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# Succession of *Aphanizomenon flos-aquae* and *Microcystis aeruginosa* in direct co-culture experiments at different temperatures and biomasses*

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**Abstract** Cyanobacterial blooms have become a serious global environmental issue due to their potential risk for releasing detrimental secondary metabolites into aquatic ecosystems, posing a great threat to water quality management for public health authorities. *Aphanizomenon*, a common filamentous cyanobacterial genus belonging to Nostocales, is under particular concern because its several members are able to form harmful blooms. Furthermore, succession of bloom between *A. flos-aquae* and *Microcystis* occurs in many natural lakes. To evaluate the competitiveness of *A. flos-aquae* vs. *M. aeruginosa*, two sets of experiments at different ratios of biomass at 15 °C and 25 °C were conducted. Results show that at 15 °C, the two species were able to coexist, and *A. flos-aquae* showed a specific higher growth rate, and its growth was promoted by the presence of *M. aeruginosa*. At 25 °C, the growth of *A. flos-aquae* was inhibited by the biomass of *M. aeruginosa*, and *M. aeruginosa* suppressed *A. flos-aquae* in competition. Additionally, the vegetative cell size of *A. flos-aquae* was significantly influenced by the co-culture with *M. aeruginosa*, whereas the filament length of *A. flos-aquae* was not significantly affected. This study confirms that temperature is the dominating factor on the succession of *A. flos-aquae* and *M. aeruginosa* of a different biomass.

**Keyword**: cyanobacterial bloom; *Aphanizomenon flos-aquae*; *Microcystis aeruginosa*; succession; temperature; biomass

## 1 INTRODUCTION

Cyanobacteria, a category of earth's most primitive oxygenic photoautotrophs, are able to adapt to complicated and varied environments on the earth (Paerl and Otten, 2013). Aggravated water eutrophication due to modern anthropogenic activities promoted the proliferation of cyanobacteria, increased the frequency of cyanobacterial bloom outbreak in many freshwater ecosystems (Gehringer and Wannicke, 2014), and damage local aquatic ecosystems, which poses great threats to water quality and challenge to the management (Glibert et al., 2005; Best, 2019; Mushtaq et al., 2020). *Microcystis*, *Dolichospermum*, *Planktothrix*, *Raphidiopsis*, and *Aphanizomenon* are regarded as the most common bloom-forming cyanobacteria genera (Paerl and Otten, 2016). *Aphanizomenon* belongs to the order Nostocales, and contains several members causing harmful blooms (Cires and Ballot, 2016; Codd et al., 1999). It was reported that *Aphanizomenon* species could produce several types of toxins, including paralytic shellfish poisoning (PSP) toxins, cylindrospermopsins (CYNs), microcystins (MCs), and anatoxins (ATXs) (Rapala et al., 1993; Sabour et al., 2005; Pearson et al., 2010; Rzymski et al.,

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2011; Cirés and Ballot, 2016). Furthermore, it has been reported that these species can produce odor compounds (Suurnäkki et al., 2015).

The seasonal succession of dominant species is the typical characteristic of phytoplankton assemblages in freshwater ecosystems (Tsukada et al., 2006; Moustaka-Gouni et al., 2007; Messineo et al., 2010). Eukaryotic algae are usually dominant species of phytoplankton in spring, and shortly after is replaced by cyanobacteria in summer (Wang et al., 2021). In addition, cyanobacterial communities undergo successions among different genera (Wu et al., 2016; Shan et al., 2019). Succession of dominant species is controlled by multiple biotic and abiotic factors, including temperature, nutritional levels, light intensity, hydrological conditions, allelopathy, and grazing pressure (Bormans et al., 2005; Smayda, 2008; Paerl and Paul, 2012; Tan et al., 2019)

Several field studies have reported that the seasonal succession between *A. flos-aquae* and *Microcystis* species is a common phenomenon in lakes, such as Dianchi Lake, China (Liu et al., 2006b; Wu et al., 2016) and Ford Lake in the southeastern Michigan (McDonald and Lehman, 2013), showed that *Microcystis* could become a dominant over *A. flos-aquae* in a warming water. *Microcystis* has developed several traits to form its ecologically competitive advantages, such as colony formation (Yang et al., 2006), ability to regulate buoyancy (Brookes and Ganf, 2001), secondary metabolites production (Ma et al., 2015), luxury phosphorus uptake (Shen and Song, 2007), high affinity for dissolved inorganic nitrogen (Takamura et al., 1987), and fast growth at warmer temperatures (Carey et al., 2012; Paerl and Otten, 2013). However, *A. flos-aquae* also posses some of the above-stated features, such as presence of gas vesicles and the formation of fascicles as aggregates in waters (Liu et al., 2006a; Yang et al., 2006). These shared traits between *A. flos-aquae* and *Microcystis* indicate that their niches overlap to some extents. *A. flos-aquae* as a common type of filamentous heterocystous cyanobacteria, owns some unique traits such as nitrogen fixation ability and cellular differentiation into heterocysts and akinetes, which is distinct from unicellular *Microcystis*. Therefore, these unique traits that differentiate their niches and reflect relative fitness may provide the potential for the dominance of *A. flos-aquae* or coexistence between *A. flos-aquae* and *Microcystis*.

