Strong effects of amoebae grazing on the biomass and genetic structure of a *Microcystis* bloom (Cyanobacteria)

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Summary

Despite its importance for bloom toxicity, the factors determining the population structure of cyanobacterial blooms are poorly understood. Here, we report the results of a two-year field survey of the population dynamics of *Microcystis* blooms in a small hypertrophic urban pond. Microscopic enumeration of *Microcystis* and its predators and parasites was combined with pigment and microcystin analysis and denaturing gradient gel electrophoresis of the ITS rDNA region to assess population dynamics and structure. Two main *Microcystis* morpho- and ITS types were revealed, corresponding to *M. aeruginosa* and *M. viridis*. In both years, high population densities of naked amoebae grazing on *Microcystis* coincided with rapid decreases in *Microcystis* biomass. In one year, there was a shift from heavily infested *M. aeruginosa* to the less-infested *M. viridis*, allowing the bloom to rapidly recover. The preference of amoebae for *M. aeruginosa* was confirmed by grazing experiments, in which several amoeba strains were capable of grazing down a strain of *M. aeruginosa*, but not of *M. viridis*. Zooplankton and chytrid parasites appeared to be of minor importance for these strong and fast reductions in *Microcystis* biomass. These findings demonstrate a strong impact of small protozoan grazers on the biomass and genetic structure of *Microcystis* blooms.

Introduction

Cyanobacterial blooms, strong proliferations of cyanobacterial populations often leading to scum-formation, are a common phenomenon in freshwater bodies worldwide. Their frequency is expected to further increase due to eutrophication and climate change, as they are favoured by hypertrophic conditions and high temperatures (Jöhnk et al., 2008; Paerl and Huisman, 2008). While they can be present year-round in tropical climates (Dejenie et al., 2009), they are largely restricted to summer and autumn in colder climates (Fogg et al., 1973). Due to their widespread toxicity, high biomass build-up and negative impacts on aquatic food webs and human use of freshwaters (Codd et al., 2005), bloom-forming cyanobacteria are regarded as nuisance and harmful species (Chorus and Bartram, 1999). Most cyanobacterial species consist of a number of functionally divergent genotypes, including differences in toxin production (Schembri et al., 2001; Carrillo et al., 2003; Briand et al., 2008). Bloom populations may differ strongly in genetic composition, both in space and time, with obvious consequences for bloom toxicity and persistence under environmental change (Kardinaal et al., 2007a). Factors controlling this turnover in population structure are only beginning to be addressed, however.

In natural blooms, the turnover of genotypes is generally attributed to changes in abiotic conditions, including irradiance, nutrients, temperature, pH and CO2 concentration (e.g. Yagi et al., 1994; Shapiro, 1997; Vezie et al., 2002; Kardinaal et al., 2007a,b; Briand et al., 2008; Imai et al., 2009), while the role of biotic interactions has been hardly considered so far. Recently however, it was shown that interactions between strains can be very strong and may depend on colonization order (van Grembergh et al., 2009a), suggesting that priority effects may contribute to genotype turnover. In addition, grazing by zooplankton can act as a main factor determining the fate of phytoplankton biomass (e.g. Sommer et al., 1986), community (e.g. Cottingham, 1999; van Grembergh et al., 2008) and population structure (Vanormelingen et al., 2008). However, many cyanobacteria are a relative poor food source for zooplankton due to toxin production (DeMott, 1999; Rohrlack et al., 2001) and morphological
and size constraints (Fulton and Pael, 1987; Lampert, 1987). Whether zooplankton can exert a significant influence on cyanobacterial blooms remains a topic of much debate (e.g. Tillmians et al., 2008). Even so, there is some experimental evidence for grazing by large Daphnia spp., influencing cyanobacterial bloom population structure (Sarnelle and Wilson, 2005; van Gremberghe et al., 2009b).

During bloom conditions, parasites and grazers other than zooplankton might be important. Viruses (Tucker and Pollard, 2005; Honjo et al., 2006) and bacteria (Caiola and Pellegrini, 1984; Choi et al., 2005) can have rapid and devastating effects on cyanobacterial populations. Also, a range of specialized unicellular eukaryotes feeding on various species of cyanobacteria has been identified, including heterotrophic flagellates (Nishibe et al., 2002; Zhang et al., 2008), chytrid fungi (Davis et al., 2003; Sige et al., 2007), ciliates (Canter et al., 1990; Fialkowska and Pajdak-Stos, 1997) and amoebae (Cook and Ahearn, 1976; Wright et al., 1981; Laybourn-Parry et al., 1987; Nishibe et al., 2004). At least one flagellate species has been shown to be able to degrade microcystin-LR, one of the most common cyanobacterial toxins (Zhang et al., 2008). Effects of these protozoan grazers on natural bloom dynamics are only rarely addressed and have shown conflicting results. Naked amoebae were shown to be able to control the biomass of a natural Anabaena bloom (Cook and Ahearn, 1976). However, two other studies, dealing with grazing by testate amoebae and heterotrophic flagellates on natural Microcystis populations, could not reveal any significant effect on cyanobacterial biomass (Nishibe et al., 2002; 2004). Therefore, the consequences of protozoan grazing for the genetic structure of cyanobacterial blooms largely remain to be elucidated.

Microcystis is one of the most common bloom-forming cyanobacteria worldwide (Visser et al., 2005). Based on colony morphology, several morphospecies have been recognized. However, the lack of genetic divergence based on 16S rDNA and DNA-DNA hybridizations suggests that the morphological diversity concerns a single species, Microcystis aeruginosa (Otsuka et al., 2001). This conclusion is strengthened by the relatively low divergence (Otsuka et al., 1999; Haande et al., 2007) and lack of phylogenetic structure (I. van Gremberghe, in preparation) for the ITS rDNA spacer region. Population-level studies have revealed that natural Microcystis-bloom populations are often genetically diverse and typically comprise multiple morphologically and biochemically different genotypes (Wilson et al., 2005; Yoshida et al., 2008; Moisander et al., 2009), which either coexist or replace each other during the growth season (Kardinaal et al., 2007b; Briand et al., 2009; Rinta-Kanto et al., 2009).

