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Variation in the growth of *Microcystis aeruginosa* depending on colony size and position in colonies

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Abstract – Growth of colonial *Microcystis aeruginosa* was investigated by performing an incubation experiment. Colonies of *M. aeruginosa* were separated based on size (colony diameter <100 μm, 100–200 μm and >200 μm) by filtration. Additionally, the cells around the surface of the colonies were separated from those inside the colonies by short-term ultrasonic treatment followed by filtration. Experimental results indicate that *M. aeruginosa* grew continuously throughout a 35-day incubation period in a nutrient-rich medium at specific growth rates between 0.045 and 0.310 d^{-1}. On day 14, larger colonies exhibited insignificantly higher specific growth rates. However, on day 35, the specific growth rates of colonies with diameters less than 100 μm insignificantly exceeded those of larger colonies. Internal cells of the colonies tended to grow faster than peripheral cells. Furthermore, the specific growth rates of the cells that comprised colonies with diameters of below 200 μm exceeded those of peripheral cells. These results suggest a potential growth strategy of *M. aeruginosa* in maintaining a high growth rate, eventually leading to the dominance of large colonies, which have notable ecological advantages over smaller ones.

Key words: *Microcystis aeruginosa* / colony size / cell position in colonies / specific growth rate / incubation experiment

Introduction

The dominance of toxic cyanobacteria and their formation of water blooms have been important problems in freshwater environments. Bloom-forming cyanobacteria generally exist as massive or filamentous colonies. Among the many features associated with large colonial forms of cyanobacteria include quick vertical migration (Kromkamp and Walsby, 1990), effective uptake of phosphorus (Shen and Song, 2007), protection from ultraviolet radiation (Sommaruga *et al.*, 2009) and invulnerability to grazers (Ghadouani *et al.*, 2003; Yang *et al.*, 2008). These features are closely related to the ecological advantages of bloom-forming cyanobacteria over unicellular species in water ecosystems. Many studies have demonstrated that bloom formation of cyanobacteria is primarily supported by the cell growth rather than recruitment from the sediment (Brunberg and Blomqvist, 2003; Karlsson-Elfgren and Brunberg, 2004; Ihle *et al.*, 2005). Such blooms usually start from small colonies, with larger colonies subsequently increasing and forming dense blooms (Reynolds *et al.*, 1981). Therefore, elucidating the growth performance of colonies is priority concern in the study of cyanobacterial blooms.

Still, the growth of cyanobacterial colonies largely remains unclear. As is reasonably assumed, microenvironment of cells is variable, depending on their position in a colony, and the primary location of cell growth within a colony has not yet been elucidated. Peripheral cells may outperform internal cells in the uptake of nutrients or inorganic carbon in low ambient concentrations (Paerl, 1983). Additionally, peripheral cells may have the advantage in capturing light. However, if colonies are exposed to excessive irradiance that is detrimental for the growth or photosynthesis (Whitelam and Codd, 1983; Köhler, 1992), internal cells may hold an advantage over peripheral cells because the peripheral cells may serve as a protective barrier.

This study investigates the dependence of the growth performance of colonial *M. aeruginosa* on colony size (colony diameter of <100 μm, 100–200 μm and >200 μm) and position in the colony (close to the edge *vs.* within the colonies). The specific growth rates of *M. aeruginosa* cells in each fraction were determined based on the frequency of dividing cells (FDC) technique.

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Materials and methods

Organism and standard culture condition

The non-axenic *M. aeruginosa* strain used in this study was originally isolated from Feitsui Reservoir (Taipei, Taiwan) in May 2008. Cells of the strain tightly aggregate with each other and form spherical or elongated colonies. The strain was maintained under the standard condition of 24 °C under 40 μmol photons.$m^{-2}.s^{-1}$ irradiance with a 12 h:12 h light-dark cycle. The C medium (Watanabe and Ichimura, 1977) was used with the following modifications: the sole sources of nitrogen, phosphorus, calcium and potassium were $NaNO_3$ (2260 μmol.L^{-1}), Na_2HPO_4 (174 μmol.L^{-1}), $CaCl_2{\cdot}2H_2O$ (635 μmol.L^{-1}) and KCl (989 μmol.L^{-1}), respectively, and the pH was adjusted to 7.6 with 400 mg.L^{-1} of *N*-2-hydroxyethlpiperazine-*N*'-2-ethanesulfonic acid (HEPES).

