RESEARCH ARTICLE

Covariation between zooplankton community composition and cyanobacterial community dynamics in Lake Blaarmeersen (Belgium)

Ineke van Gremberghe[1], Jeroen Van Wichelen[1], Katleen Van der Gucht[1], Pieter Vanormelingen[1], Sofie D'hondt[1], Christophe Boutte[2], Annick Wilmotte[2] & Wim Vyverman[1]

[1]Laboratory of Protistology and Aquatic Ecology, Department of Biology, Ghent University, Ghent, Belgium; and [2]Institute of Chemistry, University of Liège, Liège, Belgium

Correspondence: Ineke van Gremberghe, Laboratory of Protistology and Aquatic Ecology, Department of Biology, Ghent University, Krijgslaan 281 S8, 9000 Ghent, Belgium. Tel.: +32 9 264 85 42; fax: +32 9 264 85 99; e-mail: ineke.vangremberghe@ugent.be

Received 13 June 2007; revised 12 September 2007; accepted 20 October 2007.
First published online 17 December 2007.

DOI:10.1111/j.1574-6941.2007.00422.x

Editor: Riks Laanbroek

Keywords
denaturing gradient gel electrophoresis; cyanobacteria; 16S rRNA gene; zooplankton; *Synechococcus*; mesotrophic lake.

Abstract

The cyanobacterial community composition in the mesotrophic Lake Blaarmeersen was determined by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments during two consecutive years to assess the importance of different classes of explanatory variables (bottom-up and top-down factors, physical variables and phytoplankton) in cyanobacterial community dynamics. The most dominant cyanobacteria in Lake Blaarmeersen were *Synechococcus* (three genotypes), *Limnothrix redekei* and *Anabaena/Aphanizomenon*. Analyses of Similarity revealed that the cyanobacterial community in Lake Blaarmeersen differed significantly between the growing season and the winter season as well as between the epilimnion and hypolimnion during the stratified periods. Mantel tests revealed significant correlations between the DGGE data and bottom-up factors, physical variables, the phytoplankton community composition and, interestingly, the zooplankton community composition. In general, the zooplankton community composition (especially the cladoceran community) was more important in structuring the cyanobacterial community than the total zooplankton biomass. This study shows that grazing zooplankton communities can have a relatively strong impact on the cyanobacterial community dynamics and that this impact can be equally important as bottom-up processes regulated by nutrient concentrations and/or physical variables.

Introduction

Cyanobacteria have been attracting considerable attention in the last decade, as these autotrophic prokaryotes often dominate phytoplankton communities in nutrient-rich freshwater lakes during warm weather conditions. Because many freshwater lakes worldwide have been affected by eutrophication, blooms of particular cyanobacterial species have become a common and recurrent phenomenon (Mur *et al.*, 1999; Codd *et al.*, 2005; Zurawell *et al.*, 2005; Jayatissa *et al.*, 2006).

Freshwater cyanobacterial communities are usually composed of biochemically and ecologically distinct species. For instance, different species and strains produce distinct secondary metabolites that can be toxic for eukaryotes (e.g. Milutinovic *et al.*, 2003; Pflugmacher, 2004; Viaggiu *et al.*, 2004; Rohrlack *et al.*, 2005), some cyanobacteria fix nitrogen while others do not (Ni *et al.*, 1990; Downing *et al.*, 2001; Fiore *et al.*, 2005) and particular species exhibit different optima in relation to environmental parameters (Huisman & Hulot, 2005). Therefore, it is interesting to know the environmental conditions that tend to lead to the dominance of certain species. Whereas several studies revealed the influence of nutrients and physical variables (e.g. light, temperature, turbulence . . .) on the cyanobacterial community composition (e.g. Jacoby *et al.*, 2000; Salmaso, 2000; Pandey & Pandey, 2002; Noges *et al.*, 2003), the potential importance of top-down factors considering cyanobacterial community dynamics is poorly studied. This is probably due to the fact that cyanobacteria are thought to be less susceptible to grazing by zooplankton in comparison with other phytoplankton groups because they can form large

colonies or filaments (Lampert, 1987; Gliwicz & Lampert, 1990; DeMott *et al.*, 2001). Although some colonies and filaments are indeed too large to be consumed even by macrozooplankton, smaller cyanobacteria, being unicellular or consisting of small colonies or filaments, can easily be grazed. Several studies have reported grazing by *Daphnia* (e.g. DeBernardi *et al.*, 1981; Holm & Shapiro, 1984; Rohrlack *et al.*, 1999; Work & Havens, 2003), *Bosmina* (Work & Havens, 2003), copepods (Haney, 1987; DeMott & Moxter, 1991; Koski *et al.*, 2002; Work & Havens, 2003), rotifers (Starkweather & Kellar, 1983; Gilbert, 1996), ciliates (Brabrand *et al.*, 1983) and flagellates (Nishibe *et al.*, 2002) on a range of cyanobacterial species. Some zooplankton species graze more efficiently on cyanobacteria than others (Lampert, 1987). Copepods for example can select cyanobacteria based on size and chemical characteristics like toxicity and nutritional value (DeMott & Moxter, 1991; Engström *et al.*, 2000). Therefore, in lakes with moderate to high nutrient concentrations where the cyanobacterial community is diverse, it is likely that both zooplankton abundance and composition play a role in cyanobacterial community dynamics. Previous studies indicate that grazing by zooplankton can influence the phytoplankton and the bacterioplankton community composition considerably (Bergquist *et al.*, 1985; Degans *et al.*, 2002; Muylaert *et al.*, 2002; Sarnelle, 2005). Although the effect of zooplankton grazing might be expected to be less pronounced concerning cyanobacteria (Sellner *et al.*, 1993), more information is needed about the relationships between zooplankton and cyanobacterial community dynamics.

This study aims to evaluate the impact of top-down factors relative to other important explanatory variables on seasonal changes in the cyanobacterial community in Lake Blaarmeersen. The cyanobacterial community in the lake was monitored for 2 years using denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments. Furthermore, the cyanobacterial community composition was related directly to top-down, bottom-up and other explanatory variables.

Materials and methods

Study site and sample collection

Lake Blaarmeersen (51°25′ N, 3°41′ E) is a temperate monomictic sand-pit lake with a high recreational value in the city of Ghent. Since 1982, the lake and the surrounding area has been opened to the public for recreation purposes. In the years following its construction in 1976, it was an oligotrophic, macrophyte-dominated lake but, like many other lakes in Belgium, eutrophication has resulted in a gradual increase of phytoplankton biomass, including cyanobacteria (Geysen & Coppejans, 1985; several unpublished studies). Lake Blaarmeersen has a surface area of almost 20 ha and a maximal depth of 15 m.

Samples from Lake Blaarmeersen were taken fortnightly in 2003 (but monthly in winter) and monthly in 2004 at a depth of 0.5 and 7.5 m (some extra samples were taken at a depth of 1.5 and 3.5 m) at a fixed position in the lake where the water depth is maximal (15 m). Phytoplankton samples were taken with a vertical point water sampler. These samples were fixed with the alkalic lugol-formalin-sodiumthiosulphate method (Sherr & Sherr, 1993). On each occasion, a depth-integrated (0, 2.5, 5, 7.5, 10 m) zooplankton sample was gathered with a Schindler–Patalas sampler at the same locality. Zooplankton samples were fixed with a sugar-saturated formaldehyde solution (end concentration 4%) (Haney & Hall, 1973). Water samples for DNA extraction were filtered over a 25 mm 0.2 μm GSWP membrane filter (Millipore) and frozen as soon as possible at $-20\,^{\circ}C$. A depth profile for oxygen concentration, temperature, conductivity and pH was made with a conductivity–temperature-depth (CTD) multi-meter (YSI 650 MDS). An underwater light profile was made with a Licor 1400 Data logger and two sensors (Aquamatic AOPL UV912). Water transparency was also measured with a Secchi-disk. For determination of dissolved nutrient concentrations (nitrate, ammonium and orthophosphate), water was filtered over a GF/F glass fibre filter and stored frozen until analysis using a Skalar auto-analyser (Grasshoff, 1976; Koroleff, 1976). Suspended matter was measured gravimetrically after filtration of a known volume of water on dried preweighed GF/F filters.

Microscopical determination and counting

Phytoplankton (including filamentous cyanobacteria) was identified according to Tikkanen & Willénn (1992) and counted with an inverted microscope (Zeiss Axiovert), expressed as numbers per millilitre and converted into biomass ($\mu g\,C\,L^{-1}$) by determining the average biovolume (μm^3) of each taxon (Menden-Deuer & Lessard, 2000). Phytoplankton was identified up to genus or species level. For the samples from 2004, an extra counting of picocyanobacteria was carried out using epifluorescence (Zeiss Axioplan microscope) with black polycarbonate filters. Zooplankton was identified and counted as individuals per litre with a binocular loupe and abundance was converted into biomass using published length-weight regressions (Bottrell *et al.*, 1976). Copepods were identified as calanoids, cyclopoids, copepodites and nauplii, cladocerans were identified up to genus level and rotifers were identified up to genus and species levels.

DNA extraction

Genomic DNA from the filters containing water samples was isolated as described by Zwart *et al.* (1998), which

includes the beat-beating method with phenol extraction and ethanol precipitation. After extraction, the DNA was purified on a Wizard column (Promega).

