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RESEARCH PAPER

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Effects of temperature, and availability of nitrogen and phosphorus on the abundance of Anabaena and Microcystis in Lake Biwa, Japan: an experimental approach

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Abstract Under optimal nutrient conditions, both *Microcystis* sp. and *Anabaena* sp. isolated from Lake Biwa grew optimally at 28–32°C but differed in maximal growth rates, phosphate uptake kinetics, maximal phosphorus quotas, and growth responses to nitrogen and phosphorus limitation. The maximal growth rates of *Microcystis* and *Anabaena* were 1.6 and 1.25 divisions day^{-1}, respectively. With phosphate and nitrate in the growth-limiting range, the growth of *Microcystis* was optimal at an N : P ratio of 100 : 1 (by weight) and declined at lower (nitrogen limitation) and higher (phosphorus limitation) ratios. In contrast, *Anabaena* growth rates did not change at N : P ratios from 1000 : 1 to 10 : 1. Starting with cells containing the maximal phosphorus quota, *Microcystis* growth in minus-phosphorus medium ceased in 7–9 days, compared with 12–13 days for *Anabaena*. The phosphate turnover time in cultures starved to their minimum cell quotas was 7.9 min for *Microcystis* and 0.6 min for *Anabaena*. *Microcystis* had a higher K_s (0.12 μg P l^{-1} 10^{-6} cells) and lower V_{max} (9.63 μg P l^{-1} h^{-1} 10^{-6} cells), than *Anabaena* (K_s 0.02 μg P l^{-1} h^{-1} 10^{-6} cells; V_{max} 46.25 63 μg P l^{-1} h^{-1} 10^{-6} cells), suggesting that *Microcystis* would not be able to grow well in phosphorus-limited waters. We conclude that in spite of the higher growth rate under ideal conditions, *Microcystis* does not usually bloom in the North Basin because of low availability of phosphorus and nitrogen. Although *Anabaena* has an efficient phosphorus-uptake system, its main strategy for growth in low-phosphorus environments may depend on storage of phosphorus during periods of abundant phosphorus supply, which are rare in the North Basin.

Key words Anabaena · Microcystis · Lake Biwa · Temperature · Nitrogen and phosphorus ratios

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Introduction

The two basins of Lake Biwa differ in morphometry and trophic state (Pollingher 1990). The North Basin is larger, deeper, and less productive than the South Basin, although spatial differences exist in both basins, and shallow bays are typically more eutrophic than offshore areas. The North Basin is considered to be mesotrophic and the South Basin eutrophic. Blooms of blue-green algae (cyanobacteria) have been reported periodically from parts of the South Basin. For example, populations of *Anabaena* reached 3800 colonies ml^{-1} in 1985 and 1986; *Microcystis* reached 400 colonies ml^{-1} in 1987 and 1988 (Shiga Prefectural Institute 1995); and a large population of *Anabaena affinis* (2400 colonies ml^{-1}) and a smaller population of *Microcystis aeruginosa* (52 colonies ml^{-1}) developed in Akanoi Bay in 1995 (Yodogawa Water Quality Conference 1995). In contrast, *Microcystis* populations in the North Basin are typically very low. For example, in 1998 the maximum densities of *M. aeruginosa* plus *M. wesenbergii* were 1000 cells ml^{-1} at an inshore station (Watanabe 1999).

The timing of the appearance of *Anabaena* blooms in the South Basin has been linked to nitrogen depletion (Tezuka 1988), the onset of the blooms occurring about 2 weeks after dissolved inorganic nitrogen (DIN) was depleted. During the blooms total phosphorus (TP) and particulate phosphorus (PP) were higher than before suggesting that the low N : P ratios reported to select for nitrogen-fixing cyanobacteria (Schindler 1977) are responsible for the blooms in the South Basin. However, Tezuka (1988) pointed out that *Anabaena* blooms do not occur in the North Basin, although DIN depletion also occurs there. He attributed the difference to a shortage of phosphorus in the North Basin, where TP seldom exceeds 10 μg l^{-1} in contrast to the South Basin, where values >20 μg l^{-1} were reported during the *Anabaena* blooms. Frenette et al. (1996) reported that phytoplankton biomass and production in the North Basin were limited by phosphorus and not nitrogen in the fall of 1993. The ratio of total dissolved nitrogen to particulate phosphorus was around 100 but increased to about 400 after a typhoon.

