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Effect of dilution rate on competitive interactions between the cyanobacterium *Microcystis novacekii* and the green alga *Scenedesmus quadricauda* in mixed chemostat cultures

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We examined the competition between the cyanobacterium Microcystis novacekii (Kom.) Comp. and the green alga Scenedesmus quadricauda (Turpin) Brébisson using unialgal and mixed chemostat cultures with various supply rates of culture medium where NH_4^+-N limited algal growth. In unialgal cultures, both species grew at all of the dilution rates examined (0.1, 0.3 and 0.8 day^{-1}): steady-state cell densities were 1×10^4 to 8×10^4 cells mL^{-1} for M. novacekii and 0.5×10^5 to 2.1×10^5 cells mL^{-1} for S. quadricauda. Microcystis novacekii was dominant in mixed cultures at a dilution rate of 0.1 day^{-1}, where the steady-state cell density was 1×10^4 to 7×10^4 cells mL^{-1} for M. novacekii and 1×10^2 to 5×10^2 cells mL^{-1} for S. quadricauda. Scenedesmus quadricauda was dominant in mixed cultures at the higher dilution rates (0.3 and 0.8 day^{-1}), where the final cell density was 0.5×10^2 to 6.4×10^2 cells mL^{-1} for M. novacekii and 0.2×10^5 to 7×10^5 cells mL^{-1} for S. quadricauda. This result indicates that the dilution rate affects the competitive interaction. We conclude that it is necessary to consider water exchange in the study of mechanisms of cyanobacterial blooms.

INTRODUCTION

Algal blooms occur regularly in eutrophic waters, causing both economic and ecological disturbance. Blue-green algal (cyanobacterial) blooms, in particular, have significant effects on the quality of water resources for drinking as well as for agriculture (Collins, 1978; Watanabe, 1994). Cyanobacterial blooms cause a rancid smell in drinking water, the decay of the bloom leads to a reduction in dissolved oxygen content and, furthermore, some species produce toxins (Collins, 1978; Lampert, 1981; Nizan *et al.*, 1986; Carmichael, 1994; Codd, 2000). Therefore, it is desirable to control blooms of cyanobacteria.

To be able to control the growth of cyanobacteria it is necessary to understand the mechanisms of bloom development. It has been suggested that high loads of nutrients to lakes lead to blooms (Fogg, 1969; Gibson and Smith, 1982). However, cyanobacteria do not necessarily bloom when the nutrient load is high. Dominance by chlorophytes has been reported in shallow hypertrophic Danish lakes (Jensen *et al.*, 1994). The cyanobacterium *Microcystis* tends to bloom in stagnant waters rather than in flowing waters (Pearl, 1988). One possible explanation for this is that the growth of algae is suppressed differently under various water-flow conditions owing to the competitive interaction with other algae for nutrients.

Kuwata and Miyazaki evaluated theoretically the effect of dilution rate on competition between *Microcystis novacekii* and the green alga *Scenedesmus quadricauda* in nitrogen-limited chemostat cultures, using numerical simulations (Kuwata and Miyazaki, 2000). According to their results, *Microcystis novacekii* should dominate at lower dilution rates, while *S. quadricauda* dominates at

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higher dilution rates. However, this prediction has not been confirmed experimentally. It is necessary to examine experimentally the effect of water turnover on the competition between *Microcystis* and other algae, and to determine whether higher turnover leads to a decrease in the cyanobacteria.

In this study, the competitive interaction between the cyanobacterium *M. novacekii* and the green alga *S. quadricauda* was analysed using chemostat cultures under various dilution rates, and the effect of water turnover on competition between *Microcystis* and other algae was discussed.

METHOD

The cyanobacterium *M. novacekii* and the green alga *S. quadricauda* were used. *Microcystis novacekii* (Tsukuba Algal Collection TAC-19) was kindly supplied by Dr M. Watanabe of the Tsukuba Botanical Garden, National Science Museum and the *S. quadricauda* had been maintained in our laboratory (Watanabe and Miyazaki, 1996). Stock cultures for each species were maintained at room temperature at an irradiance of 20 μmol photons m^{-2} s^{-1} in a 14 h light:10 h dark cycle in modified WC medium (Guillard and Lorenzen, 1972) including MOPS (3-[*N*-morpholino]propanesulfonic acid) as a buffering agent to regulate pH at 7.8, and ammonium as a nitrogen source. Silicate was removed from the medium. The cells of *M. novacekii* were present in the form of unicells.

