TEMPERATURE MAY BE THE DOMINATING FACTOR
ON THE ALTERNANT SUCCESSION OF Aphanizomenon
flos-aquae AND Microcystis aeruginosa IN DIANCHI LAKE

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ABSTRACT

This field investigation studied the effects of environmental factors on the seasonal dynamics and alternant succession of two cyanobacterial species, Aphanizomenon flos-aquae and Microcystis aeruginosa from November 2008 to May 2009 in Dianchi Lake of China. Temperature, pH, conductance, and transparency were measured in Fubao Bay of Dianchi Lake every week. At the same time, dissolved oxygen and inorganic carbon, total nitrogen and phosphorus, chlorophyll-a and phytoplankton biomass were field-investigated. The results analyzed by CCA (canonical correspondence analysis) indicated that temperature is the most correlative impact factor on A. flos-aquae and M. aeruginosa’s seasonal succession. Total nitrogen, total phosphorus and transparency are the second correlation factors. Temperature gradient experimentation in laboratory indicated that A. flos-aquae could grow under all of the 4 temperature conditions: 10, 15, 20 and 25 °C, but growth of A. flos-aquae at 10 and 15 °C was slower than that at 20 and 25 °C. M. aeruginosa could not grow at 10 °C and very slowly at 15 °C, but faster than A. flos-aquae at 20 and 25 °C. The result of the laboratory experimentation is coincident with the field investigation. It suggests that temperature may be the dominating factor on the alternation succession of A. flos-aquae and M. aeruginosa in Dianchi Lake.

KEYWORDS: Aphanizomenon flos-aquae, Microcystis aeruginosa, alternant succession, temperature

INTRODUCTION

Cyanobacterial blooms have become a worldwide environmental problem [1]. As we all know, high-frequency occurrences of cyanobacterial blooms reduce the efficiency of the water, destroy the balance of the ecosystem, and also produce many kinds of cyanobacterial toxins which may be harmful to public health. Consequently, the knowledge of phytoplankton community variations may contribute to a better understanding of driving factors of key species, helpful for formation of algal blooms. Many investigators studied cyanobacterial blooms by different aspects and methods [2-5]. Now, most of the studies consider that the occurrences of some cyanobacterial blooms are decided by physiological, morphological and ecological attributes of the species in the given environment [6], but the environmental conditions, such as temperature, ray radiation and nutrition, are important to regulate the key species [7-10]. Temperature was thought to be the most important factor influencing the phytoplankton’s growth and succession [11, 12].

Dianchi Lake (24°40’~25°02’N, 102°36’~103°40’E) is a typical eutrophic lake and one of the 6 largest fresh lakes in Yungui-plateau of southwest China. It has a surface area of about 300 km² as well as mean depths of 4.4 m. Dianchi Lake is located in a subtropical region. The annual mean temperature is 15 °C, and the annual average sunlight is 2400 h. Due to heavy pollution and nature conditions, the lake all the year round suffers from extensive algal blooms in recent years. In Dianchi Lake, cyanobacterial blooms are dominant, especially M. aeruginosa and A. flos-aquae alternatively succeeding. M. aeruginosa community occurred from June to November every year, and its biomass reaches peak value in summer. When temperature is declining in winter, biomass of M. aeruginosa decreases gradually, reaching a minimum in January [13]. With M. aeruginosa’s declining, A. flos-aquae community begins to develop from February and reaches a maximum in March, then generally persisting to May. The seasonal variation of the forming cyanobacterial species is very obvious and well-regulated in Dianchi Lake. In this eutrophic lake, when nutrition and light are not the limiting factors, what are those to drive the succession of both A. flos-aquae and M. aeruginosa? This study investigated environmental factors affecting the alternate succession of A. flos-aquae and M. aeruginosa, and we hypothesized temperature may be the most dominating factor on this succession in Dianchi Lake.
MATERIALS AND METHODS