Here, we report the results of a high-resolution two-year field survey of the population dynamics of a Microcystis bloom in a small urban pond (Westveld Pond in Gent, Belgium). Microscopical counts of Microcystis and its predators and parasites were combined with pigment and microcystin analysis, denaturing gradient gel electrophoresis (DGGE) of the ITS rDNA, and complemented with in vitro grazing experiments. We show that grazing by naked amoebae resulted in a rapid decline of Microcystis biomass in both years. In one year, there was also a rapid shift from an amoebae-grazing sensitive-, to an amoebae-grazing resistant Microcystis morphotype, allowing the bloom to rapidly build up again. The selectivity of amoebae-grazing was confirmed by laboratory grazing experiments.

Results

Phytoplankton and Microcystis bloom dynamics

The limnological parameters measured during the growth seasons of 2007 and 2008 are summarized in Table 1. Water temperature and Secchi depth are shown in Fig. 1A.

In both years, as soon as the water temperature stabilized around 20°C, phytoplankton biomass started to increase (Fig. 1B), reaching maximal chlorophyll concentrations of 781 µg l⁻¹ (2007) and 957 µg l⁻¹ (2008). As a result, the Secchi depth decreased to values of around 10–15 cm during the phytoplankton bloom period (Fig. 1A).

Cyanobacteria dominated the phytoplankton during almost the whole study period in 2007 (29 to 83% of the total phytoplankton biomass, Fig. 1B). Microcystis was by far the most dominant cyanobacterial taxon, while other cyanobacteria (mainly Aphanocapsa and Geitlerinema) had only a limited contribution (Fig. 1C).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
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<tbody>
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<td>PO₄-P (µg l⁻¹)</td>
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<td>127</td>
</tr>
<tr>
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<td>55</td>
</tr>
<tr>
<td>NH₄-N (µg l⁻¹)</td>
<td>49</td>
<td>&lt;1</td>
<td>1334</td>
</tr>
<tr>
<td>SiO₂ (µg l⁻¹)</td>
<td>140</td>
<td>&lt;1</td>
<td>2 000</td>
</tr>
<tr>
<td>TN (mg N l⁻¹)</td>
<td>6.6</td>
<td>2.2</td>
<td>11.3</td>
</tr>
<tr>
<td>TP (µg P l⁻¹)</td>
<td>792</td>
<td>192</td>
<td>1 280</td>
</tr>
<tr>
<td>SPM (mg l⁻¹)</td>
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<td>19</td>
<td>297</td>
</tr>
<tr>
<td>temperature (°C)</td>
<td>16.3</td>
<td>4.2</td>
<td>22.2</td>
</tr>
<tr>
<td>Dissolved O₂ (mg l⁻¹)</td>
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<td>0.95</td>
<td>17.6</td>
</tr>
<tr>
<td>% O₂</td>
<td>92</td>
<td>12</td>
<td>185</td>
</tr>
<tr>
<td>pH</td>
<td>8.1</td>
<td>6.3</td>
<td>9.9</td>
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<tr>
<td>Conductivity (µS cm⁻¹)</td>
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<td>90</td>
<td>218</td>
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<tr>
<td>Chlorophyll a (µg l⁻¹)</td>
<td>397</td>
<td>45</td>
<td>1 151</td>
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<tr>
<td>Cladocerans (N l⁻¹)</td>
<td>3</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>Copepods (N l⁻¹)</td>
<td>30</td>
<td>2</td>
<td>110</td>
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<td>Copepod nauplii (N l⁻¹)</td>
<td>106</td>
<td>0</td>
<td>500</td>
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<tr>
<td>Rotifers (N l⁻¹)</td>
<td>6899</td>
<td>40</td>
<td>54 000</td>
</tr>
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</table>

a. Only data for 2008 available.
There was a dense and extensive *Microcystis* bloom population, regularly producing scums, from June to October 2007, with a temporary decrease in bloom biomass near the end of June. The gradual decline of the *Microcystis* bloom in autumn coincided with decreasing water temperatures. In 2008, cyanobacteria were less dominant (0–57%), and *Microcystis* only dominated the cyanobacterial community with a high bloom biomass from June to August, following a spring bloom of *Limnothrix redekei* and *Geitlerinema* sp. (Fig. 1B and C). After a sudden crash of the *Microcystis* population around the middle of August 2008, *Euglena* sp. and *Anabaena planctonica* became dominant, at times forming a floating surface layer.
Microcystis population structure

Two *Microcystis* morphotypes could be distinguished, *M. aeruginosa* and *M. viridis* (Fig. 2). *Microcystis aeruginosa* cells were smaller (4.3 ± 0.6 μm length, 3.9 ± 0.6 μm width), greenish and loosely aggregated in relatively large colonies of on average 120 cells (Fig. 2A). During periods of maximal biomass, macro-colonies of up to 5000 cells were present. The *M. aeruginosa* colonies were surrounded by a very thin mucilage matrix (visible after adding methylene blue to the samples). *Microcystis viridis* had larger, brownish cells (6.2 ± 0.6 μm length, 5.5 ± 0.4 μm width), which were aggregated in smaller colonies with an average of about 50 cells (Fig. 2B), although at times colonies up to 400 cells could be observed. These colonies were surrounded by a dense, extended mucilage matrix. These two morphotypes showed contrasting dynamics (Fig. 3A and D), with *M. aeruginosa* dominating in June 2007 (maximal biomass: 35.4 mg C l⁻¹ on 18 June) and from June to August 2008 (maximal biomass: 35.8 mg C l⁻¹ on 18 July). *Microcystis viridis* increased in biomass and dominated between July and November 2007 (maximal biomass: 19.3 mg C l⁻¹ on 16 August), following a sudden decrease in *M. aeruginosa* biomass near the end of June 2007. *Microcystis viridis* was always present in small numbers in 2008, although most colonies looked unhealthy with many lysed cells, and did not form a bloom after the population crash of *M. aeruginosa* around the middle of August.

These contrasting dynamics were also seen by DGGE of the ITS rDNA (Fig. 3B and E). Eight different ITS types could be detected by sequencing bands, of which only two reached high relative abundances in the population. One
Fig. 3. A. Biomass of the two main Microcystis morphotypes and amoebae population densities in Westveld Pond in 2007.

B. The DGGE profile of the Microcystis ITS rDNA population structure for the same period, numbers indicate excised bands for sequence analysis.

C. Proportion of colonies infested by amoebae for the two Microcystis morphotypes separately and microcystin concentrations (μg microcystin-LR equivalents per litre; ELISA immunoassay) during 2007.

D, E and F. Corresponding results for 2008.

