined using the method of Lorenzen (1967). Information about total phytoplankton biomass and the concentrations of dissolved and particulate organic carbon (DOC, POC) at the above described depth layers was provided by the Zurich Water Supply Company for the first sampling date in each month (in sum for five sampling dates).

Calculation of neutral buoyancy depth and depth of photosynthetic compensation point—Records of solar irradiance at the water surface at station Mythenquai were obtained from the Web site of the water police Zurich for each sampling day (http://www.stadt-zuerich.ch/pd/de/index.html). Values were recorded at 10-min intervals and were obtained in W m⁻², which is equivalent to J s⁻¹ m⁻². The daily insolation at the water surface (*Q₀*) was calculated from the sum of the 10-min records (each record was first multiplied by 600, i.e., 60 s × 10 min) and converted to mol m⁻² by the ratio of 2.116 μmol J⁻¹ (theoretical ratio given in Walsby et al. 2004). The daily insolation at each depth (*Q₂*) in 1-m intervals was calculated as

\[
Q_2 = Q_0 \times E_2 \times E_0^{-1}
\]

where *E₂* is the irradiance at depth *z* and *E₀* is the irradiance just below the water surface. Walsby et al. (2004) described the neutral buoyancy depth of *P. rubescens* as the depth where the daily insolation (*Q₈*) is 0.28 mol m⁻². We determined the sampling depths above (*z₁*) and below (*z₈*) where *Q₁ > Q₈ > Q₈₋₁*. The exact neutral buoyancy depth (*z₈*) is the depth where the daily insolation is *Q₈* and was calculated by logarithmic interpolation according to Walsby et al. (2004):

\[
z₈ = z₁ + \Delta z \times [\ln(Q₁) - \ln(Q₈)] \times [\ln(Q₁) - \ln(Q₁₋₁)]^{-1}
\]

where *Δz* was the sampling depth interval (1-m steps).

The same procedure was applied for the calculation of the depth of photosynthetic compensation point (*z₈*). Davis and Walsby (2002) defined an irradiance of 0.8245 μmol m⁻² s⁻¹ as compensation point for growth in a 12 h:12 h light:dark cycle at a temperature of 15°C. That value was determined for an isolate (*P. rubescens* B9972) from Blelham Tarn, English Lake District. From this we calculated the daily photon insolation for the compensation point (*Q₈* in mol m⁻²):

\[
Q₈ = 0.8245 \text{ μmol m}^{-2} \text{ s}^{-1} \times 10^{-6} \times 43,200 \text{s} \text{ (equivalent to 12 h)}
\]

We determined a value of 0.0356 mol m⁻² for *Q₈* and then
Applied the above formulae to calculate $z_C$ by replacing $Q_N$ with $Q_C$.

Abundances of heterotrophic bacteria and cyanobacteria—One to two milliliters of formaldehyde fixed samples were stained with 4′,6-diamidino-2-phenylindole (DAPI, 1 $\mu$g mL$^{-1}$ sample [Porter and Feig 1980]), filtered onto black polycarbonate filters (0.22-$\mu$m pore size, Osmonics), and placed on cellulose nitrate support filters (0.45-$\mu$m pore size, Sartorius). To quantify heterotrophic bacteria, at least 400 DAPI-stained cells per filter were counted at ultraviolet excitation (Zeiss filter set 01) with an epifluorescence microscope (Zeiss AxioImager.M1) at $\times1600$ magnification. Single celled coccolid cyanobacteria (presumably *Synechococcus* spp.) were counted by their autofluorescence under green light excitation (Zeiss filter set 15) at a magnification of $\times630$. Filaments of *P. rubescens* were counted with the same filter set at $\times100$ magnification.

Biovolume and biomass of heterotrophic bacteria—Twenty images of DAPI-stained preparations were recorded with a highly sensitive charge-coupled device (CCD) camera (Vosskühler) at a magnification of $\times1250$. Images were saved as eight-bit files (256 gray values) and had a dimension of 1380 $\times$ 1040 pixels. Size determination of heterotrophic bacterial cells was automated and performed with the image analysis software AxioVision 4.6 (Zeiss) following the workflow described in Posch et al. (2009). Total biovolume (mm$^3$ L$^{-1}$) of heterotrophic bacteria was determined by multiplying the mean cell volume ($MCV_{\text{BAC}}$ in $\mu$m$^3$) by abundance (bacteria L$^{-1}$). $MCV_{\text{BAC}}$ was converted to cellular carbon content ($CC_{\text{BAC}}$ in fg carbon bacterium$^{-1}$) applying the allometric conversion formula $CC_{\text{BAC}} = 218 \times MCV_{\text{BAC}}^{0.86}$ (Loferer-Krössbacher et al. 1998). Bacterial biomass ($\mu$g carbon L$^{-1}$) was calculated by multiplying $CC_{\text{BAC}}$ by bacterial abundance (bacteria L$^{-1}$).