PCR amplification for DGGE

16S rRNA gene fragments were amplified using the nested PCR protocol described by Zwart *et al.* (2005). The specific cyanobacterial primers cya-b-F371 and cya-R783 and the general primers F357 (GC) and R518 were used in the first and second PCR, respectively. In the first PCR, a high specificity was obtained and in the second PCR the PCR fragment was shortened to obtain better profiles by DGGE. The first PCR was performed using the following reaction mixtures: 2 μL of template DNA, 0.5 μM of each primer, 200 μM of each deoxynucleoside triphosphate, 2.5 U of Taq DNA polymerase (Ampli Taq), 10 × PCR buffer [Tris/HCl: 100 mM, pH: 8.3; KCl: 500 mM; $MgCl_2$: 15 mM; Gelatine: 0.01% (w/v)] and 400 ng of bovine serum albumin. The mix was adjusted to a final volume of 50 μL with sterile water. The PCR program started with a denaturation step of 5 min at 94 °C. A touchdown procedure was performed consisting of 20 cycles in which the annealing temperature decreased by 1 °C every second cycle from 65 to 56 °C. Cycle step times were one minute each for denaturation (94 °C), annealing and extension (72 °C). A final extension step was performed for 10 min at 72 °C. After the cyanobacteria-specific amplification and purification with a QiaQuick PCR purification kit (QiaGen), 1 μL of PCR product was transferred to a new 50 μL reaction mixture containing the general primers described above. The PCR program started with a denaturation step of 5 min at 94 °C. A touchdown procedure was performed consisting of 20 cycles in which the annealing temperature decreased by 1 °C every second cycle from 65 to 56 °C and five additional cycles in which the annealing temperature was 55 °C. A final extension step was performed for 30 min at 72 °C. The presence of PCR products and their concentration was determined by analysing 5 μL of each PCR product on 1.66% agarose gels, staining with ethidium bromide and comparing them with a molecular weight marker (SmartLadder; Eurogentec).

DGGE profiling

DGGE was essentially performed as described by Muyzer *et al.* (1993). Equal amounts of PCR products were loaded onto 8% (w/v) polyacrylamide gels [1 mm thick, in 1X TAE (20 mM Tris acetate (pH 7.4), 10 mM acetate, 0.5 mM disodium EDTA)]. The denaturing gradient contained 35–60% denaturant [100% denaturant corresponded to 7 M urea and 40% (v/v) formamide]. Electrophoresis was performed for 16 h at 75 V and the temperature was set at 60 °C. Finally, the gels were stained with ethidium bromide and photographed on a UV transillumination table with a CCD camera. Furthermore, a small piece of gel from the middle of the target band was excised from the DGGE gel and incubated in 50 μL of sterile TE buffer (10 mM Tris, pH 7.6, 1 mM EDTA) for 24 h at 4 °C. The eluent was then reamplified and purified by DGGE one or two times. The resulting PCR products were purified using a QiaQuick PCR purification kit (QiaGen). Finally, sequencing was performed with the ABI Prism sequencing kit and the resulting sequencing reaction products were analysed on an automatic sequencer (ABI Prism 3100).

DGGE-profile analysis

On every gel, three standard lanes were analysed in parallel to the samples. These standard lanes were composed of several bands positioned distinctly in the gel. As these bands should always be formed at the same denaturant concentration in the gel, their position was used to compare the patterns formed in different gels. Digitalized DGGE images were analysed using the software package BIONUMERICS 4.5 (Applied Maths BVBA). The program detects the bands and groups the bands into band classes, based on their position in the gel. Sequence information of the bands was used to check the grouping of bands into band classes manually. Heteroduplex bands and very weak bands were removed. Further, a matrix was compiled, based on the band intensity or the presence or absence of bands in band classes. The band intensities were then converted into relative intensities (the relative contribution of each band to the total band signal in the lane).

Cloning of rRNA genes

16S rRNA gene PCR products from the sample taken in Lake Blaarmeersen on 18/07/03 at a depth of 0.5 m were cloned and sequenced as described in Boutte *et al.* (2005). The DNA was amplified with the primers CYA359F (Nübel *et al.*, 1997) and 23S30R (Taton *et al.*, 2003). The PCR conditions and PCR program were as described in Boutte *et al.* (2005). The TOPO TA Cloning Kit (Invitrogen) was used for cloning, following the manufacturer's instructions. Colonies were screened by a direct PCR with the primers CYA359F and CYA784R using an annealing temperature of 60 °C instead of 54 °C to increase the specificity of the detection. Each positive clone was sequenced using primer 1092R. Sequences were obtained from Genome Express (Meylan). Sequencing reactions were performed using an ABI Prism system 3730 XL^{TM}. The sequenced fragment has a length of 700–800 bp.

Sequence analysis and database deposits

The 16S rRNA gene sequences were screened against GenBank/EMBL using the algorithm BLAST (Altschul *et al.*,

1990) to identify the cyanobacteria. The sequence data have been submitted to the EMBL database under accession numbers AM410055–AM410064 for the DGGE bands and AM411877–AM411904 for the clones.

Statistical analysis

Multivariate statistics were used to investigate the relation between cyanobacterial community composition and explanatory variables. The similarity of DGGE profiles (only the dominant cyanobacteria *Synechococcus*, *Limnothrix redekei* and *Anabaena/Aphanizomenon* were selected from the DGGE profile and their relative band intensities were used), the cyanobacterial community obtained from counting (relative density), phytoplankton community composition (absolute biomass of taxa without cyanobacteria), zooplankton community composition (absolute biomass of taxa) and community composition of copepods, cladocerans and rotifers (absolute biomass of the three groups) was determined using the Bray–Curtis Index of Similarity (Primer 5, Clarke & Gorley, 2001). For each of these datasets, a similarity matrix was prepared using log $(x+1)$-transformed data. The Bray–Curtis similarity matrix of the DGGE profile was used to create a dendrogram, using weighted group average linkage in cluster analysis (Primer 5, Clarke & Gorley, 2001). Dissimilarity matrices of environmental data were calculated using Euclidean distances (Primer 5, Clarke & Gorley, 2001). Two matrices were constructed separately: one including the dissolved nutrients (nitrate, ammonium, total dissolved nitrogen and orthophosphate) and one including the physical variables (temperature, Secchi depth, oxygen level, pH and conductivity). Nutrient data were fourth root transformed and Secchi depth and conductivity were log $(x+1)$ transformed. Dissimilarity matrices of total zooplankton biomass, biomass of *Daphnia* and *Bosmina*, total cyanobacterial biomass and phytoplankton biomass were calculated using Euclidean distances (Primer 5, Clarke & Gorley, 2001) on log $(x+1)$-transformed data.

Analyses of Similarity (ANOSIM) using 999 permutations were run to test for statistically significant differences in cyanobacterial assemblage (Primer 5, Clarke & Gorley, 2001). ANOSIM generates a test statistic R, and the magnitude of R is indicative of the degree of separation between groups, with a score of 1 indicating complete separation and 0 indicating no separation. Monte–Carlo randomization of the group labels was used to generate null distributions in order to test the hypothesis that within-group similarities are higher than would be expected if sample DGGE profiles were grouped at random.

Mantel tests (Mantel, 1967; Mantel & Valand, 1970) were used to investigate the relation between cyanobacterial community composition and abiotic/biotic variables. In the Mantel test, the null hypothesis is that distances in a matrix A are independent of the distances, for the same objects, in another matrix B. Testing of the null hypothesis is performed by a randomization procedure in which the original value of the statistic is compared with the distribution found by randomly reallocating the order of the elements in one of the matrices. The statistic used for the measure of the correlation between the matrices is the Pearson correlation coefficient. Simple Mantel tests evaluate whether the association between two independent similarity matrices, describing the same set of entities, is stronger than one would expect from chance. Partial Mantel tests are used to determine the relationship between two matrices while controlling the effect of a third matrix. Simple and partial Mantel tests were carried out by the ZT software tool (Bonnet & Van de Peer, 2002) using Bray–Curtis similarity matrices for community data and Euclidean distance dissimilarity matrices for environmental data and total biomass. The program MANTELTESTER (http://manteltester.berlios.de) was used to automate the Mantel tests.

Spearman rank correlation coefficients were calculated using STATISTICA 6.0 for Windows (StatSoft Tulsa http://www.statsoft.com) to obtain extra information about positive or negative correlations among the different cyanobacterial genotypes (relative intensities of single DGGE band classes) and to obtain information about positive or negative correlations between each cyanobacterial genotype and biomass of *Daphnia* and *Bosmina*. In addition, Spearman rank correlation coefficients were calculated between the total cyanobacterial biomass and the biomass of *Daphnia* and *Bosmina*. All data were log $(x+1)$ transformed before the analyses.

Results

Physico-chemical characteristics

Lake Blaarmeersen is a typical temperate, monomictic lake. The water layers are well mixed during winter and thermal stratification occurs predominantly in summer. As a result, during the study period the water was well oxygenated during complete mixing but as soon as thermal stratification started, an oxycline developed first in deeper water and later more at the surface, dividing the water body into a well-oxygenated epilimnion and a poorly oxygenated hypolimnion. In summer, severe oxygen depletion (with values $< 1\,\mathrm{mg\,O_2\,L^{-1}}$) was observed. During winter turnover, the euphotic depth was lower than the mixing depth and the primary producers could encounter light limitation when they were transported passively in the deeper darker zones. In late spring, a typical spring clear water phase was visible when the amount of suspended matter was lowest and