Chemostat cultures were kept at 25°C under continuous irradiance of 150 μmol photons m^{-2} s^{-1}. The culture medium contained 10 μM ammonium chloride as a nitrogen source, such that nitrogen limited the growth of both algae (Watanabe and Miyazaki, 1996). This medium was continuously supplied to the 1 L culture vessels at a dilution rate of 0.1, 0.3 or 0.8 day^{-1}. Unialgal culture experiments were conducted once for each dilution rate. Mixed-culture experiments were performed in duplicate. *Microcystis novacekii* was cultivated for 2–3 weeks and *S. quadricauda* for 3–5 days before the competition experiments. The cultivated cells were inoculated into the culture vessels at densities of $\sim10^3$–10^4 cells mL^{-1} for *M. novacekii* and ~10–10^3 cells mL^{-1} for *S. quadricauda*. The cultures were stirred continuously with magnetic stirrers. Samples (8 mL) were removed from the culture vessels at predetermined times, using a syringe, and were fixed with a few drops of Lugol's solution (100 g of KI, 50 g of I_2, 95 mL of acetic acid, 1000 mL of H_2O). Cells in the fixed samples were counted under an inverted microscope (Zeiss Axiovert 135). A minimum of 400 cells per sample were counted.

RESULTS

In the unialgal cultures at a dilution rate of 0.1 day^{-1}, *M. novacekii* and *S. quadricauda* grew well (Figure 1A and B). The cell density of *M. novacekii* was $2.3 \times 10^4 \pm 0.8 \times 10^4$ (mean ± SD) cells mL^{-1} during the experiment (Figure 1A). *Scenedesmus quadricauda* increased during the first 4 days and then became constant, with a density of $6.6 \times 10^4 \pm 0.2 \times 10^4$ cells mL^{-1} (Figure 1B).

In the mixed cultures at 0.1 day^{-1}, *M. novacekii* dominated (Figure 1C). In the first mixed culture (Figure 1C-1), *M. novacekii* increased for the first 2 days and then levelled off at $1.9 \times 10^4 \pm 0.9 \times 10^4$ cells mL^{-1}. The *S. quadricauda* increased immediately after inoculation but fell to $<1.7 \times 10^2$ cells mL^{-1} after day 7. In the second experiment (Figure 1C-2), the cell density of *M. novacekii* was constant at $\sim5.6 \times 10^3$ cells mL^{-1} for the first 2 weeks. The density then increased gradually and reached 2.5×10^4 cells mL^{-1} on day 20. The cell density of *S. quadricauda* was $4.2 \times 10^2 \pm 1.3 \times 10^2$ cells mL^{-1} after day 9 in the second experiment. At steady state, *M. novacekii* in the mixed cultures showed a cell density similar to that in the unialgal culture, while *S. quadricauda* in the mixed culture showed a lower density than in the unialgal culture, indicating that *M. novacekii* suppressed the growth of *S. quadricauda* in competition at a dilution rate of 0.1 day^{-1}.

In the unialgal cultures at a dilution rate of 0.3 day^{-1}, both *M. novacekii* and *S. quadricauda* grew well (Figure 2A and B). The cell density of *M. novacekii* was $1.4 \times 10^4 \pm 0.6 \times 10^4$ cells mL^{-1}. The *S. quadricauda* increased during the first 4 days and then became constant, with a density of $1.5 \times 10^5 \pm 0.4 \times 10^5$ cells mL^{-1}.

In the mixed cultures at a dilution rate of 0.3 day^{-1}, *S. quadricauda* was a superior competitor. In the first mixed culture (Figure 2C-1), the cell density of *M. novacekii* decreased to 2.0×10^2 cells mL^{-1} during the first 5 days. Then the density gradually decreased to 8.8×10^1 cells mL^{-1} by day 14. *Scenedesmus quadricauda* increased during the first 4 days. The population density reached 4.2×10^4 cells mL^{-1} on day 5. After day 5, the density ranged between 2.1×10^4 and 11.3×10^4 cells mL^{-1}, with an average of $4.8 \times 10^4 \pm 3.3 \times 10^4$ cells mL^{-1}. In the second mixed-culture experiment (Figure 2C-2), the cell density of *M. novacekii* showed a pattern of decrease similar to that in the first experiment. After day 12, the density reached $2.2 \times 10^2 \pm 2.0 \times 10^2$ cells mL^{-1}. *Scenedesmus quadricauda* increased for the first 5 days and became constant with a density of $4.2 \times 10^4 \pm 2.4 \times 10^4$ cells mL^{-1} after day 5. The density of *S. quadricauda* after day 5 was >100-fold that of *M. novacekii* at steady-state growth. The densities of *M. novacekii* in the mixed cultures were 1/100th the size of that in the unialgal culture

