Effect of light availability on *Microcystis aeruginosa* blooms in shallow hypereutrophic Lake Kasumigaura

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*Microcystis aeruginosa* causes surface blooms in eutrophic lakes in the tropical and temperate zones. We investigated which factors suppressed blooms of *M. aeruginosa* in Lake Kasumigaura, a shallow hypereutrophic lake in the temperate zone of Japan, from 1999 to 2007 by quantifying the 16S rRNA gene of *M. aeruginosa* using real-time PCR. These results showed that *M. aeruginosa* existed in the lake water throughout the year. The abundance of *M. aeruginosa* usually increased from March to July and decreased from August to February. The factors determining the maximum density of the year were the overwintering population and the growth rate in summer (in June and July). The overwintering population was affected by abundance during the previous summer. The growth rates of *M. aeruginosa* in summer were correlated significantly with the extinction coefficient and mean daily photosynthetically active radiation irradiance. The mean daily irradiance in summer from 1999 to 2007 was 77% of that from 1983 to 1986, when blooms of *M. aeruginosa* occurred. Therefore, the most important factor controlling the growth of *M. aeruginosa* in Lake Kasumigaura after 1999 appears to be the amount of light supplied to the water column.

KEYWORDS: *Microcystis aeruginosa*; real-time PCR; Lake Kasumigaura; PAR irradiance; extinction coefficient

INTRODUCTION

Lake Kasumigaura is the second largest lake in Japan and is located 60 km northeast of Tokyo. The lake is used for multiple purposes, including aquaculture, fishing, irrigation, drinking water and recreation. Lake Kasumigaura is near the Pacific Ocean, and the wind tends to be strong throughout the year (annual mean wind velocity is 8.4 m s⁻¹ at the Lake Kasumigaura water research station; Fig. 1). Surface blooms of *Microcystis aeruginosa* occurred in Lake Kasumigaura from the latter half of the 1960s until the latter half of the 1980s. After 1978, summer blooms of *M. aeruginosa* occurred yearly. The blooms in 1983, 1984 and 1986 were especially large, with more than 4.0 × 10⁷ μm³ mL⁻¹ of *M. aeruginosa* recorded in summer. During this period, the lake became hypereutrophic, resulting in damage to cultured carp, a foul odor and diminished aesthetic value of the scenery. However, blooms of *M. aeruginosa* disappeared suddenly in 1987 (Takamura et al., 1992). Since then, no surface bloom of *M. aeruginosa* has been observed. The concentration of microcystin, a hepatotoxin produced by *M. aeruginosa*, has been decreasing since the *M. aeruginosa* blooms disappeared (Shirai et al., 1991). In August 2005, however,
at several locations in Lake Kasumigaura, many colonies of *M. aeruginosa* were visible on the surface of the water.

To predict when blooms may appear in the future, it is important to elucidate the factor(s) controlling the growth of *M. aeruginosa* in Lake Kasumigaura. Takamura et al. (Takamura et al., 1992) reported that the disappearance of *M. aeruginosa* blooms was caused by a change in the ratio of total nitrogen to total phosphorus (TP) (namely, phosphorus limitation) in the water column from 1987 to 1989. After 1991, however, the phosphorus concentration clearly increased, but no bloom was observed, suggesting that the phosphorus concentration is not the factor controlling the appearance of blooms in the lake. Therefore, the factor restraining the appearance of the *Microcystis* blooms after 1991 remains unclear.

Various factors have been reported to affect the species composition of cyanobacteria in eutrophic lakes, including nutrient concentrations (Interlandi et al., 1999), water temperature (Jöhnk et al., 2008), inoculum size (Robson and Hamilton, 2004; Cao et al., 2005), grazing (Lampert, 1987) and underwater irradiance (Havens et al., 1998; Holland and Walsby, 2008). In Lake Kasumigaura, large-scale dredging of the sediment began in 1992, and from 1996 onward the annual mean transparency was less than 60 cm. Therefore, we believe that the growth of *M. aeruginosa* during the summer is controlled by underwater light availability.