Field investigation

High frequency field investigation was carried out between November 2008 and May 2009 in Fubao Bay of Dianchi Lake at sampling sites 1 (24°55'55.14"N, 102°41'8.72"E), 2 (24°55'38.48"N, 102°41'4.62"E) and 3 (24°55'22.28"N, 102°41'1.57"E). Aim of the field investigation was to find out how environmental factors change during the alternate succession of *A. flos-aquae* and *M. aeruginosa*, and what is the most dominating factor. Water samples were collected weekly from November to February, and four days a time from February to May during the quick growth of *A. flos-aquae*. Temperature, pH, conductance, transparency, dissolved oxygen, dissolved inorganic carbon, total nitrogen, total phosphorus, chlorophyll-a and phytoplankton's biomass were measured. Each site was divided into surface-layer and under-layer (surface-layer was 0.5 m under water surface, and underlayer was 0.5 m above the bottom). Dissolved oxygen was measured *in situ* using a YSI 550A dissolved oxygen meter. Temperature, conductance and pH were measured by a YSI 63 pH/conductivity/salinity/temperature meter. Total phosphorus (TP) was analysed by ammonium molybdate-ascorbic acid method with persulfate digestion in disposable polycarbonate bottles in an autoclave at 120 °C for 45 min, and absorbance measured at 700 nm. Total nitrogen (TN) was digested with alkaline potassium persulfate and absorbance measured at 220 and 275 nm. Water transparency was measured with a 20-cm diameter black and white Secchi disk. For chlorophyll-a measurements, 100 ml water sample was filtered through a Whatman GF/C glass fiber filter. The filters were extracted using 95% alcohol in the dark for 24 h at 4 °C, and then, the extractive part was centrifuged at 4500 rpm and the absorbance of supernatant was measured at 665 and 649 nm. For phytoplankton’s biomass, a 1 L water sample was preserved with Lugol’s iodine solution immediately after sampling and concentrated to 50 ml after sedimentation for 48 h [14]. After complete mixing, 0.1 ml of concentrated sample was counted directly in a 0.1 ml counting chamber under the microscope at 400X magnification. The algal cells were counted on a cell-by-cell basis. In addition, a series of CCA (canonical correspondence analyses) was performed using CANOCO 4.5 [15] to elucidate the relationships between the phytoplankton and environmental variables along the study period.

Laboratory experimentation

Based on the field investigation’s results, a temperature gradient experimentation in laboratory was designed to testify if temperature is the most dominating factor on the succession of key species. Four temperatures were designed: 10, 15, 20 and 25 °C. *A. flos-aquae* and *M. aeruginosa* were cultured under all temperatures with a photon supply of about 25 µmol photons m$^{-2}$s$^{-1}$ on a 24 h photoperiod, respectively. Each condition had 3 comparison experimentations. OD$_{665}$ and chlorophyll-a (Ultrospec 3000 UV/VIS spectrophotometer) as well as chlorophyll fluorescence (Phyto-PAM fluorometer [16]) were measured every 2 days. Chlorophyll-a was measured by measuring OD$_{665}$ and OD$_{649}$ after extraction by 95% alcohol for 24 h [17].
RESULTS

Change of environmental factors during variation of both *A. flos-aquae* and *M. aeruginosa*

Temperature

During the field investigation in Fubao Bay, the water body temperature continuously declined from November 2008, and reached the lowest value (9.1 °C) at the end of January 2009, but then increased again (Fig. 2). For each sampling site, temperature of both surface and under layer were identical, but temperatures among the 3 sites were different (site 1: highest, site 3: lowest, difference between 3 sites: about 0.5 °C). With decreasing temperature, biomass of *M. aeruginosa* declined, and after the lowest temperature, with slow temperature increase, *A. flos-aquae* biomass increased quickly. This indicated that temperature was correlated closely to succession of *A. flos-aquae* and *M. aeruginosa* (Figs. 3 and 4). Dates of *M. aeruginosa* community decline or *A. flos-aquae* community increase lagged behind temperature decline (about 10-15 days after the appearance of lowest temperature).

TP, TN and N/P

The change of total N and P in Fubao Bay was similar with temperature, which fell firstly and then rose, but not so obviously as temperature. In addition, the ratio of N/P was analyzed at site 3 (Fig. 5), showing that the trend of N/P change was similar with temperature. Total N and P concentrations changed a likely biomass of the phytoplankton. It may be due to the fact that most of N and P was stored intracellularly in phytoplankton.

CCA was used to elucidate the relationships between the phytoplankton and environmental variables, and the results indicate that temperature is most correlative with alternate succession of *A. flos-aquae* and *M. aeruginosa*. Secondly, total N, conductance, total P, and transparency are that in turns (Fig. 6).

Biomass change of phytoplankton

Result of *M. aeruginosa* colony counting indicated the amount was declining from November 2008 to February 2009 (Fig. 7). Finally, *M. aeruginosa* community collapsed at the end of January 2009 when water body temperature declined to the lowest (ca 9.1 °C). Then, after transitory recruitment, the amount of *M. aeruginosa* colonies rose rapidly from March 2009, showing that the community of *M. aeruginosa* developed.