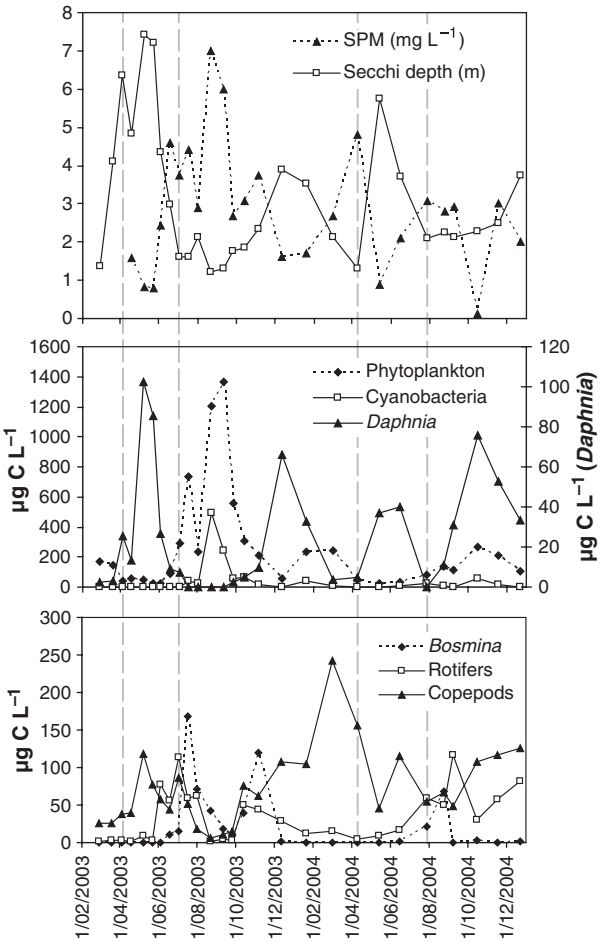

Fig. 1. Seasonal variation in water clarity expressed as the amount of suspended matter (SPM) or Secchi depth (top), total phytoplankton biomass (including cyanobacteria), cyanobacterial biomass and biomass of *Daphnia* (middle), and biomass of *Bosmina*, rotifers and copepods (bottom) in Lake Blaarmeersen in 2003 and 2004 [for SPM, phytoplankton and zooplankton biomass (µg C L^{-1}), the data from the epilimnion (0.5 m) are used].

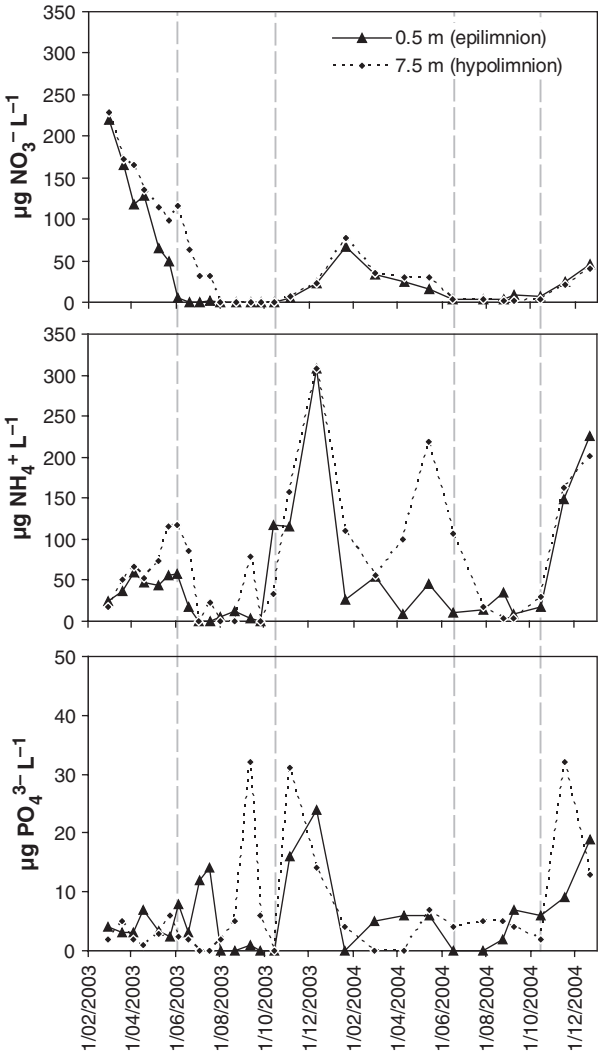

Fig. 2. Seasonal fluctuations in the concentration of dissolved nutrients in the water column of Lake Blaarmeersen in 2003 and 2004 in the epilimnion and hypolimnion.

Secchi depth and euphotic depth were maximal. This was probably induced by the high grazing pressure on phytoplankton, mainly by large cladocerans (*Daphnia*) at that time (see Fig. 1). The pH values were higher in times when there was more phytoplankton production (mainly in summer at the surface of the water column). The total ion concentration was somewhat higher at the bottom of the lake during the stratified period due to the presence of the sediment layer. The highest concentrations of dissolved nutrients (total dissolved nitrogen: up to 300 µg L^{-1}, orthophosphate: up to 30 µg L^{-1}) were observed in autumn and winter when organic matter became mineralized and the water column was mixed up to the bottom. During summer, dissolved nutrients became depleted in the epilimnion (see Fig. 2).

Zooplankton community

In 2003 as well as in 2004, the zooplankton community was mainly dominated by predatorous as well as herbivorous rotifers, but the macrozooplankton community also reached high numbers. The herbivorous cladoceran *Daphnia* was encountered mainly in spring ('clear water phase') in both years and in winter 2003, but the highest density was found in October 2004 (up to 50 individuals L^{-1}). *Bosmina* was very abundant in summer and autumn 2003 (up to 170 individuals L^{-1}), a lower density was seen in summer 2004. Other cladocerans detected in Lake Blaarmeersen were *Ceriodaphnia* (low density in autumn 2003) and *Leptodora* (low density in summer and autumn 2004). Copepods were encountered mostly in spring and winter in both years but

with maximum abundance in early spring 2004, just before the 'clear water phase' (up to 180 individuals L^{-1}). Calanoid copepods were present in spring 2003, after which they were replaced by cyclopoid copepods. High numbers of rotifers were encountered in early summer 2003, just after the 'clear water phase' (more than 2000 individuals L^{-1}). In August and September, rotifers were scarce, whereas in October, November and December they were abundant again. The same pattern was seen in 2004, although the total density was lower in comparison with 2003. The most abundant rotifers in Lake Blaarmeersen were *Polyarthra* sp., *Keratella cochlearis*, *Keratella quadrata*, *Asplanchna priodonta*, *Synchaeta* sp., *Kellicottia longispina* and *Pompholyx complanata*. To compare the grazing capacity of different zooplankton groups and species, biomass is more appropriate than numbers; therefore, the zooplankton community composition was studied using biomass data (see Fig. 1).

Phytoplankton community

Similar values were obtained for the total biomass of phytoplankton at a depth of 0.5 m (epilimnion) and 7.5 m (hypolimnion), except in summer 2003 where the biomass was lower in the hypolimnion compared with the epilimnion. High biomass of phytoplankton (up to 1300 $\mu g\,C\,L^{-1}$) was especially seen in summer and autumn (see Fig. 1). The phytoplankton that dominated most frequently in Lake Blaarmeersen were cryptophytes, but dinophytes, chlorophytes, cyanobacteria and diatoms also reached high numbers in particular periods. In 2003, in early spring (end February), a bloom of small centricate diatoms (*Stephanodiscus* sp.) was detected, which was followed by a spring bloom (just before the 'clear water phase') of cryptophytes (*Cryptomonas* sp.). After the 'clear water phase', in June and July, high numbers of dinophytes (*Ceratium furcoides*, *Peridinium* sp.) were encountered, whereas in late summer filamentous phytoplankton (chlorophytes: *Mougeotia* sp. and cyanobacteria: *Anabaena* sp., *Aphanizomenon flos-aquae* and *Limnothrix redekei*) was present in the lake. In autumn, there was again a bloom of cryptophytes. In 2004, the same pattern was observed, although there were some differences in the summer period because cyanobacteria, diatoms, dinophytes and chlorophytes all reached high numbers at that time.

In general, cyanobacteria had a rather low contribution to the total phytoplankton biomass and reached a maximal contribution during summer and autumn (see Fig. 1). During the sampling period, the biomass of cyanobacteria was similar or lower in the hypolimnion compared with the epilimnion, except in late summer and early autumn of 2004.

Cyanobacterial community

A significant correlation ($r = 0.145$; $P = 0.008$) was found by a simple Mantel test (ZT, Bonnet & Van de Peer, 2002) between the cyanobacterial community composition data (relative contribution of the most dominant groups: *Synechococcus*, *Limnothrix redekei* and *Anabaena/Aphanizomenon*; see further) obtained by counting and obtained by DGGE for the growing season. The rather low correlation was probably mainly due to the fact that *Synechococcus* was underestimated in the counting data of 2003 because no extra counting with a fluorescence microscope was performed for these samples. Furthermore, at several sampling dates during winter and spring no cyanobacteria were detected by the microscope in contrast to DGGE by which it was still possible to detect them. Considering these aspects, DGGE was used to study the cyanobacterial community composition.

Figure 3 shows the DGGE profile obtained by cyanobacteria-specific PCR amplification of DNA from samples taken in Lake Blaarmeersen from April 2003 to December 2004 at a depth of 0.5 and 7.5 m (and a few samples at a depth of 1.5 and 3.5 m). Owing to the fact that cyanobacteria were not dominant in the lake, many chloroplast sequences of other phytoplankton groups were also amplified (see Table 1). However, the sequence information of the bands was used to select the dominant cyanobacterial taxa and all the statistical analyses (see further) were performed on the community composition of *Synechococcus* (band class 10, 14 and 18), *Limnothrix redekei* (band class 16) and *Anabaena/Aphanizomenon* (band class 19) (see Fig. 3 and Table 1). The dominance of these cyanobacteria was confirmed by the counting data (these taxa reached the highest absolute biomass). Additionally, more 16S rRNA gene sequence information of these three cyanobacterial taxa was obtained by cloning to confirm and complement the DGGE data (see the 'Materials and methods' section for accession numbers of the sequences).