Long-term and seasonal monitoring of environmental parameters and the accurate quantification of *M. aeruginosa* abundance are necessary to clarify which factor is suppressing the growth of *M. aeruginosa* in Lake Kasumigaura. In this study, to obtain accurate abundance data, we monitored the concentration of *M. aeruginosa* 16S rDNA from 1999 to 2007 (9 years) by real-time PCR (RT-PCR), and we measured various

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**Fig. 1.** The location of St. 3 in Lake Kasumigaura, where water samples were collected and mean daily irradiance and water temperature were measured in this study. The arrow indicates the constriction at the mouth of Takahamairi Bay.
environmental parameters in Lake Kasumigaura over this period as well.

**METHOD**

**Study area**

Lake water was collected at St. 3 (36°7.11′N, 140°22.85′E, at the center of Takahamairi Bay; Fig. 1) in Lake Kasumigaura. The water level of Lake Kasumigaura is regulated at a rather high level from 1963 by a water gate constructed downstream. Takahamairi Bay is shallow (average depth, 3.18 m depends on a map measured in 1991) and the most eutrophic site in the lake, and the movement of water is delayed at a constriction (arrow in Fig. 1). The differences of water temperature between the surface and the bottom were mainly less than 1°C at St. 3 even during summer.

**Sample collection**

St. 3 is the designated monitoring station of the United Nations Global Environment Monitoring System Water Trend Monitoring Project, and the monthly water quality data are compiled in the Lake Kasumigaura database administered by the National Institute for Environmental Studies (NIES), Japan (http://db.cger.nies.go.jp/gem/inter/GEMS/database/kasumi/top.html). Monthly sampling was carried out around noon from April 1999 to December 2007. Lake water samples were collected from the surface to 2-m depth with a 2-m-long column sampler (diameter, 50 mm; RIGO, Tokyo, Japan). The 500-mL polyethylene bottle was placed in an ice cooler and immediately brought back to the NIES laboratory. A sample used to analyze nutrients and the filters were stored at -80°C until DNA extraction.

**DNA extraction**

DNA was extracted from microorganisms on a membrane filter with the FastDNA SPIN Kit (MP Biomedicals LLC, Illkirch, France) in accordance with the manufacturer’s instructions. Each membrane filter was cut into about 10 pieces, and all pieces were placed in a centrifuge tube with 1 mL of the kit’s isolation solution. Homogenization was performed with FastPrep (MP Biomedicals LLC). Homogenizing conditions were set at a speed of 4.0 for 20 s with the 0.25-inch Sphere + Garnet Matrix. The material was centrifuged at 14,000 × g, and 600 μL of supernatant was collected. At the end of the procedure, 100 μL of the DNA extract was acquired through the processing of one membrane filter. DNA extraction was carried out from two pieces of filter and DNA concentration used a mean value.

**Real-time quantitative PCR**

SYBR Green I PCR amplification was performed with a LightCycler 480 instrument (Roche, Mannheim, Germany). For the RT-PCR assay, we used the forward primer Micro233f (5′-GTAATGGCCTGAGAAGAGC-3′) modified from Micro229f (Tomioka et al., 2008) and the cyanobacteria-specific reverse primer Cyanor342r (5′-GCTGGCCCTCCCGTAGGAGT-3′). This primer set amplified a 104-bp portion of the 16S rDNA of M. aeruginosa. The 20-μL reaction mixture contained 1× LightCycler 480 SYBR Green I Master (Roche), 10 pmol of each primer and DNA extract. Denaturation was performed for 5 min at 95°C, followed by 40 cycles of repeated denaturation (10 s at 95°C), annealing (10 s at 60°C) with fluorescence acquisition (wavelength, 530 nm) and extension (10 s at 72°C). The temperature transition rate was 4.4°C s⁻¹ for denaturation and extension and 2.2°C s⁻¹ for annealing. A negative standard of Milli-Q water instead of the DNA solution was prepared in all experiments. After RT-PCR, a melting curve analysis was performed by continuous measurement of fluorescence while heating from 65 to 97°C at a transition rate of 0.11°C s⁻¹. The crossing point was determined with the second derivative max method using LightCycler 480 software version 1.5 (Roche). Each DNA extract sample was measured three times, and the mean 16S rDNA concentration was used for subsequent analyses.

