Effects of mixing intensity on colony size and growth of *Microcystis aeruginosa*

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**Abstract** – Mixing is an integral environmental factor that affects lake ecosystems. For the cyanobacterium *Microcystis*, colony size is important with respects to migration velocity, how cells respond to grazing pressure, light attenuation, nutrient uptake and growth. To understand how mixing shapes colony size and the growth of *Microcystis*, we measured the effects of different current velocities (0, 0.16, 0.32, 0.64, and 1.28 m s⁻¹) on *M. aeruginosa* in Lake Taihu. After 24 h of continuous mixing, the mean colony sizes of *M. aeruginosa* in the controls, 0.16, 0.32, 0.64, and 1.28 m s⁻¹ groups were 23.6, 50.1, 92.9, 67.8, and 37.3 μm, respectively. Colony sizes of *M. aeruginosa* in all treatment groups were significantly larger than those in controls. As well, the concentration of soluble extracellular polysaccharides and bound extracellular polysaccharides of *M. aeruginosa* in all treatment groups were significantly higher than those in controls. Except for the highest level of mixing (1.28 m s⁻¹), the growth rate of *M. aeruginosa* was significantly higher than that in controls. This study suggested that mixing intensity over short time periods can significantly influence colony size and the growth of *M. aeruginosa*.

**Keywords:** *Microcystis* / colony size / mixing / EPS / Lake Taihu / growth

1 Introduction

The formation of cyanobacterial blooms is one of the most troubling symptoms of eutrophication (Sommaruga et al., 2009). Cyanobacterial blooms alter ecological processes and decrease the economic value of impacted waters. Controlling blooms induced by eutrophication remains a challenge facing water quality and water supply managers globally (Harke et al., 2016).

Many cyanobacterial blooms in eutrophic waters are dominated by the non N₂-fixing colonial genus *Microcystis*. Field investigations have demonstrated that large colonies of *Microcystis* are a main component of many blooms. In eutrophic Lake Taihu (China), investigations have found that large colonies (colony size > 38–50 μm) were dominant during *Microcystis* bloom events (Cao and Yang, 2010; Zhu et al., 2014; Qin et al., 2018), while small colonies (colony size < 50 μm) were dominant in the water column during non-blooming periods (Wu and Kong, 2009; Cao and Yang, 2010). Large *Microcystis* colonies also dominate during bloom periods in other eutrophic lakes (Sabart et al., 2013). Studies have shown that larger colonies of *Microcystis* have advantages in upwards floating speed (Xiao et al., 2012), the ability to resisting grazing stress (Oliver and Ganf, 2000), the ability to capture light (Kirk, 1975; Robarts and Zohary, 1984), and nutrient uptake (Shen and Song, 2007). Larger colonies formation by *Microcystis* better enables cells to access optimal light and nutrient environments by floating on the water surface as a thick “scum” (Reynolds, 2006; Yamamoto et al., 2011; Qin et al., 2018). The above investigations have suggested that *Microcystis* colony size was of an important facts affecting blooms formation in eutrophic fresh waters.

In fresh waters, *Microcystis* populations mostly exist as colonies during blooms (Wu and Kong, 2009; Cao and Yang, 2010; Qin et al., 2018). However, in lab, *Microcystis* colonies usually transform to unicells or paired-cells in growth medium. How *Microcystis* unicells might transform to colonies remains unclear. Studies have demonstrated many factors that can induce unicell to colony transformation of *Microcystis*, including biotic and abiotic factors, e.g., zooplankton grazing (Burket et al., 2001), bacteria (Wang et al., 2016b), microcystins (Sedmak and Elersek, 2006), high light intensity
Microcystis remains unknown.