The most abundant cyanobacterium in the DGGE profile is *Synechococcus*, which is represented by three genotypes (band classes 10, 14 and 18; see Fig. 3 and Table 1). The genotype on position 10 was present in Lake Blaarmeersen during the whole sampling period at both depths, whereas the genotype on position 18 was only found in summer and autumn and the genotype on position 14 was only detected in the summer of 2004 in the hypolimnion. The cyanobacterium *Limnothrix redekei* (band class 16; see Fig. 3 and Table 1) was dominant in 2003 in summer in the hypolimnion and in autumn at both depths. The nitrogen-fixing cyanobacterium *Anabaena/Aphanizomenon* was detected in late summer 2003 in the epilimnion (band class 19; see Fig. 3 and Table 1). The relative contributions of *Synechococcus* genotypes, *Limnothrix redekei* and *Anabaena/Aphanizomenon* at a depth of 0.5 m and 7.5 m detected by DGGE are also visualized in Fig. 4. ANOSIM (Primer 5, Clarke & Gorley, 2001) revealed significant differences in the cyanobacterial community composition between the 'growing season' (late

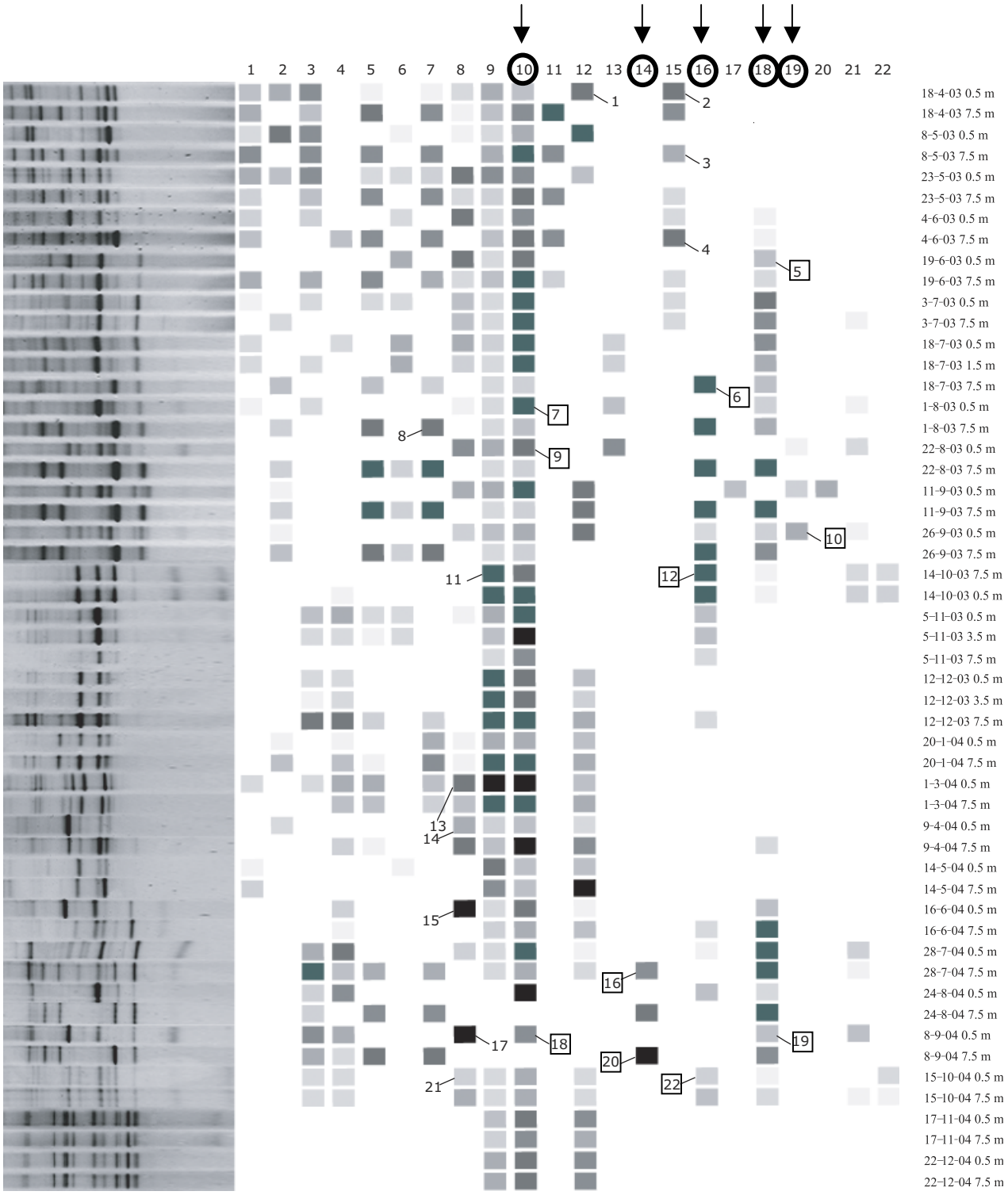

Fig. 3. Photograph of the original DGGE gels (left) and digitalized DGGE profile (right) obtained by cyanobacteria-specific PCR amplification of DNA from samples taken in Lake Blaarmeersen in 2003 and 2004. Sampling dates and depths are indicated at the far right side of the figure. Bands are grouped into band classes (numbers of band classes are shown on top of the figure; see also Table 1). Individual bands that were sequenced are indicated and numbered (see also Table 1). Heteroduplex bands were removed from the band classes. Band classes representative for the most dominant cyanobacteria and used in the statistical analyses are indicated with arrows on top of the figure and circles around the band class number. Sequenced bands in these band classes have squares around their band number.

Table 1. Closest relatives of sequenced bands from DGGE profile found in GenBank/EMBL (for numbers of bands and band classes see Fig. 3, accession numbers of sequences in EMBL: AM410055–AM410064)

Number band class	Number band	Sequence length (bp)	Closest match	Accession number of closest match	Percentage similarity (bp/bp)
7	8	88	Chloroplast (Diatom)	AY858017	100 (87/87)
8	13, 14, 15, 17, 21	136	Chloroplast	DQ363182	100 (129/129)
9	11	137	Chloroplast (Diatom)	AY678496	99 (132/133)
10	7, 9, 18	136	*Synechococcus*	AY436570	100 (118/118)
12	1	114	Chloroplast (Cryptophyte)	AY453067	100 (113/113)
14	16, 20	136	*Synechococcus*	AF448068	99 (129/130)
15	2, 3, 4	122	Chloroplast (Diatom)	AF418973	100 (121/121)
16	6, 12, 22	134	*Limnothrix redekei*	AJ544070	100 (128/128)
18	5, 19	120	*Synechococcus*	AY224199	100 (117/117)
19	10	121	*Aphanizomenon flos-aquae*	AY038035	100 (118/118)
			Aphanizomenon gracile	AJ293124	100 (118/118)
			Anabaena mendotae	AJ293107	100 (118/118)
			Anabaena affinis	AF247591	100 (118/118)

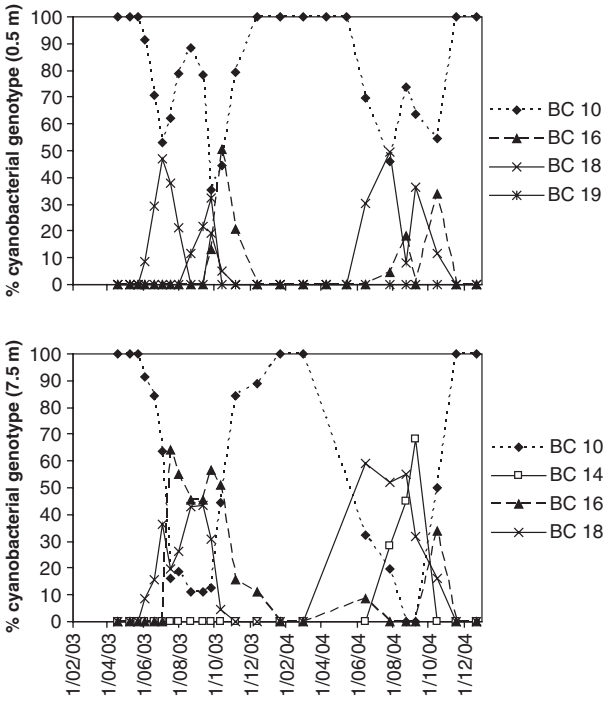

Fig. 4. Seasonal variation in the dominance of cyanobacterial genotypes (relative contribution) at a depth of 0.5 m (top) and 7.5 m (bottom) in Lake Blaarmeersen in 2003 and 2004 (BC10, BC 14 and BC18 = *Synechococcus*, BC16 = *Limnothrix redekei* and BC19 = *Anabaena/Aphanizomenon*; BC stands for band class; see also Fig. 3 and Table 1).

spring, summer and early autumn) and the 'winter season' (late autumn, winter and early spring) ($R = 0.47$; $P = 0.001$) and significant differences in the cyanobacterial community composition between a depth of 0.5 and 7.5 m ($R = 0.239$; $P = 0.001$) during the growing season, while no significant differences in the cyanobacterial community composition between a depth of 0.5 and 7.5 m were found during the winter season. The Bray–Curtis cluster (Primer 5, Clarke & Gorley, 2001) of the cyanobacterial community (see Fig. 5) is composed of two subclusters: one that groups the 'growing season samples' and one that groups the 'winter season samples'. Three samples taken in summer 2004 in the hypolimnion are grouped separately due to the presence of a *Synechococcus* genotype (band class 14). In the winter season, there was little variation in the cyanobacterial community and especially one *Synechococcus* genotype (band class 10) was found.