The copy number of M. aeruginosa 16S rDNA in sampled water was calculated as follows:

\[
\text{copies per mL} = \frac{\text{copies per } \mu\text{L DNA extract} \times 100}{0.6 \times 20},
\]

where 100 (μL) is the volume of the DNA extract, 0.6 (mL) is the volume of supernatant and 20 (mL) is the volume of the water sample, and the recovery of 16S rDNA was assumed to be 100%. Approximately 10 copies of rDNA are equivalent to one M. aeruginosa cell.
(Tomioka et al., 2008) and cell diameter of M. aeruginosa was from 3 to 9 μm.

**Specific growth rate of Microcystis aeruginosa**

Specific growth rates were calculated using the difference in the natural logarithm of M. aeruginosa 16S rDNA concentration divided by the number of days between adjacent sampling dates as follows:

\[
\mu_M = \frac{\ln C_{M+1} - \ln C_M}{N},
\]

where \(\mu_M\) is the specific growth rate, \(C_M\) is M. aeruginosa 16S rDNA copy number per mL on the monthly sampling date, \(C_{M+1}\) is M. aeruginosa 16S rDNA copy number per mL on the next sampling date, and \(N\) is the number of days between the sampling dates.

**Extinction coefficient**

The vertical profile of photon flux density (μmol m\(^{-2}\) s\(^{-1}\)) in the range of 400–700 nm (photosynthetically active radiation, PAR) at depths of 0.5 and 1 m at St. 3 was measured using a quantum photometer (LI-192SR, Li-Cor, Lincoln, NE, USA), and the data are available in the Lake Kasumigaura database administered by NIES. The extinction coefficient (\(\alpha\)) was calculated from the photon flux density at these depths using the following equation (Kirk, 1975):

\[
\alpha = \frac{\ln I_{0.5} - \ln I_1}{0.5},
\]

where \(I_{0.5}\) is photon flux density at 0.5 m and \(I_1\) is photon flux density at 1 m. The extinction coefficients used for analysis (\(\alpha_M\)) were means of one monthly sampling date and the next (see Supplementary data, Appendix).

**Mean daily irradiance and water temperature**

The global solar irradiation (\(R\), MJ m\(^{-2}\) day\(^{-1}\)) and the daily mean water temperature (°C) at a point 150 m from the bank of the Lake Kasumigaura water research station at a depth of 20 cm were obtained from the meteorological database of the Lake Kasumigaura water research station of NIES (http://www-cger.nies.go.jp/kasumi/index.html, in Japanese) (Fig. 1). The global solar radiation was measured using a neopyrheliometer (MS-42, EKO Instruments Co., Tokyo, Japan). Water temperature was measured using a thermometer (E-733, Yokogawa Denshikiki Co., Tokyo, Japan).

Daily PAR at the top of the water column (\(I_0\), μmol m\(^{-2}\) s\(^{-1}\)) assuming that light was supplied in the same intensity for 24 h was calculated as follows:

\[
I_0 = R \times \left(\frac{10^6}{24} \times \frac{42.9}{100} \times \frac{93.5}{100}\right) \times 4.75\]


where \(R\) is the global solar irradiation. The daytime global solar radiation includes 42.9% of PAR (Thimijan and Heins, 1983) and the average reflectance loss value is 6.5% (Wetzel, 2001). The conversion coefficient from global solar irradiance (μJ m\(^{-2}\) s\(^{-1}\)) in the PAR to photon flux (\(I_0\), μmol m\(^{-2}\) s\(^{-1}\)) is 4.57 (Thimijan and Heins, 1983).

The average daily PAR incident irradiance (\(I_{0M}\)) during the period between adjacent monthly sampling dates was calculated as follows:

\[
I_{0M} = \frac{1}{N} \sum_{i=1}^{N} I_{0i},
\]

where \(N\) is the number of days between one monthly sampling date and the next (see Supplementary data, Appendix). The average value of water temperature (\(T_M\)) was calculated in a similar way (see Supplementary data, Appendix).