Culture experiment

Prior to the experiment, *M. aeruginosa* was cultured in a culture bottle that contained 1000 mL of a modified C medium under the standard condition, but under irradiance with 100 μmoL photons.$m^{-2}.s^{-1}$ with continuous stirring (100 rpm) and in a stream of filtered air (0.22 μm pore filter, 600–720 mL.min^{-1}). After 12 days of preculture, a 10 mL subsample was added to a new bottle that contained 1400 mL of medium. Incubation was initiated 3 h after the start of the light period, with continuous stirring and a stream of filtered air, as described above. The experiments were run in duplicate. The two bottles are referred to hereinafter as bottles A and B, with the respective results individually analyzed as well.

Monitoring the growth of *Microcystis aeruginosa*

Fifteen milliliter samples were taken every 3 h over four 21-h periods during the 35-day incubation. The samples were preserved immediately with paraformaldehyde at a final concentration of 2%. A 6 mL subsample was filtered through 200 and 100 μm mesh nylon screens in a stepwise manner to fractionate the colonies based on their size-classes, *i.e.*, <100 μm, 100–200 μm and >200 μm. Another 6 mL subsample in a test tube was placed in an ultrasonic cleaner (Power Sonic 410, Hwashin Technology, Seoul, South Korea) at a frequency of 40 kHz for 2 s to exfoliate the cells around the colony surface. According to preliminary flow cytometry analysis using unialgal *M. aeruginosa* strains NIES-1090, 1157 and 1355, which were supplied from the National Institute for Environmental Studies in Tsukuba, Japan, the ultrasonic treatment did not destroy the paraformaldehyde-preserved cells (data not shown). Therefore, as is assumed here, the ultrasonic treatment adopted in this work does not destroy the cells of the *M. aeruginosa* strain used herein. Numerous solitary cells were obtained after the ultrasonic treatment while the colonies remained, implying that the treatment exfoliates peripheral cells without completely dispersing colonies into their constituent cells. The water solution was then filtered through a nylon screen with a pore diameter of 20 μm to yield two fractions, of peripheral cells (filtrate) and internal cells, which were present as colonies (on the nylon screen). A drop of Lugol's iodine solution was added to each fraction, which was then placed in the ultrasonic cleaner for approximately 1 min to disperse the colonies completely into their constituent particles (single cells, dividing cells and paired cells). The states of over 400 cells were observed under an inverted microscope (Axio Observer A1, Carl Zeiss, Göttingen, Germany), and FDC was calculated as $D/(S+D+2P)$, where S, D and P denote the number of single cells, dividing cells and paired cells, respectively. The specific growth rate (μ) of *M. aeruginosa* in each fraction was determined using the equation proposed by Yamamoto and Tsukada (2009):

$$\mu = \frac{1}{n-1}\sum_{i=1}^{n-1}\frac{|f_{i+1}-f_i|}{T_i} \quad (1)$$

where $n=8$ (number of samples during 21 h), $T_i=3$ h (interval between ith and $i+1$th samplings), and f_i denotes the FDC value at the ith sampling. Equation (1) is thus reduced to

$$\mu = \sum_{i=1}^{7}|f_{i+1}-f_i|. \quad (2)$$

Equation (2) gives the specific growth rate in units of d^{-1}.