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dominated in June 2007 and throughout the 2008 growth season (bands 3=10=14=17=20=22=26=29=30, also referred to as ‘ITS 3’, see below), and the second one from July to November 2007 and in the 2007–08 winter sample (bands 1=8=11=13=16=19=24=25=28, also referred to as ‘ITS 1’, see below). The other six ITS types (bands 4, 5, 2=6=9, 7, 12=15=18, 21=23=27=31) correspond to weaker bands and thus less abundant ITS types. These dynamics suggest that the two Microcystis morphotypes correspond to two different ITS types, which was confirmed by the molecular analysis of 34 Microcystis strains, isolated from the same pond (Table 2). All 14 M. viridis strains corresponded to the ITS type (ITS 1, see Table 2), which was dominant in the same period as the M. aeruginosa morphotype. Four M. aeruginosa strains belonged to another ITS type, (ITS 2, see Table 2) and 2 M. aeruginosa strains corresponded to an ITS type similar to bands 12, 15 and 18 (ITS 4, see Table 2).

**Microcystin concentration**

Both Microcystis morphotypes produced microcystins (Table 2). High concentrations of these toxins were also detected in the water samples during dominance of each morphotype, with a maximal total (intracellular plus extracellular) concentration of 168 (15/06/2007) and 99 (22/07/2008) μg (MC-LR equivalents) l⁻¹ during M. aeruginosa dominance and 251 μg (MC-LR equivalents) l⁻¹ (17/09/2007) during M. viridis dominance (Fig. 3C and F). In 2007,
a strong reduction in total microcystin concentration was observed, just before the *M. aeruginosa* bloom collapse.

**Predators and parasites of Microcystis**

The zooplankton community (Fig. 1E) was dominated by rotifers, both in terms of density (91%) and biomass (62%). Cyclopoid copepods and small cladocerans (mainly *Bosmina longirostris* and *Daphnia cucullata*) were present but never reached a high biomass (2–340 and 0–31 μg C l⁻¹ respectively). From the 29 rotifer taxa that could be identified, *Brachionidae* including *Brachionus calyciflorus pala, B. angularis* and *B. diversicornis* were dominant. A distinct maximum could only be observed in June prior to the *M. aeruginosa* bloom development in both years (7309 and 1748 μg C l⁻¹ on 08/06/2007 and 03/06/2008 respectively). During and after Microcystis dominance, zooplankton was virtually absent.

*Microcystis* colonies infected by a parasitic chytrid were detected in 97% of the samples. This chytrid was identified as *Chytridium microcystidis* Rohde & Skuja (Karling, 1977), based on the presence of an epibiotic, sessile and operculate zoosporangium with a rhizoidal axis at its basal periphery that forms an extended system of branched rhizoids invading the mucilage matrix of which the endings encircle and penetrate *Microcystis* cells (Fig. 2D). In general, the infection percentage was somewhat higher for *M. viridis*, but the pattern was more or less similar for both *Microcystis* morphotypes, especially in 2007 (Fig. 1D). Before the bloom, the infection percentage reached 73% for *M. viridis* colonies and 32% for *M. aeruginosa* colonies. During the *M. aeruginosa* bloom, very low infection values were observed for both *Microcystis* morphotypes. However, after the *M. aeruginosa* bloom period, the percentage of infection reached a maximal value of 76% for *M. aeruginosa* and even 85% for *M. viridis* during its bloom build-up phase. For the rest of the season, the chytrid infection gradually decreased. In 2008, the infection percentage never reached values higher than 15% for *M. aeruginosa*, while for *M. viridis* a maximal value of 52% was observed.

Naked amoebae (Fig. 2C and D), sometimes attached to *Microcystis* colonies, were observed in almost every sample, usually in low numbers. However, coinciding with a respective 40- and 30-fold decrease in *M. aeruginosa* biomass in both years, the abundance of these amoebae increased exponentially in a matter of days (Fig. 3A and D), reaching maximal densities of 67 000 and 24 500 amoebae ml⁻¹ on 28/06/2007 and 14/08/2008 respectively. During these maxima up to 82% and 60% of the *M. aeruginosa* colonies were infested with amoebae (Fig. 3C and F). At the end of June 2008, another high infestation percentage (46%) of *M. aeruginosa* colonies was visible (Fig. 3F), which was due to another small and cyst-forming amoeba (see below). However, the population density of this amoeba was not high and a profound effect on *M. aeruginosa* biomass was not apparent. The infestation percentages of *M. viridis* colonies during these amoebae maxima (Fig. 3C and F) were always much lower (40% and 16% respectively). In 2007, as soon as the *M. aeruginosa* bloom collapsed, *M. viridis* increased in biomass, in spite of the presence of amoebae at densities still around 5000 ml⁻¹ (Fig. 3A).

The morphology of the majority of the isolated amoeba strains and of the amoebae encountered in the field samples (Fig. 2C and D) resembled the dactylopodial morphotype of the *Gymnamoebae*, and more particularly the genus *Korotnevella* (Smirnov and Goodkov, 1999; O’Kelly et al., 2001). In addition, amoebae strains isolated on 25/06/2008 were of the reticulate morphotype, resembling *Gephyrramoeba*, while one strain (A17WVB) isolated on 04/07/2008 had a fan-shaped morphology, resembling *Vanella* (Table 2). The 18S rDNA sequences from three strains with a dactylopodial morphology (A16WVB, A21WVB, A54WVB) showed the largest similarity in GenBank (91–93%) with *Korotnevella* strains. For the two sequenced strains with a reticulate morphology (A1WVB, A2WVB), closest resemblance (86%) was found with an unidentified amoeba (J. Van Wichelen, in preparation).

**Amoebae food preference**

The preference of the amoebae for *M. aeruginosa* was tested by in vitro experiments in which monocultures of *M. aeruginosa* and *M. viridis* were grown in the absence or presence of one of four amoeba strains (Fig. 4). Growth of *M. aeruginosa* was heavily suppressed by the presence of each of the amoeba strains (Fig. 4A, Table 3). From day 6 onwards, all amoeba treatments had a significantly lower *M. aeruginosa* biomass compared with controls (Table 4). In contrast, no effects of the presence of amoeba strains on *M. viridis* growth were observed (Fig. 4B and Table 3). Amoeba densities at the end of the experiment differed significantly between *M. aeruginosa* and *M. viridis* treatments (Fig. 4D). While amoeba numbers had increased strongly when feeding on *M. aeruginosa*, they remained at the same density (reticulate strains, mainly cysts) or decreased in density below the detection limit (dactylopodial strains) when given *M. viridis* as food. No significant difference in microcystin concentration per unit *Microcystis* biomass between controls and amoeba treatments was found at the end of the experiment, both for *M. aeruginosa* and *M. viridis* (Fig. 4C).