Biovolume and biomass of *P. rubescens* filament densities, an appropriate sample volume (1–10 mL) was filtered onto 5-$\mu$m pore-sized white polycarbonate filters (25 mm diameter, Sterico) in order to obtain approximately 500 filament per filter. Biovolume determinations were made for samples with an abundance of at least 20 filaments mL$^{-1}$. Samples were filtered at partial vacuum (not exceeding 100 mm Hg) to avoid fragmentation of filaments. Images of preparations were recorded with a CCD camera (AxioCam MRm, 12 bit grayscale, 1388 $\times$ 1040 pixel, Zeiss) mounted on an epifluorescence microscope (AxioImager.Z1, Zeiss) equipped with a motorized stage. Fluorescent images were acquired using green light excitation (Zeiss filter set 43) and a $\times10$ EC Plan-Neofluar objective. Large composite images were generated by the software module MosaiX for the image analysis software Axio Vision 4.6.3 (Zeiss). From each preparation, 11 $\times$ 15 adjacent images (corresponding to an area of approximately 1 $\times$ 1 cm) were recorded to produce a composite image. Quantification and length measurement of individual filaments on these images was performed by image analysis as described in Zeder et al. (2010). The image analysis works even for crossing filaments or filaments running side by side. Since the width of filaments was found to be rather constant, a mean width of 5.7 $\mu$m was used for further calculations (Walsby et al. 1998). We considered the shape of a *P. rubescens* filament as a cylinder with two spherical ends. The volume of the cylinder was calculated by multiplying the measured length by the mean cross section (25.5 $\mu$m$^2$) of a filament. By adding the volume of the two spherical ends (97 $\mu$m$^3$) to the cylinder volume, the filament volume was determined (for details on the equation see Posch et al. 2009). Total biovolume (mm$^3$ L$^{-1}$) of *P. rubescens* was determined by multiplying the mean filament volume with abundance (filaments L$^{-1}$). Calculations of *P. rubescens* biomass ($\mu$g C L$^{-1}$) were based on a value of 270 $\mu$g of dry weight mm$^{-3}$ and a carbon content of 45% of dry weight (Zotina et al. 2003).

Cumulative biomass (carbon) of heterotrophic bacteria and *P. rubescens*—Depth-related volumetric data of Lake Zurich are based on isobath areas measured at 1-m intervals (data from D. M. Livingstone, Eawag). Volumes for 1 m depth layers were calculated with the truncated cone equation according to Wetzel (2001). Since the water column was sampled in 2.5-m intervals between 0 and 20 m (eight depths, 35% of total lake volume), the volumetric contribution of each such sampling layer was calculated. For the estimation of cumulative biomass (kg of carbon) in each depth layer, the biomass estimates (mg carbon L$^{-1}$) were multiplied with the water volumes of the respective sampling depth layer. It is problematic to calculate whole system parameters based on a single sampling location. Nevertheless, our sampling location was near the sampling station of the Zurich Water Supply Company (WVZ). Based on the monthly profiles taken for 40 yr, the WVZ regularly calculates the whole lake budgets of major nutrients and biological parameters and presents these data in 10$^3$ kg lake$^{-1}$.

CARD-FISH—Five to ten milliliters of paraformaldehyde-fixed samples from five depths (2.5, 7.5, 10, 12.5, 20 m) were filtered onto white polycarbonate filters (0.2-$\mu$m pore size, Millipore) placed on a support filter (Sartorius). Filters were rinsed with distilled water, air dried, and stored at $-20^\circ$C until further processing. CARD-FISH was performed as previously described (Sekar et al. 2003; the permeabilization step was modified by using proteinase K instead of achromopeptidase). The following horseradish peroxidase–labeled probes were used (Amann and Fuchs 2008): EUB I–III for all Bacteria, probe BET42a for the Betaproteobacteria, probe CF319 (Cytophaga–Flavobacterium–Bacteroides), and probe HGC69a for Actinobacteria. For the signal amplification step, custom fluorescein labeled tyramides (Invitrogen) were used. Filter sections were counterstained with DAPI (1 $\mu$g mL$^{-1}$) and evaluated with a fully automated epifluorescence microscope system as previously described by Salcher et al. (2008).