The *A. flos-aquae* community began to develop before February in Fubao Bay, but rapidly dominated from middle...
FIGURE 6 - CCA of the relationships between the phytoplankton (A. flos-aquae and M. aeruginosa) and environmental variables (temperature, pH, conductance, transparency, dissolved oxygen, dissolved inorganic carbon, total nitrogen, total phosphorus).

FIGURE 7 - Total amount of M. aeruginosa colonies in Fubao Bay in winter of February, when the water temperature was about 13 °C. Furthermore, A. flos-aquae bloom appeared in April with a mass of clubbed colonies in water (Fig. 3). At the end of April, A. flos-aquae bloom disappeared and biomass of A. flos-aquae decreased quickly when the temperature was 19.4 °C. At the same time, M. aeruginosa community appeared fleetly. M. aeruginosa substituted A. flos-aquae to be the dominant species again in May 2009.

Effect of temperature on growth of A. flos-aquae and M. aeruginosa

Effect of temperature on growth of A. flos-aquae and M. aeruginosa

Laboratory experimentation indicated temperature-affected growth of A. flos-aquae and M. aeruginosa (Fig. 8). A. flos-aquae can grow under 10, 15, 20 and 25 °C, but the growth at 10 and 15 °C is slower than at 20 or 25 °C. Moreover, A. flos-aquae has a period of delay in growth about 10 days at low temperatures, such as 10 and 15 °C. M. aeruginosa cannot grow at 10 °C, grows very slowly at 15 °C, but grows faster than A. flos-aquae at 20 or 25 °C. This indicates that results of laboratory experimentation are coincident with those of field investigation. Temperature is the driving factor on the succession of A. flos-aquae and M. aeruginosa. A. flos-aquae can grow at 10 °C, but M. aeruginosa cannot. Furthermore, A. flos-aquae has a delay in growth over a period of ca. 10 days at low temperature. Gu et al. [18] also demonstrated that A. flos-aquae’s growth needs a period of delay of about 6 to 8 days [18]. These experimental results demonstrate that growth dominance of M. aeruginosa is at high temperature and that of A. flos-aquae at low temperature. Consequently, A. flos-aquae was the dominant species in winter, and then, with rising temperature, M. aeruginosa became the dominant species again.

Effect of temperature on photosynthesis of A. flos-aquae and M. aeruginosa

Measure of chlorophyll fluorescence showed that value of Fv/Fm (maximum quantum yield of photosystem II) declined at beginning of inoculation at 10 °C for A. flos-aquae, and then slowly rose after 5 to 6 days. But for M. aeruginosa, the value of Fv/Fm declined at last for 5 to 6 days, reaching zero in the end (Fig. 9). Measure of chlorophyll fluorescence indicated temperature-affected photosynthetic activity of M. aeruginosa, but for A. flos-aquae, the influence was not so distinct. To M. aeruginosa, the values of Fv/Fm and ETR (electron transport rate) were high at 20 and 25 °C, indicating that its photosynthetic activity was not influenced at high temperature (Fig. 10). However, A. flos-aquae can grow at: 10,
15, 20 and 25 °C, although its photosynthetic activity declined at low temperature, but only inconspicuously. In fact, Fv/Fm and ETR values are two important indicators about photosynthetic efficiency and photosynthetic activity of PS II function. Due to the decrease of photosyn-

FIGURE 8 - *M. aeruginosa* and *A. Flos-aquae*’s growth curve under 10 °C (A), 15 °C (B), 20 °C (C) and 25 °C (D).

FIGURE 9 - *M. aeruginosa* and *A. Flos-aquae*’s Fv/Fm curve under 10 °C (A), 15 °C (B), 20 °C (C) and 25 °C (D).
thetic efficiency, growth of phytoplankton cell will be slower, and also, the competitive advantage will be weaker, but finally succession will occur. An inhibition of photosynthetic efficiency may interfere with the organization of pigments and reaction centres of PS II at low temperature [19]. From the result of both field investigation and laboratory experimentation, it can be deduced that temperature was the dominating factor during the succession of A. flos-aquae and M. aeruginosa. Moreover, temperature may be work on photosynthetic activity of phytoplankton to affect the growth and succession.

**DISCUSSION**

Both A. flos-aquae and M. aeruginosa are species forming blooms in eutrophic water bodies. Because of many different physiological, morphological and ecological characteristics, they evolved different adaptive strategies to the environment [20-28]. A. flos-aquae and M. aeruginosa become the dominating species at different seasons, and alternate succession among both occurs in Dianchi Lake every year. The knowledge of variation of phytoplankton communities in Dianchi Lake may contribute to a better understanding of driving factors of the succession of the key species in Dianchi Lake.

