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Growth of bloom-forming cyanobacteria under low nutrient conditions

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Introduction

In 1994, toxic *Microcystis aeruginosa* (cyanobacteria) blooms occurred for the first time in two harbors in the North Basin of Lake Biwa, Japan (YOSHIDA et al. 1996). Since then, there have been many other reports of inshore blooms of this species, and low numbers of *Microcystis* colonies are now found throughout the North Basin, the main body of the lake, during late summer each year (KUMAGAI et al. 1999). Since Lake Biwa supplies drinking water to almost 14 million people, it is important to assess the risk of toxic cyanobacterial blooms. This requires an improved understanding of the mechanisms of *Microcystis* bloom initiation and development.

The ability of *Microcystis* colonies to migrate vertically through the water column is well established (e.g. REYNOLDS & WALSBY 1975, REYNOLDS et al. 1987). The colonies are able to photosynthesize at the surface during the daytime and move into nutrient-rich deeper waters at night (GANF & OLIVER 1982). This migration ability gives *Microcystis* a competitive advantage relative to other phytoplankton during periods of thermal stratification in temperate lakes. Because of this association with stratification, it was thought that such blooms occurred only in shallow stratified eutrophic lakes and not in deeper oligotrophic lakes (see REYNOLDS 1973). The deep North Basin of Lake Biwa thus seems an unusual habitat for *Microcystis*. It is obvious that *Microcystis* colonies overwinter on the sediment and recruit into the water column as the water temperature increases in spring or summer (PRESTON et al. 1980). Nevertheless, colonies of this bloom-forming species are unlikely to be recruited from the bottom sediment in the offshore region of the North Basin of Lake Biwa because the depth is more than 50 m (TSUJIMURA et al. 2000) and the pressure effects on gas vacuoles would be considerable. When a *Microcystis* bloom occurs, it tends to remain near the water surface and is more stable (KUMAGAI et al. 2000). It is more likely that offshore populations are recruited from enriched, shallow inshore sites and it is therefore important to know to what extent these inshore populations can continue to grow once they are transported into offshore waters by the wind and currents (ISHIKAWA et al. 1999).

The relationship between the phytoplankton growth and ambient nutrient supply is commonly described by the Monod equation (MONOD 1949). However, luxury nutrient uptake is well known in phytoplankton, whereby the cells can store nutrients in excess of their immediate growth requirements, for example nitrogen as cyanophycin and phosphorus as polyphosphate bodies (KROMKAMP 1987, SIMON 1987). Under poor nutrient conditions the phytoplankton use these stores to maintain biomass and to continue to grow (RHEE 1973). The Droop equation (DROOP 1973, OKADA et al. 1982, ISTVÁNOVICS et al. 1993) describes growth rate as being dependent on the stored nutrient quota in the cell. However, this model cannot be directly applied to field data since phytoplankton are seldom under continuous steady-state conditions. If there are time lags between nutrient uptake, storage, remobilization of stored reserves and cell division, the growth rate will be slower than that predicted by either the Monod or Droop models.

Recently an Artificial Neural Network model successfully predicted cyanobacterial blooms in Lake Kasumigaura, Japan (RECKNAGEL 1997, YABUNAKA et al. 1997). The novel back-propagation method may be useful in simulating future phytoplankton growth in that eutrophic lake. However, the suitability of this method in predicting blooms in lakes with ongoing eutrophication, as is the situation in the North Basin of Lake Biwa, is uncertain since nutrient distributions in such lakes are variable and unsteady.

The overall aim of the present research project was to evaluate the transport and growth of *Microcystis* populations that originate in the inshore waters of Lake Biwa under high nutrient conditions and that are subsequently advected offshore where nutrients are low. The first step of the study was to estimate

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the capacity of *M. aeruginosa* for growth during transport. Experiments in the field and laboratory used low-nutrient offshore water to test the growth of inshore *Microcystis* colonies on their stored intercellular nutrients.

Methods

Field incubation experiment

On 21 August 2000 there was a cyanobacterial bloom over several inshore sites in the North Basin of Lake Biwa, Japan. At this time a water sample was taken at the Offshore Station (35° 21' N, 136° 12' E; maximum depth 50 m) of Nagahama in the North Basin from 0.5-m depth using a Van Dorn sampler. The water was filtered through a Whatman GF/F glass fiber filter (0.7-μm pore size) to remove particles larger than bacteria, then the filtered water was poured into transparent 10-L NALGENE Polycarbonate bottles. Nutrients were determined as follows: ammonium (NH_4-N) was assayed by the method described by SAGI (1966), nitrate (NO_3-N) was analyzed with an AACS-II Auto Analyzer (Bran Luebbe), and soluble reactive phosphorus (SRP) was estimated using the molybdenum mixed reagent method (MURPHY & RILEY 1962), samples for particulate carbon (PC) and nitrogen (PN) were filtered onto GF/F filters and analyzed using a CHN Analyzer PE2400II (Perkin Elmer), and particulate phosphate (PP) was analyzed by the molybdenum mixed reagent method after persulfate digestion.