**Amoebae growth and grazing rates**

The growth and grazing rate of the amoebae on *M. aeruginosa* were assessed by placing *M. aeruginosa*
cultures (strain M31) in the dark for 40 h in the presence of high densities of amoebae (strain A16WVB). *Microcystis* population growth during this time interval was very limited since only a minor increase (6.3%) in *Microcystis* biomass was observed in the absence of amoebae (data not shown), allowing the inference of amoebae grazing rates. The amoebae showed a growth rate of 1.29 divisions day\(^{-1}\), which was higher than the estimated growth rates for the field data during the amoeba population density peaks (Table 5). The amoebae had a calculated grazing rate of 9.23 ± 1.78 pg (*Microcystis* C amoeba\(^{-1}\) h\(^{-1}\)), which also falls in the range estimated for the field data, where a higher grazing rate was estimated at the onset of each *M. aeruginosa* bloom collapse, which afterwards gradually declined to a lower grazing rate than the one observed in the experiment (Table 5).

**Discussion**

Although amoebae are an inherent part of pelagic freshwater microbial food webs, they are generally overlooked or ignored (Sims et al., 2002). This is mainly because of their sporadic occurrence at high population densities in the water column (e.g. Arndt, 1993; Mathes and Arndt, 1995; Weisse and Müller, 1998), generally in association with suspended particles (Rosgeron and Gwaltney, 2000; Rogerson et al., 2003). In Westveld Pond, the maximal observed population densities of amoebae were much higher than previously reported from any plankton community (Murzov and Caron, 1996; Anderson, 1997; Rogerson and Gwaltney, 2000; Anderson, 2007). In both sampling years, the pronounced, but very short-lived, peak in the abundance of *Microcystis*-grazing amoebae, coincided with dramatic decreases in *Microcystis* biomass, which demonstrates that herbivorous amoebae can have a strong impact on the biomass of natural phytoplankton populations. Microscopic observations of *Microcystis* bloom samples from other lakes in Belgium, Sweden and Denmark in 2009 have also revealed the presence of *Microcystis*-grazing amoebae (J. Van Wichelen, unpublished). This suggests that the importance of pelagic amoebae for cyanobacterial bloom dynamics is seriously underestimated. One reason could be the relatively low sampling frequency in most monitoring studies. A monthly or even biweekly sampling frequency is likely to miss an amoebae population peak, as herbivorous amoebae densities can change dramatically within a few days (this study, see also Canter and Lund, 1968). As a consequence, a strong influence of grazing amoebae on the dynamics of natural phytoplankton populations has only rarely been reported (e.g. Canter and Lund, 1968; Bailey-Watts and Lund, 1973; Cook and Ahearn, 1976; Van Wichelen et al., 2006). In the case of *Microcystis*, it was only shown thus far that laboratory cultures could be completely destroyed by *Microcystis*-associated amoebae (Reynolds et al., 1981).

Grazing by amoebae not only caused dramatic decreases in *Microcystis* biomass, it was also highly selective. The susceptibility of *M. aeruginosa* and the apparent resistance of *Microcystis viridis* were confirmed by the *in vitro* experiments. Microcystins apparently were not the main factor involved in this specificity, since both

**Table 3.** Results of repeated measures ANOVA, testing for the effects of time and amoebae grazing (factor ‘treatment’) and their interaction on the biomass of *Microcystis aeruginosa* and *Microcystis viridis* respectively, during the amoebae grazing experiments.

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>P</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Time</td>
<td>5</td>
<td>25 692.402</td>
<td>180.533</td>
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<tr>
<td>Treatment</td>
<td>4</td>
<td>24 515.900</td>
<td>90.908</td>
<td>&lt; 0.001</td>
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<tr>
<td>Time × Treatment</td>
<td>20</td>
<td>7 600.555</td>
<td>53.407</td>
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<td><strong>Microcystis viridis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>5</td>
<td>19 837.803</td>
<td>3307.741</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Treatment</td>
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<td>35 937</td>
<td>1.000</td>
<td>0.294</td>
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<tr>
<td>Time × Treatment</td>
<td>20</td>
<td>4 889</td>
<td>0.815</td>
<td>0.684</td>
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</table>

Significant values (P < 0.05) are in bold.

**Table 4.** Pairwise comparisons of *Microcystis aeruginosa* biomass between controls without amoebae (C) and each of the amoeba-grazing treatments at different time points during the grazing experiment, using post hoc Tukey tests following a one-way ANOVA (two factor levels, control and amoebae).

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<tbody>
<tr>
<td>A1WVB versus C</td>
<td>0.703</td>
<td>0.502</td>
<td>0.998</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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<tr>
<td>A2WVB versus C</td>
<td>0.960</td>
<td>0.031</td>
<td>0.510</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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<td>A16WVB versus C</td>
<td>0.426</td>
<td>0.327</td>
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<td>0.002</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>A21WVB versus C</td>
<td>0.971</td>
<td>0.098</td>
<td>0.128</td>
<td>0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Only P-values are shown, significant values (P < 0.05) are in bold.
Microcystis morphotypes produced microcystins, whether amoebae were present or not. Density dependence of amoebae grazing was probably not a cause for the inability of the amoebae to graze down \textit{M. viridis} in the field, since the maximal biomass of \textit{M. viridis} found was higher than the \textit{M. aeruginosa} biomass at which grazing effects became obvious, both in the field and during the experiments. The resistance of \textit{M. viridis} to amoebae grazing

![Fig. 4. Growth of \textit{M. aeruginosa} strain M31 (A) and \textit{M. viridis} strain W24 (B) in the absence or presence of different amoeba strains, as revealed by absorbance measurements. (C) Microcystin concentrations (\(\mu\text{g microcystin-LR equivalents per liter; ELISA immunoassay}\)) per unit \textit{Microcystis} biomass in M31 (black bars) and W24 (white bars) cultures with or without (controls) different strains of \textit{Microcystis}-grazing amoebae at the end of the experiment. Microcystin concentrations are not shown for two of the amoeba treatments due to the near-absence of \textit{Microcystis} cells. (D) amoeba population densities (logarithmic scale) at the end of the experiment in M31 (black bars) and W24 (white bars) \textit{Microcystis} cultures. The black line represents the amoeba density at the start of the experiment.