Clone libraries—On 01 October 2007, we prefilted 250 mL of unfixed water samples from 10, 12.5, and 15 m depth over 5-$\mu$m pore size filters to remove larger...
organisms, filtered them onto white polycarbonate membranes (Millipore, Type GTP, 0.2-µm pore size, 47-mm diameter), which were stored frozen until further processing. DNA was extracted with the Powerbead soil isolation kit (Mobio), and almost complete 16S ribosomal RNA (rRNA) encoding genes were amplified with the primers GM3f and GM4r (Muyzer et al. 1995). Polymerase chain reaction (PCR) products were purified with the QIAquick PCR purification kit (Qiagen), inserted into TOPO vectors (TOPO TA cloning kit for sequencing; Invitrogen), and cloned into competent Escherichia coli cells according to the manufacturer’s instructions. After screening of the clones for right-sized inserts, plasmid preparations were done with the QIAprep Spin Miniprep Kit (Qiagen). The sequencing reactions were accomplished with the primer GM1f (Muyzer et al. 1993) and the ABI BigDye chemistry on an ABI 3730 Genetic Analyzer (Applied Biosystems). Partial sequences were first analyzed by BLAST (www.ncbi.nlm.nih.gov/BLAST) and the ribosomal database project (http://rdp.cme.msu.edu) for their phylogenetic affiliations. Nearly full-length sequences of all unique sequence types were obtained by additional sequencing with vector primers M13f and M13r (Messing 1983). Partial sequences were assembled with the DNA baser software (HeracleSoftware) and checked for chimeric origin using Mallard and Pintail (Ashelford et al. 2005). Phylogenetic analyses were performed with the ARB software package (Ludwig et al. 2004) using the ARB reference database SILVA SSU 98. All sequences were automatically aligned with the SONS (Schloss and Handelsman 2006). All sequences were deposited to European Molecular Biology Laboratory (EMBL) with accession numbers FN665702-FN665785 and FN668013-FN668374.

Statistical analysis—Pearson’s correlation coefficients were calculated to identify significant relationships between microbial and environmental data. All abundance, biovolume, and environmental data were log (x + 1) transformed, and percentage data were arcsine transformed prior to analysis. The program SPSS was used for statistical analyses.

Results

Thermal stratification and development of P. rubescens—A stable thermal summer stratification formed at the beginning of the study period (Fig. 1A). The vertical extension of the metalimnion (water layer with temperature gradients > 1 °C m⁻¹ depth) was characterized by regular oscillations, reflecting internal waves (seiches) known to occur in Lake Zurich. Decreasing water temperatures at the beginning of October caused an extension of the epilimnetic mixing depth, leading to a complete disruption of thermal summer stratification at the end of the study (Fig. 1A).

Dissolved oxygen was gradually depleted in the metalimnion, and concentrations of < 2 mg L⁻¹ were measured at the end of thermal stratification (Fig. 1B). Highest abundances of P. rubescens (Fig. 1C) occurred in the upper zone of the metalimnion during summer situation (except 10 September), only partly reflecting the neutral buoyancy depth. From 22 August onward, a large proportion of the population was even found below the depth of the photosynthetic compensation irradiance (Fig. 1C). During stratification, filament abundances mirrored biovolume dynamics (Fig. 1D). Owing to autumnal mixis, filaments became entrained in the epilimnion and were distributed almost homogeneously. This resulted in lower filament densities throughout the water column; however, their biovolume remained high as a result of the increasing mean length of individual filaments (Fig. 1E). For the depth zone from 10 m to 15 m, the mean length was determined as 415 ± 37 µm for the period 03 July to 08 October, and increased to a value of 546 ± 55 µm for the remaining investigation period. In contrast, average abundance decreased from 232 ± 111 x 10³ to 160 ± 35 x 10³ filaments L⁻¹ for the two periods, respectively (Fig. 1C). However, average biovolume in the 10- to 15-m zone was 2.7 mm³ L⁻¹ during stratification and increased to 3.1 mm³ L⁻¹ during autumnal mixis.