The average value of mean daily irradiance in the water column (\(I_{\text{mean}M}\); see Supplementary data, Appendix) was calculated as follows (Ferrero et al., 2006):

\[
I_{\text{mean}M} = \frac{I_{0M} \times [1 - \exp(-\alpha_M \times z)]}{\alpha_M \times z},
\]

where \(I_{0M}\) is the average daily PAR incident irradiance between adjacent monthly sampling dates, \(\alpha_M\) is the extinction coefficient and \(z\) is the mean depth (3.18 m) of Takahamairi Bay.

**Nutrients**

Nutrient data, including orthophosphate phosphorus (PO\(_4\)-P), dissolved total phosphorus (DTP), TP, dissolved inorganic nitrogen (DIN), dissolved total nitrogen (DTN) and total nitrogen (TN), were measured on each sampling date and are available in the Lake Kasumigaura database. PO\(_4\)-P and DIN were analyzed using a continuous flow analyzer (BRAN + LUETBE, AACS-II, Germany). DTP and TP were analyzed after persulfate digestion in an autoclave at 121°C for 30 min under acid conditions, and DTN and TN were
analyzed after persulfate digestion in an autoclave at 121°C for 30 min under alkaline conditions (Takamura et al., 1992). The nutrient concentrations used for analysis were means of one monthly sampling date and the next (see Supplementary data, Appendix).

**Statistical analyses**

Simple linear regression analyses were employed to test for statistical significance of the relationships between *M. aeruginosa* growth rate versus light exposure, extinction coefficient, water temperature and nutrient concentrations using the standard least squares method of S-plus version 6.0 J (Insightful Corp., Seattle, WA, USA).

**RESULTS**

**Seasonal variations of Microcystis aeruginosa density and specific growth rate**

The densities of *M. aeruginosa* from 1999 to 2007, measured as 16S rDNA copies mL$^{-1}$, are shown in Fig. 2a, and the specific growth rates between adjacent sampling dates are illustrated in Fig. 2b. *Microcystis aeruginosa* was detected in Lake Kasumigaura on every sampling date during the 9-year study, even during the winter season. The density of *M. aeruginosa* exhibited a regular trend of seasonal variation, with the rDNA concentration increasing from March to July and decreasing from August to February. That is, the specific growth rate of *M. aeruginosa* from August through February ($\mu_{\text{Aug}}, \mu_{\text{Sept}}, \mu_{\text{Oct}}, \mu_{\text{Nov}}, \mu_{\text{Dec}}, \mu_{\text{Jan}}$ and $\mu_{\text{Feb}}$) tended to be negative, such that the minimum density was observed regularly in March. When the maximum density of the previous year was high, the density in spring of the next year tended to be high as well. The density of *M. aeruginosa* increased slowly from March through May (the average of $\mu_{\text{Mar}}, \mu_{\text{Apr}}$ and $\mu_{\text{May}}$ for the 9 years was 0.021 day$^{-1}$) and increased rapidly in June and July (the average of $\mu_{\text{Jun}}$ and $\mu_{\text{Jul}}$ was 0.040 day$^{-1}$). Accordingly, the highest density of *M. aeruginosa* was usually recorded in August.

The highest maximum density in August during the 9-year study ($9.9 \times 10^5$ copies mL$^{-1}$) was observed in 2001. The average of $\mu_{\text{Jun}}$ and $\mu_{\text{Jul}}$ in 2001 (0.046 day$^{-1}$) was in the middle range of all $\mu_{\text{Jun}}$ and $\mu_{\text{Jul}}$ values, and the density in March 2001 ($3.5 \times 10^4$ copies mL$^{-1}$) was the highest in March among the 9 years. The second largest maximum density in August ($5.2 \times 10^5$ copies mL$^{-1}$) was observed in 2005. The average of $\mu_{\text{Jun}}$ and $\mu_{\text{Jul}}$ in 2005 was 0.052 day$^{-1}$, which was the second largest average value of $\mu_{\text{Jun}}$ and $\mu_{\text{Jul}}$ over the 9 years. In contrast, the lowest density of *M. aeruginosa* in August ($2.5 \times 10^4$ copies mL$^{-1}$) was observed in 2003. The average of $\mu_{\text{Jun}}$ and $\mu_{\text{Jul}}$ in 2003 was −0.013 day$^{-1}$, and the density in March 2003 ($9.79 \times 10^2$ copies mL$^{-1}$) was the second lowest value during the 9 years. The density in August 2004 was