There have been many reports about blooms formed by *A. flos-aquae* in winter (Jones, 1979; Baker, 1981; Üveges et al., 2012). Wu et al. (2010) described that *A. flos-aquae* bloomed in the winter and early-spring in Dianchi Lake, showing its tolerance to lower temperature; they also studied the effect of environmental factors on the seasonal succession of *Microcystis* and *A. flos-aquae* in the lake, and suggested that temperature is the most influential factor on the initiation of rapid growth and succession between *A. flos-aquae* and *Microcystis*. Ma et al. (2015) demonstrated that *Microcystis* strains are able to suppress the growth of *A. flos-aquae* as shown in *A. flos-aquae* co-culture with *Microcystis* filtrate, and this allelopathic effects may partially explain the driving factor for the seasonal succession from *A. flos-aquae* to *Microcystis* species. Apparently, studies in field and laboratory experiments have revealed the mechanism for the succession between *Microcystis* and *A. flos-aquae* to some extents (Wu et al., 2010, 2016; Ma et al., 2015), but did not set an experimental system on nor explore the direct co-culture of them. Since the indoor co-culture can simulate more precisely the mechanism of the competition between the two species, and help understanding the details of bloom succession for better control of eutrophic water bodies and cyanobacteria blooms. Furthermore, the influence of biomass shall be considered in study of the succession of different cyanobacterial species since the biomass is not equal in the natural waters.

In this study, to address the dominant environmental factor on the succession and dominance priority between *A. flos-aquae* and *M. aeruginosa*, we performed an *A. flos-aquae-M. aeruginosa* co-culture at 15 °C and 25 °C in different initial biomass gradients. In the co-culture experiment, the characteristics of growth and succession of the two species were studied and the morphological change of *A. flos-aquae* was observed.

## 2 MATERIAL AND METHOD

### 2.1 Strain and culture condition

Two strains of *A. flos-aquae* CHAB7452 and *M. aeruginosa* CHAB7427 were isolated for this study from Meiliang Bay, Taihu Lake, China in May 2019 (Fig.1). Strain CHAB7452 was originally in large fascicle-like colonies and composed of several straight trichomes, and the bundle filaments could irreversibly transform into solitary trichomes under laboratory conditions. Strain CHAB7427 was unicellular.

The cultures of the both cyanobacterial strains were maintained in BG11 medium in 250-mL flasks

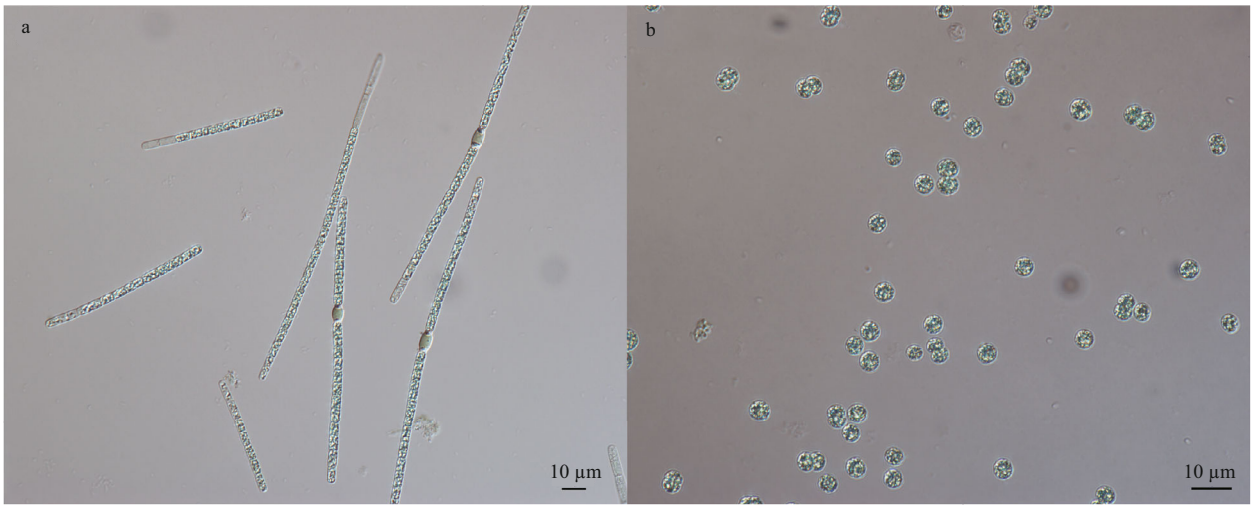


**Fig.1 Photomicrographs of *A. flos-aquae* strain CHAB7452 (a) and *Microcystis aeruginosa* strain CHAB7427 (b)**

containing 120 mL of the medium, at 25 °C with a 12-h:12-h light:dark cycle under a constant white light intensity of 30 μmol/($m^2$·s). The cultures were manually shaken thrice daily during incubation until the exponential phase.