Quantitative importance of P. rubescens—The quantitative role of P. rubescens in relation to total phytoplankton was explored with respect to their contribution to DOC and POC, chlorophyll a, and total algal biomass (Fig. 2). POC concentrations were significantly correlated with the biomass of P. rubescens (Fig. 2A, linear regression, r² = 0.88, p < 0.0001, n = 54). Depending on filament densities, P. rubescens biomass formed up to 70% of POC (Fig. 2B, y = 7.1 x x 0.44, r² = 0.82, p < 0.0001, n = 54). In contrast, DOC concentrations remained stable at 1.44 ± 0.09 mg C L⁻¹ (average ± 1 standard deviation) during the investigation period, and there was no significant correlation with P. rubescens abundance or biomass. The dominance of P. rubescens in the water column was also reflected by a significant correlation of filament abundance with total Chl a concentrations (Fig. 2C, linear regression, r² = 0.83, p < 0.0001, n = 54). This relationship mirrored the high contribution of the cyanobacterium to total algal biomass (Fig. 2D, y = 84.9 x (1 − 0.99), r² = 0.85, p < 0.0001, n = 54), i.e., up to 85% of the phototrophic biomass was made by P. rubescens in early winter.

Dynamics of coccolid cyanobacteria and heterotrophic bacteria—In early summer, heterotrophic bacteria and coccolid cyanobacteria (presumably Synechococcus spp.) were the quantitatively dominant prokaryotes in the epilimnion (Fig. 3A). During autumnal mixis, P. rubescens increasingly expanded into the upper water body, paralleled by a drastic decline of coccolid cyanobacteria. During the whole investigation period, the prokaryotic biomass of the metalimnion was mainly dominated by P. rubescens, and no coccolid cyanobacteria could be detected in this water layer (Fig. 3A). Heterotrophic bacterial abundances gradually decreased throughout the water column between
July and November (Fig. 3B). There was a significant negative correlation between the abundances and biovolumes of heterotrophic bacteria and *P. rubescens* (Table 1). Actinobacteria were the dominant group within the heterotrophic bacterial assemblage (range, 15% to 52%; mean, 29% of cells detected by CARD-FISH with the bacterial probe EUB I–III). The highest contribution of Actinobacteria to total *Bacteria* was observed at the onset of the *P. rubescens* bloom (Fig. 3C). Betaproteobacteria (Fig. 3D) and members of the Cytophaga–Flavobacteria (CF) subclade of *Bacteroidetes* (Fig. 3E) formed smaller parts of the total bacterial assemblage with mean values of 12% and 8%, respectively. The number of Betaproteobacteria decreased throughout the study period, whereas CF
formed two distinct peaks in the epilimnion and reached maximal numbers in and below the *P. rubescens* growth zone at the end of the summer stratification (Fig. 3E). The abundances of *Actinobacteria* and *Betaproteobacteria* were negatively correlated with those of *P. rubescens* (Table 1). In contrast, the proportions of CF were positively related to both *P. rubescens* abundance and biovolume.

**Total biomass of heterotrophic bacteria and *P. rubescens* in the upper 20 m of Lake Zurich**—Total biomasses (kg carbon) of heterotrophic bacteria and *P. rubescens* in the upper 20 m water column were estimated from the volume of the respective depth layers and the biomass of organisms (µg carbon L⁻¹). At the beginning of thermal summer stratification *P. rubescens* biomass was only around half (41 × 10³ kg of carbon) of that of heterotrophic bacteria (99 × 10³ kg of carbon, Fig. 4). From August 2007 on, the biomass of the cyanobacterium steadily increased, reaching four times higher values than heterotrophic bacteria within only 1 month. During summer stratification most biomass of *P. rubescens* was found in the metalimnion (Fig. 4A). During autumnal mixis, the contribution of *P. rubescens* biomass in the upper 8 m equaled or even exceeded that of the metalimnetic population. In contrast, heterotrophic bacterial biomass continuously decreased during the season, resulting in a negative relationship with *P. rubescens* biomass (Fig. 4B). At the end of the study period, bacterial biomass estimates in the upper 20 m of the lake reached a minimum of 46 × 10³ kg of carbon.