Mixing is an integral environmental factor that affects lake ecosystems. Several studies have reported that mixing was an important factor affecting colony size of colonial Microcystis (Regel et al., 2004; Wang et al., 2016; Xiao et al., 2016; Yang et al., 2017; Li et al., 2018). However, the quantitative effect of mixing on colony size of Microcystis remains unclear. To understand this, we established simulation experiments to measure the effects of different mixing intensity (0, 0.16, 0.32, 0.64, and 1.28 m s⁻¹) on M. aeruginosa isolated from Lake Taihu. The result of this research shed light on quantitative effect of mixing on colony size and growth of Microcystis and potentially blooms in shallow lakes like Lake Taihu.

2 Materials and methods

Single colony of Microcystis aeruginosa, one of the dominant species of Microcystis in Lake Taihu (China), was isolated from lake water in Meiliang Bay (dominated by Microcystis bloom) in Lake Taihu in September 2016 and maintain in BG-11 medium (Rippka et al., 1979). After November 2016, unialgal cultures of M. aeruginosa were transferred to modified BG-11 medium (where TN = 50 mg L⁻¹ and TP = 2.5 mg L⁻¹). Until the beginning of our experiment, the M. aeruginosa cultures persisted as a mixture of single-cells, paired-cells and small colonies for a period of five months (~4.83 × 10⁶ cells mL⁻¹). At the beginning of the experiment, 150 mL inoculums of the exponentially growing M. aeruginosa (~4.83 × 10⁶ cells mL⁻¹) were transferred to 500 mL Erlenmeyer flasks containing 200 mL of modified BG-11 medium. Considering the current velocities of Lake Poyang (0.075–1.34 m s⁻¹) (Lai et al., 2015), Lake Chaohu (0.002–0.109 m s⁻¹) (Wang et al., 2016a), and Lake Taihu (0.005–0.077 m s⁻¹) (Zhou et al., 2016), different mixing intensities were designed as following: 0, 50, 100, 200, and 400 rpm, which approximate current velocities of 0, 0.16, 0.32, 0.64, and 1.28 m s⁻¹ (Camacho et al., 2007; Rodriguez et al., 2009), respectively. For treatments, continuous mixing was maintained for 24 h while the 0 rpm groups were considered as the controls. All treatments were maintained in triplicate. Next, treatment groups were put into 500 mL flasks then put on four shaker incubators (50, 100, 200, and 400 rpm) for 24 h at 25 °C under dark to simulate the effect of the mixing induce by wind-wave on M. aeruginosa. Mixing was generated on four horizontally oscillating shaking incubators. After shaking treatments, cultures were maintained in a “quiescent state” (i.e., no shaking). Controls were kept quiescent during the entire experimental period. Finally, after continuing mixing for 24 h, all groups were put in incubator at 25 °C under cool white fluorescent lights at an intensity of 40.5 mol m⁻² s⁻¹ with a light-dark period of 12:12 h. The total concentration of nitrogen and phosphorus in all groups nutrient were TN = 50 mg L⁻¹ and TP = 2.5 mg L⁻¹ at the start of the shaking.

Samples were collected at 0, 1, 3, 5, 7, 9, and 11th days into this experiment to measure EPS (extracellular polysaccharide), colony size and abundance of M. aeruginosa. The content of soluble extracellular polysaccharide (sEPS) and bound extracellular polysaccharide (bEPS) were quantified spectrophotometrically by the anthrone method (Herbert et al., 1971) using glucose as standard. Samples of M. aeruginosa (5 mL) were preserved with Lugol's iodine solution; these samples were concentrated to 1 mL after 5 mL of M. aeruginosa settled for 48 h. M. aeruginosa colonies in the concentrated samples were measured (400x magnification) with a Nikon E200 microscope and QCapture Pro software (QImaging, Surrey BC, Canada). To determine mean colony size, at least 100 colonies of M. aeruginosa were measured. The abundance of M. aeruginosa was for at least 100 units for unicell and paired-cells, at least 100 colonies for 3–10 cell colony⁻¹ and 10–100 cells colony⁻¹ and 100 colonies for >100 cells colony⁻¹. The concentrations of Chla were determined by spectrophotometry.