![Fig. 2. Temporal variations in (a) the biomass of Microcystis aeruginosa measured as 16S rDNA copies mL$^{-1}$ based on RT-PCR and (b) the specific growth rate of M. aeruginosa at St. 3 in Lake Kasumigaura from April 1999 to December 2007.](https://academic.oup.com/plankt/article-abstract/33/8/1263/1441122/Effect-of-light-availability-on-Microcystis)
1.5 × 10^5 copies mL⁻¹, which was the third lowest value for August among the 9 years. The density in March 2004 (1.73 × 10^5 copies mL⁻¹) was the lowest value during the study period. Our data indicate that the density in spring was lower because the growth yield of the previous year was low. As a result, in spite of the highest growth rate (0.149 day⁻¹) being observed in July 2004, the maximum density in the summer of 2004 was low. These results indicate that the maximum densities in summer depend on the abundance in spring and the growth rates in June and July. Therefore, we investigated which environmental factor(s) influence the specific growth rate in summer.

**Relationships between specific growth rates and environmental factors**

Figure 3 illustrates the relationships between the specific growth rate and environmental factors during sampling in June, July and August, when *M. aeruginosa* exhibited a high-specific growth rate. Mean daily PAR irradiance and specific growth rate were significantly positively correlated (*P* = 0.009; Fig. 3c, Table I), whereas the extinction coefficient and specific growth rate showed a significant negative correlation (*P* = 0.008; Fig. 3b). The specific growth rate and daily incident irradiance showed a weak relationship (*P* = 0.07; Fig. 3a). The specific growth rate and TP showed a significant negative correlation (*P* = 0.03; Fig. 3g). No significant relationships were found between the specific growth rate and water temperature or nutrient concentrations other than TP. Insufficient phosphorus usually inhibits the growth of *M. aeruginosa*, although in this study we found a negative correlation. Therefore, TP was not a limiting factor for the growth of *M. aeruginosa* during the 9-year study period.

In Fig. 3, the specific growth rate for July 1999 (μ_july 1999) is shown as an open square and that for July 2007 (μ_july 2007) as an open triangle; these values were low despite the extinction coefficient being low during these 2 months. The specific growth rate for June 2006 (μ_june 2006), shown as an open circle, was high, although mean irradiance was low that month. In July 2007, the incident irradiance was low and mean irradiance was relatively low. Thus, the low specific growth rate for July 2007 can be explained by the low mean irradiance. However, the low and high specific growth rates in July 1999 and June 2006, respectively, could not be explained by the mean daily irradiance or other environmental factors analyzed in this study. Therefore, we looked for differences between environmental factors on the actual day of sample collection instead of using mean values. The dissolved oxygen concentration in the bottom water on the sampling day after growth in August 1999 was 5.2 mg L⁻¹, whereas the value in July 2006 was 1.7 mg L⁻¹. These oxygen concentrations show there were differences in the water mixing state between the sampling day in August 1999 and that in July 2006. Moreover, the day before sampling in August 1999 and June 2006 were rainy. These findings suggest that water stability before the sampling day may have a direct influence on the density of *M. aeruginosa*.

**DISCUSSION**

With global climate change, there is growing concern that the extent of *M. aeruginosa* blooms will increase with rising temperatures (Paerl and Huisman, 2008). Thus, it is important to understand this species’ bloom dynamics by quantifying the annual variation of *M. aeruginosa* over a long-term period. In this study, we accurately measured *M. aeruginosa* abundance from 1999 to 2007 using the RT-PCR method, which allowed us to track the monthly variation in *M. aeruginosa* 16S rDNA concentration in Lake Kasumigaura. The density of *M. aeruginosa* in the lake usually increased from March to July and decreased from August to February.

Because blooms occur in the summer, it is important to clarify which factors determine the maximum density of *M. aeruginosa* during that season. When there was a relatively high density of *M. aeruginosa* in spring and the specific growth rates in June and July were high, the density of *M. aeruginosa* in August was high. Furthermore, when the maximum density of the previous year was high, the density in the following spring tended also to be high. These findings suggest that the growth rates in June and July affect the maximum abundance of *M. aeruginosa* in summer. Therefore, we examined the association between environmental parameters and the specific growth rate from June to July and from July to August. The specific growth rate was significantly correlated with the extinction coefficient and the mean daily PAR irradiance during these two periods.