The residual subsamples in each 21-h period were mixed to determine the cell density and the colony size. At least 20 images were captured using a digital CCD camera (AxioCam MRm, Carl Zeiss, Göttingen, Germany), and the lengths of 101–121 colonies along the longest axis were measured using AxioVision 4.7 software (Carl Zeiss, Göttingen, Germany).

Results

Figure 1 plots the growth and range of the diameters of *M. aeruginosa* colonies. Although *M. aeruginosa* grew continuously in both bottles, the growth slowed with time. Despite the higher cell density of *M. aeruginosa* that was grown in bottle B than that in bottle A throughout the incubation period, the final yields on day 35 were almost equal (2.50×10^6 cells.mL^{-1} in bottle A, 2.52×10^6 cells.mL^{-1} in bottle B). The specific growth rates (d^{-1}), as calculated from the change in the natural logarithms of the population densities over seven or 14-day intervals, were 0.263 (days 0–14), 0.227 (days 14–21), 0.130 (days 21–28) and 0.094 (days 28–35) in bottle A, and 0.310 (days 0–14), 0.184 (days 14–21), 0.129 (days 21–28) and 0.045 (days 28–35) in bottle B. The colony diameters in bottles A and B ranged between 9.8 and 830 μm with a mean of 138 μm and between 11.8 and 1030 μm with a mean of 148 μm, respectively. The mean colony diameters in bottle A on days 14, 21, 28 and 35 were 128, 91.0, 140