3 Statistical analyses

One-way analysis of variance (ANOVA) was used to test the differences in EPS, abundance, and colony size of M. aeruginosa between treatments and controls. All analyses were made using the SPSS19.0 computer programs.

4 Results

4.1 The colony size of M. aeruginosa

In this experiment, the mean colony sizes of M. aeruginosa in controls, 0.16, 0.32, 0.64, and 1.28 m s⁻¹ groups were 23.2 (±1.8), 35.7 (±1.2), 51.6 (±2.6), 40.8 (±4.3), and 25.7 (±2.4) μm, respectively (Fig. 1a). ANOVA showed that the mean colony sizes of M. aeruginosa in 0.16, 0.32, 0.64 m s⁻¹ groups were significantly larger than those in controls (P < 0.01), but no significant difference between mean colony size of M. aeruginosa in controls and the 1.28 m s⁻¹ groups was found (P > 0.05). In the first day in this experiment, the colony size of M. aeruginosa in 0.16, 0.32, 0.64, and 1.28 m s⁻¹ groups increased after continuing mixing for 24 h. The colony size of M. aeruginosa in controls, 0.16, 0.32, 0.64, and 1.28 m s⁻¹ groups was 23.6 (±1.8), 50.1 (±8.6), 92.9 (±4.8), 67.8 (±10.9), and 37.3 (±3.9) μm, respectively (Figs. 1b and 2). An ANOVA showed that the colony sizes of M. aeruginosa in all treatment groups were significantly larger than that in controls (P < 0.05) in the first day in this experiment. The colony size of M. aeruginosa in all treatment groups gradually decreases with the time, while the colony size of M. aeruginosa in control keep steady (around 23 μm) (Fig. 1b). At the 11th day of the experiment, the colony size of M. aeruginosa in controls, 0.16, 0.32, 0.64, and 1.28 m s⁻¹ groups was 23.0 (±3.7), 28.2 (±4.7), 33.9 (±3.3), 25.2 (±1.6), and 22.1 (±2.1) μm, respectively (Fig. 1b).

4.2 The EPS of M. aeruginosa

EPS (extracellular polysaccharides) have been shown to be important in colony formation of Microcystis (Yang et al., 2008). In this study, there was no significant difference between the mean value of sEPS (soluble extracellular polysaccharide) and bEPS (bound extracellular polysaccharide) among treatments; the mean values were 12.0 mg L⁻¹ and 3.0 mg L⁻¹, respectively (Table 1). The EPS content was significantly higher in treatments than that in controls (Table 1). ANOVA showed that the differences in EPS, abundance, and colony size of M. aeruginosa were statistically significant (P < 0.05). However, there was no significant difference between mean colony size of M. aeruginosa in controls and the 1.28 m s⁻¹ groups was found (Table 1). The colony size of M. aeruginosa in controls, 0.16, 0.32, 0.64, and 1.28 m s⁻¹ groups was 23.6 (±1.8), 50.1 (±8.6), 92.9 (±4.8), 67.8 (±10.9), and 37.3 (±3.9) μm, respectively (Figs. 1b and 2). An ANOVA showed that the colony sizes of M. aeruginosa in all treatment groups were significantly larger than that in controls (P < 0.05) in the first day in this experiment. The colony size of M. aeruginosa in all treatment groups gradually decreases with the time, while the colony size of M. aeruginosa in control keep steady (around 23 μm) (Fig. 1b). At the 11th day of the experiment, the colony size of M. aeruginosa in controls, 0.16, 0.32, 0.64, and 1.28 m s⁻¹ groups was 23.0 (±3.7), 28.2 (±4.7), 33.9 (±3.3), 25.2 (±1.6), and 22.1 (±2.1) μm, respective (Fig. 1b).
polysaccharides), and bEPS (bound extracellular polysaccharides) of *M. aeruginosa* in controls and that in 0.16, 0.32, 0.64, and 1.28 m s\(^{-1}\) groups during this experiment (*P* > 0.05). However, on the first day after continuing mixing for 24 h, the concentration of sEPS for *M. aeruginosa* in 0.16 m s\(^{-1}\) (1.70 pg cell\(^{-1}\)), 0.32 m s\(^{-1}\) (1.78 pg cell\(^{-1}\)), 0.64 m s\(^{-1}\) (1.70 pg cell\(^{-1}\)), and 1.28 m s\(^{-1}\) (1.70 pg cell\(^{-1}\)) were significantly higher than that in controls (1.44 pg cell\(^{-1}\)) (*P* < 0.01) (Fig. 3). Also, the concentration value of bEPS of *M. aeruginosa* in 0.16 m s\(^{-1}\) (0.38 pg cell\(^{-1}\)), 0.32 m s\(^{-1}\) (0.45 pg cell\(^{-1}\)), 0.64 m s\(^{-1}\) (0.39 pg cell\(^{-1}\)), and 1.28 m s\(^{-1}\) (0.36 pg cell\(^{-1}\)) groups were significantly higher than that in controls (0.26 pg cell\(^{-1}\)) (*P* > 0.05). The concentration of sEPS and bEPS for *M. aeruginosa* in all groups gradually decreased with the time after the first day in this experiment (Fig. 3).

