Dominance of *Microcystis* with Special Reference to Carbon Availability in Lake Water

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The mechanism of dominance by *Microcystis* spp. (Cyanophyceae) was examined with special reference to the concentration of dissolved inorganic carbon (DIC), in *in situ* experiments using lake water. Lake water collected from mesotrophic Lake Biwa was enriched with nitrogen and phosphorus nutrients prior to the experiments, and use of a NaHCO₃-Na₂CO₃ buffer was successful in allowing a change of DIC concentrations for phytoplankton without lowering the pH. The experiments consisted of a control (no DIC addition) and a treatment. There were no significant differences in levels or patterns of change in pH values, nutrient (nitrogen and phosphorus) or chlorophyll concentrations between the two systems. *Microcystis* became dominant in the control system, while *Scenedesmus* sp. dominated in the DIC system. This suggested that the low DIC concentration in surrounding water favored dominance by *Microcystis* although this cyanobacterium needed some other carbon sources in order to proliferate. The buoyancy regulation of *Microcystis* may be the strategy by which it floats up to the surface layer, where there is abundant CO₂, and dominates when the DIC concentration in the water column is low.

Key words: *Microcystis*, DIC concentration, carbon limitation, Chlorophyceae

Cyanobacterial blooms are a common symptom of lake eutrophication, and numerous studies have examined the mechanisms by which they are initiated. The chemical environment for cyanobacterial dominance has also been investigated intensively. Previous studies have demonstrated that N₂-fixing cyanobacteria such as *Anabaena* are at an advantage under N limited conditions compared to non N₂-fixing phytoplankton, and the cyanobacteria tend to dominate when the N:P ratio in lake water is low^{13,24}. In contrast, the bloom formation mechanisms of *Microcystis*, the most notorious bloom-forming cyanobacterium, have not yet been fully explained. Although dominance by *Microcystis* does require plenty of nitrogen and phosphorus^{24,25}, supplement-

ing these nutrients also enhances the growth of other algae such as Chlorophytes^{24,25}. Since *Microcystis* blooms are a nuisance in many lakes all over the world, it is important to elucidate the mechanisms of their formation.

Another important factor responsible for cyanobacterial blooms is CO₂ availability^{8–12,17–20}. King^{8,9} hypothesized that cyanobacteria, though superior competitors of low CO₂ concentrations compared with other phytoplankters, would predominate after a further reduction in the CO₂ concentration. In an experimental system, the dominant phytoplankton shifted from cyanobacteria to chlorophytes after CO₂ addition¹⁸, and the succession was reversible when the pH was raised. Klemer and colleagues^{11,12} examined the role of carbon availability in buoyancy regulation which makes cyanobacteria superior to other phytoplankters in CO₂ uptake. Cyanobacteria have a carbon concentrating mechan-

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ism which elevates the CO_2 level around the active site of the primary photosynthetic carboxylating enzyme Rubisco, allowing the organisms to conduct efficient photosynthesis and may provide them with an advantage over other phytoplankters^{1,2}).

Although the phytoplankton succession due to addition of CO_2 was obvious in Shapiro's study¹⁸, the effect was synergistic because in addition to changing the carbon concentration, it changed the pH. Shapiro¹⁸ examined phytoplankton succession after addition of CO_2 dissolved in an alkaline solution to keep the pH high and observed the same result as CO_2 addition, though his results varied between experiments (Table 1 in Shapiro¹⁸). Further, most studies which have examined cyanobacterial dominance in relation to carbon concentration, or pH changes, refer to dominance by N_2 -fixing species^{3,8,12,17,18,20}. The CO_2 concentration indirectly affected the dominance of the N_2 -fixing cyanobacterium *Aphanizomenon flos aquae* caused by an alteration in pH³. However, the available information about this aspect of non- N_2 -fixing genera, such as *Microcystis*, is still limited.

In the present study, we examined dominance by *Microcystis* with special reference to the carbon concentration in *in situ* experiments using lake water.

A 160 liter sample of surface water was collected from an enclosure placed in Akanoi Bay of Lake Biwa¹⁶ using a bucket, on 17 August 1997. The bay is the most eutrophic area of the lake^{15,23} and the concentrations of nutrients and conditions of other environmental variables in the enclosed waters are described elsewhere¹⁶.

We poured 40 liters into each of four 70 liter polychlorinated vinyl buckets (diameter, ca. 40 cm; depth, ca. 60 cm) and added N and P nutrients or dissolved inorganic carbon (DIC) as described below. Removal of herbivores was impossible due to serious overlap of sizes between *Microcystis* colonies and possible herbivores such as planktonic crustaceans and rotifers. Two buckets served as the DIC system, and the other two as the controls. For the DIC system, the concentration of DIC was raised using a NaHCO_3 - Na_2CO_3 buffer solution which is commonly used in biochemical studies²². NaHCO_3 and Na_2CO_3 solutions both of which had a carbon concentration of 2.4 g C l^{-1} were prepared, and 44.8 ml of the former and 35.2 ml of the latter solution was added to the DIC system to adjust the pH at 9.8, which is the same as that of the lake water. The initial DIC concentration in the addition system was 9.2 mg C l^{-1} , and that of the control, 4.4 mg C l^{-1} . NH_4NO_3 and KH_2PO_4 were added to each bucket at final concentrations of $2000 \text{ } \mu\text{g N l}^{-1}$ and $100 \text{ } \mu\text{g P l}^{-1}$, respectively. The DIC and control systems each consist-