### 2.2 Design of the experiment

The co-cultured experiment included 7 groups, and each group consisted of triplicate cultures at 15 °C and 25 °C. The first two groups, Groups A and B, were set for controls corresponding to monocultures of *A. flos-aquae* (CHAB7452) and *M. aeruginosa* (CHAB7427), respectively. The remaining groups, from C to G, were set for test groups of co-culture of *A. flos-aquae* (CHAB7452) and *M. aeruginosa* (CHAB7427) in different initial ratios of biomass with a total initial biomass of 24.3 mg/mL in 250-mL flasks as listed below:

A: 100% *Aphanizomenon* (A 100%) (A:M=100:0);

B: 100% *Microcystis* (M 100%) (A:M=0:100);

C: 95% *Aphanizomenon* (A 95%) and 5% *Microcystis* (M 5%) (A:M=95:5);

D: 75% *Aphanizomenon* (A 75%) and 25% *Microcystis* (M 25%) (A:M=75:25);

E: 50% *Aphanizomenon* (A 50%) and 50% *Microcystis* (M 50%) (A:M=50:50);

F: 25% *Aphanizomenon* (A 25%) and 75% *Microcystis* (M 75%) (A:M=25:75);

G: 5% *Aphanizomenon* (A 5%) and 95% *Microcystis* (M 95%) (A:M=5:95).

All the flasks were placed in an incubator under the same cultivation conditions except for temperature and manually shaken three times a day and their positions in the incubator were randomly adjusted during incubation. For each group, 10-mL subsamples were collected from the flasks during the experiment, from which 5 mL was fixed with Lugol's iodine solution (1% final concentration) for cell counting and the rest 5 mL was used for the morphological observation. Fresh sterile BG11 media were supplemented after sampling to maintain constant 120-mL culture volumes.

### 2.3 Growth measurement

The subsamples were taken for cell counting under a microscope (Olympus CX21, 400× magnification, Olympus, Tokyo, Japan), and specific growth rate ($\mu$) was calculated by the equation:

$$\mu=(\ln C_2-\ln C_1)/(t_2-t_1),$$

where $C_1$ and $C_2$ are the biomass (mg/L) at time $t$ for *A. flos-aquae* and *M. aeruginosa*.

Analyses of the morphology were based on photomicrographs taken with a Nikon eclipse 80i microscope (Japan) equipped with a DS-Ri1 digital camera (Nikon, Japan) photomicrographic system at 400× magnification. The cell width, length, and filament length of *A. flos-aquae* were measured and analyzed using NIS-Elements D 3.2, in which 360 vegetative cells and 360 trichomes were randomly chosen for the morphological analysis.

### 2.4 Statistical analysis

All experiments were conducted in triplicate; data were presented in mean±standard deviation. Statistical differences were evaluated by Kruskal-Wallis test in SPSS v19.0 software for windows. Differences with $P$ values less than 0.05 were considered significant.

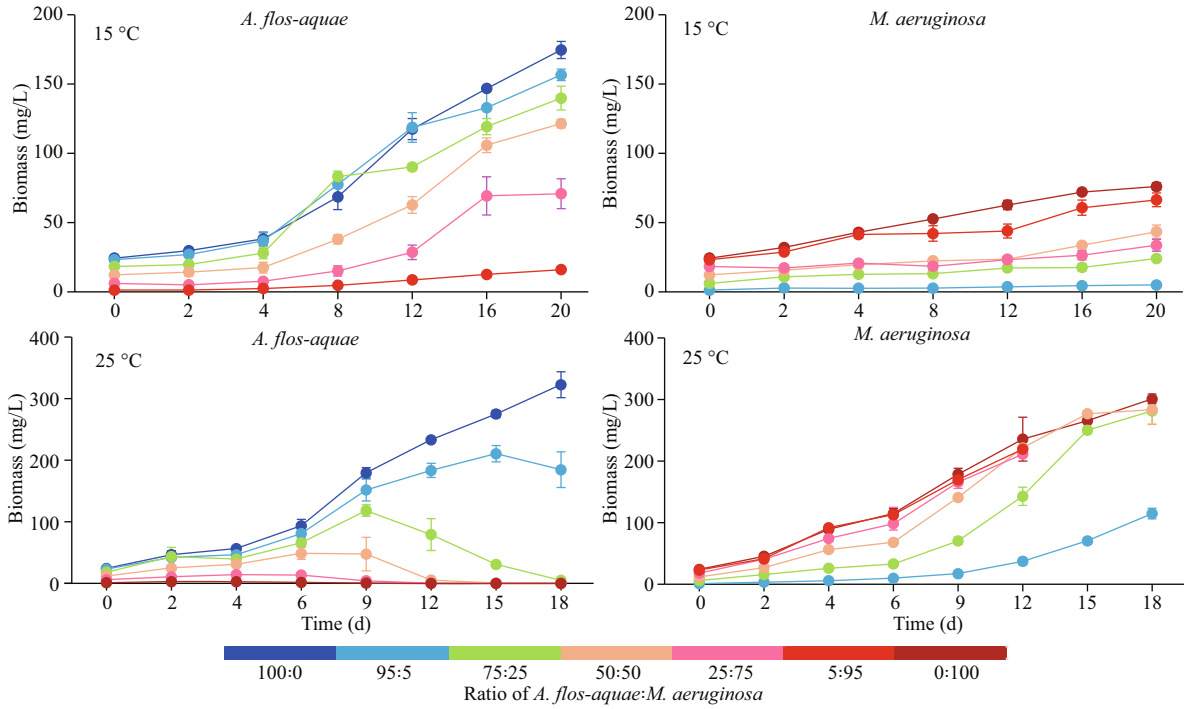


**Fig.2 The growth curves of the *A. flos-aquae* and *M. aeruginosa* at 15 °C and 25 °C**

Figures were generated using Microsoft Excel and R v3.5.1.