Microcystis aeruginosa occurs in both basins, but blooms have been reported mainly from the South Basin, particularly in shallow bays such as Akanoi Bay. In view of increasing nitrogen and phosphorus loadings into the lake, there is a concern that in the North Basin *Microcystis* blooms will expand from Nagahama Harbor and Shiozu Bay into other parts as the lake becomes more eutrophic, especially since *Microcystis* is widespread in sediments and can readily resuspend into overlying water, particularly during typhoons (Watanabe 1999).

In a previous study of the potential for algae and cyanobacteria to grow following resuspension from Akanoi Bay sediments (Nalewajko and Murphy 1998), two species of *Anabaena* were predominant in several types of media, but *Microcystis* was not apparent, possibly because nutrient conditions in our media were not suitable for this species. Our objective here is to define the optimal temperature and irradiance requirements for growth of Lake Biwa isolates of *Anabaena* and *Microcystis*, compare their performance under a range of nitrogen and phosphorus availability, and examine the phosphorus-uptake kinetics of the two taxa.

Methods

Anabaena sp. and *Microcystis aeruginosa* were isolated from Lake Biwa by Judy Acreman, University of Toronto Culture Collection, and grown in Chu 10 medium (Nichols 1973). Although the cultures were not axenic, examination in an epifluorescence microscope after acridine orange staining revealed very few bacteria during the exponential growth phase.

Ilumination was provided by cool white fluorescent lights. Irradiance was measured with a Biospherical Instruments QSL-100 photometer. Growth was measured as optical density at 550 nm in a Spectronic 20 instrument or by enumeration in a Wild inverted microscope at ×400 magnification. The latter method was used for counts of heterocyst frequency in *Anabaena*.

Phosphate turnover time (P-tt) in cultures and phosphate uptake at a range of concentrations were measured using carrier-free ^{32}P—PO_4 (Dupont Canada), as described in Twiss and Nalewajko (1992), and Michaelis-Menten parameters were calculated from Lineweaver-Burk transformations of the data (Riggs 1963).

In experiments on the effects of N:P ratios on growth, stock solutions of K_2HPO_4 and $Ca(NO_3)_2$ used in preparation of Chu 10 media were diluted 10-fold. The phosphorus concentration was held constant, and nitrogen was added to Chu 10 medium to get a range of N:P ratios from 1000:1 to 10:1. Triplicate cultures of 250 ml each were set up at each N:P ratio. Growth was followed by measuring the optical density every day for 15 days. Growth rates (divisions per day) were calculated from the exponential phase of growth, usually from day 2 to 10. Stock cultures for Michaelis-Menten assays and for experiments on growth as a function of N:P ratios were grown first in Chu 10 with only 10% of the usual phosphorus concentration, and then in minus-phosphorus Chu 10 medium until P-tt in the cultures reached the previously determined shortest value: 7.9 min for *Microcystis* and 0.6 min for *Anabaena.*

Chlorophyll *a* concentrations corrected for phaeophytin were determined on acetone-extracted samples (Parsons et al. 1984).

Results

In Chu 10 medium, there was a slight difference in the optimal temperature for growth and the range over which growth was observed in the two species (Fig. 1). *Anabaena* grew best (1.25 divisions day^{-1}) between 28°C and 32°C and showed a sharp decrease at 35°C. *Microcystis* had a higher maximal growth rate (1.6 divisions day^{-1}) also at 28°C to 32°C but did not show a marked decrease in growth at 35°C. For both species at temperatures in the 25°C to 35°C range, an irradiance of 130 μmol m^{-2} s^{-1} was optimal.