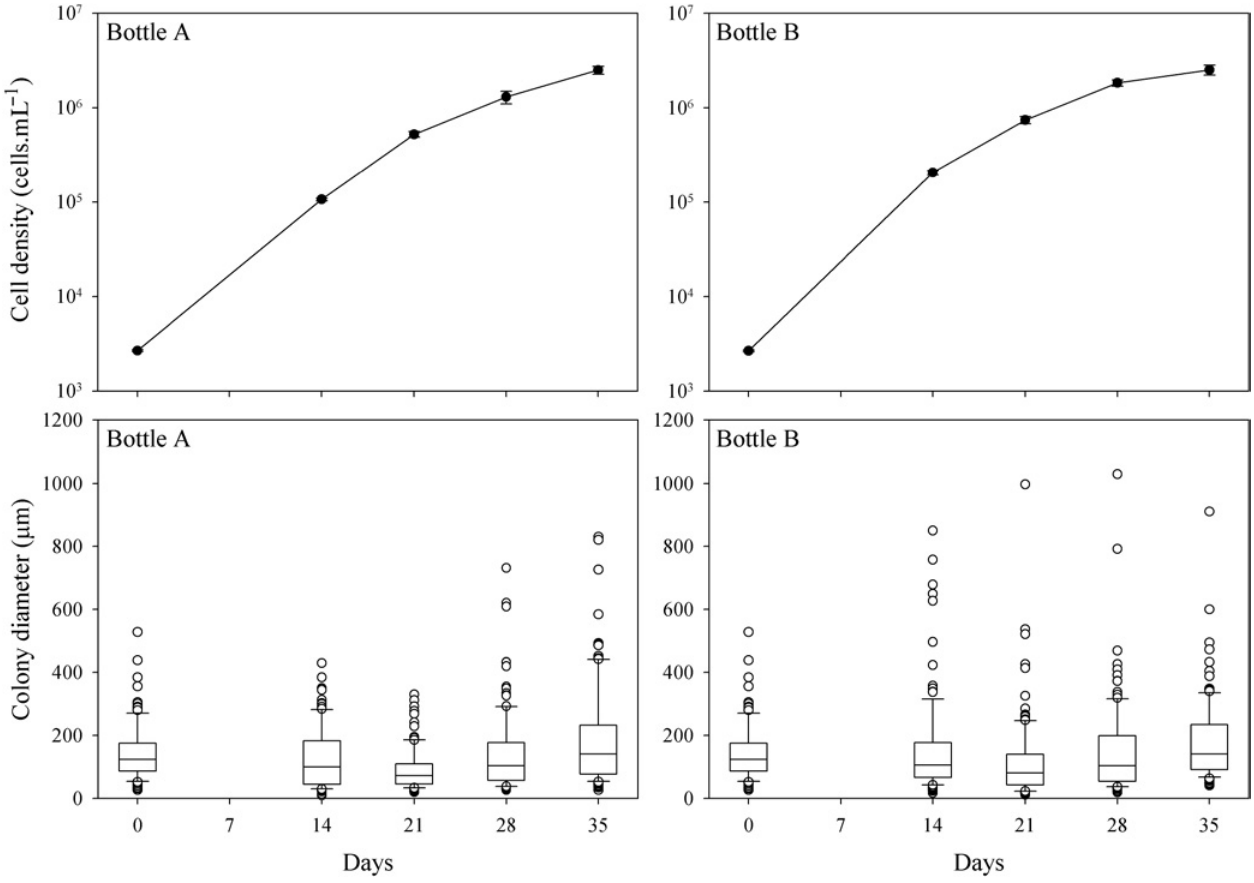

Fig. 1. Growth and ranges of colony diameter of *Microcystis aeruginosa*. Cell densities are expressed as mean ± SD ($n = 3$). In the lower panels, the 25th, 50th and 75th percentiles are indicated by the horizontal lines and the fifth and 95th percentiles are indicated by error bars. Open circles represent outliers.

and 193 μm, respectively. A similar variation trend in the mean colony diameter of *M. aeruginosa* was observed in bottle B; the mean colony diameters on days 14, 21, 28 and 35 were 155, 116, 146 and 176 μm, respectively.

Figures 2 and 3 plot the dependence of variation in FDC of *M. aeruginosa* on colony size and cell position. The FDC was always below 0.18, and largely fluctuated between 0.05 and 0.15. Higher FDC values in bottle A were often observed during the light period, regardless of colony size or cell position. The increase in FDC during the light period was less clear when the cells were separated based on their position in colonies on days 28 and 35. Similar changes in the FDC of *M. aeruginosa* were observed in bottle B, yet were rather obscure compared with the results in bottle A.

Table 1 presents the specific growth rates of *M. aeruginosa* depending on colony size or position of the cells in the colonies. The values were close to those calculated directly from changes in cell density, as described above. Specific growth rates increased insignificantly with colony size on day 14 (ANOVA, $F_{2,\ 3} = 1.95$, $P > 0.05$). The specific growth rates of colonies with diameters below 100 μm were insignificantly higher than those of the colonies with diameters above 100 μm on day 35 (ANOVA, $F_{2,\ 3} = 2.83$, $P > 0.05$). The specific growth rates of internal cells significantly exceeded those of peripheral cells (paired t-test, $n = 8$, $P < 0.05$), except in bottle A on day 28, for which the opposite result was obtained. Moreover, peripheral cells exhibited significantly lower specific growth rates than the cells that formed colonies with diameters of below 100 μm (paired t-test, $n = 8$, $P < 0.05$) and 100–200 μm (paired t-test, $n = 8$, $P < 0.01$).

Discussion

This study has demonstrated that changes in the FDC of *M. aeruginosa* and the specific growth rate varied with colony size and the position of cells in colonies, presumably because of the associated variation in cellular microenvironments. The nitrogen and phosphorus concentrations are typical growth-limiting factors in general photosynthetic organisms. However, in this study, the initial nitrogen and phosphorus concentrations were sufficiently high, and *M. aeruginosa* was grown with continuous stirring. Under such conditions, nutrients are assumed to diffuse uniformly, including inside large colonies, and the concentrations of nutrients are assumed to remain constant inside the bottle. We thus believe that