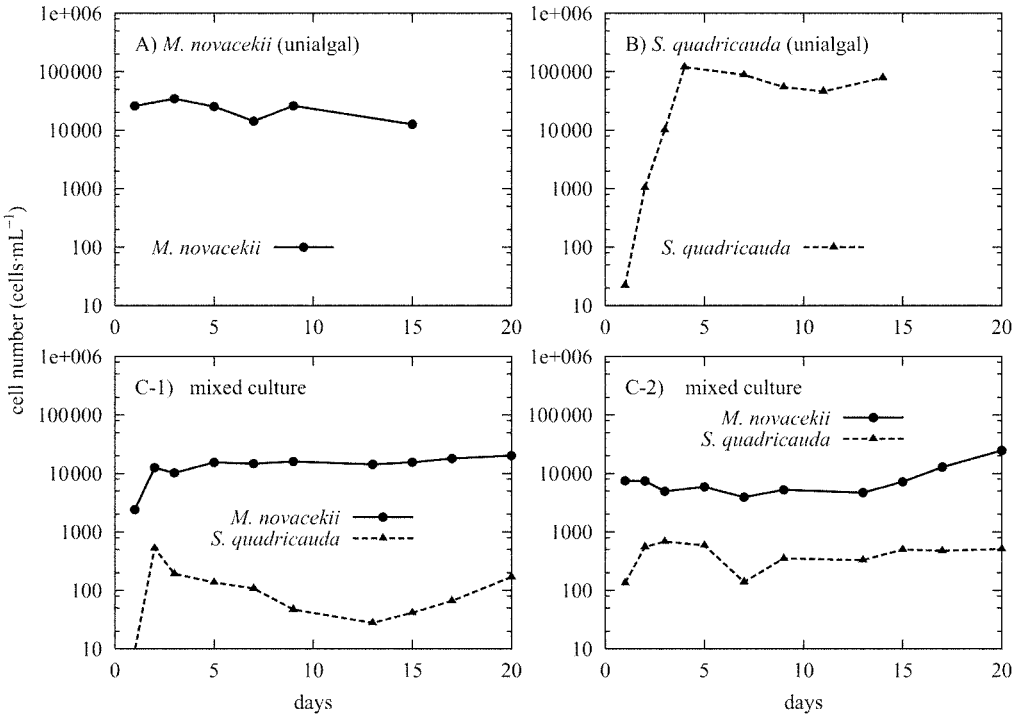

Fig. 1. Growth of *M. novacekii* and *S. quadricauda* in chemostat cultures with the dilution rate 0.1 day^{-1}.