Influence of biotic and abiotic factors on the cyanobacterial community dynamics

Simple and partial Mantel tests (ZT, Bonnet & Van de Peer, 2002) were performed to investigate the relationship between the cyanobacterial community composition (relative contributions of *Synechococcus* genotypes, *Limnothrix redekei* and *Anabaena/Aphanizomenon*) as determined by DGGE and the community composition of zooplankton and phytoplankton (without cyanobacteria). In addition, the influence of dissolved nutrients and physical conditions (temperature, Secchi depth, oxygen level, pH and conductivity) was investigated (see Table 2). The simple Mantel tests revealed that the zooplankton and phytoplankton community composition as well as the dissolved nutrient concentrations and physical conditions of the lake are significantly related to the cyanobacterial community composition, while all these components are also related to each other. Partial Mantel tests revealed that each component independently influenced the cyanobacterial community dynamics. The highest correlations were found with dissolved nutrients, zooplankton community composition and physical variables, and a slightly lower correlation was found with the phytoplankton community composition. For the

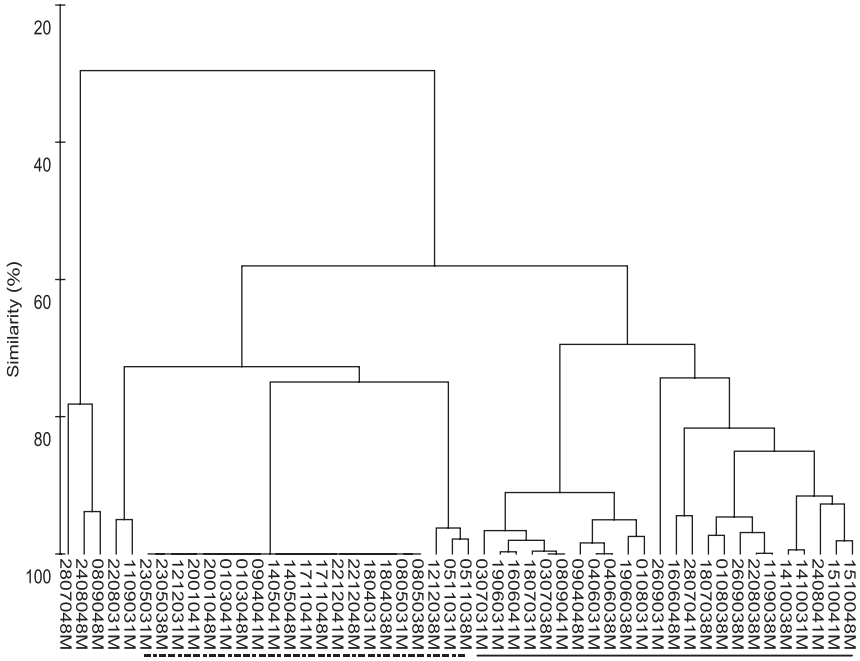

Fig. 5. Bray–Curtis cluster of the cyanobacterial community composition (band classes 10, 14, 16, 18 and 19; see Fig. 3 and Table 1) of the samples taken in Lake Blaarmeersen in 2003 and 2004. Full line: samples taken in the 'growing season' (late spring, summer and early autumn), interrupted line: samples taken in the 'winter season' (late autumn, winter and early spring), 1 M = 0.5 m, 8 M = 7.5 m.

Mantel tests described here, the band intensities in the DGGE profile were used (relative contributions of cyanobacterial taxa per sample). In addition, Mantel tests were also performed on the presence/absence DGGE data (presence or absence of cyanobacterial taxa per sample). In general, the correlations that were found were comparable with the correlations found when using band intensities, although they were slightly lower (not shown).

Simple Mantel tests also revealed that the zooplankton community composition influenced the total cyanobacterial biomass ($r = -0.346$; $P < 0.0001$) but the total biomass of zooplankton did not influence the cyanobacterial community dynamics ($r = -0.120$; $P > 0.05$). Furthermore, the zooplankton composition based on the absolute biomass of the three major groups (copepods, cladocerans and rotifers) has no significant correlation with the cyanobacterial community composition when controlling for nutrients ($r = 0.064$; $P > 0.05$) and physical variables ($r = 0.115$; $P > 0.05$). The different zooplankton groups were also correlated separately to the cyanobacterial community composition and total cyanobacterial biomass by simple and partial Mantel tests (see Table 3). The community composition of all three zooplankton groups has a significant correlation with the cyanobacterial community composition and biomass. However, when controlling for the phytoplankton community, only the cladoceran community has a significant correlation with the cyanobacterial community composition. The rotifer community has no significant correlation with the cyanobacterial biomass when controlling for phytoplankton biomass, cladoceran community and nutrients (see Table 3). *Bosmina* and *Daphnia* were the most abundant cladocerans in Lake Blaarmeersen (see Fig. 1), and simple Mantel tests showed that the biomass of both species has a significant correlation with the cyanobacterial community composition and biomass. However, only *Bosmina* seemed to influence the cyanobacterial community dynamics independently (see Table 4).

Spearman rank correlation coefficients were also calculated between the relative intensities of single DGGE band classes and biomass of *Daphnia* and *Bosmina* (STATISTICA 6.0) to know which species influenced each other in a positive or negative way. Table 5 shows that band class 10 (*Synechococcus* sp.) has a negative correlation with band classes 14 (*Synechococcus* sp.), 16 (*Limnothrix redekei*) and 18 (*Synechococcus* sp.) and band class 18 has a positive correlation with band classes 14 and 16. Further, *Daphnia* is positively correlated to band class 10 and negatively to band classes 16, 18 and 19 while *Bosmina* is negatively correlated to band class 10 and positively to band classes 16 and 18. In addition, Spearman rank correlation coefficients show that the cyanobacterial biomass has a negative correlation with the biomass of *Daphnia* ($r = -0.457$; $P < 0.001$) and a positive correlation with the biomass of *Bosmina* ($r = 0.689$; $P < 0.0001$).

Discussion

Lake Blaarmeersen was characterized by a diverse cyanobacterial community dominated by the unicellular nontoxic cyanobacterium *Synechococcus*. The three *Synechococcus* genotypes probably represent different ecotypes because they were present in the lake during different periods and

Table 2. Results of Mantel tests relating the cyanobacterial community composition to the community of zooplankton and phytoplankton and abiotic variables

	Cyanobacterial community	Zooplankton community	Phytoplankton community	Nutrients	Physical variables
Cyanobacterial community		0.306****	0.218***	−0.335****	−0.280****
Zooplankton community	0.253*** controlled for phytoplankton community 0.237*** controlled for nutrients 0.282**** controlled for physical variables		0.341****	−0.272****	−0.135**
Phytoplankton community	0.127* controlled for zooplankton community 0.139* controlled for nutrients 0.167** controlled for physical variables	0.295**** controlled for cyanobacterial community 0.288**** controlled for nutrients 0.322**** controlled for physical variables		−0.275****	−0.220****
Nutrients	−0.275*** controlled for zooplankton community −0.293*** controlled for phytoplankton community −0.274*** controlled for physical variables	−0.189** controlled for cyanobacterial community −0.197** controlled for phytoplankton community −0.245*** controlled for physical variables	−0.220*** controlled for cyanobacterial community −0.202** controlled for zooplankton community −0.224*** controlled for physical variables		0.302****
Physical variables	−0.253**** controlled for zooplankton community −0.243**** controlled for phytoplankton community −0.199*** controlled for nutrients	−0.054 (NS) controlled for cyanobacterial community −0.065 (NS) controlled for phytoplankton community −0.057 (NS) controlled for nutrients	−0.169*** controlled for cyanobacterial community −0.186**** controlled for zooplankton community −0.149*** controlled for nutrients	0.230*** controlled for cyanobacterial community 0.278**** controlled for zooplankton community 0.258**** controlled for phytoplankton community	

Results of simple Mantel tests are shown above the diagonal, results of partial Mantel tests below the diagonal. Correlations are presented as Pearson's correlation coefficients:
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.
NS, not significant.

in different strata of the water column. It is unlikely that the three different band classes represent intragenomic sequence variation in 16S rRNA genes of *Synechococcus* (Palenik *et al*., 2003) because the three genotypes did not occur in the same samples and negative correlations were found between the genotypes. In Lake Loosdrecht (the Netherlands), Zwart *et al*. (2005) also detected three different genotypes of *Synechococcus*, represented by three bands on different positions in the DGGE gradient. The relative intensities of these bands varied in spring, summer and autumn, indicating that they may belong to different ecotypes. None of the *Synechococcus* genotypes found in Lake Blaarmeersen has 100% sequence similarity with the genotypes from Lake Loosdrecht. The genus *Synechococcus* is polyphyletic (Honda *et al*., 1999; Robertson *et al*., 2001) and more sequence information is needed to determine the phylogenetic relationships between the different genotypes. The dominance of the filamentous nontoxic cyanobacterium *Limnothrix redekei* in the hypolimnion during the stratification period and in autumn agrees with other evidence that this species is better adapted to lower temperatures and light intensities than other cyanobacteria (e.g. Mur & Schreurs, 1995; Havens *et al*., 1998). The nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon* were detected as a single band by DGGE. By sequencing a fragment of the 16S rRNA gene, it is not possible to distinguish between these two taxa (Kolmonen *et al*., 2004). Furthermore, phylogenetic studies have shown that *Anabaena* and *Aphanizomenon* probably belong to one genus (Castenholz, 2001; Gugger *et al*., 2002). Microscopical determination confirmed that both cyanobacteria were present in Lake Blaarmeersen; *Anabaena* was mainly detected in August 2003 and *Aphanizomenon* was mainly detected in September 2003. *Anabaena* and *Aphanizomenon* are known to form surface blooms in summer when the water column is stable (Salmaso, 2000). Some of the other bands in the DGGE profile were identified as chloroplast sequences. One should pay attention to the fact that primers specific for cyanobacterial 16S rRNA genes also amplify most of the chloroplast 16S rRNA genes (Kolmonen *et al*., 2004; Stiller & McClanahan, 2005; Zwart *et al*., 2005; Ouellette