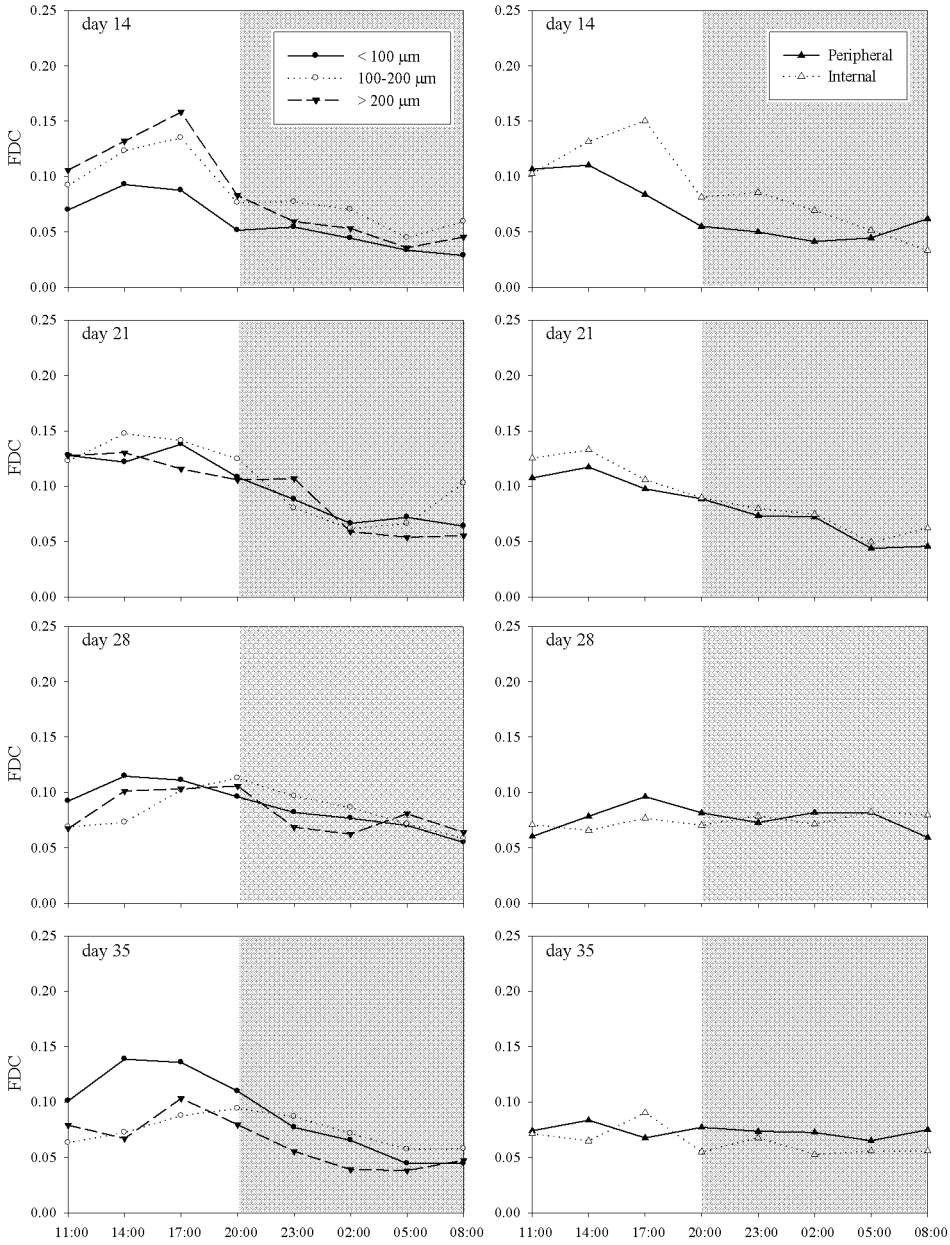

Fig. 2. Changes in FDC of *Microcystis aeruginosa* in bottle A. Gray zone indicates dark period.