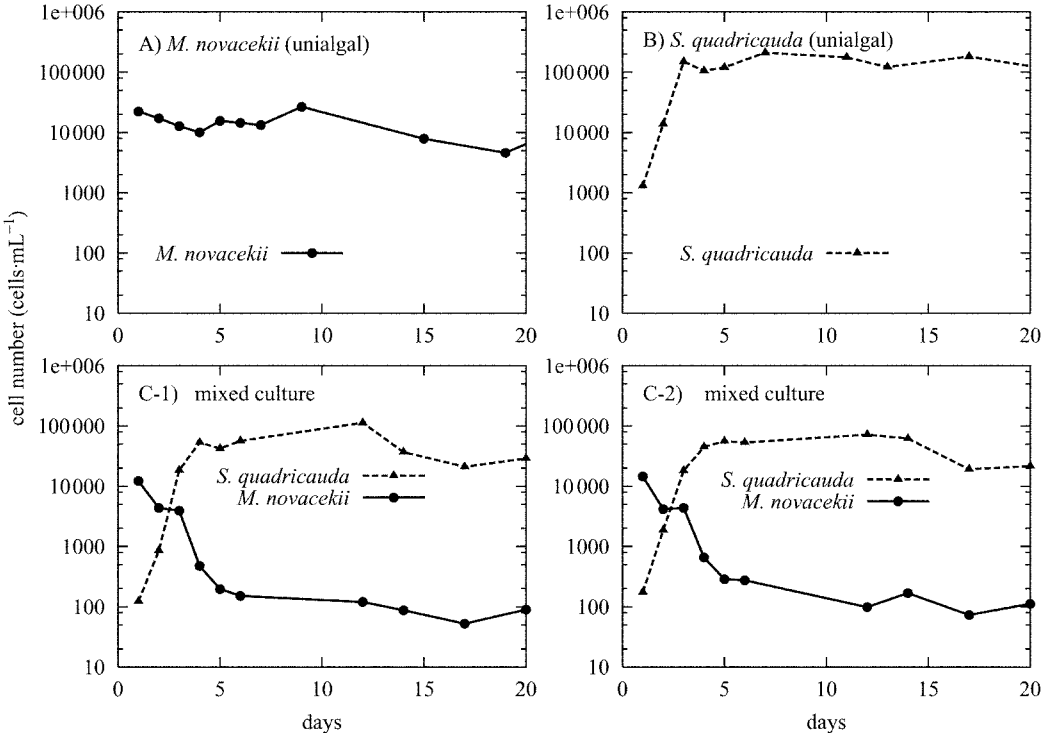

Fig. 2. Growth of *M. novacekii* and *S. quadricauda* in chemostat cultures with the dilution rate 0.3 day^{-1}.

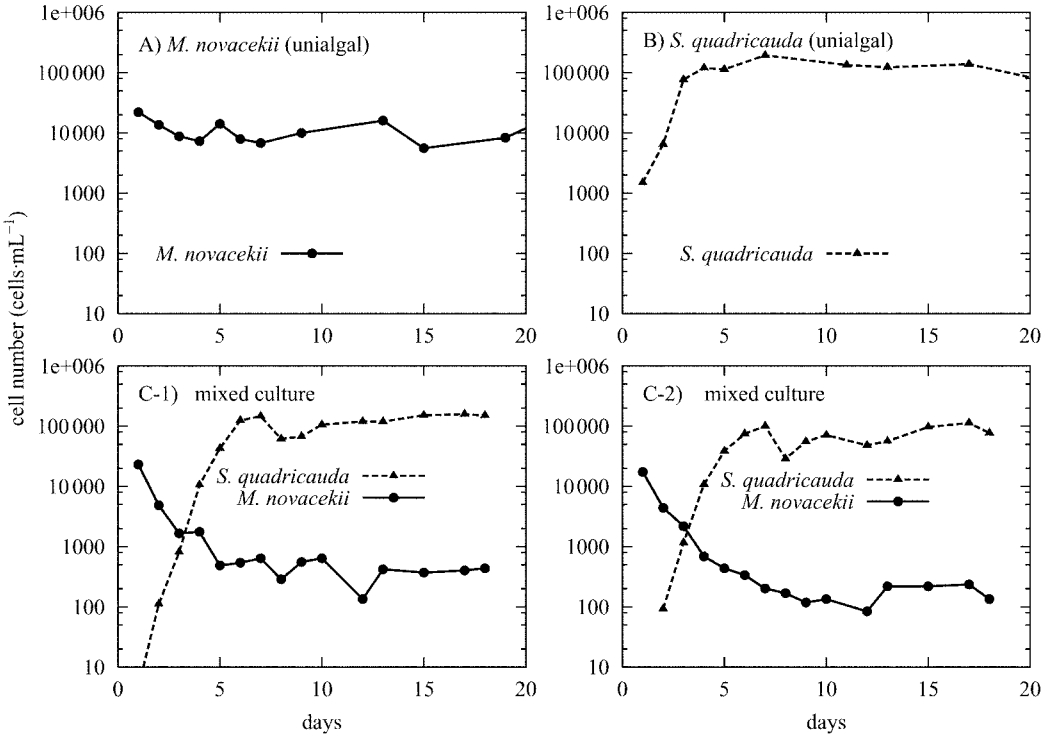

Fig. 3. Growth of *M. novacekii* and *S. quadricauda* in chemostat cultures with the dilution rate 0.8 day^{-1}.

at steady state. In contrast, the densities of *S. quadricauda* in the mixed cultures were similar to that in the unialgal culture. This shows that the growth of *M. novacekii* was depressed by the presence of *S. quadricauda* at a dilution rate of 0.3 day^{-1}.

In the unialgal cultures at a dilution rate of 0.8 day^{-1} both *M. novacekii* and *S. quadricauda* grew well (Figure 3A and B). The cell density of *M. novacekii* was almost constant, being $1.1 \times 10^4 \pm 0.5 \times 10^4$ cells mL^{-1}. *Scenedesmus quadricauda* increased for the first 4 days and then became constant with a density of $1.3 \times 10^4 \pm 0.4 \times 10^5$ cells mL^{-1}.