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Table 5. Amoebae growth and grazing rates on \textit{Microcystis aeruginosa}, estimated for selected dates with high amoebae abundance in Westveld Pond and calculated for amoeba strain A16WVB during an in vitro grazing experiment.

<table>
<thead>
<tr>
<th>Field data</th>
<th>Microcystis biomass (mg C L(^{-1}))</th>
<th>Amoebae density (N ml(^{-1}))</th>
<th>Growth rate (divisions day(^{-1}))</th>
<th>Grazing rate (pg C amoeba(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>18/06/2007 (T0)</td>
<td>35.42</td>
<td>2 600</td>
<td>0.59 (T0-72)</td>
<td>22.24</td>
</tr>
<tr>
<td>21/06/2007 (T72)</td>
<td>27.22</td>
<td>8 900</td>
<td>0.55 (T72-192)</td>
<td>6.21</td>
</tr>
<tr>
<td>26/06/2007 (T192)</td>
<td>7.37</td>
<td>59 500</td>
<td>0.09 (T192-240)</td>
<td>2.14</td>
</tr>
<tr>
<td>28/06/2007 (T240)</td>
<td>0.886</td>
<td>67 000</td>
<td>0.47 (T20-240)</td>
<td>7.26</td>
</tr>
<tr>
<td>08/08/2008 (T0)</td>
<td>19.81</td>
<td>5 800</td>
<td>0.29 (T0-72)</td>
<td>12.47</td>
</tr>
<tr>
<td>11/08/08 (T72)</td>
<td>12.70</td>
<td>10 500</td>
<td>0.41 (T72-144)</td>
<td>7.41</td>
</tr>
<tr>
<td>14/08/08 (T144)</td>
<td>3.88</td>
<td>24 500</td>
<td>0.35 (T0-144)</td>
<td>8.52</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In vitro</th>
<th>Microcystis biomass (mg C L(^{-1}))</th>
<th>Amoebae density (N ml(^{-1}))</th>
<th>Growth rate (divisions day(^{-1}))</th>
<th>Grazing rate (pg C amoeba(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>30.81 ± 1.61</td>
<td>13 834</td>
<td>1.29</td>
<td>9.23 ± 1.78</td>
</tr>
<tr>
<td>T40</td>
<td>19.10 ± 0.35</td>
<td>61 333 ± 5 140</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The growth and grazing rates were estimated for different time intervals (hours) between the start of the amoebae population increase (T0) and maximal amoebae densities in the field (T240 and T144 for 2007 and 2008, respectively), or between the start (T0) and the end of the experiment (T40).
may be caused by the physical barrier provided by the thick mucilage layer, its biochemical composition or some other factor.

Rapid switches between grazing-resistant and grazing-sensitive Microcystis genotypes have the potential to directly influence predator-prey or parasite-host cycles, as shown in laboratory experiments (Yoshida et al., 2003). Strong fluctuations in prey densities can even be dampened by rapid switches between defended and sensitive genotypes when the cost of defence is small (Yoshida et al., 2007). In our study system, the increase in abundance of the grazing-resistant Microcystis genotype was not rapid enough to prevent a temporary Microcystis population crash, although its increasing dominance allowed the bloom to persist throughout the rest of the summer period. The same pattern was found in a natural bloom population of the Raphidophyceae microalga Heterosigma akashiwo and its viral parasite (Tarutani et al., 2000). These periodic population crashes might be caused by a rather small inoculum (due to a large cost of defence) and/or a relatively low growth rate of the defended genotypes (compared with the growth rate of the amoebae).

Zooplankton grazers were apparently less important in determining Microcystis biomass and genetic composition in Westveld Pond as peaks in their abundance did not coincide with observable changes in the Microcystis population. This is not very surprising given that the zooplankton community consisted mainly of rotifers of the genus Brachionus, which are generally incapable of using Microcystis as a food source (Rothhaupt, 1991; Geng and Xie, 2008). Instead, rotifer densities declined during the most intense cyanobacterial bloom periods. More surprising was the apparently minor importance of chytrid fungi for the strong reductions in Microcystis biomass, especially given their sometimes very high infection rates before and after the M. aeruginosa bloom. Chytrid fungi can cause a strong numerical reduction of their host population and, by doing so, influence primary production and phytoplankton species succession (Holfeld, 1998). However, although parasitism could have been a substantial loss factor, M. aeruginosa was apparently able to outgrow its parasite during bloom conditions in Westveld Pond, in contrast to M. viridis whose biomass maxima coincided with high chytrid infection rates, although without preventing M. viridis to develop a bloom in 2007. It should be noted that the high infection percentages reported here concern colonies and not individual cells; only one to several tens of cells in a parasitized Microcystis colony were infected by the developing zoosporangia, leaving many cells of the colony intact. However, it is possible that subtle host–parasite co-evolution was occurring within each Microcystis morphotype, in response to the chytrid infections, if genetic variation in infectivity and resistance is present in the parasite and host respectively (see, e.g. Decaestecker et al., 2007; De Bruin et al., 2008). Additionally, the inability of the amoebae during the first abundance peak in 2008 to influence Microcystis biomass may have been due to rapid Microcystis microevolution, although the relatively low Microcystis density, in comparison with the maximal Microcystis biomass observed in both years, might also play a role. The fact that the two amoeba strains isolated from that abundance peak were able to graze down an M. aeruginosa strain isolated after the amoeba peak suggests that it might rather be the second possibility. In any case, potential genetic variation for resistance and infectivity in Microcystis and amoebae/chytrid fungi, respectively, possibly leading to co-evolution, should be further investigated.

Other potentially important predators or parasites were not monitored during this study. Microcystis-consuming heterotrophic flagellates or ciliates were probably never present in high abundances as they were never observed in the live samples investigated microscopically after every sampling occasion. Two other potential parasites of the cyanobacteria, cyanophages and bacteria (Fogg et al., 1973), were also not monitored. A possible reason for the inability of M. viridis to form a bloom in 2008 could be a viral infection, since many colonies in that year showed signs of severe cell lysis, a feature that can be caused by cyanophages (Tucker and Pollard, 2005).