M. aeruginosa cells take up a sufficient amount of nutrients, independently of the colony size or the cell position in the colonies. Inorganic carbon can be a limiting factor in the growth of *M. aeruginosa* in nutrient-rich batch cultures (Yamamoto and Nakahara, 2005); however, in this study, *M. aeruginosa* grew in a continuous supply of air. Therefore, both nutrients and inorganic carbon may be reasonably excluded as potential growth-limiting factors for *M. aeruginosa*.

The availability of light also significantly affects the growth and photosynthetic activities of cyanobacteria. An earlier laboratory study involving a unicellular *M. aeruginosa* strain demonstrated that the *M. aeruginosa* at 90 µmol photons.m^{-2}.s^{-1} grew rapidly, reaching the stationary phase soon thereafter; however, *M. aeruginosa* continuously grew at 30 µmol photons.m^{-2}.s^{-1} at a low rate, but for a longer period (Yamamoto and Nakahara, 2005). Zevenboom and Mur (1984) also demonstrated the ability of *M. aeruginosa* to grow successfully under low irradiance conditions. Such growth features of *M. aeruginosa* with respect to irradiance appear to reasonably explain the results of this study. The specific growth rates of the internal cells were higher than those of the peripheral cells; this seems to be because the internal cells could maintain higher growth ability than the peripheral cells, because irradiance at the center of the colonies was adequately reduced by the peripheral cells.

The above hypothesis may explain why the natural populations of *M. aeruginosa* can adapt to a strong irradiance to a certain extent. That is, the high photosynthetic activity of the natural populations of *M. aeruginosa* at strong irradiance, as observed by Paerl *et al.* (1985),