M. aeruginosa colonies were collected using a plankton net (mesh size 20 μm) at the surface of Nagahama Harbor (Inshore Station, 35° 22' N, 136° 16' E) and the colonies were washed using the filtered lake water, photographed and cell numbers counted. Four treatment bottles were each inoculated with three small colonies ca. 150 μm in diameter. The other isolated *Microcystis* colonies were filtered onto a GF/F filter and PC, PN and PP in the colonies were analyzed. The four bottles were tied to the field incubation buoy (Fig. 1), which was installed at the offshore station. Water temperature at 0.5-m depth was measured from 10:00 h on 23 August until 10:00 h on 24 August at 6-h intervals using an F-probe (Centre for Water Research; University of Western Australia). Irradiance measurements were taken at the Meteorological Station at Hikone (35° 16' N, 136° 14' E). The field incubation was started at 19:00 h on 23 August. Two bottles were removed from the buoy after 2 and 4 days. The large phytoplankton in the recovered water samples was concentrated to 2–3 mL by filtering through a plankton net (mesh size 10 μm) and fixed with Lugol's iodine solution. The growth of colonies was estimated by microscopic counts.

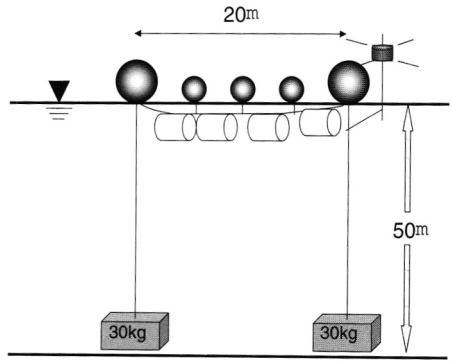

Fig. 1. The incubation set-up for the field experiment in Lake Biwa.

Laboratory incubation experiment

Water samples were collected as for the field incubation experiment at the same offshore station on 8 September 2000. The lake water was filtered and stored in a refrigerator. *M. aeruginosa* colonies were collected from the inshore station on 22 and 27 September. These were brought back to the laboratory immediately, and one colony was inoculated into each well of an IWAKI 24-well micro-plate (well diameter 16 mm) containing 1 mL of the filtered lake water, with 22 replicates for each experiment. On September 22 there were 80–13,320 cells colony^{-1}, and on September 27 there were 200–1712 cells colony^{-1}. Incubations were at 28 °C under ca. 100 μmol photons m^{-2} s^{-1} with a 12:12 h light/dark cycle. The growth and development of *M. aeruginosa* were monitored once a day for 4 days by counting the cell numbers and taking 1304 × 1024-pixel digital images using a high resolution CCD camera (Hitachi KP-F100) mounted on a stereoscopic microscope adjusted to 90 × (OLYMPUS SZX-ILL B100).

Results

Field incubation experiment

There were some differences in the physical and chemical environment between inshore and offshore stations (Table 1). Water temperature was more variable at the inshore station but the mean values were similar to the offshore site. The lowest temperatures were recorded at 04:00 h and the highest at 10:00 h at both stations. NH_4-N, NO_3-N and SRP concentra-

Table 1. Physiological and chemical conditions at inshore and offshore stations during the field incubation period (August 21–24, 2000).

	Water depth (m)	Water temp. (°C)	Nutrient concentration						PC:PN:PP ratio	Irradiance at Hikone Meteorological Station (MJ m^{-2} day^{-1})
			NH_4-N (μM)	NO_3-N (μM)	SRP (μM)	PC (μg L^{-1})	PN (μg L^{-1})	PP (μg L^{-1})		
Inshore station	2.2	28.4–29.2	2.55	3.58	0.45	764	114	27	37:6:1	21.8–23.7
Offshore station	50	28.0–29.1	0.24	0.13	0.01	424	42	3	125:12:1	

tions at the offshore station were more than an order of magnitude lower than at the inshore site. PC, PN, and PP concentrations offshore were also much lower than inshore. PC:PN:PP ratios were 37:6:1 inshore and 125:12:1 offshore, mostly due to a very low PP value offshore. PC, PN and PP concentrations in *Microcystis* from the inshore station were 3.7×10^{-6} μg $cell^{-1}$, 5.1×10^{-7} μg $cell^{-1}$ and 6.5×10^{-8} μg $cell^{-1}$, respectively (C:N:P, 155:18:1 by atom). Irradiance during the field incubation period was 21.8–23.7 MJ m^{-2} day^{-1}, and there was no rainfall over the 4-day period.