**Diversity and phylogenetic affiliation of heterotrophic prokaryotes**—Three clone libraries from 10, 12.5, and 15 m depth from October 2007 yielded 485 16S rRNA gene sequences, whereof 37 were discarded as chimeras and two as mitochondrial DNA. The remaining 446 sequences were grouped in 138 operational taxonomic units (OTUs, similarity level 97%, Fig. 5). However, pronounced undersampling was still indicated by the Chao1 estimator (348 predicted phylotypes, coverage 42%). The library from 15 m depth (metalimnetic oxygen minimum zone) showed the highest ratio of predicted vs. observed phylotypes (64%), although less than half as many sequences were analyzed from this depth layer than in the two other libraries (79 vs. 186 and 181 sequences). Most sequences were affiliated with *Actinobacteria* (142, 44 OTUs) and *Bacteroidetes* (137, 55 OTUs), fewer with *Alphaproteobacteria* (91, 7 OTUs) and *Betaproteobacteria* (40, 16 OTUs). Other sequences were affiliated with *Gammaproteobacteria* (2, 2 OTUs), *Cyanobacteria* (11, 1 OTU), *Thermomicrobia* (7, 2 OTUs), and candidate division OP1 (1).
The community overlap between the three water depths was low (Fig. 5) with only 13 core OTUs present in all libraries. Twelve more OTUs were shared between the libraries from 10 and 12.5 m, nine between 12.5 and 15 m, respectively, and only two more OTUs were shared between 10 and 15 m. The 12.5-m depth library (zone of *P. rubescens* maximum) had the highest number of unique sequence types (46), followed by 10 m (39), whereas only 17 OTUs were exclusively found in a 15-m depth.

Only 37% of the expected diversity of *Actinobacteria* was covered by our sequencing. Most sequences from this group were from the typical freshwater lineages acI (92, 28 OTUs) and acIV (48, 14 OTUs). *Bacteroidetes* (coverage: 58% of Chao1 estimate) mainly fell into the classes *Flavobacteria* (34 OTUs, 87 sequences) and *Sphingobacteria* (21 OTUs, 50 sequences). Sequenced *Betaproteobacteria* were affiliated with the LD28-*Methylphilus* clade (beta IV, five OTUs, 16 sequences), with the R-BT-*Limnohabitans*...
Table 1. Pearson correlation coefficients of *P. rubescens* related parameters with picocyanobacteria, heterotrophic bacteria, the proportions of three subgroups of *Bacteria*, and with major limnological parameters (oxygen, water temperature, chlorophyll *a*, and spherical light intensity). CF = *Cytophaga–Flavobacteria*, Picocyano = picocyanobacteria.

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<td>0.61***</td>
<td>0.63***</td>
<td>0.58***</td>
<td>—</td>
</tr>
<tr>
<td>CF cluster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Bacteria</td>
<td>0.33**</td>
<td>0.23</td>
<td>—</td>
<td>-0.28</td>
<td>0.57***</td>
<td>0.37***</td>
</tr>
<tr>
<td>Abundance</td>
<td>—</td>
<td>0.22</td>
<td>0.31</td>
<td>0.49***</td>
<td>—</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*p < 0.01.
**p < 0.005.
***p < 0.001.

cluster of beta I (1 OTU, 7 sequences), with other uncultured beta I bacteria (7 OTUs, 12 sequences), with *Polynucleobacter* sp. (1 OTU), and with *Nitrospira* sp. (1 OTU). The diversity of *Betaproteobacteria* was very well covered in 10 and 12.5 m depth (72% and 75% of Chao1 estimates, respectively), whereas the 15-m layer was clearly undersampled.

Nine OTUs from the acI clade and five from the acIV cluster were found exclusively in the *P. rubescens* layer, and so were more than one third (37%) of all OTUs affiliated with *Bacteroidetes*. Specifically, these OTUs were related to *Flavobacteria* (6), the *Flexibacter aggregans* clade (7 out of 17), *Sphingobacterium* sp., *Flectobacillus* sp., and other sphingobacterial OTUs that have been detected in freshwaters, microbial mats, biofilms, sediments, and soils. Unique betaproteobacterial OTUs in 12.5 m depth were affiliated with *Leptothrix* (1), *Rhodoferax* (2), *LD28* (1), and the ammonium oxidizing *Nitrospira* (1).

Discussion

Spatiotemporal dynamics of *P. rubescens*—*P. rubescens* was detected in Lake Zurich more than 110 yr ago and has since then been intensely studied with respect to its physiology, toxicity (Kurmayer and Jüttner 1999; Blom et al. 2006), and population dynamics (Walsby and Schanz 2002; Walsby et al. 2004). The densities of these cyanobacteria substantially declined during the period of strongest eutrophication (1960–1970), but they reappeared with decreasing phosphorus loads, eventually accounting for half of total phytoplankton biomass in summer and for up to 80% in autumn and winter (Micheletti et al. 1998). Our study shows that *P. rubescens* currently still represents the dominant primary producer in Lake Zurich (Fig. 2C,D). However, in comparing our results with previous studies, the epilimnetic water temperature in summer 2007 seemed to be colder than average summer water temperatures. A maximum of only 22°C was reached, compared with 26°C in several previous years (Micheletti et al. 1998); thus in 2007 the epilimnion was probably less stable and there was more entrainment during the summer months than in other years.