4.3 The abundance and growth of *M. aeruginosa*

The mean abundance of *M. aeruginosa* in controls, 0.16, 0.32, 0.64, and 1.28 m s\(^{-1}\) groups was 4.05, 4.79, 5.17, 4.48, and 4.30 \(\times\) 10\(^4\) cells mL\(^{-1}\) (Fig. 4a), respectively. The mean abundance of *M. aeruginosa* in 0.16, 0.32, and 0.64 m s\(^{-1}\) groups was significantly higher than those in controls and in the 1.28 m s\(^{-1}\) groups (*P* < 0.01) (Fig. 4a). The growth rates of *M. aeruginosa* in controls, 0.16, 0.32, 0.64, and 1.28 m s\(^{-1}\) groups was 0.227 (±0.006), 0.271 (±0.007), 0.298 (±0.006), 0.240 (±0.007), and 0.220 (±0.007) (Fig. 5b), respectively. The growth rates of *M. aeruginosa* in 0.16, 0.32, 0.64 m s\(^{-1}\) groups were significantly higher than that in controls and in 1.28 m s\(^{-1}\) groups (*P* < 0.05), while no significantly different between the growth rates of *M. aeruginosa* in 1.28 m s\(^{-1}\) groups and that in controls (*P* > 0.05). The variations of abundance and Chla of *M. aeruginosa* in this study were showed in Figure 5. The abundance of *M. aeruginosa* in 0.32 m s\(^{-1}\) groups (8.87 \(\times\) 10\(^4\) cells mL\(^{-1}\)) was the highest among all groups at the end of experiment. Similar were found in Chla of *M. aeruginosa* in 0.32 m s\(^{-1}\) groups (2212 μg L\(^{-1}\)) (Fig. 5).

Before mixing, no colonies with >100 cells of *M. aeruginosa* were found in any treatment groups or the controls (Tab. 1). After mixing for 24 h, the proportion of cells within >100 cell colonies relative to total abundance of *M. aeruginosa* increased from 0 to 9.68 in 0.16 m s\(^{-1}\) groups, from 0 to 32.13 in 0.32 m s\(^{-1}\) groups, from 0 to 28.29 in 0.64 m s\(^{-1}\) groups, and from 0 to 15.64% in 1.28 m s\(^{-1}\) groups on the first day, respectively. However, no colonies with >100 cells of *M. aeruginosa* were found in control groups on the first day. In contrast, on the first day, the proportion of cells in 10–100 cell colonies of *M. aeruginosa* significantly decreased in all treatment groups (Tab. 1) after mixing, e.g., in the 0.32 m s\(^{-1}\) groups, the cell abundance proportion of 10–100 cells colony to total abundance of *M. aeruginosa* decreased from 57.00% to 30.13% (Tab. 1). This suggested that >100 cell colonies of *M. aeruginosa* may have come from the aggregation of 3–100 cells colony by mixing, especially the 10–100 cells colony.