## 3 RESULT

### 3.1 Effect of A:M biomass ratios on the growth of the two cyanobacterial strains at two different temperatures

At 15 °C, biomasses of *A. flos-aquae* and *M. aeruginosa* all increased during the whole experiment period (Fig.2). After co-culture in different biomass ratios of *M. aeruginosa*, all the biomass of *A. flos-aquae* in each group increased from initial values of 24.300, 23.085, 18.225, 12.150, 6.075, and 1.215 mg/L in Groups A (A:M=100:0), C (A:M=95:5), D (A:M=75:25), E (A:M=50:50), F (A:M=25:75), and G (A:M=5:95), respectively, to final biomass values of 174.489, 156.529, 139.758, 121.345, 70.754, and 15.941 mg/L, respectively (Fig.2). *A. flos-aquae* in Groups G (A:M=5:95), with minimal initial biomass, showed the greatest increase in biomass of 13.12 times. The growth pattern of *M. aeruginosa* was similar to that of *A. flos-aquae*. The biomass of *M. aeruginosa* in all of the groups reached the peak value at the end of the experiment, reaching 75.973, 4.913, 23.995, 43.366, 33.507, and 66.332 mg/L in Groups B (A:M=0:100), C (A:M=95:5), D (A:M=75:25), E (A:M=50:50), F (A:M=25:75), and G (A:M=5:95), respectively (Fig.2). *M. aeruginosa* in Groups C (A:M=95:5) also had the lowest initial biomass and the greatest increase of 4.04 times in the experiment.

**Table 1 The specific growth rates ($\mu$) (/d) of *A. flos-aquae* and *M. aeruginosa* at 15 °C**

| Group | *A. flos-aquae* | *M. aeruginosa* |
|---|---|---|
| A (100%A) | $0.099\pm0.002^{cd}$ | – |
| B (100%M) | – | $0.057\pm0.002^{bc}$ |
| C (95%A, 5%M) | $0.096\pm0.001^{d}$ | $0.069\pm0.008^{a}$ |
| D (75%A, 25%M) | $0.102\pm0.003^{c}$ | $0.069\pm0.001^{a}$ |
| E (50%A, 50%M) | $0.115\pm0.001^{b}$ | $0.063\pm0.006^{ab}$ |
| F (25%A, 75%M) | $0.122\pm0.008^{ab}$ | $0.030\pm0.007^{d}$ |
| G (5%A, 95%M) | $0.129\pm0.006^{a}$ | $0.053\pm0.004^{cd}$ |

Different superscript letters indicate significant difference among groups ($P<0.05$). – means no data.

The specific growth rates ($\mu$) of *A. flos-aquae* in Groups E, F, and G were significantly higher than that of Group A ($P<0.05$) (Table 1). However, there were no significant differences in Groups A from Groups C and D ($P>0.05$) (Table 1). In the test groups, the $\mu$ value of *A. flos-aquae* in Group C was significantly lower than other groups ($P<0.05$) (Table 1), and those of *M. aeruginosa* in Groups C and D were significantly higher than that of Group B, whereas Group F was significantly lower than Group B ($P<0.05$) (Table 1). In the test groups, there were no significant differences among Groups C, D, and E ($P>0.05$) (Table 1). However, the $\mu$ value of *M. aeruginosa* in the three groups were significantly higher than those of Groups F and G ($P<0.05$) (Table 1).