Nicklisch and Kohl (Nicklisch and Kohl, 1983) estimated the half-saturation constant of *M. aeruginosa* by a Monod-type model at 25 and 27.5°C as 33.1 and 45.9 W m⁻² (151 and 208 μmol m⁻² s⁻¹), respectively. They used a colony-forming strain and continuous light. For microcystin-producing *M. aeruginosa* PCC 7806, however, Hesse et al. (Hesse et al., 2001) reported that the half-saturation constant was 8.79 μmol m⁻² s⁻¹ and the light saturation point was 31.8 μmol m⁻² s⁻¹ at 20°C and a 12/12 h light/dark cycle. The lower values were measured with single-cell cultures and the higher ones with colony-forming strains. During the study period,
Fig. 3. Correlations ($n = 18$) of the specific growth rate of Microcystis aeruginosa with environmental parameters from June to July and from July to August during 1999 to 2007: (a) daily incident irradiance, (b) extinction coefficient, (c) mean daily irradiance, (d) water temperature, (e) orthophosphate phosphorus concentration, (f) dissolved total phosphorus concentration, (g) total phosphorus concentration, (h) dissolved inorganic nitrogen concentration, (i) dissolved total nitrogen concentration and (j) total nitrogen concentration. Values that do not fit the approximate equation for specific growth rate and the extinction coefficient are shown as an open square (μJ$_{\text{PAR},1999}$) and open triangle (μJ$_{\text{PAR},2007}$); the outlier from the mean irradiance equation is shown as an open circle (μJ$_{\text{PAR},2006}$). Relationships of specific growth rate with environmental factors were fitted by linear regressions. Non-significant regressions ($P > 0.05$) are given by a broken line.
The mean water temperature from June to August in Lake Kasumigaura was 25.1°C and the average mean irradiance was 24.2 μmol m⁻² s⁻¹ (range, 12.9–42.3 μmol m⁻² s⁻¹). Therefore, this would suggest that insufficient light limited the growth of *M. aeruginosa* in summer during the 9 years. Using other *M. aeruginosa* strains, Hesse and Kohl (Hesse and Kohl, 2001) reported that the light saturation points ranged from 50 to 100 μmol m⁻² s⁻¹ at 20°C and a 12/12 h light/dark cycle. Recalculation of our mean irradiance of 24.2 μmol m⁻² s⁻¹ to a 12 h light period results in a doubled value. However, even this value is considerably below the relevant saturation value of about 100 μmol m⁻² s⁻¹, which should be higher than this at 25°C. Based on the findings of these latter studies, it appears that the light supply during summer in Lake Kasumigaura limited the growth of *M. aeruginosa* and may induce the transition of the strains of *M. aeruginosa* (for example, from a toxic strain to a non-toxicity strain).

**Table I: Relationships between environmental parameters and specific growth rate of *Microcystis aeruginosa* from July to August in 1999 to 2007**

<table>
<thead>
<tr>
<th>Environmental parameters</th>
<th>Equation</th>
<th>$R^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident PAR</td>
<td>$y = 0.00032x - 0.070$</td>
<td>0.19</td>
<td>0.07</td>
</tr>
<tr>
<td>Extinction coefficient</td>
<td>$y = -0.036x + 0.204$</td>
<td>0.37</td>
<td>0.008*</td>
</tr>
<tr>
<td>Mean PAR</td>
<td>$y = 0.0035x - 0.047$</td>
<td>0.36</td>
<td>0.009*</td>
</tr>
<tr>
<td>Water temperature</td>
<td>$y = 0.0068x - 0.130$</td>
<td>0.10</td>
<td>0.2</td>
</tr>
<tr>
<td>PO₄-P</td>
<td>$y = -0.019x + 0.059$</td>
<td>0.06</td>
<td>0.3</td>
</tr>
<tr>
<td>DTP</td>
<td>$y = -0.0088x + 0.052$</td>
<td>0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>TP</td>
<td>$y = -0.028x + 0.200$</td>
<td>0.27</td>
<td>0.03*</td>
</tr>
<tr>
<td>DIN</td>
<td>$y = -0.0011x + 0.066$</td>
<td>0.13</td>
<td>0.1</td>
</tr>
<tr>
<td>DTN</td>
<td>$y = -0.0030x + 0.054$</td>
<td>&lt;0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>TN</td>
<td>$y = -0.0012x + 0.140$</td>
<td>0.06</td>
<td>0.3</td>
</tr>
</tbody>
</table>

