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RESEARCH ARTICLE OPEN ACCESS

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 polysaccharide 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 N_2-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 others 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 (Burkert *et al.*, 2001), bacteria (Wang *et al.*, 2016b), microcystins (Sedmak and Eleršek, 2006), high light intensity

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(Xiao, 2011), temperature and phosphorus (Zhu *et al.*, 2016; Duan *et al.*, 2018), and the presence of heavy metals (Bi *et al.*, 2013). Although a lot of research has been conducted, the mechanism of colony formation of *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.*, 2016c; 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 (~4.83×10^6 cells mL^{-1}). At the beginning of the experiment, 150 mL inoculums of the exponentially growing *M. aeruginosa* (~4.83×10^6 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; Rodríguez *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 control ($P < 0.05$) 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).

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

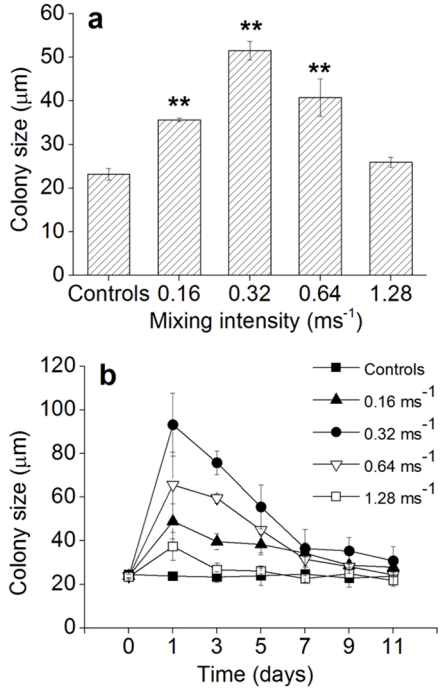

Fig. 1. Mean colony size and colony size variations of *M. aeruginosa* in controls, 0.16, 0.32, 0.64, and 1.28 m s^{-1} groups in this experiment. Error bars represent ± SD (*$P < 0.05$, **$P < 0.01$, $n = 3$). a = mean colony size of *M. aeruginosa*; b = colony size variations of *M. aeruginosa* with time.

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^{-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 4.30×10^6 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/d (±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 (8.87×10^6 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 *Microcysits* 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

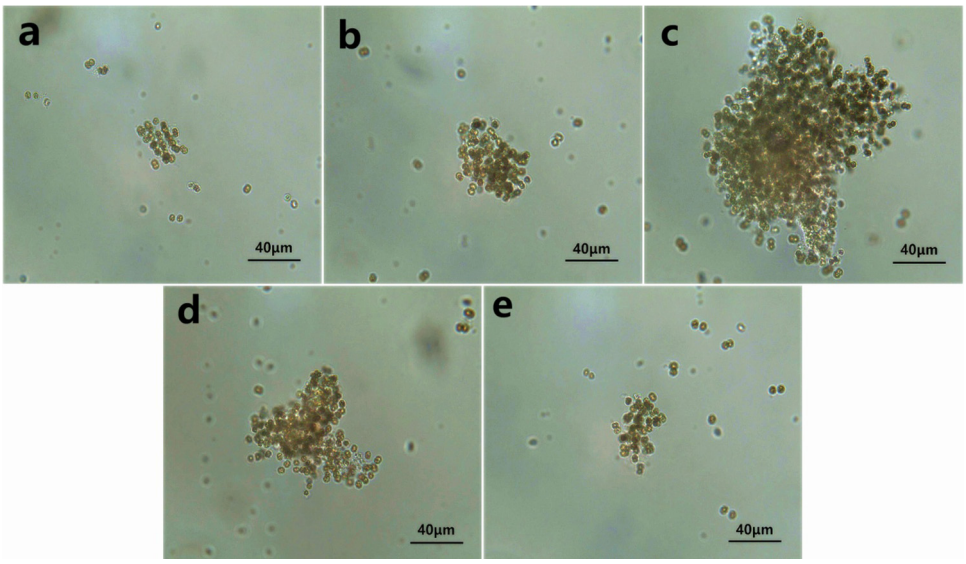

Fig. 2. Images of typical *M. aeruginosa* cells in controls, 0.16, 0.32, 0.64, and 1.28 m s^{-1} groups after continuing mixing for 24 h in the first day in this experiment. a = controls; b = 0.16 m s^{-1}; c = 0.32 m s^{-1}; d = 0.64 m s^{-1}; e = 1.28 m s^{-1}.