The growth of the two species responded differently to N:P ratios (Fig. 2). *Anabaena* growth remained constant at ratios from 1000:1 to 10:1, but *Microcystis* showed a clear optimum at 100:1 and very little growth at 1000:1 (phosphorus limitation) and 10:1 (nitrogen limitation). In *Anabaena* (Fig. 3), total chlorophyll concentrations were lower in cultures grown at 1000:1 than at the other N:P ratios, but differences among the latter were not significant. In *Microcystis*, chlorophyll concentrations were extremely low at 10:1 and 1000:1 and were higher and similar at all other N:P ratios. Heterocyst frequency in *Anabaena* ranged from 2% to 4.5% of vegetative cells and did not show a clear trend with N:P ratios in the medium.

Populations of *Anabaena* cells grown in full Chu 10 medium for 8 days, then centrifuged, washed with minus-phosphorus Chu 10 medium, and resuspended in sterilized

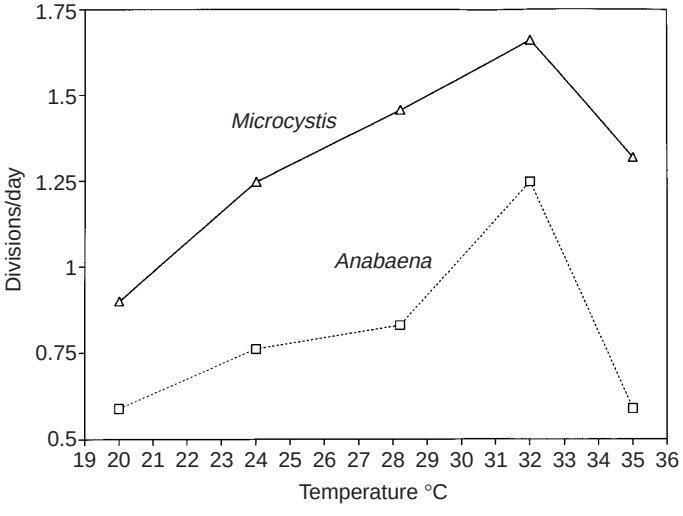

Fig. 1. Growth rates of *Microcystis* and *Anabaena* at various temperatures. Cultures were grown in Chu 10 medium for 15 days. Growth rates were calculated from the exponential growth phase (days 2–10)

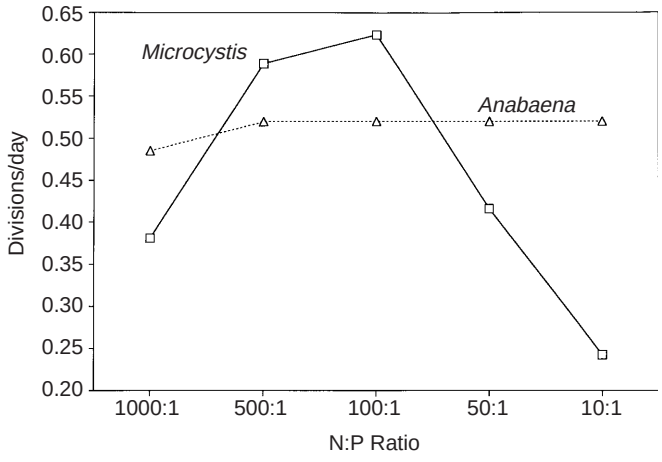

Fig. 2. Growth of *Microcystis* and *Anabaena* at various N:P ratios in Chu 10 medium. Growth rates were calculated as in Fig. 1