PO₄-P, orthophosphate phosphorus; DTP, dissolved total phosphorus; TP, total phosphorus; DIN, dissolved inorganic nitrogen; DTN, dissolved total nitrogen. *Significant relationships.
by the change in light supply in the water (Kardinaal et al., 2007).

The mean daily irradiance in summer from 1999 to 2007 was 24.2 μmol m$^{-2}$ s$^{-1}$, whereas it was 31.3 μmol m$^{-2}$ s$^{-1}$ from 1983 to 1986 (when blooms occurred) and 34.1 μmol m$^{-2}$ s$^{-1}$ from 1987 to 1990 (when blooms suddenly stopped occurring; Table II). Other factors, such as water temperature and nutrient concentrations, were not different between periods when blooms occurred and our study period. The PO$_4$-P concentration from 1987 to 1990, when M. aeruginosa blooms suddenly stopped occurring, was one-third of that when blooms occurred. Together, these findings suggest that the factor limiting the growth of M. aeruginosa in Lake Kasumigaura changed from phosphorous (Takamura et al., 1992) to light from 1990 to 1999, and underwater light levels may have controlled the abundance of M. aeruginosa in Lake Kasumigaura during the summers of 1999 to 2007. Furthermore, Zevenboom and Mur (Zevenboom and Mur, 1984) report that growth rate increased as photoperiod lengthened, for Microcystis grown at saturating light. Nicklisch et al. (Nicklisch et al., 2008) described that photoperiod affected the light-growth rate in all algal species which they studied. High extinction coefficients reduce effectiv photoperiod underwater. Short photoperiod may have restricted the growth of M. aeruginosa.

Turbidity decreases light levels in the water column, and increased turbidity in a lake can be caused by calcite deposition (Vanderploen et al., 1987) and sediment resuspension by wind (Reynolds, 1984; Hawley and Eadie, 2007). For example, a high-turbidity plume persisted for more than a month after a storm in Lake Michigan (Eadie et al., 2002). In Lake Kasumigaura, Seki et al. (Seki et al., 2006) reported that sediment stirred up by wind caused a turbidity increase, because recently deposited sediment is resuspended easily by wind in this shallow lake. In contrast, Utagawa and Takamura (Utagawa and Takamura, 2007) reported that increased calcite particles were the cause of increased turbidity in Lake Kasumigaura, although they were unable to clarify the reasons for the recent increase in calcite deposition. If turbidity in the lake were to decrease in the future, blooms of M. aeruginosa may occur again. Therefore, it is important to clarify the mechanisms underlying the recent increases in turbidity.

In this study, the specific growth rates in July 1999 and June 2006 could not be explained by the mean irradiance. In most years during the study period, the specific growth rate in August was negative, except for 1999, 2000 and 2007 in Fig. 2b, although the mean irradiance and water temperature were still high from August to September (see Supplementary data, Appendix). These phenomena might be explained by the turbulence structure of the lake. Many studies have shown that M. aeruginosa grows best when living at the water surface (e.g. Köhler, 1992), whereas mixing by wind and circulation is disadvantageous for M. aeruginosa (Visser et al., 1996; Huisman et al., 2004; Backer et al., 2008). The water of the Lake Kasumigaura was unstable in the summer and diatoms almost did not dominate from 1999 to 2007 (data not shown). Even in summer, the incident irradiance was around 340 μmol m$^{-2}$ s$^{-1}$ in Lake Kasumigaura (Table II). In addition, Wiedner et al. (Wiedner et al., 2003) reported that the growth of M. aeruginosa PCC 7806 was not inhibited by PAR up to 403 μmol m$^{-2}$ s$^{-1}$ which was the highest value tested. Thus, the benefit of increased light availability by floating on the surface outweighs the potential damage caused by photoinhibition. Reynolds (Reynolds, 1984) assumed that isothermal mixing occurs in September in a shallow lake with a mean depth of 4.8 m. Moreover, the wind tends to be strong on this lake. Thus, circulation and wind are very likely to be a factor inhibiting the increase of M. aeruginosa in Takahamairi Bay, where the mean water depth is 3.18 m. Changes in the turbulence structure of natural waters, for instance, as driven by climate change, may promote blooms of M. aeruginosa (Huisman et al., 2004).