Tsujimura et al. [30] researched the growth of A. flos-aquae isolated from Lake Biwa between 5-32 °C, and demonstrated that A. flos-aquae can already grow at 8 °C; but optimum temperature range was 23-29 °C. They also found that A. flos-aquae cultured at 5 °C for 25 days retained the ability to grow. Our results of field investigations showed that the time of A. flos-aquae community development was water temperature kept lower (Figs. 2 and 3). In laboratory experimentation, A. flos-aquae can grow under 10, 15, 20 and 25 °C, but M. aeruginosa cannot grow at 10 °C, but grow very slowly at 15 °C. This indicates that A. flos-aquae growth during winter is due to its low temperature tolerance rather than low temperature preference. M. aeruginosa could not grow at low temperature. Compared with M. aeruginosa, the values of Fv/Fm and ETR in A. flos-aquae, reflecting the organism’s photosynthetic activity, were higher at 10 °C (Figs. 9 and 10). This physiological feature enables A. flos-aquae to dominate easily during lower temperature in high densities by accumulating at the surface [31].

Collapse of M. aeruginosa community in winter was for its low temperature intolerance, whereas increasing of A. flos-aquae during winter was due to its low temperature tolerance. Figs. 9 and 10 display that the photosynthetic ability of M. aeruginosa is higher than that of A. flos-aquae at high temperature conditions, which enables M. aeruginosa to regulate its quick growth.

As we all know, temperature is a dominating environmental factor affecting photosynthetic ability. For example,
Guo [32] investigated orange’s photosynthetic activity at a low temperature (10 °C). They found that its net photosynthetic rates, its light saturation point and apparent photosynthetic quantum yield decreased at low temperature, but light compensation point and CO₂ compensation point increased at the same time. Martin et al. [33] found that photosynthetic activity of tomato’s leafage declined at 10 °C for the damnification of chloroplast and stoma. Zhang et al. [34] researched on Haematococcus’s photosynthetic oxygen evolution rate at 10-35 °C, and found it increasing from 10-20 °C, and decreasing from 20-35 °C, and the maximal photosynthetic oxygen evolution rate appeared at 20 °C. Deng et al. [35] found that photosynthesis of Scrippsiella trochoidea and Alexandrium tamarense were sensitivity to temperature change, but photosynthetic activity cannot be affected by temperatures between 17-25 °C, whereas out of this region, it decreased rapidly. Warner et al. [36] demonstrated that fluctuations in the photosynthetic capacity of tropical corals were highly correlated to seasonal patterns of both temperature and light. Increasing temperature [37] and light intensities [38] under laboratory conditions have also been shown to increase net photosynthetic rates of ZK and ZC in A. elegantissima. Su et al. [39] observed similar trends for Potamogeton crispus L. (a submerged plant of Potamogetonaceae). Our results of laboratory experimentation show that temperature has an important effect on photosynthetic ability of A. flos-aquae and M. aeruginosa. A. flos-aquae and M. aeruginosa have different photosynthetic ability under the same temperature, but the former has stronger photosynthetic ability than the latter under low temperature, which contributes to a competition of A. flos-aquae community in low temperature season.

At the same time, though temperature is a dominating environmental factor on succession of A. flos-aquae and M. aeruginosa in Dianchi Lake, the role of the other environmental factors, such as total N and P as well as N/P ratio, should not be neglected on phytoplankton’s succession (Figs. 5 and 6). Synergistic effects with temperature may be occurring. However, it is not known what are the functioning chemical factors either influencing the process of succession or the pattern of succession in an eutrophic lake.

During the fieldwork, we found that the biomass of A. flos-aquae did not appear immediately when the lowest temperature was reached, and both the date of M. aeruginosa community decline and the date of A. flos-aquae community increase were delayed behind temperature decline (about 10-15 days after the appearance of lowest temperature). It is thought that the growth of A. flos-aquae needs accumulated temperature. Due to absence of the research about accumulated temperature on phytoplankton, a method of botanical accumulated temperature calculation was used in this study. According to botanical method, 10 °C is the minimum temperature for growth. So, accumulated temperature for A. flos-aquae’s growth was calculated from February 4th to 20th, and its value was 213 °C. Of course, this conclusion was a comparative cursory for the absent research about accumulated temperatures on phytoplankton nowadays. So, accumulated temperature should be concerned in future studies on the succession of A. flos-aquae and M. aeruginosa in Dianchi Lake.

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