Micheletti et al. (1998) reported that the productive period of *P. rubescens* in Lake Zurich was mainly limited to metalimnetic growth during the summer stratification. Our results suggest that there was net increase both during the periods of thermal stratification and of autumnal epilimnetic mixis. This was revealed by two independent approaches, the precise measurement of filament bio-volumes and lengths (Fig. 1D; Zeder et al. 2010) and by weighting the biomass estimates by the respective water volumes of the different depth layers (Fig. 4A). Repeated short-lived epilimnetic maxima of *P. rubescens* during the summer stratification (Fig. 1C) were likely the effect of longer periods of low daily insolation (Walsby et al. 2004). In such situations the mixing depth can exceed the depth of cyanobacterial neutral buoyancy (Fig. 1C) so that the population will partly get entrained in the epilimnion. The introduction of filaments in zones of higher irradiance and turbulence typically led to pronounced aggregation. This might explain their rapid disappearance from the mixed epilimnetic layer within 1–2 weeks (Figs. 1C,D, 4A), e.g.,
via faster sinking rates of aggregates or wind driven drift to the banks. The decreasing levels of irradiance (Fig. 1C) were probably also responsible for the massive epilimnetic growth of cyanobacteria during autumn (Figs. 1C,D, 4). Interestingly, this autumnal population of _P. rubescens_ was less prone to aggregation and less rapidly eliminated from the epilimnion, potentially hinting at successions of specific genotypes.

The determination of mean length of filaments allowed for additional distinction of the two growth phases: While the metalimnetic biomass maxima of _P. rubescens_ were mainly due to higher filament numbers (Fig. 1C), the autumnal biomass production was due to the significantly higher filament length (Fig. 1E). This pattern most likely reflected contrasting growth strategies. Knowledge about the relationship between cyanobacterial population growth and filament length is scarce. Typically, shorter filament lengths are associated with poor growth conditions such as aging cultures (Seki et al. 1981) and nutrient stress (P limitation, Poulíčková et al. 2004; and N limitation, Adamec et al. 2005). During summer stratification, _P. rubescens_ filaments are highly concentrated into the relative thin metalimnetic layer leading to self shading and possible intraspecific competition. Autumnal circulation creates a dilution of filament abundances (Fig. 1C). The advantages of being closer to the surface, thus getting increased light conditions, together with the release of intraspecific competition pressure could have been the reason for the observed increasing filament length and biomass (Figs. 1E, 4).

**Niche differentiation of _P. rubescens_ and coccoid cyanobacteria**—We observed a clear negative relationship between the spatial and temporal abundance distributions of _P. rubescens_ and picocyanobacteria (probably *Synechococcus* spp.) (Fig. 3A; Table 1). During the summer stratification...

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**Fig. 4.** (A) Depth-integrated total biomass (kg carbon) of heterotrophic bacteria and _P. rubescens_ in the upper 20 m of Lake Zurich. The biomass of _P. rubescens_ is presented for three depth layers: epilimnion, metalimnion, and hypolimnion. (B) Correlation between the total biomass of heterotrophic bacterial and _P. rubescens_ in the upper 20 m of Lake Zurich. The dotted line links subsequent sampling dates, and the solid line indicates the linear regression.
tion both groups coexisted by occupying different vertical niches in the water column, with picocyanobacteria being mainly restricted to the epilimnion and *P. rubescens* stratified in the metalimnion. Owing to their small size and high surface to volume ratio, picocyanobacteria have a competitive advantage at the nutrient-depleted conditions of the epilimnion in summer (Winder 2009); e.g., *Synechococcus* has a higher affinity for orthophosphate and higher uptake rates than eukaryotic algae (Lavallee and Pick 2002). Interestingly, the repeated introduction events of *P. rubescens* filaments into the epilimnion were paralleled by immediate declines of picocyanobacteria (Figs. 1C, 3A). This negative effect might be related to decreasing insolation at such occasions (Table 1), since the spherical light intensities were correlated negatively with the abundances of *P. rubescens* but positively with those of picocyanobacteria (Table 1). However, the abrupt total disappearance of the latter suggests other reasons besides light limitation, e.g., protistan predation (Pernthaler et al. 1996) or allelopathic interaction (Sukenik et al. 2002). So far, there is no evidence for the release of allelopathic substances by *P. rubescens*. On the other hand, the observed negative correlation might also reflect a negative effect of picocyanobacteria on *P. rubescens* as a result of decreasing light intensity in the metalimnion.