ed of buckets containing lake water thus enriched. Hence, solar radiation and water temperature were almost natural except for the absence of sediments. We secured these buckets within the lake enclosure using ropes, incubated them there for 18 days and took subsamples using a ladle from each bucket to monitor water temperature, pH, DIC concentration, chlorophyll concentration, inorganic nitrogen and phosphorus concentrations and phytoplankton cell densities every other day.

Water temperature and pH were measured using a pH meter (TOA Co. Ltd., HM-12P). A portion of each water sample was filtered through a Whatman GF/F glass fiber filter. For the filtrate, the DIC concentration was determined using a Total Carbon Analyzer (Shimadzu, TOC-500), concentrations of ammonium ($\text{NH}_4\text{-N}$), nitrite ($\text{NO}_2\text{-N}$) and nitrate ($\text{NO}_3\text{-N}$) nitrogen were determined by the ionchromatographic method (Johnson 1986⁷), and the dissolved inorganic phosphorus (DIP) concentration was determined by the method of Murphy & Riley (1962)¹⁴.

To determine concentrations of chlorophyll, a 20 to 70 ml portion of each water sample was filtered through a Whatman GF/F filter. Chlorophyll in seston retained on the filter was extracted using 10 ml of acetone:dimethyl sulfoxide (DMSO)=1:1 solution²¹, and the amount of chlorophyll was determined using a fluorometer (Turner Design Co. Ltd., Model 10-AU-005, Field Fluorometer⁴). Another 120 to 170 ml portion of the water sample was fixed with acid Lugol's solution at a final concentration of 1%, and the phytoplankton concentrated by natural sedimentation. Phytoplankton cells were enumerated with a haemocytometer under a microscope at $\times 200$ or $\times 400$ magnification. Algal cell numbers were converted into biovolume using the cell volume of each phytoplankter⁶.

We examined the statistical differences in abundance of dominant phytoplankters, with the Mann-Whitney U-test using StatView ver. 5.0 (SAS Institute Inc.).

A sharp increase in water temperature (Fig. 1A) in the buckets was detected from the first (27.8°C) to second day (30.2°C) and thereafter, the temperature gradually decreased. Large fluctuations of pH were found from the initial day (9.8) to day 4 (ca. 11), and thereafter the pH generally decreased in both systems (Fig. 1B).

As would be expected, DIC concentrations were higher in the DIC vessels than the controls. Concentrations in both decreased from about day 2 to day 7 (Fig. 1C) after which the control levels stayed fairly constant while the concentration increased slightly in the DIC system, from day 11 onwards. Concentrations of dissolved inorganic nitrogen ($\text{DIN}=\text{NH}_4+\text{NO}_2+\text{NO}_3$) (Fig. 1D) and phosphorus (DIP)

Fig. 1. Changes in water temperature (A), pH (B), and dissolved inorganic carbon (DIC) (C), dissolved inorganic nitrogen (DIN) (D), dissolved inorganic phosphorus (DIP) (E) and chlorophyll (F) concentrations in the control (open circles) and the DIC (solid circles) systems. Vertical bars which indicate differences between duplicates are shown when they exceed the size of the symbol.

(Fig. 1E) declined sharply in both control and DIC systems from the start of the experiment to day 7 (DIN) and day 4 (DIP), and did not show any significant increase thereafter.

Trends in the change in the chlorophyll concentration in the two systems were also similar (Fig. 1F). The concentration increased rapidly from day 4 to day 7, decreased from day 9 to day 11 and then fluctuated. Thus, we could find no significant differences in levels or patterns of change in water temperature, pH, nutrient and chlorophyll concentrations between the DIC and control systems, although the maximum concentration of chlorophyll in the former system was higher than that of the control (Fig. 1F).

The dominant phytoplankton species at the start of the experiment were *M. aeruginosa*, *Planktosphaeria gelatinosa* and *Ceratium hirundinella*. The species diversity decreased during incubation and, finally, about 95% of the phytoplankton biomass was *Microcystis* spp. plus *Scenedesmus* spp. in both systems (Fig. 2). Hence, Figure 2

shows marked changes in the abundance of these two genera. In the DIC system, they both increased from the start to day 7, but the abundance of *Microcystis* spp. dominated from day 7 to 11 and remained at low levels from day 14 onwards, while *Scenedesmus* spp. continued to increase and predominated at the end of the experiment (Fig. 2B). In the control system, the abundance of these two dominant phytoplankters increased, and finally, *Microcystis* spp. seemed to be more abundant than *Scenedesmus* spp. despite that the abundances of the two dominant phytoplankton genera were highly variable (Fig. 2A). We examined the differences in abundance between these two dominant phytoplankton genera for day 7 to 11 during *Microcystis* dominance and day 14 to 18 during *Scenedesmus* dominance, using the abundances of the two dominant phytoplankton genera and the Mann-Whitney U-test. In both cases, the differences were significant at $p < 0.01$ and $p < 0.02$, respectively. For the control system, we also examined the differ-

Fig. 2. Changes in biovolume densities of *Microcystis* spp. (open circles) and *Scenedesmus* spp. (solid circles) in the control (A) and DIC (B) systems. Vertical bars which indicate differences in biovolume densities between duplicates are shown when they exceed the size of the symbol.

ences in abundance between these two dominant phytoplankton genera from day 14 onwards, and found the difference was not significant ($p > 0.1$).