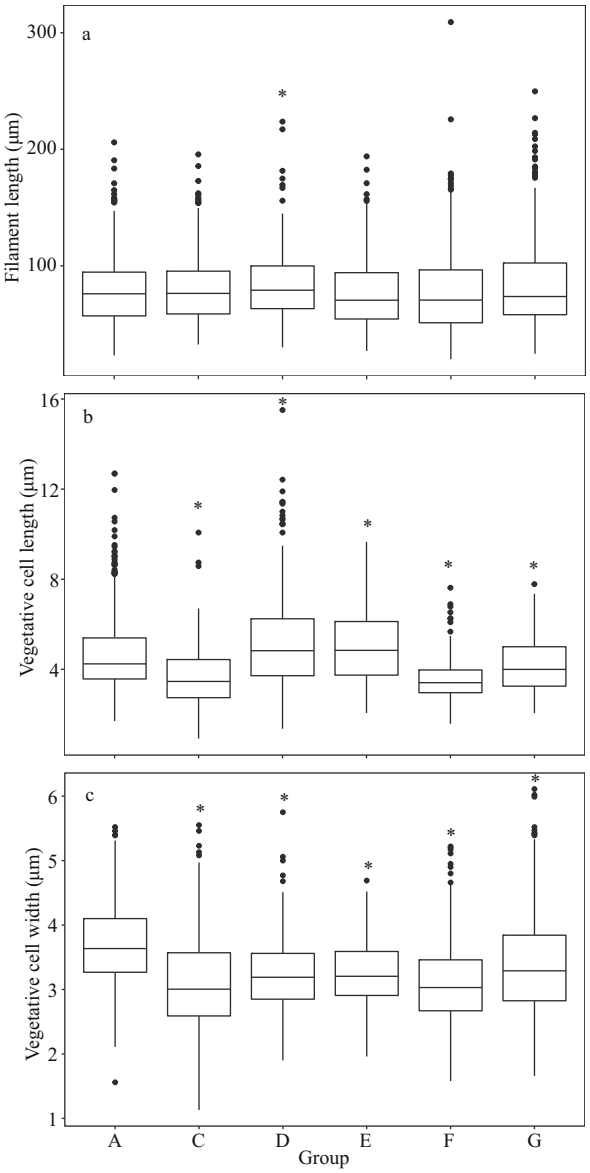


**Fig.3 The morphological characteristics of *A. flos-aquae* in each group on Day 20 at 15 °C**

a, b, and c represent the filament length, vegetative cell length and width respectively; * stands for significant difference between test group and control group (Group A) ($P$<0.05).

At 25 °C, the results are different from those at 15 °C. The biomass of *M. aeruginosa* in all groups was sustained to grow over the experiment period, whereas the biomass of *A. flos-aquae* presented a trend of earlier increase to later decrease except for Group A (100% A) (Fig.2). The whole experiment lasted 18 days; however, the filaments of *A. flos-aquae* were hardly seen in the Groups F and G until Day 12. The biomass of *A. flos-aquae* dropped to 5.070 (on Day 18), 0.280 (on Day 18), 0.282 (on Day 12), 0.019 mg/L (on Day 12) from the initial values of 18.225, 12.150, 6.075, and 1.215 mg/L in Groups D, E, F, and G, respectively (Fig.2). The highest biomass of *M. aeruginosa* reached 300.931 (on Day 18), 114.811 (on Day 18), 281.483 (on Day 18), 283.501 (on Day 18), 211.178 (on Day 12), and 219.325 mg/L (on Day 12) in Groups B, C, D, E, F, and G, respectively (Fig.2).

### 3.2 Effect of A:M biomass ratios on the morphological features of *A. flos-aquae*

The length and width of the vegetative cell were significantly different among groups at 15 °C (Fig.3b–c) ($P$<0.05). The lengths were 4.76±1.85, 3.65±1.24, 5.16±1.97, 5.00±1.57, 3.54±0.86, and 4.20±1.19 μm in Groups A, C, D, E, F, and G, respectively (Fig.3b); and the widths were 3.70±0.61, 3.09±0.79, 3.21±0.56, 3.25±0.50, 3.12±0.64, and 3.41±0.82 μm for Groups A, C, D, E, F, and G, respectively (Fig.3c). The vegetative cell widths in the test groups were significantly lower than that of control group. In addition, there is no significantly difference between control group and test group in the filament length except for Group D (Fig.3a).

The vegetative cell length and width were significantly different among groups at 25 °C (Fig.4b–c) ($P$<0.05). Comparing with the control group (Group A), the filament length in Groups E and F was significantly lower, whereas there are no significantly differences with other test groups (Fig.4a) ($P$>0.05). The vegetative cell lengths were 3.23±0.83, 4.13±1.16, 3.74±1.17, 3.01±0.78, 3.02±0.57, and 3.11±1.15 μm for Groups A, C, D, E, F, and G, respectively (Fig.4b).

The vegetative cell widths in the test groups were significantly lower than that of control group (3.49±0.75 μm) except Group C (3.73±0.98 μm), similar to the result at 15 °C ($P$<0.05) (Fig.4c).

### 3.3 Effect of initial A:M biomass ratios on final biomass percentages of the two cyanobacterial strains

At 15 °C, when the experiment ended, the percentage of *M. aeruginosa* as invader dropped to 3.04% from 5.00% in Group C (A:M=95:5), compared to the percentage of *A. flos-aquae* as invader up to 19.38% in Group G (A:M=5:95) (Fig.5). In Groups D (A:M=75:25), E (A:M=50:50), and F (A:M=25:75), the percentages of *M. aeruginosa* went up first and then fell to 14.65%, 26.33%, and 32.14%, respectively, whereas the percentages of *A. flos-aquae* increased up to 85.35%, 73.67%, and 67.86%, respectively on Day 20 (Fig.5). The direct competition experiments