**Interactions between *P. rubescens* and heterotrophic bacteria**—In contrast to the body of knowledge about *P. rubescens*, information about heterotrophic bacteria in Lake Zurich is scarce (Köster and Jüttner 2000; Zeder et al. 2009). A positive correlation is often observed between primary production and heterotrophic bacterial biomass (Simon et al. 1998), and cyanobacterial exudates can represent an important proportion of the organic carbon supply of aquatic bacteria (Wang and Priscu 1994). However, previous studies reported very low DOC excretion rates by *P. rubescens* (Feuillade et al. 1988), and these cyanobacteria even seem to have a mechanism to reassimilate their own exudates (Chang 1981). This questions the role of *P. rubescens* as a carbon source for heterotrophic bacteria. In our study we found stable DOC concentrations in Lake Zurich during the entire cyanobacterial bloom period (Fig. 2A) and a significant negative correlation between the abundances, biovolumes (Table 1), and depth-integrated biomasses (Fig. 4B) of *P. rubescens* and heterotrophic bacteria. In contrast, picocyanobacteria and heterotrophic bacteria were positively correlated (Table 1), as was also reported by Eiler and Bertilsson (2004).

In general, heterotrophic bacteria and primary producers are known to compete for the same nutrients (Mindl et al. 2005; Løvdal et al. 2007). However, the competitive

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**Fig. 5.** Diversity and similarity of microbial 16S rDNA collected from the three depth layers (10, 12.5, and 15 m depth) on 01 October 2007. (A) Overlap of the numbers of bacterial OTUs (97% sequence similarity level) in the three depth layers and of OTUs affiliated with *Actinobacteria, Bacteroidetes*, and *Betaproteobacteria*, respectively (analyzed separately). (B) Number of OTUs, sequences (in brackets), and the estimated OTU richness at 97% sequence similarity (Chao1 coverage) of *Bacteria, Actinobacteria, Bacteroidetes*, and *Betaproteobacteria* in the different depth layers.

<table>
<thead>
<tr>
<th>B</th>
<th>Total community</th>
<th>Actinobacteria</th>
<th>Bacteroidetes</th>
<th>Betaproteobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OTUseqs (sequences)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 m</td>
<td>66 (186)</td>
<td>19 (39)</td>
<td>25 (47)</td>
<td>10 (21)</td>
</tr>
<tr>
<td>12.5 m</td>
<td>80 (181)</td>
<td>28 (67)</td>
<td>33 (55)</td>
<td>8 (17)</td>
</tr>
<tr>
<td>15 m</td>
<td>41 (79)</td>
<td>16 (36)</td>
<td>18 (35)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>138 (446)</td>
<td>44 (142)</td>
<td>55 (137)</td>
<td>16 (40)</td>
</tr>
<tr>
<td></td>
<td>Chaó coverage (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 m</td>
<td>45.2</td>
<td>51.4</td>
<td>43.7</td>
<td>72.0</td>
</tr>
<tr>
<td>12.5 m</td>
<td>40.1</td>
<td>44.0</td>
<td>45.9</td>
<td>75.3</td>
</tr>
<tr>
<td>15 m</td>
<td>62.2</td>
<td>64.1</td>
<td>84.0</td>
<td>53.3</td>
</tr>
<tr>
<td>Total</td>
<td>41.9</td>
<td>37.1</td>
<td>58.4</td>
<td>51.6</td>
</tr>
</tbody>
</table>
interactions between P. rubescens and heterotrophic bacteria might be even more complex if the photoheterotrophic capabilities of these cyanobacteria are taken into account (Feuillade et al. 1988; Zotina et al. 2003; Walsby and Jüttner 2006). The uptake rates of amino acids by P. rubescens are particularly high at low light levels even below the photosynthetic compensation irradiance, and they are sufficient to even allow for slow filament growth under such conditions (Zotina et al. 2003; Walsby and Jüttner 2006). During the summer metalimnetic bloom in 2007 a considerable proportion of the P. rubescens population was situated below the light compensation depth (Fig. 1C). This indicates that heterotrophic uptake is relevant for the survival of these filaments. The biomass-specific incorporation rates of P. rubescens for free dissolved amino acids are about 10 times lower than those of heterotrophic bacteria (Zotina et al. 2003; Salcher et al. 2010). However, since P. rubescens represented 10 times more biomass than bacteria in the metalimnion (Fig. 4), these cyanobacteria should nevertheless be considered as an important competitor for organic carbon.