Scenedesmus quadricauda was the superior competitor in the mixed chemostat culture at a dilution rate of 0.8 day^{-1} (Figure 3C). The cell density of *M. novacekii* decreased immediately after the start of the experiments, while the density of *S. quadricauda* increased rapidly and was higher than that of *M. novacekii* after the culture reached a steady state. The density of *S. quadricauda* ($1.2 \times 10^5 \pm 0.36 \times 10^5$ cells mL^{-1}) was 1000 times as high as that of *M. novacekii* ($1.6 \times 10^2 \pm 0.6 \times 10^2$ cells mL^{-1}) at steady state in mixed culture (Figure 3C-1). The decrease in the growth of *M. novacekii* suggests that this species was inferior, compared with *S. quadricauda* at a dilution rate of 0.8 day^{-1}. Similar results were obtained in the other mixed-culture experiment, although there were slight differences in the fluctuations of population densities (Figure 3C-2).

The growth data in this study are presented as cell numbers (cells mL^{-1}), although the data might be more representative if expressed as a biovolume. *Microcystis novacekii* has a cell volume of $\sim 2.8 \times 10^{-17}$ m^3, according to the formulae given by Hillebrand *et al.* (Hillebrand *et al.*, 1999). Similarly, *S. quadricauda* has a cell volume of $\sim 1.1 \times 10^{-16}$ m^3, thus cells of *S. quadricauda* have cell volumes approximately four times those of *M. novacekii*. Figures 1–3 could be redrawn using biovolume. Then, the difference in growth between *S. quadricauda* and *M. novacekii* would be smaller in the mixed cultures, although the relative dominance would not change. Since this study's focus is not on the absolute dominance in the culture vessels *per se*, but on the changes in relative abundance caused by the difference in culture conditions, the interpretation of the results is not affected by the chosen means of comparison.

DISCUSSION

Microcystis novacekii grew well in the unialgal cultures at all the dilution rates, indicating that the supply rates of the culture media had no effects on the growth of *M. novacekii* in steady states under the conditions examined. The suppression of blooms of blue-green algae in flowing water may not be the result of direct effects of water

exchange on their growth but of other effects such as competitive interactions.

In the mixed cultures, the dominance of *M. novacekii* or of *S. quadricauda* depended on the dilution rates (Figures 1–3), indicating that the supply rates of the media influenced the competitive outcome. *Microcystis novacekii* was dominant at low dilution rates (0.1 day^{-1}) but not at the higher rates (0.3 and 0.8 day^{-1}), indicating that *Microcystis* dominates under conditions in which water exchange rates are low. These conditions agree with observations of *Microcystis* blooms in the field occurring in stagnant lakes and reservoirs with low exchange rates.

Hyenstrand *et al.* reported the growth responses of the green alga *Scenedesmus* and the cyanobacterium *Synechococcus* (Hyenstrand *et al.*, 2000). The cyanobacterium was favoured under conditions in which inorganic nitrogen was supplied in small pulses at a high frequency in the culture where *Scenedesmus* and *Synechococcus* were separated by a permeable dialysis membrane. These conditions might approximate those of the mixed culture at a dilution rate of 0.1 day^{-1}. The slow, small supply of nutrients seems to favour the cyanobacterium, compared with the green alga.

Many studies on cyanobacterial blooms have reported that the dominance of *Microcystis* results from changes in ratios of nutrients such as phosphorus, nitrogen or silicate (Holm and Armstrong, 1981; Smith, 1983; Sakshaug and Olsen, 1986; Takamura *et al.*, 1992). However, the present study shows experimentally that changes in dilution rates lead to the replacement of the dominant species, without necessarily changing the ratio of nutrients. Therefore, it is necessary to take water exchange rates into account when assessing the dynamics of *Microcystis* blooms.