5 Discussion

In this study, we found that mixing intensities (0.16–0.64 m s\(^{-1}\)) favor increased colony sizes for *M. aeruginosa*. Wang *et al.* (2016c) reported that colony size of *Microcystis* significantly increased after mixing driven by Typhoon Soulik in Lake Taihu. Yang *et al.* (2017) found that simulating mixing (24 h) significantly enlarged the colony sized of *Microcystis* in Lake Taihu. However, O’Brien *et al.* (2004) reported that *Microcystis* colonies collected from field broke up into smaller colonies (<200 μm) after mixing. Xiao’s (2016) study showed that no colonies conformation was found after mixing. Li *et al.* (2018) reported that three species of *Microcystis* colonies collected from Lake Taihu broke up into smaller colonies after mixing. Overall these previous observations have resulted in conflicting results concerning the effects of mixing on colony formation by *Microcystis*.

One important variable to be considered is the starting conditions of *Microcystis* for each of these studies. In the current experiment, the mean colony size of *M. aeruginosa* was 23.6 μm at the onset of experiment, while it was above 200 μm in O’Brien’s (2004) study. In Xiao’s (2016) study, the *Microcystis* was only single-cells, while those were a mixture of single-cells, paired-cells and small colonies of *M. aeruginosa* in this study. Similarly in our study the mixing used was continuous over 24 h, while it was 30 min in Li’s (2018) study. In total the above studies showed that whether the colonies of *Microcystis* aggregate or disaggregate after mixing may depend on the mixing intensity, the mixing time and colony size of *Microcystis*.

EPS are mainly found in mucilage or the cell's sheath, and it can affect the “stickiness” of the cell surface, contributing to
Colony formation in *Microcystis* (Yang *et al.*, 2008; Li *et al.*, 2013; Zhu *et al.*, 2014). Research has shown that the concentration of EPS in *Microcystis* colonies was significantly higher than in single cells (Li *et al.*, 2013). Small colonies of *Microcystis* may come from the division of mother cell and adhesion via EPS (Kessel and Eloff, 1975). It is thought that sEPS (soluble extracellular polysaccharides) may increase cell adhesiveness, while the bEPS (bound extracellular polysaccharides) may prevent daughter cells from separating after cell division (Li *et al.*, 2013). In this study, the concentrations of bEPS and sEPS of *M. aeruginosa* in 0.16, 0.32, 0.64, and 1.28 m s⁻¹ were significantly higher than that in controls after...
continuing mixing for 24 h ($P < 0.05$). The increased bEPS and sEPS after mixing may explain why colony size of *M. aeruginosa* enlarged in all treatment in this study, especially in 0.32 m s$^{-1}$ groups.

Many studies have shown that stress conditions can lead to EPS production and releasing by *Microcystis* spp., including grazing of plankton (Yang *et al.*, 2008), high concentration of calcium (Wang *et al.*, 2011) and microcystin-RR (Gan *et al.*, 2012). Gan *et al.* (2012) found that microcystin-RR induced EPS in the culture medium and up regulated genes related to polysaccharide biosynthesis, but had no effect on the cell growth rate. Li *et al.* (2007) found that allelopathy material ecahyl–2–methyl acetoacetate (EMA) produced by reed could raise the respiration rate of *M. aeruginosa*, causing the CO$_2$ concentration raising in its culture flask, lowering the photosynthesis action rate of *M. aeruginosa*. In this study, mixing is one of stress conditions, so EPS content in the mixing treated groups was much higher than that of the control after mixing for 24 h in the dark and no significant difference was observed in both cell density and Chla. Also, we infer that respiration rate in all treated groups would raise to satisfy the energy requirement of EPS production and releasing of *Microcystis* spp. in the mixing treated groups.