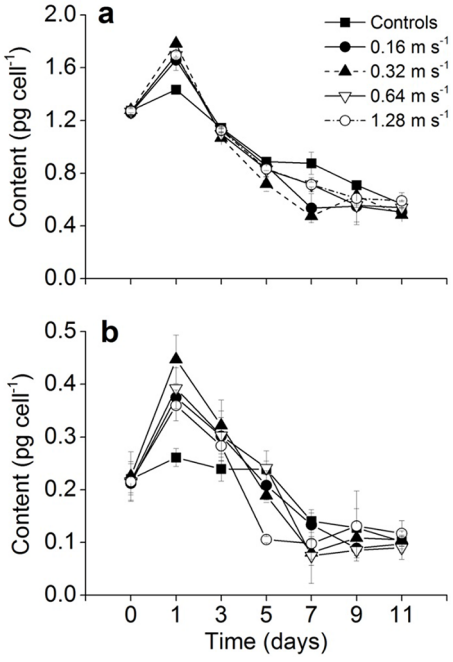

Fig. 3. Extracellular polysaccharides of *M. aeruginosa* in controls, 0.16, 0.32, 0.64, and 1.28 m s^{-1} groups in this experiment. sEPS = soluble extracellular polysaccharides; bEPS = bound extracellular polysaccharides; a = sEPS of *M. aeruginosa*; b = bEPS of *M. aeruginosa*.

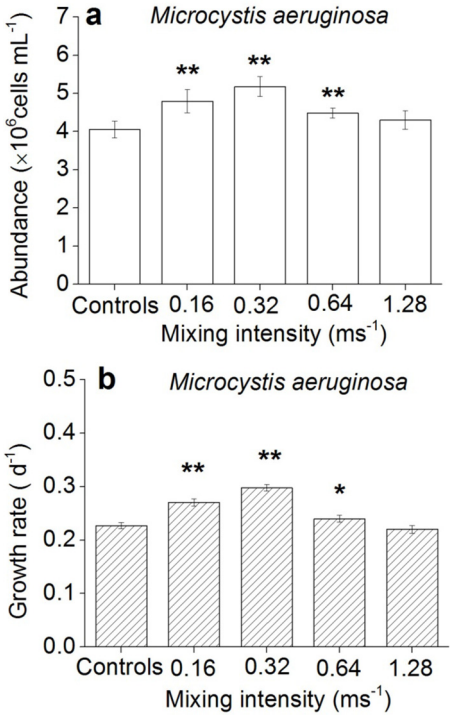

Fig. 4. Mean abundance and growth rates of *M. aeruginosa* in controls, 0.16, 0.32, 0.64, and 1.28 m s^{-1} groups in this experiment. Error bars represent ± SD (*$P < 0.05$, **$P < 0.01$, $n = 3$). a = mean abundance of *M. aeruginosa*; b = growth rates of *M. aeruginosa*.