After 2 days, some of the colonies had broken into small fragments and the number of colonies increased. The total number of cells had increased from 1249 (day 0) to 3020 (day 2) in one bottle and from 1336 to 4069 cells in the duplicate bottle. After 4 days, the *M. aeruginosa* colonies had begun to deteriorate and the cell numbers showed a net change from 1689 (day 0) to 1856 (day 4) in one bottle and from 4514 to 2048 in the duplicate. The relative increase of cell numbers in the incubation bottles normalized to the initial values was thus higher after 2 days compared to 4 days (Fig. 2). The relative cell densities for this period were 2.40 and 3.05× that of day 0, indicating 1.27 and 1.61 cell divisions over the 2 days in the incubation bottles. Nutrient levels in the bottles were measured on each date. The SRP concentration after 2 days decreased by 0.01 μM but showed no difference after 4 days relative to day 0. The NO_3-N concentration decreased by 0.07 μM after 2 days, but increased by 0.04 μM relative to day 0 after 4 days. The NH_4-N concentration increased by 0.51 μM in both the 2- and 4-day bottles.

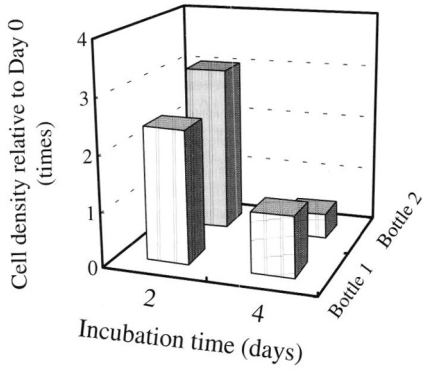

Fig. 2. Density of *Microcystis* cells relative to day 0 in the bottles during the field experiment in Lake Biwa.

Laboratory incubation experiment

Initial nutrient concentrations in the filtered lake water were 1.57 μM NH_4-N, 0.11 μM NO_3-N, and 0.01 μM SRP. The number of cells per colony increased over the first days of the experiment for both the 22 September and 27 September samples. In four colonies collected on 22 September, the mean cell number of *M. aeruginosa* increased for the first 2 days but then decreased over the subsequent 2 days (Fig. 3a). The colonies collected on 27 September grew more slowly at first and the number of cells then decreased after 3 days (Fig. 3b). The variation among colonies, each of which was in separate wells, was greater in the 22 September

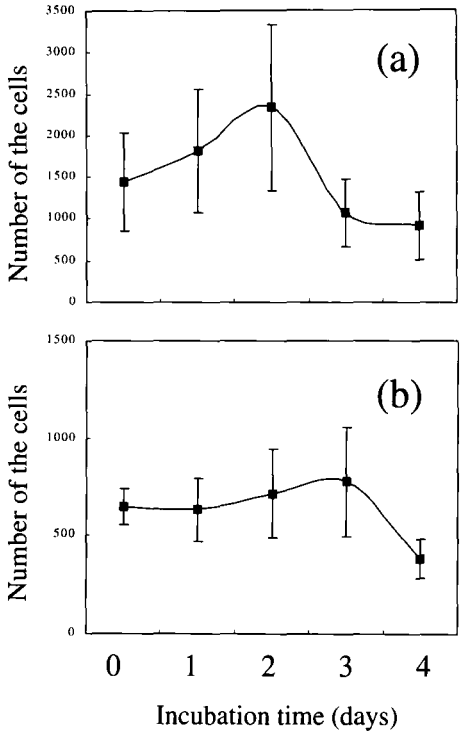

Fig. 3. Cell concentrations of *Microcystis* (mean ± SE) during the well plate incubations. Colonies collected on 22 September (a), and on 27 September (b).

experiment compared to 27 September. The maximum relative growth density after 2 days was at a ratio of 4.75 times, relative to day 0 in the 22 September assay, compared to 3.71 times after 3 days in the 27 September group. The mean relative growth rates up to the time of peak cell densities were in the ratio of 1.94 ± 0.27 (SE) times and 1.43 ± 0.21 (SE) times, equivalent to 2.25 and 1.81 times cell divisions over the initial days of experiments.