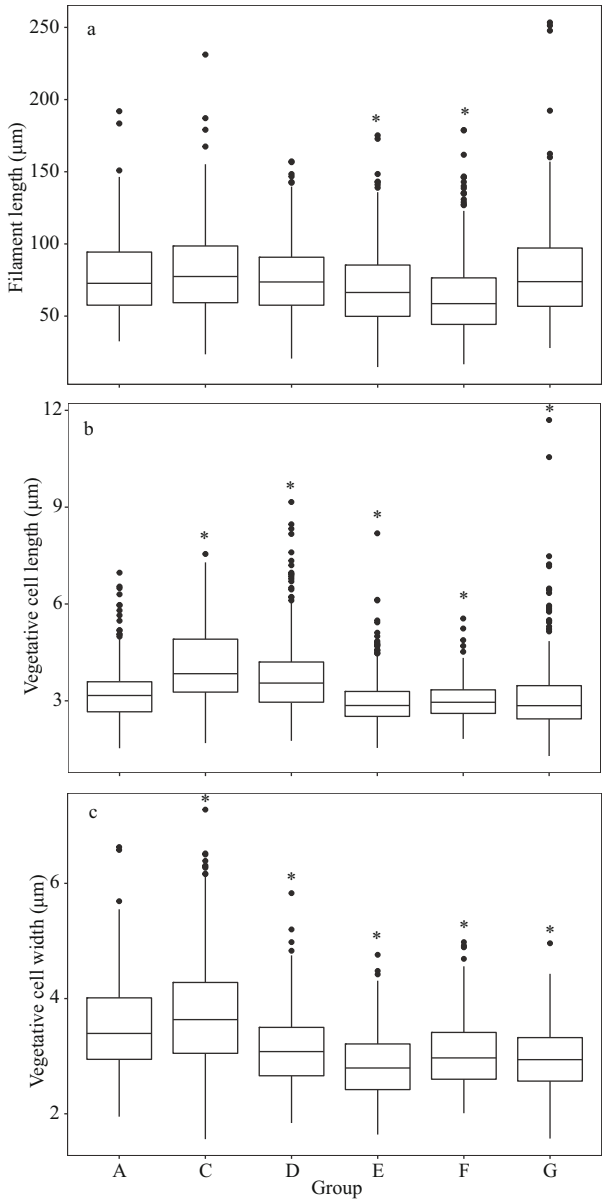


**Fig.4 The morphological characteristics of *A. flos-aquae* in each group on Day 6 at 25 °C**

* stands for significant difference between test group and control group (Group A) ($P$<0.05).

between *A. flos-aquae* and *M. aeruginosa* at 15 °C indicated that both two species were able to coexist and *A. flos-aquae* completely dominated at the end of the experiment period in the competitive groups. Thus, *A. flos-aquae* showed a stronger competitive ability at 15 °C over *M. aeruginosa*.

At 25 °C, the relative contribution of *M. aeruginosa* in total biomass as invader reached 38.34% on Day 18 from initial 5.00% in Group C (A∶M=95∶5), while the percentage of *A. flos-aquae* as invader dropped to nearly zero in Group G (A∶M=5∶95) at the end of experiment (Fig.6). Similarly, in the Groups D (A∶M=75∶25), E (A∶M=50∶50), and F (A∶M=25∶75), the percentages of *M. aeruginosa* in total biomass increased from 25.00%, 50.00%, 75.00% (Day 0) to 98.23% (Day 18), 99.90% (Day 18), 99.87% (Day 12), completely dominated at the end of the experiment (Fig.6). The competitive outcomes indicated that *A. flos-aquae* was unable to coexist with *M. aeruginosa* in the competitive groups, and *M. aeruginosa* showed a strong competitive ability at 25 °C.

In the competition experiments between *A. flos-aquae* and *M. aeruginosa* under two temperatures, the competitive outcomes were different, and the relative dominance of *A. flos-aquae* and *M. aeruginosa* was significantly affected by water temperature (Figs.5–6).

## 4 DISCUSSION

Understanding the mechanisms of cyanobacterial bloom formation and seasonal succession will help to harness cyanobacterial blooms. Succession of dominant bloom-forming species with seasonal change has frequently occurred in many eutrophic waters worldwide. A large number of studies based on field survey have reported the succession from blooms of *A. flos-aquae* to *Microcystis* as a typical and common case in the whole bloom period. Researches into the mechanism of the succession have been also conducted based on mainly the monoculture of *A. flos-aquae* or *Microcystis*, and co-culture of *A. flos-aquae* and of *Microcystis* filtrates. It was shown that both *Microcystis* and *A. flos-aquae* were able to reach optimal growth at 25 °C, but *A. flos-aquae* showed was a stronger competitor against *Microcystis* at 15 °C. On the other hand, the growth of *A. flos-aquae* was significantly inhibited by *Microcystis* culture at 25 °C in a culture-filtrate assay system (Ma et al., 2015). Apparently, the results from this study could partially explain the reason for the seasonal succession from blooms of *A. flos-aquae* to those of *Microcystis*, and the mechanisms need to be verified in more experiments. Moreover, competition experiments between the two cyanobacterial species shall consider the initial biomass contribution from each of them.