Table 3. Results of simple and partial Mantel tests relating the cyanobacterial community composition and total cyanobacterial biomass to the community of the different zooplankton groups

	Copepod community	Cladoceran community	Rotifer community
Cyanobacterial community	0.186**	0.300****	0.151*
	0.118* controlled for cladoceran community	0.266**** controlled for copepod community	0.066 (NS) controlled for copepod community
	0.128* controlled for rotifer community	0.279**** controlled for rotifer community	0.096 (NS) controlled for cladoceran community
	0.128 (NS) controlled for phytoplankton community	0.250*** controlled for phytoplankton community	0.124 (NS) controlled for phytoplankton community
	0.139* controlled for nutrients	0.204*** controlled for nutrients	0.139* controlled for nutrients
	0.175* controlled for physical variables	0.261**** controlled for physical variables	0.147* controlled for physical variables
Cyanobacterial biomass	−0.317****	−0.361****	−0.098*
	−0.250**** controlled for cladoceran community	−0.305**** controlled for copepod community	0.080* controlled for copepod community
	−0.313**** controlled for rotifer community	−0.350**** controlled for rotifer community	−0.027 (NS) controlled for cladoceran community
	−0.274**** controlled for phytoplankton biomass	−0.311**** controlled for phytoplankton biomass	−0.062 (NS) controlled for phytoplankton biomass
	−0.286**** controlled for nutrients	−0.293**** controlled for nutrients	−0.085 (NS) controlled for nutrients
	−0.317**** controlled for physical variables	−0.364**** controlled for physical variables	−0.097* controlled for physical variables

Correlations are presented as Pearson's correlation coefficients:
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.
NS = not significant.

Table 4. Results of simple and partial Mantel tests relating the cyanobacterial community composition and total cyanobacterial biomass to the biomass of *Daphnia* and *Bosmina*

	Biomass of *Daphnia*	Biomass of *Bosmina*
Cyanobacterial community	−0.155**	−0.314****
	−0.006 (NS) controlled for *Bosmina* biomass	−0.276*** controlled for *Daphnia* biomass
	−0.113* controlled for phytoplankton community	−0.280*** controlled for phytoplankton community
	−0.053 (NS) controlled for nutrients	−0.240*** controlled for nutrients
	−0.127* controlled for physical variables	−0.289**** controlled for physical variables
Cyanobacterial biomass	0.217***	0.311****
	0.081* controlled for *Bosmina* biomass	0.242*** controlled for *Daphnia* biomass
	0.149** controlled for phytoplankton biomass	0.280**** controlled for phytoplankton biomass
	0.141** controlled for nutrients	0.252**** controlled for nutrients
	0.216*** controlled for physical variables	0.311**** controlled for physical variables

Correlations are presented as Pearson's correlation coefficients:
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.
NS, not significant.

et al., 2006). That this does not always happen is illustrated by the fact that several sequences in the databank Genbank/EMBL are indicated as belonging to cyanobacteria (especially clones) whereas in fact they represent chloroplasts. As a result, the DGGE profiles do not reflect only the cyanobacterial diversity but a combination of cyanobacteria and chloroplasts. Therefore, microscopical counts and cloning were additionally performed to provide extra information and complement the DGGE data presented here.

This study confirms the influence of several physical variables on the cyanobacterial community dynamics (e.g. Dokulil & Teubner, 2000; Salmaso, 2000; Pawlik-Skowronska *et al*., 2004), as well as the influence of dissolved nutrient concentrations (e.g. Pandey & Pandey, 2002; Pawlik-Skowronska *et al*., 2004). On the other hand, two other classes of environmental variables, the zooplankton and phytoplankton community composition, are also correlated to the cyanobacterial community composition. While the

Table 5. Results of Spearman rank order correlations relating the relative abundance of single cyanobacterial genotypes (= relative intensity of respective DGGE bands) and biomass of *Daphnia* and *Bosmina* to each other (BC = band class, see Fig. 3 and Table 1)

	BC 10	BC 14	BC 16	BC 18	BC 19	*Daphnia*	*Bosmina*
BC 10 (*Synechococcus*)		−0.397**	−0.619****	−0.860****	−0.091 (NS)	0.517***	−0.705****
BC 14 (*Synechococcus*)	−0.397**		−0.173 (NS)	0.373**	−0.065 (NS)	−0.058 (NS)	0.131 (NS)
BC 16 (*Limnothrix redekei*)	−0.619****	−0.173 (NS)		0.296*	−0.023 (NS)	−0.293*	0.549****
BC 18 (*Synechococcus*)	−0.860****	0.373**	0.296*		−0.116 (NS)	−0.414**	0.512***
BC 19 (*Anabaena/Aphanizomenon*)	−0.091 (NS)	−0.065 (NS)	−0.023 (NS)	−0.116 (NS)		−0.327*	0.169 (NS)
Daphnia	0.517***	−0.058 (NS)	−0.293*	−0.414**	−0.327*		−0.660****
Bosmina	−0.705****	0.131 (NS)	0.549****	0.512***	0.169 (NS)	−0.660****	

Correlations are presented as Spearman rank correlation coefficients:
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; NS, not significant.

zooplankton and phytoplankton communities are generally not independent of each other (Shapiro, 1995; Sarnelle, 2005; Abrantes *et al*., 2006), a significant and independent relationship was found with the cyanobacterial community composition, suggestive of direct effects. The rather low but significant correlation between the phytoplankton and the cyanobacterial community composition might reflect mechanisms of phytoplankton succession and competition (Huszar & Caraco, 1998; Domingues *et al*., 2005; Huisman & Hulot, 2005). The correlation between the zooplankton and cyanobacterial community composition is relatively high. When this relationship is analysed further, it appears that the genus or species identity of zooplankton is more important than the major zooplankton group (copepods, cladocerans and rotifers) to which they belong. The importance of the different zooplankton species present in the lake was confirmed by Mantel tests on the community composition of the copepods, cladocerans and rotifers, respectively. Only the cladoceran community composition had a strong and unique influence on the cyanobacterial community dynamics. However, this finding needs to be interpreted with caution because of the lower taxonomic resolution of the copepod data (calanoids, cyclopoids, copepodites and nauplii) compared with those of cladocerans and rotifers (which were identified at the species or genus level), which may have obscured significant relationships. *Daphnia* and *Bosmina* were the most important grazing cladocerans in Lake Blaarmeersen and mainly *Bosmina* considerably influenced the cyanobacterial community dynamics. While *Daphnia* is a nonselective grazer, *Bosmina* grazes selectively (DeMott & Kerfoot, 1982) and can consume small as well as large food particles (Bleiwas & Stokes, 1985). Additionally, *Bosmina* might also indirectly influence the cyanobacterial community composition by grazing on heterotrophic nanoflagellates (DeMott & Kerfoot, 1982) because heterotrophic nanoflagellates graze in turn on small cyanobacteria (Nishibe *et al*., 2002). In principle, it is also possible that the cyanobacterial community influences the zooplankton abundance and composition, because some cyanobacteria are toxic, have a low nutritional value or form large colonies and filaments that can negatively affect several zooplankton species (Ghadouani *et al*., 2003; Murrel & Lores, 2004; Ruokolainen *et al*., 2006). However, the total biomass of cyanobacteria in Lake Blaarmeersen was low in comparison with other phytoplankton taxa and toxic cyanobacterial species were scarce, indicating that the influence of the cyanobacterial community on the zooplankton abundance and composition was limited. The positive correlation found between total cyanobacterial biomass and *Bosmina* biomass and the negative correlation between total cyanobacterial biomass and *Daphnia* biomass is in agreement with previous studies (Romo *et al*., 1996; DeMott *et al*., 2001; Abrantes *et al*., 2006) and might reflect a negative influence of filamentous cyanobacteria on *Daphnia*; however, it can also be the result of grazing of large *Daphnia* species on all cyanobacteria whereas *Bosmina* grazes more selectively.

The cyanobacterial community was composed of unicellular as well as filamentous cyanobacteria. Probably, the unicellular cyanobacteria are more easily grazed by different zooplankton species in comparison with the filamentous forms. Despite its low food quality, the picocyanobacterium *Synechococcus* is grazed by several zooplankton species (Park *et al*., 2002; Martin-Creuzburg *et al*., 2005). When grazed together with higher quality food, zooplankton is much less negatively affected (DeMott *et al*., 1998; Von Elert & Wolffrom, 2001). Probably, the importance of zooplankton differs from lake to lake and is dependent on several biotic and abiotic characteristics. When the nutrient status is too high, the cyanobacterial community will become very dominant and will be composed by one or a few species. Under these circumstances, the influence of zooplankton on cyanobacteria will be very limited because zooplankton does not grow well (Ahlgren *et al*., 1990; Ghadouani *et al*., 2003; Müller-Navarra *et al*., 2004).

Several studies using a traditional microscopical approach studied the interactions between phytoplankton

(including cyanobacteria) and zooplankton community composition (e.g. van Donk *et al.*, 1990; Schriver *et al.*, 1995; Romo *et al.*, 1996; Jeppesen *et al.*, 1998; Romo *et al.*, 2004), and showed that zooplankton can play a role in structuring the phytoplankton community. However, in these studies no direct correlations between the cyanobacterial and zooplankton community composition are calculated as cyanobacteria are considered to be a part of the phytoplankton community. Therefore, it is difficult to compare the zooplankton–cyanobacteria interactions found in this study with these traditional microscopical studies.