While amoebae are clearly important in determining Microcystis population dynamics in Westveld Pond, this does not mean that abiotic factors are not important as well. First, water temperature influences the length and timing of the growth season in which Microcystis blooms might develop. Second, M. aeruginosa probably has a competitive advantage earlier in summer, when the water temperature is higher and the photoperiod longer. In both years only M. aeruginosa developed a bloom in spite of the presence of resting stages from both Microcystis types on the sediments during winter. Microcystis viridis became dominant only later in summer after the almost complete disappearance of M. aeruginosa, in accordance with the observations of Takamura and Watanabe (1987). This is probably linked with lower optimal temperature and irradiance preferences of M. viridis in comparison with other Microcystis species (Yagi et al., 1994; Imai et al., 2009). However, around the time of the amoebae-induced shift between the two Microcystis morphotypes, there were no significant changes in water temperature or other abiotic variables.

Concerning the identity of the amoebae, the dominant species may belong to Korotnevella, based on morphology and the similarity between its SSU rDNA and those of other amoebae in GenBank. However, at least two other Microcystis-grazing amoebae, presumably a Vanella sp. and a Gephyramoeba sp., were isolated. A testate

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amoeba grazing on *Microcystis* was reported previously (Nishibe *et al*., 2004). This suggests a rather high diversity of amoeba capable of feeding on *Microcystis*. More detailed phylogenetic analyses based on SSU rDNA and a higher-resolution species-level marker will be necessary to delineate and describe the different amoeba species. Their taxonomy and food preference is currently being addressed (J. Van Wijchen, in preparation).

In conclusion, in this study we provide evidence that amoeba occurring at high population densities for very short periods are one of the main factors determining the biomass and genetic composition of a natural *Microcystis* bloom. The finding that these amoeba were able to graze down certain dominant and even toxigenic cyanobacterial genotypes makes them an ideal target as a potential biological control agent for *Microcystis* blooms. Our research therefore contributes to the increasing knowledge about the potential of biological control mechanisms to reduce *Microcystis* bloom development and persistence (see Sigee *et al*., 1999; Gumbo *et al*., 2008).

**Experimental procedures**

**Study site description**

Westveld Pond (51°04′05.9″N, 3°46′42.7″E) is a highly eutrophic urban pond, located in a small park area in the city of Gent (Flanders, Belgium). It has a maximal surface area of 2024 m$^2$ and a maximum depth of about 2 m. The pond is of Gent (Flanders, Belgium). It has a maximal surface area of eutrophic urban pond, located in a small park area in the city about 500 kg ha$^{-1}$ (unpublished data provided by the City Council of Gent). The pond harboured a dense fish community (estimated at 2008. On each sampling occasion, water temperature, phytoplankton growth season, water samples were collected weekly or twice a week under bloom conditions. During winter (December to March), only one sample was taken in January 2008. On each sampling occasion, water temperature, oxygen concentration (absolute and relative), pH and conductivity were measured at one location in the pond with a YSI 600R multi-parameter probe. Integrated water samples of the entire water column were taken using a tube sampler at three locations, pooled and thoroughly mixed in a plastic 100 l container. A 250 ml phytoplankton subsample was fixed with Lugol’s iodine, formaldehyde and sodium thiosulfate (Sherr and Sherr, 1993). Five to 30 litres of mixed water was filtered over a 70 μm mesh size net to concentrate zooplankton. Zooplankton samples were fixed with sugared formaldehyde to a final concentration of 5% v/v (Haney and Hall, 1973).

Water samples for DNA extraction, nutrient and pigment analysis were transported to and further processed in the laboratory.

**Nutrient analysis**

Glass-fibre (Whatman GF/C)-filtered water samples (100 ml), pre-incinerated (500°C) glass fibre filters through which between 15 and 200 ml of water sample was passed and 50 ml unfiltered water samples in centrifuge tubes (BD Falcon) to which 0.2 ml H$_2$SO$_4$ (2.5 M) was added, were kept frozen at −20°C for the analysis of dissolved nutrients, particulate organic nitrogen (PON) and total phosphorus (TP) respectively. All analyses were carried out according to standard methods (Eaton *et al*., 2005), using a Skalar auto-analyser for dissolved nutrients and TP and a nitrogen/carbon analyser (Carlo Erba NA 1500) for PON.

**Pigment analysis**

Glass fibre filters (Whatman GF/F), through which between 15 and 200 ml of water sample was passed, were stored in 8 ml 90% HPLC grade acetone. After two 15 min ultrasonication procedures, separated by an overnight period at 4°C in the dark, HPLC analysis was carried out using the Wright and colleagues (1991) gradient elution method, with a Waters system comprising a Waters 996 PDA detector and a Waters 470 fluorescence detector. Calibration was made using commercial external standards (DHI, Denmark). Cyanobacterial biomass was determined from HPLC phytoplankton pigment concentrations using CHEMTAX, a matrix factorization program, which estimates the contribution of each specified phytoplankton pigment class to the total chlorophyll a concentration (Mackey *et al*., 1996). The initial ratio matrix used in this study was derived from Sarmento and Descy (2008) and CHEMTAX processing was run until stability of the pigment ratios in the output ratio matrix was reached.

**Microscopy**

Enumeration of *Microcystis* colonies and single cells, other cyanobacteria and amoebae was carried out by counting 1 ml aliquots of the fixed phytoplankton samples in a Sedgewick–Rafter counting chamber (PYSER-SGI, USA) under a Leitz Diaplan light microscope. Two different *Microcystis* morphotypes were distinguished on the basis of colony morphology (see Results) and counted at a 40× magnification along randomly chosen transects (each 20 mm$^2$) or counting squares (1 mm$^2$), depending on their density, to a total minimum of at
least 100 units. *Microcystis* colonies infested with grazing amoebae were distinguished in order to determine the percent of infested colonies to the total amount of colonies observed for each *Microcystis* morphotype. The abundance of *Microcystis* single cells and suspended and attached amoebae was measured by counting all individuals on 10 randomly chosen squares of 1 mm² at a magnification of 100×. Depending on population densities, other colonial and filamentous cyanobacteria were also counted using randomly chosen squares, transects or the whole counting chamber, to a total of at least 100 units. At all times, care was taken to also include any floating specimens by changing the focus towards the coverslip. The average number of cells per *Microcystis* colony for each sample was obtained by counting all the cells from at least 10 colonies for both morphotypes in a drop of phytoplankton sample on a microscope slide at a magnification of 400×. The average number of cells per colony was multiplied by the number of colonies in each sample to obtain the mean *Microcystis* cell density for each colony morphotype. The size of 200 cells from a mixed sample (2007: 21/06, 23/07, 13/08, 17/09; 2008: 24/06, 15/07, 19/08, 15/09; 1 ml each) was measured for each *Microcystis* morphotype, using Axiovision 4.4 software (Zeiss, Jena, Germany), while for the other cyanobacteria at least 10 individual units (cells or colonies) per sample were measured for biovolume calculations (Hillebrand et al., 1999). By using the average cell volume, the C-biomass for each taxon was calculated according to Menden-Deuer and Lessard (2000).