Therefore, we designed an experiment of co-culture system in which *A. flos-aquae* with *M. aeruginosa* were mixed at 15 °C and 25 °C in different biomasses, to clarify the competition and dominance of the two strains. *A. flos-aquae* and *M. aeruginosa* showed different behaviors in competitiveness at 15 °C and 25 °C. At 15 °C, the two species were more than

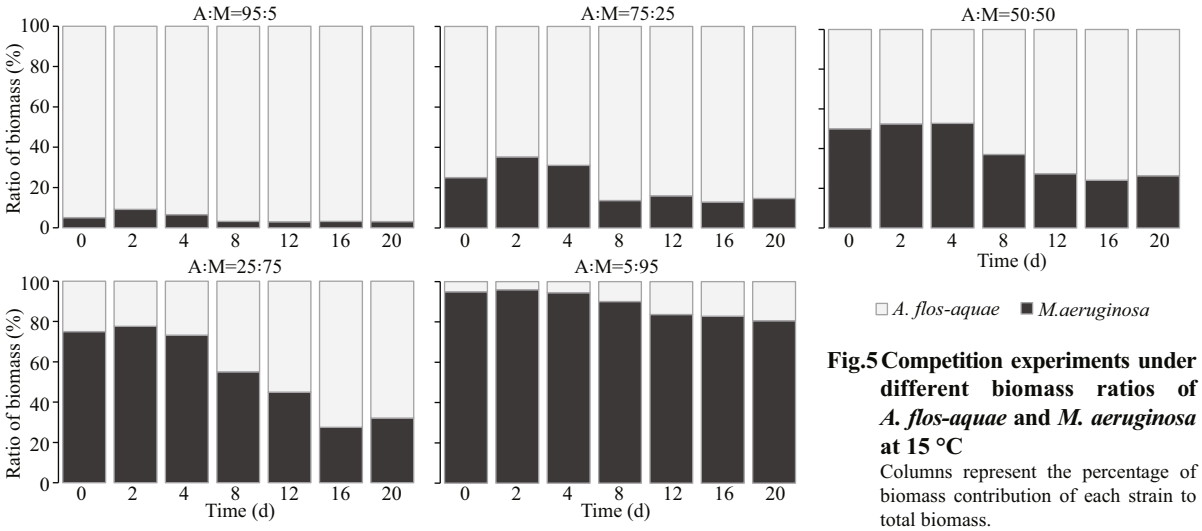


**Fig.5 Competition experiments under different biomass ratios of *A. flos-aquae* and *M. aeruginosa* at 15 °C**

Columns represent the percentage of biomass contribution of each strain to total biomass.

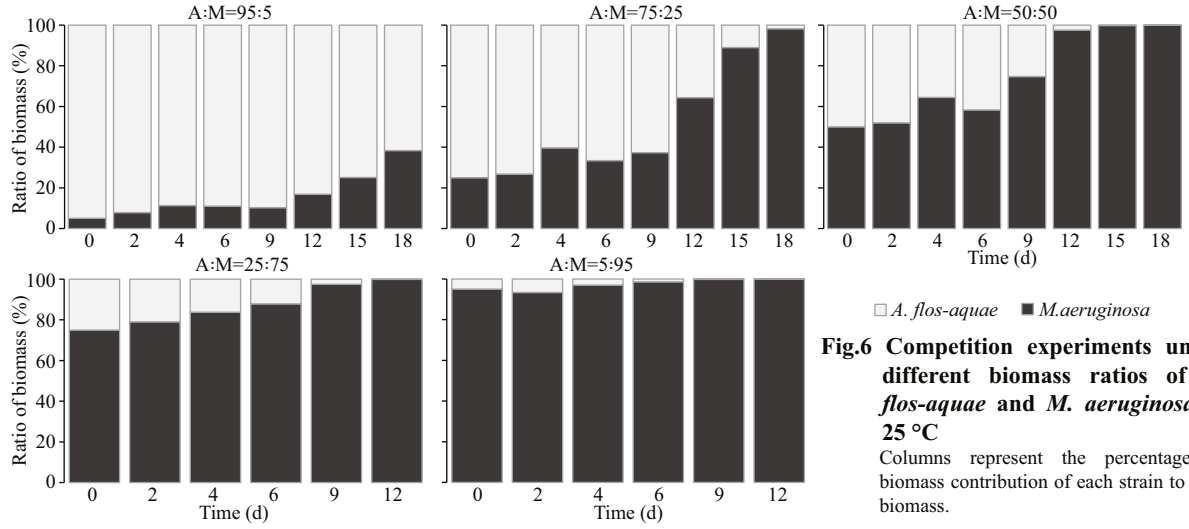


**Fig.6 Competition experiments under different biomass ratios of *A. flos-aquae* and *M. aeruginosa* at 25 °C**

Columns represent the percentage of biomass contribution of each strain to total biomass.

coexistence, and *A. flos-aquae* had a higher specific growth rate when its growth was stimulated by the presence of *M. aeruginosa*. However, at 25 °C, the growth of *A. flos-aquae* was inhibited by the invasion of *M. aeruginosa*, and *M. aeruginosa* could suppress *A. flos-aquae* in the competition experiments at last.