The goal of this study was to investigate the contribution of top-down variables relative to other explanatory variables in explaining cyanobacterial community dynamics in a natural setting. However, a significant relation between cyanobacterial community composition and community composition of potential predators may arise not only from a direct trophic interaction but also through indirect effects. Elser & Urabe (1999) showed that different zooplankton species differ in nutrient recycling, which can influence the concentrations of dissolved nutrients, which can in turn influence the cyanobacterial community dynamics (Callieri *et al.*, 2004). However, this indirect effect of zooplankton was mainly excluded by calculating partial Mantel tests, which allow controlling for confounding effects of covarying variables. Despite these tests, this study cannot completely rule out the possibility that part of the correlation found is due to temporal changes in zooplankton and cyanobacterial community. Therefore, to confirm the importance of a direct relationship between zooplankton and cyanobacteria due to grazing, more field studies and experimental studies in which variables can be manipulated independently of each other are needed. The results from this study, however, strongly suggest that, while in general studies on the ecology of cyanobacteria rely mostly on the impact of bottom-up forces and physical conditions, one should also pay attention to top-down forces, which can be as important as dissolved nutrient concentrations and/or physical variables in structuring the cyanobacterial community.

Acknowledgements

The research presented here was carried out in the framework of the Belgian project B-BLOOMS (EV/13/34B) 'Algal blooms: emerging problem for health and sustainable use of surface waters,' which was supported by the Federal Science Policy Office. The authors would like to thank Renaat Dasseville, Sam De Coster, Kevin De Keyser and Fanny De Ruysscher for helping with sampling and plankton analyses and Dirk Van Gansbeke for the nutrient analyses.

References

Abrantes N, Antunes SC, Pereira MJ & Goncalves F (2006) Seasonal succession of cladocerans and phytoplankton and their interactions in a shallow eutrophic lake (Lake Vela, Portugal). *Acta Oecolog Int J Ecol* **29**: 54–64.

Ahlgren G, Lundstedt L, Brett M & Forsberg C (1990) Lipid-composition and food quality of some fresh-water phytoplankton for cladoceran zooplankters. *J Plankton Res* **12**: 809–818.

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* **215**: 403–410.

Bergquist AM, Carpenter SR & Latino JC (1985) Shifts in phytoplankton size structure and community composition during grazing by contrasting zooplankton assemblages. *Limnol Oceanogr* **30**: 1037–1045.

Bleiwas AH & Stokes PM (1985) Collection of large and small food particles by *Bosmina*. *Limnol Oceanogr* **30**: 1090–1092.

Bonnet E & Van de Peer Y (2002) Zt: a software tool for simple and partial mantel tests. *J Statist Software* **7**: 1–12.

Bottrell HH, Duncan A, Gliwicz ZM, Grygierek E, Herzig A, Hillbrichtilkowska A, Kurasawa H, Larsson P & Weglenska T (1976) Review of some problems in zooplankton production studies. *Norw J Zool* **24**: 419–456.

Boutte C, Komárková J, Grubisic S, Znachor P, Bataille A, Zapomělová E, Gullukaya A, Jezberová J & Wilmotte A (2005) A polyphasic approach to assess the cyanobacterial diversity of summer samples from Czech reservoirs. *Algolog Studies* **117**: 177–195.

Brabrand A, Faafeng BA, Kallqvist T & Nilssen JP (1983) Biological-control of undesirable cyanobacteria in culturally eutrophic lakes. *Oecologia* **60**: 1–5.

Callieri C, Balseiro E, Bertoni R & Modenutti B (2004) Picocyanobacterial photosynthetic efficiency under *Daphnia* grazing pressure. *J Plankton Res* **26**: 1471–1477.

Castenholz RW (2001) Phylum BX. Cyanobacteria. Oxygenic photosynthetic bacteria. *The Archaea and the Deeply Branching and Phototrophic Bacteria, Bergey's Manual of Systematic Bacteriology, Vol. 1*, 2nd edn (Boone DR, Castenholz RW & Garrity GM, eds), pp. 473–599. Springer-Verlag, Berlin, Germany.

Clarke KR & Gorley RN (2001) *PRIMER 5 for Windows*. PRIMER-E Ltd, Plymouth, UK.

Codd GA, Lindsey J, Young FM, Morrison FL & Metcalfe JS (2005) Harmful cyanobacteria: from mass mortalities to management measures. *Harmful Cyanobacteria* (Huisman J, Matthijs HCP & Visser PM, eds), pp. 1–23. Springer, Dordrecht.

DeBernardi R, Giussani G & Lasso Pedretti E (1981) The significance of blue-green algae as food for filter-feeding zooplankton: experimental studies on *Daphnia* spp. fed by *Microcystis aeruginosa*. *Verhandlungen Int Vereinigung Theoretische Angewandte Limnol* **21**: 477–483.

Degans H, Zollner E, Van der Gucht K, De Meester L & Jurgens K (2002) Rapid *Daphnia*-mediated changes in microbial

community structure: an experimental study. *FEMS Microbiol Ecol* **42**: 137–149.

Dokulil MT & Teubner K (2000) Cyanobacterial dominance in lakes. *Hydrobiologia* **438**: 1–12.

DeMott WR & Kerfoot WC (1982) Competition among cladocerans – nature of the interaction between *Bosmina* and *Daphnia*. *Ecology* **63**: 1949–1966.

DeMott WR & Moxter F (1991) Foraging on cyanobacteria by copepods – responses to chemical defenses and resource abundance. *Ecology* **72**: 1820–1834.

DeMott WR, Gulati RD & Siewertsen K (1998) Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnol Oceanogr* **43**: 1147–1161.

DeMott WR, Gulati RD & Van Donk E (2001) *Daphnia* food limitation in three hypereutrophic Dutch lakes: evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. *Limnol Oceanogr* **46**: 2054–2060.

Domingues RB, Barbosa A & Galvao H (2005) Nutrients, light and phytoplankton succession in a temperate estuary (the Guadiana, south-western Iberia). *Est Coast Shelf Sci* **64**: 249–260.

Downing JA, Watson SB & McCauley E (2001) Predicting cyanobacteria dominance in lakes. *Can J Fisheries Aquat Sci* **58**: 1905–1908.

Elser JJ & Urabe J (1999) The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Ecology* **80**: 735–751.

Engström J, Koski M, Viitasalo M, Reinikainen M, Repka S & Sivonen K (2000) Feeding interactions of the copepods *Eurytemora affinis* and *Acartia bifilosa* with the cyanobacteria *Nodularia* sp. *J Plankton Res* **22**: 1403–1409.

Fiore MD, Neilan BA, Copp JN, Rodrigues JLM, Tsai SM, Lee H & Trevors JT (2005) Characterization of nitrogen-fixing cyanobacteria in the Brazilian Amazon floodplain. *Water Res* **39**: 5017–5026.

Geysen B & Coppejans E (1985) Fytohydrobiologisch onderzoek van oppervlaktewaters in Vlaanderen, 2. De vijver in het sport- en recreatiedomein Blaarmeersen (Gent). *Biol Jaarboek Dodonaea* **53**: 121–139.

Ghadouani A, Pinel-Alloul B & Prepas EE (2003) Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. *Freshwater Biol* **48**: 363–381.

Gilbert JJ (1996) Effect of temperature on the response of planktonic rotifers to a toxic cyanobacterium. *Ecology* **77**: 1174–1180.

Gliwicz ZM & Lampert W (1990) Food thresholds in *Daphnia* species in the absence and presence of blue-green filaments. *Ecology* **71**: 691–702.

Grasshoff K (1976) Determination of nitrate and nitrite. *Methods of Seawater Analysis* (Grasshoff K, ed). Verslag Chemie, Weinheim, NY, 317 p.

Gugger M, Lyra C, Henriksen P, Coute A, Humbert JF & Sivonen K (2002) Phylogenetic comparison of the cyanobacterial genera *Anabaena* and *Aphanizomenon*. *Int J System Evol Microbiol* **52**: 1867–1880.

Haney JF (1987) Field studies on zooplankton-cyanobacteria interactions. *NZ J Mar Freshwater Res* **21**: 467–475.

Haney JF & Hall DJ (1973) Sugar-coated *Daphnia*: a preservation technique for Cladocera. *Limnol Oceanogr* **18**: 331–333.

Havens KE, Phlips EJ, Cichra MF & Li BL (1998) Light availability as a possible regulator of cyanobacteria species composition in a shallow subtropical lake. *Freshwater Biol* **39**: 547–556.

Holm NP & Shapiro J (1984) An examination of lipid reserves and the nutritional-status of *Daphnia pulex* fed *Aphanizomenon flos-aquae*. *Limnol Oceanogr* **29**: 1137–1140.

Honda D, Yokota A & Sugiyama J (1999) Detection of seven major evolutionary lineages in cyanobacteria based on the 16S rRNA gene sequence analysis with new sequences of five marine *Synechococcus* strains. *J Mol Evol* **48**: 723–739.

Huisman J & Hulot FD (2005) Population dynamics of harmful cyanobacteria. *Harmful Cyanobacteria* (Huisman J, Matthijs HCP & Visser PM, eds), pp. 143–176. Springer, Dordrecht, the Netherlands.

Huszar VLD & Caraco NF (1998) The relationship between phytoplankton composition and physical-chemical variables: a comparison of taxonomic and morphological-functional descriptors in six temperate lakes. *Freshwater Biol* **40**: 679–696.

Jacoby JM, Collier DC, Welch EB, Hardy FJ & Crayton M (2000) Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*. *Can J Fisheries Aquat Sci* **57**: 231–240.

Jayatissa LP, Silva EIL, McElhiney J & Lawton LA (2006) Occurrence of toxigenic cyanobacterial blooms in freshwaters of Sri Lanka. *System Appl Microbiol* **29**: 156–164.

Jeppesen E, Sondergaard M, Jensen JP, Mortensen E, Hansen AM & Jorgensen T (1998) Cascading trophic interactions from fish to bacteria and nutrients after reduced sewage loading: an 18-year study of a shallow hypertrophic lake. *Ecosystems* **1**: 250–267.