To obtain the infection percentage by the chytrid parasite *C. microcystidis*, between 100 and 200 *Microcystis* colonies from each morphotype per sample were examined for the presence of fungal sporangia, after adding a drop of methylene blue (0.2% w/v) to increase the visibility of the fungal rhizoids and zoosporangia.

Zooplankton were identified and enumerated using a counting chamber and a Leica Wild M 10 stereo microscope. Rotifers and cladocerans were identified to species level, while copepods were only identified to order level, using the identification key of Streble and Krauter (1981). Zooplankton biomass was calculated according to Bottrell and colleagues (1976).

**Isolation and cultivation of Microcystis and amoebae**

*Microcystis* cultures were established by picking individual colonies from live samples with a sterile micropipette under a binocular microscope. They were transferred to 24-well Repli dishes and later to 250 ml Falcon culture flasks. All strains were grown in freshwater WC medium (Guillard and Lorenzen, 1972) without pH adjustment and vitamin addition, at 24°C, a photon flux density of c. 120 μE m⁻² s⁻¹ and a 12 h/12 h light/dark regime. For long-term maintenance, the culture flasks were incubated at 18°C, under a photon flux density of c. 30 μE m⁻² s⁻¹ and a 12 h/12 h light/dark regime, and re-inoculated approximately every month. In 2007, 54 *Microcystis* colonies were isolated from a single sample taken on 26/07/2007. From these, 28 cultures were established: 23 having an *M. viridis* morphology and 5 with an *M. aeruginosa* morphology. In 2008, 159 *Microcystis* colonies were isolated from seven different water samples gathered during the bloom period (22/04, 29/04, 13/05, 03/06, 17/06, 24/07 and 08/08) from which 19 cultures were established, all of the *M. aeruginosa* morphotype.

In 2008, 63 single suspended amoebae and *Microcystis* colonies with one (or in rare cases, a few) amoeba on them, were isolated using a micropipette from samples taken on 25/06 (n = 7), 04/07 (n = 16), 21/07 (n = 16) and 25/07 (n = 24). All samples were incubated for several weeks under the same conditions in which the cultures were grown before isolation of amoebae, except the last one, from which amoebae were isolated the next day (Table 2). They were put into 96-well Repli dishes, and grown on a mixture of both *Microcystis* morphotypes as food source, in the same conditions as the *Microcystis* cultures. All amoeba strains were re-isolated one to three times to eliminate algal contaminants and ensure their monoclonal status. From these, 16 cultures were established belonging to three different amoebae morphotypes according to Smirnov and Goodkov (1999). Three were of the reticulate morphotype, 12 were of the dactylopodial morphotype and 1 was fan-shaped. They were transferred to 24-well Repli dishes, re-inoculated every two to three weeks and before experimental use transferred to 250 ml Falcon culture flasks to obtain higher population densities.

**Microcystin analysis**

For the determination of microcystins in bloom samples and in *Microcystis* cultures, 1 ml of each water sample/culture was stored in a 2 ml Eppendorf vial at −20°C until analysis by enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies raised against microcystin-LR (Metcalfe et al., 2000). Since these antibodies show similar cross-reactivities with other naturally occurring microcystin structural variants, e.g. microcystins LY, LA, LW, LF, the ELISA was calibrated with purified microcystin-LR. As a result, microcystin concentrations were expressed as microcystin-LR equivalents. Samples were analysed after boiling at 100°C for 1 min to release the (thermostable) toxins from the cyanobacterial producer-cells. Microcystin concentrations are thereby termed ‘total microcystin’, and are the sum of the extracellular plus intracellular microcystin pools (Metcalfe and Codd, 2000). All analyses were performed in triplicate.

**Molecular analysis**

For *Microcystis* population profiling and characterization of cultivated *Microcystis* strains, between 2 and 15 ml of natural water sample/culture was filtered through a 25 mm 0.2 μm GSWP membrane filter (Millipore) and stored at −20°C. DNA was extracted according to Zwart and colleagues (1998) and purified on a Wizard column (Promega). For DNA from the water samples, a specific nested-PCR protocol was used to amplify only *Microcystis* ITS sequences. In a first PCR, a specific 16S rDNA primer CH for *Microcystis* (Rudi et al., 1997) was used as forward primer combined with the universal reverse 23S rDNA primer ULR (Janse et al., 2003). The composition of the reaction mix and PCR program was performed according to Janse and colleagues (2003). The resulting PCR product was purified using a QiaQuick PCR purification kit (QiaGen), diluted 10×, and used as template (2 μl in a total volume of 50 μl) for a second PCR with the...
cyanobacterium-specific 16S rDNA primer (GC)-CSIF in combination with the universal primer ULR. The composition of the reaction mix was the same as for the first PCR. The PCR program started with a denaturation step of 5 min at 94°C. After pre-incubation, 30 cycles were performed. Cycle step times were 1 min each for denaturation (94°C), annealing (65°C) and extension (72°C). A final extension step was performed for 30 min at 72°C. For the isolated Microcystis strains, 16S-23S rDNA ITS sequences were directly amplified by PCR using the primers CSIF and ULR (Jane et al., 2003).