Klemer and colleagues¹¹⁾ examined the effects of changes in carbon availability on the buoyancy of *M. aeruginosa* in chemostat systems, using various C:N weight ratios of nutrient supply ranging between 0.54 and 17. In the present study, C:N ratios at the start of the experiments in the DIC and control systems were respectively 4.5 and 2.0, suggesting moderate carbon-limitation¹¹⁾. However, the DIC concentrations in both systems increased, after a temporary decrease, and this was due to heterotrophic CO₂ release since the increase in DIC concentrations (Fig. 1C) occurred as chlorophyll concentrations decreased (Fig. 1F). Hence, even in the control systems, CO₂ might be available to phytoplankton, mainly through heterotrophic activity. Despite this, there were large differences in DIC concentrations between the control and DIC systems (Fig. 1C).

The control system of the present study suggests that low DIC availability in the surrounding water favors *Microcystis* dominance. In the DIC system, similar results were also obtained on days 7 and 9 when *Microcystis* grew rapid-

ly (Fig. 2B) at low concentrations of DIC (Fig. 1C). The enrichment of lake water using nutrients enhances growth of several phytoplankters^{18,24,25)}, and chlorophytes often dominate when carbon is added¹⁸⁾. We also detected chlorophyte dominance in the DIC system (Fig. 2B). However, it is still unclear why chlorophytes dominate under carbon-rich conditions. Watanabe & Miyazaki²⁶⁾ conducted computer simulations of the competition between *Microcystis novacekii* and *Scenedesmus quadricauda* in nitrogen limited chemostats, examining two different modes of nutrient supply: small-pulse, high frequency and large-pulse, low frequency. *M. novacekii* outcompeted *S. quadricauda* in the former mode, and *S. quadricauda* became predominant in the latter. In the present study, since the mode of nutrient supply was large-pulse and low frequency, the dominant phytoplankton would not be *Microcystis* in the control and DIC systems. Indeed, the predominant phytoplankton in the DIC system was *Scenedesmus* (Fig. 2), as Watanabe & Miyazaki²⁶⁾ found. However, in contrast to their results, the predominant phytoplankton in our low carbon control system was *Microcystis* (Fig. 2). In Watanabe & Miyazaki's²⁶⁾ study, *M. novacekii* and *S. quadricauda* were incubated in a medium with a high C:N weight ratio (at least 20.4), indicating high carbon availability for the phytoplankters. In our results, *Scenedesmus* dominated in the DIC systems where there was plenty of carbon available for phytoplankton, suggesting that a high carbon concentration is unfavorable to *Microcystis* dominance. Thus, our data partly support the results of Watanabe & Miyazaki²⁶⁾.

Microcystis has the potential for vertical migration using its buoyancy regulation. Thus, it is likely that *Microcystis* can float up to the surface layer, where there is a high concentration of DIC, and proliferate even under conditions of low DIC availability in the surrounding water. Buoyant cyanobacteria acclimated to high solar irradiance were at an advantage to intercept CO₂ diffusing in from the air⁵⁾. Flotation may be the strategy by which *Microcystis* acquires carbon and dominates when the availability of DIC in the water column is low. In the present incubation, we used buckets where wind probably had less of an effect than in natural lakes. Thus, *Microcystis* in the present study might also be a superior competitor for CO₂ due to flotation.

In Lake Biwa, *Microcystis* blooms usually occur subsequent to *Anabaena* blooms^{15,23)}. The DIC concentration decreases during *Anabaena* blooms (Nakano unpublished), suggesting carbon limitation of the phytoplankton, but *Anabaena* introduces nitrogen through N₂-fixation, and the fixed nitrogen may be released in a dissolved form available to other phytoplankton after decomposition of the *Anabae-*

na. Thus, the chemical environment after an *Anabaena* bloom may be favorable to the growth of *Microcystis* in Lake Biwa.

The initiation of a *Microcystis* bloom has still not yet been fully explained, although there are numerous studies of this issue. Our results support the hypothesis of King^{8,9)}, the results of Shapiro^{17,18)} and those of Klemer¹⁰⁾. Flotation of *Microcystis* in the surface layer may be advantageous for uptake of DIC, and this enables the cyanobacterium to dominate when concentrations of DIC in the water column are low. High levels of photosynthetic activity of phytoplankton result in low concentrations of DIC, and this is possible in eutrophic waters. Our results shed some light on why *Microcystis* dominates in eutrophic waters.

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