Kolmonen E, Sivonen K, Rapala J & Haukka K (2004) Diversity of cyanobacteria and heterotrophic bacteria in cyanobacterial blooms in Lake Joutikas, Finland. *Aquat Microb Ecol* **36**: 201–211.

Koroleff F (1976) Determination of ammonia, phosphorus and silicon. *Methods of Seawater Analysis* (Grasshoff K, ed). Verslag Chemie, Weinheim, NY, 317 p.

Koski M, Schmidt K, Engstrom-Ost J, Viitasalo M, Jonasdottir S, Repka S & Sivonen K (2002) Calanoid copepods feed and produce eggs in the presence of toxic cyanobacteria *Nodularia spumigena*. *Limnol Oceanogr* **47**: 878–885.

Lampert W (1987) Laboratory studies on zooplankton-cyanobacteria interactions. *NZ J Mar Freshwater Res* **21**: 483–490.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res* **27**: 209–220.

Mantel N & Valand RS (1970) A technique of nonparametric multivariate analysis. *Biometrics* **26**: 547–558.

Martin-Creuzburg D, Wacker A & von Elert E (2005) Life history consequences of sterol availability in the aquatic keystone species *Daphnia*. *Oecologia* **144**: 362–372.

Menden-Deuer S & Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms and other protist plankton. *Limnol Oceanogr* **45**: 569–579.

Milutinovic A, Zivin M, Zorc-Pleskovic R, Sedmak B & Suput D (2003) Nephrotoxic effects of chronic administration of microcystins -LR and -YR. *Toxicon* **42**: 281–288.

Müller-Navarra DC, Brett MT, Park S, Chandra S, Ballantyne AP, Zorita E & Goldman CR (2004) Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature* **427**: 69–72.

Mur LR & Schreurs H (1995) Light as a selective factor in the distribution of phytoplankton species. *Water Sci Technol* **32**: 25–34.

Mur LR, Skulberg OM & Utkilen H (1999) Cyanobacteria in the environment. *Toxic Cyanobacteria in Water: a Guide to Their Public Health Consequences, Monitoring and Management* (Chorus I & Bartram J, eds), pp. 15–40. E & FN Spon, London.

Murrel MC & Lores EM (2004) Phytoplankton and zooplankton seasonal dynamics in a subtropical estuary: importance of cyanobacteria. *J Plankton Res* **26**: 371–382.

Muylaert K, Van der Gucht K, Vloemans N, De Meester L, Gillis M & Vyverman W (2002) Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. *Appl Environ Microbiol* **68**: 4740–4750.

Muyzer G, Dewaal EC & Uitterlinden AG (1993) Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16s ribosomal-RNA. *Appl Environ Microbiol* **59**: 695–700.

Ni CV, Yakunin AF & Gogotov IN (1990) Effect of bound forms of nitrogen on the growth and synthesis of different *Anabaena variabilis* nitrogenases. *Microbiology* **59**: 260–263.

Nishibe Y, Kawabata Z & Nakano S (2002) Grazing on *Microcystis aeruginosa* by the heterotrophic flagellate *Collodictyon triciliatum* in a hypertrophic pond. *Aquat Microb Ecol* **29**: 173–179.

Noges T, Noges P & Laugaste R (2003) Water level as the mediator between climate change and phytoplankton composition in a large shallow temperate lake. *Hydrobiologia* **506**: 257–263.

Nübel U, Garcia-Pichel F & Muyzer G (1997) PCR primers to amplify 16S rRNA genes from cyanobacteria. *Appl Environ Microbiol* **63**: 3327–3332.

Ouellette AJA, Handy SM & Wilhelm SW (2006) Toxic *Microcystis* is widespread in Lake Erie: PCR detection of toxin genes and molecular characterization of associated cyanobacterial communities. *Microb Ecol* **51**: 154–165.

Palenik B, Brahamsha B, Larimer FW *et al.* (2003) The genome of a motile marine *Synechococcus*. *Nature* **424**: 1037–1042.

Pandey J & Pandey U (2002) Cyanobacterial flora and the physico-chemical environment of six tropical fresh water lakes of Udaipur, India. *J Environ Sci (China)* **14**: 54–62.

Park S, Brett MT, Muller-Navarra DC & Goldman CR (2002) Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for *Daphnia*. *Freshwater Biol* **47**: 1377–1390.

Pawlik-Skowronska B, Skowronski T, Pirszel J & Adamczyk A (2004) Relationship between cyanobacterial bloom composition and anatoxin-A and microcystin occurrence in the eutrophic dam reservoir (SE Poland). *Polish J Ecol* **52**: 479–490.

Pflugmacher S (2004) Promotion of oxidative stress in the aquatic macrophyte *Ceratophyllum demersum* during biotransformation of the cyanobacterial toxin microcystin-LR. *Aquat Toxicol* **70**: 169–178.

Robertson BR, Tezuka N & Watanabe MM (2001) Phylogenetic analyses of *Synechococcus* strains (cyanobacteria) using sequences of 16S rDNA and part of the phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content. *Int J System Evol Microbiol* **51**: 861–871.

Rohrlack T, Dittmann E, Henning M, Borner T & Kohl JG (1999) Role of microcystins in poisoning and food ingestion inhibition of *Daphnia galeata* caused by the cyanobacterium *Microcystis aeruginosa*. *Appl Environ Microbiol* **65**: 737–739.

Rohrlack T, Christoffersen K, Dittmann E, Nogueira I, Vasconcelos V & Borner T (2005) Ingestion of microcystins by *Daphnia*: intestinal uptake and toxic effects. *Limnol Oceanogr* **50**: 440–448.

Romo S, van Donk E, Gylstra R & Gulati R (1996) A multivariate analysis of phytoplankton and food web changes in a shallow biomanipulated lake. *Freshwater Biol* **36**: 683–696.

Romo S, Miracle MR, Villena MJ, Rueda J, Ferriol C & Vicente E (2004) Mesocosm experiments on nutrient and fish effects on shallow lake food webs in a mediterranean climate. *Freshwater Biol* **49**: 1593–1607.

Ruokolainen L, Lilley TM, Tammi M & Vuorinen P (2006) Zooplankton in relation to cyanobacteria across a geographic gradient in Archipelago sea, northern Baltic. *Boreal Environ Res* **11**: 1–11.

Salmaso N (2000) Factors affecting the seasonality and distribution of cyanobacteria and chlorophytes: a case study from the large lakes south of the Alps, with special reference to Lake Garda. *Hydrobiologia* **438**: 43–63.

Sarnelle O (2005) *Daphnia* as keystone predators: effects on phytoplankton diversity and grazing resistance. *J Plankton Res* **27**: 1229–1238.

Schriver P, Bogestrand J, Jeppesen E & Sondergaard M (1995) Impact of submerged macrophytes on fish-zooplankton-phytoplankton interactions - large-scale enclosure experiments in a shallow eutrophic lake. *Freshwater Biol* **33**: 255–270.

Sellner KG, Brownlee DC, Bundy MH, Brownlee SG & Braun KR (1993) Zooplankton grazing in a Potomac river cyanobacteria bloom. *Estuaries* **16**: 859–872.

Shapiro J (1995) Lake restoration by biomanipulation-a personal view. *Environ Rev* **3**: 83–93.

Sherr EB & Sherr BF (1993) Preservation and storage of samples for enumeration of heterotrophic protists. *Handbook of Methods in Aquatic Microbial Ecology* (Kemp PF, Sherr BF, Sherr EB & Cole JJ, eds), pp. 207–212. CRC Press, Boca Raton.

Starkweather PL & Kellar PE (1983) Utilization of Cyanobacteria by *Brachionus-Calyciflorus - Anabaena flos-aquae* (Nrc-44-1) as a sole or complementary food source. *Hydrobiologia* **104**: 373–377.

Stiller JW & McClanahan A (2005) Phyto-specific 16S rDNA PCR primers for recovering algal and plant sequences from mixed samples. *Mol Ecol Notes* **5**: 1–3.

Taton A, Grubisic S, Brambilla E, De Wit R & Wilmotte A (2003) Cyanobacterial diversity in natural and artificial microbial mats of lake Fryxell (McMurdo Dry Valleys, Antartica): a morphological and molecular approach. *Appl Environ Microbiol* **69**: 5157–5169.

Tikkanen T & Willénn T (1992) *Växtplantonflora*. Författarna Natur., Solna, 280 pp.

van Donk E, Grimm MP, Gulati RD & Klein Breteler JPG (1990) Whole-lake food-web manipulation as a means to study community interactions in a small ecosystem. *Hydrobiologia* **200/201**: 275–289.

Viaggiu E, Melchiorre S, Volpi F *et al.* (2004) Anatoxin-A toxin in the cyanobacterium *Planktothrix rubescens* from a fishing pond in northern Italy. *Environ Toxicol* **19**: 191–197.

Von Elert E & Wolffrom T (2001) Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*. *Limnol Oceanogr* **46**: 1552–1558.

Work KA & Havens KE (2003) Zooplankton grazing on bacteria and cyanobacteria in a eutrophic lake. *J Plankton Res* **25**: 1301–1306.

Zurawell RW, Chen HR, Burke JM & Prepas EE (2005) Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. *J Toxicol Environ Health-Part B-Critical Rev* **8**: 1–37.

Zwart G, Huismans R, van Agterveld MP *et al.* (1998) Divergent members of the bacterial division *Verrucomicrobiales* in a temperate freshwater lake. *FEMS Microbiol Ecol* **25**: 159–169.

Zwart G, Kamst-van Agterveld MP, van der Werff-Staverman I, Hagen F, Hoogveld HL & Gons HJ (2005) Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake. *Environ Microbiol* **7**: 365–377.