Microcystis dominance in natural lakes might be caused by factors other than competition for nutrients. *Microcystis* can maintain its position in an optimal light environment by controlling its buoyancy with gas vacuoles (Reynolds, 1972). Massive growth of *Microcystis* in the surface layers decreases the light available for other algae in deeper layers. Increases in the colony size of *Microcystis* reduce grazing by zooplankton (Lampert, 1981; Shulamit *et al.*, 1986; Fulton and Pearl, 1987). Reynolds *et al.* found that water with a massive cell density of *Microcystis* was toxic to *Asterionella*, *Eudorina* and *Chlorella* (Reynolds *et al.*, 1981). Thus, it is possible that the growth of *S. quadricauda* decreased at a dilution rate of 0.1 day^{-1} because of allelopathic metabolites produced by *M. novacekii*. At higher dilution rates, *S. quadricauda* might be able to grow because of the rapid replacement of the medium and the increased dilution of putative metabolites. However, the culture medium in which *M. novacekii* grew did not produce any inhibitory effects on the growth of *S. quadricauda* (data not shown), and we consider that the decrease in *S. quadricauda* at the dilution rate of 0.1 day^{-1} was not the result of allelopathy.

Another possible cause of *M. novacekii* dominance in the mixed cultures at lower dilution rates is the lower half saturation constant (K_m) for ammonium uptake by *M. novacekii*. A species with a lower R^* (equilibrium resource availability) excludes other species with a higher value of R^* in the competition for limiting nutrients (Tilman, 1982; Grover, 1997). Watanabe and Miyazaki estimated the half saturation constants for ammonium uptake and maximum specific growth rates to be 0.5 μM and 0.7–0.9 day^{-1} for *M. novacekii*, and 5 μM and 1.4 day^{-1} for *S. quadricauda* (Watanabe and Miyazaki, 1996). A lower half saturation constant leads to a lower R^* in chemostat studies (Tilman, 1982). Sommer demonstrated that a species with a lower half saturation constant and a lower maximal growth rate was dominant in competition at low dilution rates, and that a species with a higher half saturation constant and a higher growth rate was dominant at higher dilution rates (Sommer, 1986). Our findings agree with these conclusions, although the half saturation constant is not the only important determinant of competitive ability. The resource equilibrium availability (Tilman, 1982; Grover 1997) that is crucial in competition depends not only on the half saturation constant, which is affected by parameters of cell quota functions (Flynn, 2002), but also on the other parameters for growth and loss of the species as reviewed in Grover (Grover, 1997).

Kuwata and Miyazaki evaluated the competition between *M. novacekii* and *S. quadricauda* using a numerical simulation (Kuwata and Miyazaki, 2000). According to their results, *M. novacekii* dominates in the steady state at dilution rates of <0.65 day^{-1} in mixed chemostat cultures, while *S. quadricauda* dominates at dilution rates >0.65 day^{-1}. Our result that *M. novacekii* dominated at the lower dilution rate corresponds with their prediction, although the critical value of the dilution rate at which the dominance changed from *S. quadricauda* to *M. novacekii* was smaller (<0.3 day^{-1}) in the present study. The smaller threshold values might be because of the estimation biases of parameters such as the half saturation constant in Kuwata and Miyazaki (Kuwata and Miyazaki, 2000).

Eutrophication, high input of nutrients such as nitrogen and phosphorus, has been considered the main cause for cyanobacterial blooms (Fogg, 1969). However, water stagnation also seems to be a causative factor in the initiation of blooms. Pearl reported that physically stable conditions were necessary for cyanobacterial blooms (Pearl, 1988). He referred to temperature, wind speed and wind direction as physical factors, and related

the changes in these factors to the disappearance of the cyanobacterial blooms. Dilution rate in the present study corresponds to the exchange rate of water in the field. Low dilution rates result in more stable conditions than higher dilution rates. Our findings of *M. novacekii* being dominant at lower dilution rates and less dominant at higher dilution rates agree with the observations of Pearl (Pearl, 1988) and the dominance of chlorophytes in shallow hypertrophic Danish lakes (Jensen *et al.*, 1994). Water exchange is one of the important factors to be considered in research into the mechanisms of cyanobacterial blooms.

There are several possible ways to inhibit and decrease cyanobacteria, such as exposure of the water containing the algae to high pressure (Porat *et al.*, 1999) and the addition of inhibitory compounds (Moriwaka, 1992). Herbicidal compounds added to aquatic systems can be harmful not only to cyanobacteria but also to other algae, and to other organisms higher in the food web. The present study suggests that cyanobacterial blooms could be curbed by controlling rates of water exchange. By controlling the rates of water exchange in reservoirs and natural lakes, the algal dominance can be manipulated without significant damage to the system. An increase in the exchange rates could increase chlorophytes and decrease noxious cyanobacteria in water bodies with blooms of cyanobacteria. This management technique may lead to a greater ability to conserve water resources.

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