Microscopic analysis of the colonies showed that the edges of the *M. aeruginosa* cells became less distinct after 2 days. Most of the colonies started to decompose and were surrounded by bacteria after 4 days. Although the cell numbers increased, there was a general deterioration in the integrity of the colonies over time.

Discussion

Under nutrient-deficient conditions in the well-plate experiments and in the field experiment, *M. aeruginosa* colonies from inshore waters of the North Basin of Lake Biwa increased in cell numbers, presumably using stored nutrients. This is consistent with laboratory experiments on cyanobacteria in culture. For example, SBIYYAA et al. (1998) found that *Microcystis* continued logarithmic growth under externally P-deficient conditions for at least 8 days using stored reserves. Similarly, NAKAHARA (1993) reported that cells cultured in nutrient-rich media were able to divide a number of times using intercellular nutrients, when transferred to low-nutrient media (Table 2). It was found that the number of cells increased when transferred to the North Basin low-nutrient water, but less than that reported by NAKAHARA (1993). In a previous study regarding intercellular nutrients, the N:P atomic ratios of steady-state *M. aeruginosa* varied within the range of 24 to 15, depending on a specific growth rate from 0.1 to 0.8 day^{-1} in a P-limited chemostat (OH et al. 2000). The N:P ratio of *Microcystis* colonies at the inshore station on 21 August was 17.4:18.1 and the specific growth rate calculated from the biomass in the bottles after 2 days was 0.50 day^{-1}. This suggests that the colonies collected from Lake Biwa may not have stored nutrients to their maximum capacity.

The relative growth of *Microcystis* in the field experiments was higher than that in the plate experiments. The difference between the two methods may be due to differences in the stored nutrient quota per cell, the size of the colonies and the cell concentration. In the field experiment, 0.13–0.45 cells mL^{-1} of lake water were initially added, while incubations in well plates were inoculated at much higher concentrations, on average 1440 cells mL^{-1} on 22 September and 631 cells mL^{-1} on 27 September. External nutrients in the wells were thus likely to be depleted earlier than in the bottles. After 4 days, the colonies were decomposing in the well plates, but in the field this was more variable, with some colonies decomposing and others remaining healthy and intact. Temperatures

Table 2. Dividing times using stored nutrients in *Microcystis* colony.

Species	Sampling field	Medium	Temperature (°C)	Light (μmol photon $m^{-2} s^{-1}$)	Cell division (times)	References
M. aeruginosa	Cultured	CT*-N	25±1	40	2.2	NAKAHARA (1993)
M. aeruginosa	Cultured	CT-P	25±1	40	3.4	NAKAHARA (1993)
M. aeruginosa	Lake Biwa	Lake water	28±1	100	1.0, 0.5	This study
M. aeruginosa	Lake Biwa	Lake water	28–29	Field experiment	1.7	This study

*CT medium (WATANABE & ICHIMURA 1977).

were high, and without rapid growth the colonies may have been overtaken by bacteria and viruses. The natural light conditions, lower cell concentrations and larger container size (and lower surface to volume ratio) may have reduced this degradation effect in the field.

The epilimnetic water of Lake Biwa is transported by the stable gyre currents at a mean velocity of 0.1 m s^{-1} (KUMAGAI et al. 1998). It therefore takes 2–3 days for colonies of *M. aeruginosa* to be advected out into the offshore regions of the lake. It follows that the *Microcystis* cells could divide once or twice during their transport from inshore to offshore sites. Toxic *Microcystis* may also be able to produce microcystin as the cells are transported and divide, even under low nutrient regimes (including N-limitation). There is a linear relationship between microcystin production and cell division rates (ORR & JONES 1998).

In conclusion, these experiments demonstrated that cyanobacterial growth is possible during advection from inshore to offshore sites in Lake Biwa and that inshore populations of *Microcystis* can maintain growth for a short time under the low-nutrient conditions typical of the central northern basin of the lake. The storage quota per cell varied with the initial condition of the colonies, the nutrient conditions of the water from which they were collected, and the nutrient supply during incubation. Further experiments of this type from a variety of inshore sites would be useful in identifying the sites providing the greatest inoculum of nutrient-repleted cells for growth in the North Basin, and to further assess the risk of toxic cyanobacterial blooms in the offshore waters of Lake Biwa.

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