Denaturing gradient gel electrophoresis was performed as described by van Gremberghe and colleagues (2008) based on the protocol of Muyzer and colleagues (1993), using the D-Code system from Bio-Rad Laboratories (Hercules, CA). The denaturing gradient contained 35–40% denaturant. For the isolated Microcystis strains, ITS sequences of 47 strains were screened by DGGE to identify groups of strains with (presumably) identical sequences and 2–14 representatives of each group were then chosen for sequencing (34 strains in total, see Table 2) and some of them for the determination of microcystins. PCR products of the most dominant ITS types were pooled to make a standard for DGGE analyses of the water samples. From the DGGE profiles of the water samples, a small piece of gel from the middle of the target band was excised and incubated in 50 μl sterile TE buffer (10 mM Tris, pH 7.6, 1 mM EDTA) for 24 h at 4°C. The eluent was then re-amplified and purified on DGGE once or twice. The resulting PCR products were purified using a QiaQuick PCR purification kit (QiaGen) and sequenced. Sequencing of the ITS sequences of Microcystis was performed with the ABI-Prism sequencer (ABI-Prism 3100). In total, 31 sequences were obtained from excised bands. On every gel, three standard lanes were run in parallel with the samples to provide marker bands for gel alignments.

For characterization of the amoebae, DNA from five strains (Table 2) was extracted and sequenced as described above. The entire 18S rDNA was amplified for genus identification (Huss et al., 1999).

GenBank Accession Numbers are HM017112–HM017142 for the bands from the field samples. Accession numbers for the sequences of Microcystis and amoeba cultures are listed in Table 2.

Food preference experiments

To investigate amoebae grazing selectivity, two monoclonal Microcystis strains were selected based on morphology and ITS sequence: strain M31 with M. aeruginosa morphology and an ITS sequence identical to one of the two dominant bands on the DGGE profiles of the natural populations (ITS 3) and strain W24 with M. viridis morphology and an ITS sequence identical to the other dominant band on the DGGE profiles (ITS 1). We further refer to these strains as M. aeruginosa and M. viridis. Both Microcystis strains produced microcystins (based on ELISA). Four monoclonal amoeba cultures, two of the dactylopodial morphotype (A16WVB, A21WVB) and two of the reticulate morphotype (A11WVB, A22WVB), were selected (sampled on 25/07/2008). Since growing large numbers of amoebae on a mixture of M. aeruginosa and M. viridis strains mostly failed, the amoebae used in the experiment were grown on a mixture of M. aeruginosa strains isolated in 2007.

Grazing experiments were performed in 24-well Repli dishes filled with 2.5 ml freshwater WC-medium, inoculated with Microcystis strains and amoebae. The start biomass of the exponentially grown Microcystis strains was set at 2.5 mg C l⁻¹, a concentration similar to that in Westveld Pond before the strong increase in amoeba population densities. After concentrating the amoebae, using a nylon net (mesh size of 70, 20 and 10 μm) to remove Microcystis colonies and single cells, and after several washing steps with sterile WC-medium to remove remaining Microcystis cells, 100 μl of culture containing ±2000 amoebae was added to the wells containing the Microcystis strains. All different combinations of Microcystis and amoeba strains were set up in triplicate, and the different combinations were randomly positioned on the 24-well Repli dishes. Controls consisted of wells with only Microcystis strains, without amoebae. Instead, 100 μl of filtered WC-medium was added to these controls. The plates were placed in a cultivation room for 10 days under the same conditions as used to cultivate the Microcystis and amoeba cultures.

The influence of amoeba grazing on Microcystis biomass was assessed every second day by measuring the absorbance of the cultures at 660 nm using a Victor2 Wallac 1420 multilabel counter (Perkin Elmer). Before measurement, each culture was homogenized by gently pipetting the culture up and down. After absorbance measurements, the plates were randomly placed back on the shelf. Absorbance measurements were converted to biomass using standard curves made for each Microcystis strain before the start of the experiment. This was done by correlating biomass and absorbance using a dilution series from each culture. Cell counts were carried out on subsamples that were boiled at 100°C for 6 min to obtain unicellular cultures (Joung et al., 2006). For each Microcystis strain the size of at least 200 cells was measured to calculate the biovolume, and the average biovolume was used to calculate the average cell biomass using the equations given by Menden-Deuer and Lessard (2000). Microcystin concentrations per unit Microcystis biomass were determined before and at the end of the grazing experiments. Therefore, 1 ml samples were stored at −20°C until determination of the microcystin content with ELISA. One-milliliter subsamples from all treatments at the end of the experiment were fixed with 50 μl formaldehyde (40%) for amoeba and Microcystis cells enumeration using a Sedgewick–Rafter counting chamber.

Growth and grazing rate experiments

The grazing rate of the amoeba on M. aeruginosa was determined in 24-well Repli dishes, by inoculating 1 ml amoeba A16WVB culture (containing ±35 000 amoebae) in 1.5 ml Microcystis M31 culture. All wells contained a Microcystis biomass of c. 30 mg C l⁻¹ at the start of the experiment (Table 5). The experiments were performed in triplicate. The 24-well Repli dishes were placed in the dark in the cultivation room for 40 h in the abovementioned incubation conditions. One ml WC-medium was added to 1.5 ml of the same M31 culture in triplicate as a control to determine possible Microcystis growth in the absence of light. Microcystis biomass
Statistical analysis

To test for effects of amoebae on *Microcystis* biomass in the food preference experiments, a repeated measures analysis of variance (ANOVA) (factors time and amoeba treatment) was performed for each *Microcystis* strain separately. When an interaction between time and treatment was found, one-way ANOVA, followed by post hoc Tukey tests, was performed for each time point separately, to reveal significant differences in *Microcystis* biomass between the controls and each of the amoeba treatments. A one-way ANOVA, followed by post hoc Tukey tests, was further used to analyse the effect of amoebae on microcystin concentration per unit of biomass at the end of the experiments. Non-parametric Mann–Whitney U-tests were performed for significant differences in amoebae densities between the two *Microcystis* strains at the end of the experiments. All univariate statistical analyses were carried out with Statistica 7 for Windows.

Acknowledgements

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References


(assessed every 12 h by measuring the absorbance) and amoebae densities at the start and end of the experiment were determined as described before. The growth rate of the amoeba was calculated as (log A<sub>0</sub> – log A<sub>t</sub>)/Δt, where A<sub>0</sub> and A<sub>t</sub> refer to amoebae densities at the start and the end of the incubation period (Δt) expressed in days. Grazing rate was calculated by dividing the *Microcystis* biomass that was grazed away by the number of amoebae present during the incubation period. Therefore, a mean amoebae population density (A<sub>m</sub>) for exponential growth phase was calculated according to Heinbokel (1978) as A<sub>m</sub> = (A<sub>0</sub> – A<sub>t</sub>)/(ln A<sub>0</sub> – ln A<sub>t</sub>).


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