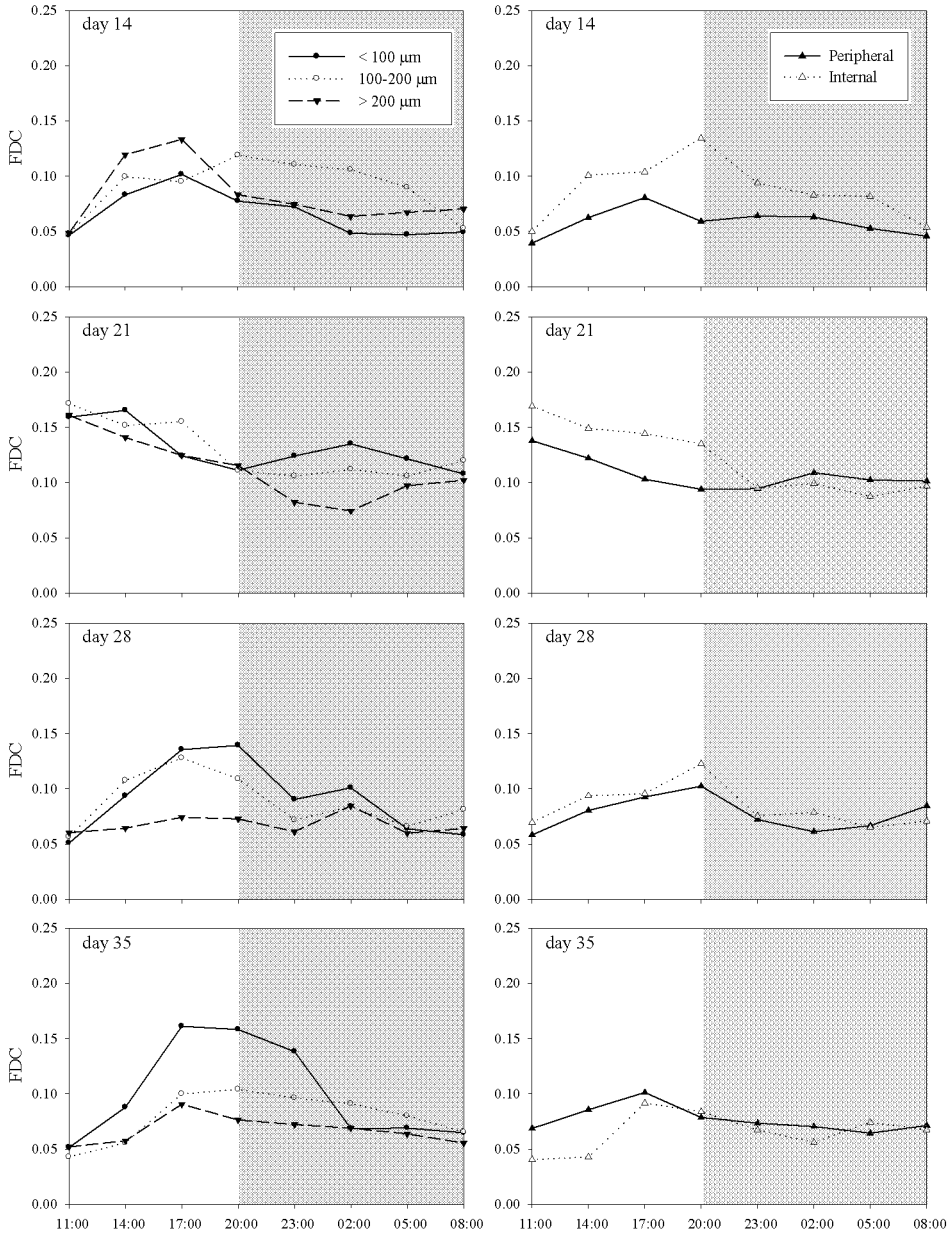

Fig. 3. Changes in FDC of *Microcystis aeruginosa* in bottle B. Gray zone indicates dark period.

Table 1. Specific growth rates (d^{-1}) of *Microcystis aeruginosa* depending on colony diameter or position of cells in colonies.

		Colony diameter (μm)			Cell position in colonies	
Days	Bottles	<100	100–200	>200	Peripheral	Internal
14	A	0.093	0.149	0.185	0.093	0.173
	B	0.112	0.146	0.161	0.086	0.165
21	A	0.108	0.151	0.082	0.085	0.103
	B	0.111	0.099	0.114	0.067	0.100
28	A	0.082	0.098	0.117	0.091	0.052
	B	0.190	0.173	0.080	0.109	0.122
35	A	0.132	0.068	0.122	0.057	0.099
	B	0.206	0.100	0.074	0.076	0.112

seems to be maintained by internal cells that are protected from strong irradiance by the peripheral cells. Furthermore, the velocity of upward migration of small colonies is lower than that of larger colonies (Kromkamp and Walsby, 1990). Therefore, when a cyanobacterial bloom is formed after a water body shifts from a turbulent state to a lentic state, large colonies are assumed to rapidly accumulate near the water surface and small colonies accumulate below a surface scum. Despite the higher risk of photoinhibition in small colonies than that in larger ones (Ibelings and Mur, 1992), surface scum attenuates the underwater irradiance, possibly facilitating the growth and photosynthesis of small colonies.

According to our results, the growth rates of cells that form small and mid-size colonies surpassed those of the peripheral cells, implying a potential growth strategy of *M. aeruginosa*. The size of a *M. aeruginosa* colony is thought to increase mainly owing to the growth of internal cells. Therefore, the growth of colonies is expected to slow down if the colonies become too large; the growth of internal cells begins to be suppressed owing to increased self-shading. However, they can overcome this suppression by separating peripheral cells as small colonies. In addition to allowing original large colonies to maintain high growth activity, this separation enhances the growth of new-born smaller colonies. Consequently, *M. aeruginosa* can generally maintain high growth activity. Furthermore, although not significantly differing in this study, small colonies may grow faster than larger colonies as the bloom progresses, subsequently increasing the mean colony size. Since large colonies accompany various ecological advantages, the increase in the percentage of large colonies is assumed to facilitate the dominance of *M. aeruginosa*. Growth performance of the colonies observed herein seems to be an underlying mechanism of the dominance of *M. aeruginosa* and its bloom formation in eutrophic environments.

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