### Table II: Means of environmental parameters between June and August samplings, when Microcystis aeruginosa density increased

<table>
<thead>
<tr>
<th>Period</th>
<th>Incident PAR (μmol m$^{-2}$ s$^{-1}$)</th>
<th>Extinction coefficient (m$^{-1}$)</th>
<th>Mean PAR (μmol m$^{-2}$ s$^{-1}$)</th>
<th>Water temp. (°C)</th>
<th>PO$_4$-P (μM)</th>
<th>DTP (μM)</th>
<th>TP (μM)</th>
<th>DIN (μM)</th>
<th>DTN (μM)</th>
<th>TN (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993–1996</td>
<td>330.0</td>
<td>3.16</td>
<td>31.3</td>
<td>25.2</td>
<td>0.98</td>
<td>1.56</td>
<td>5.65</td>
<td>24.6</td>
<td>44.2</td>
<td>80.9</td>
</tr>
<tr>
<td>1997–1999</td>
<td>333.6</td>
<td>2.83</td>
<td>34.1</td>
<td>25.2</td>
<td>0.34</td>
<td>0.98</td>
<td>3.82</td>
<td>11.1</td>
<td>ND*</td>
<td>98.7</td>
</tr>
<tr>
<td>1999–2007*</td>
<td>340.4</td>
<td>4.36</td>
<td>24.2</td>
<td>25.8</td>
<td>0.98</td>
<td>1.42</td>
<td>5.68</td>
<td>20.6</td>
<td>43.9</td>
<td>84.8</td>
</tr>
</tbody>
</table>

PO$_4$-P, orthophosphate phosphorus; DTP, dissolved total phosphorus; TP, total phosphorus; DIN, dissolved inorganic nitrogen; DTN, dissolved total nitrogen; TN, total nitrogen.

*aThe period when M. aeruginosa blooms occurred.

*bThe period after M. aeruginosa blooms suddenly ceased.

*cND, not determined.

*dThe period of the present study.

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The density of *M. aeruginosa* began to increase in March. From March to May, the water temperature was still low (mean values of $T_{\text{Max}}$, $T_{\text{Min}}$, and $T_{\text{May}}$ over the 9 years were 10.2, 15.7 and 20.0°C, respectively). Recruitment may be an important reason for the increased abundance during spring, as studies have reported the importance of recruitment from sediment (Brunberg and Blomqvist, 2003) in shallow lakes and bays influenced by wind (Verspagen et al., 2004, 2005). Further research should examine the recruitment of *M. aeruginosa* from sediment to clarify its growth dynamics during spring. On the other hand, Oberholster and Botha (Oberholster and Botha, 2007) reported an increase in density even at low temperature (about 10°C). Thus, it is necessary to pay attention to the growth activity of *M. aeruginosa* in spring.

In summary, our 9-year study of the abundance of *M. aeruginosa* in Lake Kasumigaura based on RT-PCR revealed the following: (i) *Microcystis aeruginosa* existed in the lake water throughout the year, (ii) the population size increased in spring, despite the water temperature being relatively low, (iii) the density of *M. aeruginosa* increased rapidly from June to July, and the greatest density was almost always found in August. Together, our findings suggest that the annual maximum density of *M. aeruginosa* from 1999 to 2007 was correlated with the population density in spring and the quantity of light each algal cell was able to receive. Future studies should examine the effects of velocity and direction of the wind, the densities of *M. aeruginosa* at every depth and recruitment of *M. aeruginosa* from the sediment in order to accurately predict *M. aeruginosa* blooms in Lake Kasumigaura.

**SUPPLEMENTARY DATA**

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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**REFERENCES**