Our findings in the present study showed that the variation of temperature can lead to the succession between *A. flos-aquae* and *M. aeruginosa*, and these laboratory results are similar to the seasonal succession of *A. flos-aquae* and *M. aeruginosa* in Dianchi Lake (Wu et al., 2010, 2016). Tsujimura et al. (2001) demonstrated that the optimal temperature for *A. flos-aquae* ranged 23–29 °C and the lowest temperature for *A. flos-aquae* growth was 8 °C and could survive at 5 °C for several days at dim light conditions. Üveges et al. (2012) showed that *A. flos-aquae* could grow over a wide range of temperature, and it could grow slowly in a low-temperature condition and contribute to its succession and dominance in cold ecosystems.

*Aphanizomenon flos-aquae* blooms were reported to occur in winter even under the ice cover (Üveges et al., 2012). In this study, *A. flos-aquae* showed quicker growth at 25 °C than at 15 °C. However, *A. flos-aquae* was depressed by *M. aeruginosa* in co-culture at 25 °C. On the contrary, *A. flos-aquae* did not show down the optimal growth at 15 °C, but it still had the competitive advantage over *M. aeruginosa* at 15 °C in the co-culture. Compared to the previous studies of monoculture and co-culture, this study provided more insight into the low-temperature tolerance of *A. flos-aquae* and its low-temperature intolerance and preference in high temperature with *M. aeruginosa* (Konopka and Brock, 1978; Robarts and Zohary, 1987; Wu et al., 2010; Üveges et al., 2012).

Previous studies have reported that many filamentous cyanobacteria have strong phenotypic plasticity, and their morphological characteristics

may be influenced when exogenous factors changed (Soares et al., 2013; Khan et al., 2017). The competition between different species can also influence their morphological traits (Zhu et al., 2015). It has been reported that interactions between *Raphidiopsis raciborskii* and *M. aeruginosa* could lead to significant changes in filament length, cell length, and width of *R. raciborskii* (Jia et al., 2020). In this study, *A. flos-aquae* displayed certain morphological plasticity in response to the co-culture with *M. aeruginosa* of a different biomass. All the length and width of vegetative cell were sensitive to the change in *M. aeruginosa* biomass at two temperatures. At 15 °C, the width of all test groups was significantly shorter than that of control group. At 25 °C, the vegetative cell width in all test groups was significantly smaller than that of control group except for the Group C. Therefore, the competition between *M. aeruginosa* and *A. flos-aquae* showed a tendency of decrease in the width of vegetative cell of *A. flos-aquae*, which contrasts to the result by Jia et al. (2020) showing that the width of vegetative cells of *R. raciborskii* in the test groups were significantly greater than that of control group in a competition experiment with *M. aeruginosa*. Previous studies reported that cell size is a main characteristic that influenced the ecological niches of phytoplankton (Litchman et al., 2007; Guan et al., 2020). Nutrient utilization, light utilization, as well as grazer resistance of phytoplankton were proved has a significantly correlation with cell size (Bohannan et al., 2002; Finkel et al., 2010; Litchman, 2003). To phytoplankton, the trade-offs between these functional traits can improve resource utilization efficiency and adaptability to environmental changes, thereby facilitating population growth (Violle et al., 2012; Guan et al., 2020). There was also a study reported that small cells had greater competitive advantages than large cells (Grover, 1989), thus we speculated that phenotypic variation maybe was a trade-off strategy adopted by *A. flos-aquae*. At 15 °C, the significant change in filament length occurred only in Group F (A∶M=25∶75). Similarly, at 25 °C, the filament length of *A. flos-aquae* was significantly shorter than that of control group in Groups E and F only. Such results suggested that filament length of *A. flos-aquae* was less sensitive to the changes in biomass of *M. aeruginosa* than vegetative cells, which is consistent with the study by Jia et al. (2020). Moreover, several previous studies indicated that the changes in filament length of cyanobacteria could be affected by many factors, for example, turbulence as a main factor could lead to the decrease in filament length of *Anabaena cylindrica* and *A. flos-aquae* (Thomas and Gibson, 1990; Xiao et al., 2016). However, the present study did not test the other factors on the morphological changes of filaments in *A. flos-aquae* during the co-culture competition experiments, which will be studied in the future.

## 5 CONCLUSION

Based on direct co-culture experiments, we provide a new insight into the succession between *A. flos-aquae* and *M. aeruginosa* during a whole bloom process, and confirm that temperature is the dominating factor on the alternant succession of *A. flos-aquae* and *M. aeruginosa* considering the initial biomass ratios of the both strains under specific conditions of this study. However, in the field, the succession and competition between *A. flos-aquae* and *Microcystis* were controlled by more environmental factors or combination of these factors, not only by the temperature. Thus, in order to better understand the mechanism of succession between *A. flos-aquae* and *Microcystis* species, the nutrition level, especially the nitrogen concentration or total nitrogen : total phosphorus ration should be taken into consideration in terms of $N_2$-fixing ability for *A. flos-aquae* and non-$N_2$-fixing ability for *Microcystis* in the future.

## 6 DATA AVAILABILITY STATEMENT

All data generated and/or analyzed during this study are available from the corresponding author on reasonable request.

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