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

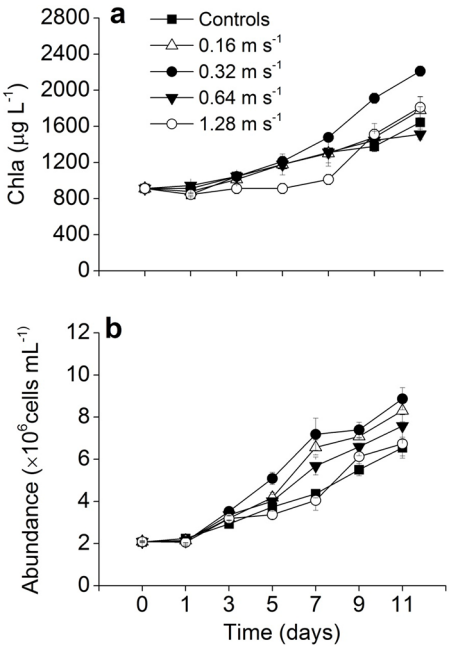

Fig. 5. Variation of abundance and Chla of *M. aeruginosa* in controls, 0.16, 0.32, 0.64, and 1.28 m s^{-1} groups in this experiment. a = Chla of *M. aeruginosa*; b = abundance of *M. aeruginosa*.

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) founded that allelopathy material eathyl–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 ($P < 0.05$), while the abundance and growth rates were not significantly different between the 1.28 m s^{-1} groups and controls ($P > 0.05$). Regel *et al.* (2004) demonstrated that low mixing intensities had no effect on the growth rate of *M. aeruginosa,* but high mixing intensity decreased growth rates. Yan *et al.* (2008) reported that certain mixing intensities stimulated the growth of *M. aeruginosa*, but high mixing intensities inhibited the growth of *M. aeruginosa* under low nutrient availability and all mixing intensities had no significant effect on the growth of *M. aeruginosa* under eutrophic nutrient. Jiang *et al.* (2012) found that low mixing intensity stimulated the growth of *M. aeruginosa* while high mixing intensity inhibited growth. Turbulence is reported to decrease the diffusive boundary layer around cells, theoretically increasing nutrient diffusion to cells (Lazier and Mann, 1989) and potentially increasing metabolic activity. Regel *et al.* (2004) found that low mixing intensities increased the esterase activity and cell viability of *M. aeruginosa*, but these were decreased at high mixing intensities. In this study, the values of ETR_{max} (the potential maximum photosynthetic rate) and I_k (half saturation light intensity) of *M. aeruginosa* in 0.16, 0.32, and 0.64 m s^{-1} groups were significantly higher than those in controls and 1.28 m s^{-1} groups ($P < 0.01$) in the 3rd day after mixing (Fig. S1). This may explain why growth rates of *M. aeruginosa* in 0.16, 0.32, 0.64 m s^{-1} groups were significantly higher than that in controls groups and in 1.28 m s^{-1} groups. Moreover, the values of MDA and SOD activities of *M. aeruginosa* in 1.28 m s^{-1} groups were significantly higher than those in controls and the 0.16, 0.32, and 0.64 m s^{-1} groups after mixing (Fig. S2). This information suggested that the hurt by high intensity mixing (1.28 m s^{-1}) on *M. aeruginosa* maybe lead to the lower growth of *M. aeruginosa*.

Taihu Lake, the third largest freshwater lake in China is shallow and hypereutrophic. *Microcystis* blooms have occurred from May to October every year since the 1950s and have been larger and more severe since the 1980s (Qin, 2008). *M. aeruginosa* has been one of the dominant species of *Microcystis* in Lake Taihu (Yang *et al.*, 2009). The mixing induced by wind-wave action in Lake Taihu is frequent due to the lakes shallow depth, large surface area and large fetch. This mixing leads to reduced transparency and nutrient resuspension into the water column from the sediments (Qin, 2008). Moreover, the mixing can increase the colony size of *Microcystis*. During the Typhoon Soulik (July 12–13, 2013), the mean current velocities was 0.0361 m s^{-1} (range 0.0063–0.1031 m s^{-1}) in Lake Taihu (Wu *et al.*, 2015). And the mean colony size of *Microcystis* in Lake Taihu significantly increased from 32.8 μm in pre-typhoon period to 69.4 μm 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.

Supplementary Material

Fig. S1. ETR_{max} (the potential maximum photosynthetic rate) and I_k (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_k, b = ETR_{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.

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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