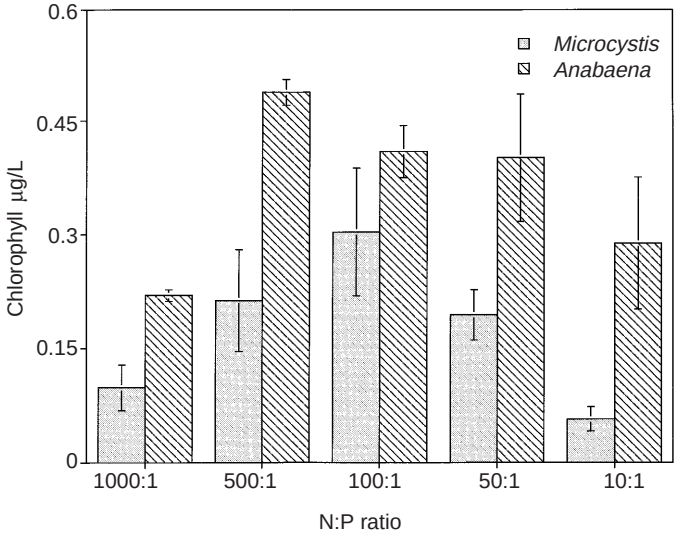

Fig. 3. Chlorophyll concentrations in the cultures used to calculate growth rates as a function of N:P ratios (Fig. 2)

minus-phosphorus Chu 10 continued to increase in numbers for 12 to 13 days. *Microcystis* cultures in identical experiments grew for only 6.8 to 9 days. After cultures reached the minimum cell phosphorus quotas, they were used in phosphate uptake experiments at a range of phosphate concentrations to determine the Michaelis-Menten parameters K_s and V_{max}. *Microcystis* had a higher K_s ($0.12\,\mu g\,l^{-1}\,10^{-6}$ cells) and lower V_{max} ($9.63\,\mu g\,l^{-1}\,h^{-1}\,10^{-6}$ cells) than *Anabaena* (K_s $0.02\,\mu g\,l^{-1}\,10^{-6}$ cells; V_{max} 46.25 $63\,\mu g\,l^{-1}\,h^{-1}\,10^{-6}$ cells).

Discussion

Microcystis, in spite of its higher maximal growth rate under nonlimiting nitrogen and phosphorus supply, would not be able to grow as fast as *Anabaena* under either nitrogen or phosphorus limitation. Unlike *Anabaena*, it cannot fix N_2 when nitrogen is in short supply. Under phosphorus limitation, it is not as efficient as *Anabaena* at phosphorus uptake, its maximal phosphorus-uptake rate is lower, and it cannot continue to grow as long as *Anabaena* in the absence of external phosphate at the expense of internally stored phosphorus. Although it is better than *Microcystis* at growing under phosphorus limitation, in our previous study (Nalewajko and Murphy 1998) we reported that this *Anabaena* species was outcompeted for phosphorus by green algae and diatoms. It is also interesting that an *Anabaena* bloom could be induced in lakewater from the South Basin simply by phosphorus enrichment (Tezuka and Nakano 1993).

In Lake Biwa, if neither nitrogen nor phosphorus were limiting, *Microcystis* would be expected to be more abundant than *Anabaena* in the fall, when water temperature is in the 28°–32°C range. In the North Basin, periodic nitrogen limitation (Tezuka 1988), and phosphorus limitation (Frenette et al. 1996), offer sufficient explanation for the absence of *Microcystis* blooms. However, the absence of *Anabaena* blooms in the North Basin is not so readily explained. One possibility is that the low prevailing phosphorus concentrations do not permit maximal uptake rates, and hence there is no opportunity for luxury phosphorus uptake and the achievement of maximal phosphorus quotas. Although nitrogen and phosphorus pulses occur after typhoons that allow deep mixing (Frenette et al. 1996), the increase in total dissolved nitrogen (TDN) is larger than the increase in phosphorus concentration measured as particulate phosphorus (PP).