Associations between P. rubescens and specific bacterial groups—Ecological interactions between cyanobacteria and heterotrophic bacteria range from parasitism through competition and commensalism to mutualism (Cole 1982). Heterotrophic bacteria can be physically attached to cyanobacterial cells. The mucilaginous colonies of Microcystis spp. often represent hotspots of bacterial abundance and activity (Worm and Søndergaard 1998). Cyanobacteria may profit from the ecto-enzymatic activity of associated bacteria, e.g., the release of amino acids or inorganic phosphate at their cell surface (Stoecker et al. 2005). Although P. rubescens occurred as a massive monospecific bloom in Lake Zurich (Fig. 2D), filaments with bacterial attachment were rarely observed. This suggests that this cyanobacterial species may actively resist colonization.

Interactions between cyanobacteria and heterotrophic bacteria are not limited to direct physical association. A wide variety of secondary metabolites are produced by cyanobacteria, which can either stimulate or inhibit the growth of different free-living heterotrophic bacteria, thereby influencing microbial community structure (Kirkwood et al. 2005; Valdor and Aboa 2007). Some bacteria are even capable of degrading cyanotoxins (Christoffersen et al. 2002). Park et al. (2001) isolated a microcystin-degrading Sphingomonas sp. from the surface water of a hypertrophic lake. Interestingly, Sphingomonas were also present in our clone libraries, and one OTU from this lineage was unique for the P. rubescens layer. However, high concentrations of secondary metabolites in the water are typically associated with the collapse of blooms due to unfavorable growth conditions, viral cell lysis (Deng and Hayes 2008) or decomposition. The persistent character of the P. rubescens bloom in Lake Zurich may thus diminish the opportunity for bacteria to either decompose filaments directly or to use compounds released by senescent P. rubescens cells.

Our analysis of the bacterial community composition at the level of large phylogenetic groups revealed only weak evidence for a particular bacterial group associated with a P. rubescens bloom (Table 1). In fact, there might be no typical microflora accompanying such blooms: Eiler and Bertilsson (2004) reported that the environmental conditions created by mass development of cyanobacteria in four different lakes produced very dissimilar bacterial communities. Nevertheless, a highly significant positive relationship ($p < 0.005$) was found between the abundances of Cytophaga-Flavobacteria (CF) (Table 1). These bacteria sporadically accumulated in the lower metalimnetic waters during the autumnal period (Fig. 3C).

CF are known for their ability to degrade biopolymers such as cellulose and chitin (Cottrell and Kirchman 2000), and isolates from the family Cytophagaceae have been obtained that are capable of lysing living cyanobacterial cells (Rashid and Bird 2001). Members of CF were also enriched in water samples with decomposing P. rubescens grown at unfavorable light and temperature conditions in the laboratory for 2 weeks (unpubl. data). In addition, a high proportion of rRNA gene sequences from our libraries was affiliated with Bacteroidetes (Fig. 5). They represented > 40% of all OTUs from the P. rubescens layer and approximately one-third of the Bacteroidetes OTUs were exclusively present in this zone (Fig. 5). More than 40% of the Bacteroidetes sequences of our libraries were affiliated with Flexibacteraceae, and seven of 17 OTUs from this lineage were present only in the layer of maximal P. rubescens densities (12.5-m depth). Eiler et al. (2006) found Flexibacteraceae that were associated with the bloom-forming cyanobacterium Gloeotrichia echinulata but were absent in water samples. It should be noted that the apparently low community overlap and the high numbers of unique OTUs in the three sampling depths (which were only 2.5 m apart from each other) might in part also be a result of undersampling. However, the diversity of sequence types affiliated with Bacteroidetes in 15 m depth (likely the zone of P. rubescens decomposition) was almost completely covered (84%), which again supports the notion that some genotypes from this group might specifically occur together with physiologically stressed cyanobacterial filaments.

In summary, our study illustrates that the persistent bloom of the dominant primary producer of Lake Zurich in metalimnetic waters and its autumnal spreading to the epilimnion may have profound consequences for other autotrophic and heterotrophic planktonic microbes. Moreover, while P. rubescens appears to be an effective competitor both for nutrients and organic carbon, there are also indications that some bacterial taxa (e.g., affiliated with Flexibacteraceae) might nevertheless profit from the presence of these cyanobacteria. Future laboratory studies with single heterotrophic bacterial isolates in combination with axenic P. rubescens isolates will be needed to get further insights into the survival or coexistence of these planktonic components.

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References


P. rubescens in Lake Zurich


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