The abundance and growth rates of *M. aeruginosa* in 0.16, 0.32, and 0.64 m s$^{-1}$ groups were significantly higher than that in controls groups and in 1.28 m s$^{-1}$ groups ($P < 0.01$). *M. aeruginosa* bloomed in pre-typhoon period to 69.4 min in post-typhoon period (Qin *et al.*, 2018). The increasing of colony size significantly increased from 32.8 min in post-typhoon period to 69.4 min in post-typhoon period within 48 h (Qin *et al.*, 2018). The increasing of colony size of *M. aeruginosa* by mixing favors upward movement of cells, enhancing exposure to light and subsequently growth and biomass accumulation (Cao and Yang, 2010; Yamamoto *et al.*, 2011; Qin *et al.*, 2018). This may explain why *M. aeruginosa* consistently becomes the dominant species of phytoplankton in Lake Taihu.

**Fig. S1.** ETR$_{\text{max}}$ (the potential maximum photosynthetic rate) and I$_{0}$ (half saturation light intensity) of *M. aeruginosa* in controls, 0.16, 0.32, 0.64, and 1.28 m s$^{-1}$ groups in this experiment. Error bars represent ± SD ($^{*}P < 0.05$, $^{**}P < 0.01$, $n = 3$). a = I$_{0}$, b = ETR$_{\text{max}}$.

**Fig. S2.** MDA and SOD activity of *M. aeruginosa* in controls, 0.16, 0.32, 0.64, and 1.28 m s$^{-1}$ groups in this experiment. Error bars represent ± SD ($^{*}P < 0.05$, $^{**}P < 0.01$, $n = 3$), a = MDA, b = SOD.

**Supplementary Material**
Table 1. The abundance proportion of different units to total abundance *M. aeruginosa* before and after continuing mixing for 24 h.

| Mix intensity (m s⁻¹) | Time (day) | Unicell (% total cells) | Two-cells (% total cells) | 3–10 cells (% total cells) | 10–100 cells (% total cells) | > 100 cells (% total cells) |
|----------------------|------------|-------------------------|---------------------------|--------------------------|----------------------------|----------------------------|
| Controls             | 0          | 7.74 ± 0.19             | 11.29 ± 0.19              | 24.26 ± 0.93             | 56.71 ± 0.80               | 0.00%                      |
|                      | 1          | 9.33 ± 0.06             | 10.67 ± 0.23              | 25.44 ± 0.06             | 54.57 ± 0.59               | 0.00%                      |
| 0.16                 | 0          | 8.23 ± 0.22             | 11.12 ± 0.17              | 23.90 ± 0.24             | 56.75 ± 0.69               | 0.00%                      |
|                      | 1          | 9.02 ± 0.06             | 11.21 ± 0.40              | 20.67 ± 0.09             | 49.42 ± 0.70               | 9.68 ± 0.38                |
| 0.32                 | 0          | 8.30 ± 0.18             | 11.14 ± 0.06              | 23.56 ± 0.12             | 57.00 ± 0.17               | 0.00%                      |
|                      | 1          | 9.78 ± 0.03             | 10.86 ± 0.05              | 17.11 ± 0.06             | 30.13 ± 0.32               | 32.13 ± 0.40               |
| 0.64                 | 0          | 8.24 ± 0.24             | 11.10 ± 0.18              | 24.02 ± 0.46             | 56.63 ± 0.59               | 0.00%                      |
|                      | 1          | 8.49 ± 0.06             | 10.74 ± 0.26              | 17.33 ± 0.15             | 35.14 ± 0.50               | 28.29 ± 0.06               |
| 1.28                 | 0          | 8.27 ± 0.18             | 11.14 ± 0.26              | 23.48 ± 0.26             | 57.10 ± 0.73               | 0.00%                      |
|                      | 1          | 11.62 ± 0.18            | 13.05 ± 0.13              | 28.48 ± 0.64             | 31.21 ± 0.06               | 15.64 ± 0.33               |

The Supplementary Material is available at https://www.limnology-journal.org/10.1051/limn/2019011/olm.

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Conflicts of interest. The authors declare that they have no conflicts of interest in relation to this article.

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