The selective influence of N:P ratios on phytoplankton community composition has been extensively examined in the context of dominance of algal taxa at the level of Division (McQueen and Lean 1987; Levich 1996), as well as the relative abundance of various cyanobacterial genera (Stockner and Shortreed 1988). In the North Basin, the TDN:PP ratio in surface water increased from about 100:1 to a maximum of 400:1 following a typhoon (Frenette et al. 1996). These ratios cannot be directly compared to ratios in our experiments, which are expressed as nitrate-N to phosphate-P, but we tentatively suggest that they correspond to ratios exceeding 1000:1.

In contrast to the North Basin, the polymictic South Basin of Lake Biwa is characterized by much higher nutrient levels and phytoplankton biomass, particularly in the shallow bays and after typhoons (Kumagai et al. 1997). In some years, various species of both *Microcystis* and *Anabaena* form large populations in the fall, but blooms are absent in other years, possibly as a result of insufficient phosphorus supply and low solar radiation (Nakanishi et al. 1992). Temperatures during July and August 1990, when *Anabaena* and *Microcystis* were abundant, reached 32°C, but in 1991, when the two species did not appear, the maximum was only 28°C. Our data show a strong dependence of growth rate on temperature (Fig. 1) but indicate that at 26°C, the lowest temperatures prevalent during these 2 months, the growth rates of *Microcystis* and *Anabaena* would still be appreciable (about 1.3 and 0.8 divisions day^{-1},

respectively) in the absence of nutrient limitation. Therefore, we conclude that phosphorus limitation and not temperature was the crucial factor that prevented growth of these cyanobacteria in 1991. The importance of climate was invoked by Watanabe (1996), who attributed the presence of abnormally large populations of *Microcystis* in the South Basin in 1994 to the unusually hot and dry weather and low water levels in the lake. Again, although our data support the idea that high water temperatures would favor *Microcystis* growth, temperature alone cannot account for the development of large blooms.

It proved impossible to positively identify the *Anabaena* isolate to species level, because it does not readily form typical filaments in Chu 10 medium. It fits in the turkestanica/orientalis complex (Komarek and Anagnostidis 1989). Similarly, the *Microcystis* can only be tentatively identified as *M. aeruginosa*, because it seldom formed colonies larger than 5 to 15 cells in Chu 10 medium, and colony morphology is diagnostic to identification to species level. However, only two species of *Microcystis* have been reported as common in Lake Biwa (Watanabe 1996): *M. aeruginosa* and *M. wesenbergii*, and because the former was far more abundant than the latter, it is likely that our isolate is in fact *M. aeruginosa.*

It is clear that several aspects of the ecology of Lake Biwa are changing. One important change involves the stability of vivianite (ferrous phosphate) in the lake sediments. In the past, vivianite was stable, but it is now dissolving in the sediments, which most likely reflects large-scale increases in organic matter and sulfur inputs into the lake. The resulting enhanced formation of sulfide in the sediments increases vivianite dissolution and has the potential to release phosphorus into the water column. Typhoon-mediated mixing probably regulates release of phosphate from the sediments, and therefore much of this process is random in space and time. However, sites near major discharges such as Nagahama City appear to be more prone to vivianite dissolution (Murphy et al., submitted to *Limnology*, this issue). The recent appearance of *Microcystis* blooms near Nagahama may reflect increased sediment release of phosphorus. The increase in *Microcystis* blooms has not paralelled the growth of the city, but it is likely that the sediments have buffered the effect of city discharges for many years. The conditions associated with vivianite dissolution are driven by microbial reactions, and nitrogen would be expected to be coreleased with phosphorus, but the observations of Frenette et al. (1996) indicated more TDN than phosphorus release. Some of the nutrient release could be mediated by algae that rest on the surface sediments and are resuspended by storms into the water column.

Water quality in Lake Biwa has been a concern since 1977, when unpleasant odors attributed to blooms of *Uroglena americana* were first reported (Pollingher 1990). These blooms typically start in the south part of the South Basin in April–June, then extend north, and collapse coincident with a temperature increase to 22°C and higher. If eutrophication continues in the North Basin, these blooms would be followed by *Microcystis* and *Anabaena* later in the year and would contribute to a further deterioration of water quality.

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