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Responses of phytoplankton functional groups to simulated winter warming

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Abstract – We investigated the seasonal dynamics of phytoplankton functional groups and the relevant environmental factors (water temperature, total nitrogen content, total phosphorous content and water transparency) in Dianchi Lake, China. We also examined the growth rates and physiological characteristics of representative species in laboratory cultures. In the field experiment, five dominant functional groups, including M (mainly consisted of *Microcystis aeruginosa*; *Microcystis wesenbergii* and *Microcystis flos-aquae*), H1(mainly consisted of *Aphanizomenon flos-aquae*), J (mainly consisted of *Scenedesmus*; *Pediastrum* and *Coelastrum*), F (mainly consisted of *Oocystis* and *Kirchneriella*) and P (mainly consisted of *Melosira*), were determined. Groups M and J were prevalent throughout the year and comprised more than 90% of the total biomass. Group M was prevalent at a relatively high temperature in summer and autumn; by contrast, group J was dominant at low temperature in winter and early spring. Co-cultivation laboratory experiments revealed that the biomass and the density of *Microcystis* sp., which is the representative species of the functional group M, were higher at 18 °C than at 13 °C. Conversely, the density and the biomass of the representative species of the functional group J (consisted of *Pediastrum duplex*, *Coelastrum microporum* and *Scenedesmus obliquus*) were higher at 13 °C than at 18 °C. At low temperatures, group M (*Microcystis* spp.) cannot successfully survive and grow at low temperatures, exhibiting various stress responses, such as inhibited photosynthetic activities and reduced phosphorous utilization. However, low temperature increased soluble carbohydrate contents of *Microcystis*, which favored the fast development of *Microcystis*, once the temperature warmed. Currently, climate warming is occurring in the Dianchi Lake basin; thus, future climate warming in winter (± 5 °C) may compromise the advantages of group J and promote the abundance of group M. The water transparency, dissolved oxygen and biodiversity in the lake would be further reduced. Furthermore, the increased microcystin and odor produced by *Microcystis* would considerably threaten the food web structure and lake ecosystem functions.

Key words: Phytoplankton / functional group / winter warming / eutrophication / responses

Introduction

Phytoplankton communities have been commonly used as indicators of climate change because these relatively short-lived organisms rapidly respond to subtle thermal changes. Occurrence frequency and bloom intensity of harmful cyanobacteria continuously increase worldwide (Figueiredo *et al.*, 2004; Taranu *et al.*, 2012). Studies have further indicated that climate warming plays important roles in promoting cyanobacterial blooms (Mooij *et al.*, 2005, 2007; Jöhnk *et al.*, 2008; Paerl and Husiman, 2008, 2009; Wagner and Adrian, 2009; Tadonléké, 2010; Paerl *et al.*, 2011) because these bloom-forming cyanobacteria exhibit a competitive advantage over other phytoplankton groups at high temperatures (Jöhnk *et al.*, 2008; Wagner and Adrian, 2009).

Previous studies mainly focused on the responses of phytoplankton to warming in terms of phenology characteristics and standing crops (McKnight *et al.*, 1996; Winder and Schindler, 2004; Elliott *et al.*, 2006; Staehr and Sand-Jensen, 2006; Huber *et al.*, 2008; Sommer and Lengfellner, 2008; Thackeray *et al.*, 2008; Elliott, 2012). Warming can influence phytoplankton directly through the bloom intensity of spring diatom blooms, algal biomasses, photosynthesis rates and indirectly by altering zooplankton community composition (Rhee and Gotham,

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1981; Raven and Geider, 1988; Elliott *et al.*, 2006; Staehr and Sand–Jensen, 2006; Huber *et al.*, 2008; Thackeray *et al.*, 2008; Jeppesen *et al.*, 2010). Thackeray *et al.* (2008) indicated that the spring peak biomass of *Cyclotella* and *Asterionella* appeared to be advancing as a result of climate warming. Xia *et al.* (2009) also reported that spring warming results in an early cyanobacterial bloom in Lake Taihu, China. Zhang *et al.* (2012) further suggested that the inter-annual variation of cyanobacterial bloom phenology in Lake Taihu is significantly correlated with the climate in this region. Cyanobacterial blooms occur earlier and last longer with the increase of temperature, sunshine hours and global radiation and the decrease of wind speed. However, the relative importance of warming and eutrophication on phytoplankton biomass remains controversial. Other studies have indicated that algae biomass responses depend on the composition of phytoplankton functional groups in aquatic ecosystems (Rigosi *et al.*, 2014).

Similar to many lakes in China, Dianchi Lake has suffered the consequences of eutrophication. Dianchi Lake is located in Yun-gui Plateau and exposed to a subtropical climate. Cyanobacterial blooms occur throughout the year; for instance, *Microcystis* blooms are most common in warm seasons and the lasting time of *Microcystis* blooms was increased in recent years. Wu *et al.* (2010) indicated that the year 2009–2010 was equipped with warm winter and the *Microcystis* bloom advanced for about 1 month (starts from March not April) and the lasting time was obviously increased. Our data showed that air temperature in Dianchi Lake increased by 0.051 °C per year from 1967 to 2011. In the next 100 years, Dianchi Lake may be warmed by approximately 5 °C (unpublished). With these changes observed in Dianchi Lake, scholars are concerned about the effect of the predicted changes in climate warming on phytoplankton, particularly in winter. This work aimed to investigate the responses of phytoplankton functional groups to simulated winter warming. We analyzed the dynamics of phytoplankton functional groups composition in Dianchi Lake in relation to concurrent environmental factors. We also designed experiments based on this survey to determine the effects of simulated winter warming on phytoplankton composition and physiology. We further evaluated the responses of dominant phytoplankton functional groups composition to future climate warming, particularly in winter warming.

Materials and methods

Field survey

Sampling site description

A field survey was conducted in Dianchi Lake, Kunming, China (24°40′N to 25°02′N, 102°36′E to 103°40′E). This region is characterized by a subtropical monsoon climate, an average winter temperature of 13 ± 2 °C and an average summer temperature of 30 ± 2 °C.

Phytoplankton collection and analysis

Field phytoplankton was collected each month from October 2009 to September 2010 across the whole Dianchi Lake at 24 sampling sites (Fig. 1). Algae were counted using Utermöhl method (Lund *et al.*, 1958; Paxinos and Mitchell, 2000). The algae prepared for counting were randomly distributed in the field of microscope. Then randomly choose about 200–300 fields to observe, so that the data were more precise, lowering down the random error. Counting error was approximately ± 10% (Venrick, 1978). The identified algae were classified into functional groups (Reynolds *et al.*, 2002; Padisák *et al.*, 2009). Algal biomass was calculated using the method reported by Hillebrand *et al.* (1999) and Sun and Liu (2003), where 1 $mm^3.L^{-1}$ = 1 $mg.L^{-1}$.

Measurement of physical and chemical parameters

An environmental monitoring system (YSI6600EDS, USA) was used to determine surface WT (0.5 m), and a Secchi disk was used to evaluate SD.

TN and TP concentrations were measured according to the Protocols for Standard Observation and Measurement in Aquatic Ecosystems of Chinese Ecosystem Research Network (CERN) (Huang *et al.*, 2000; Cai, 2007).

Data processing

In canonical correspondence analysis (CCA), data were transformed to log (x + 1) with Canoco 4.5 for

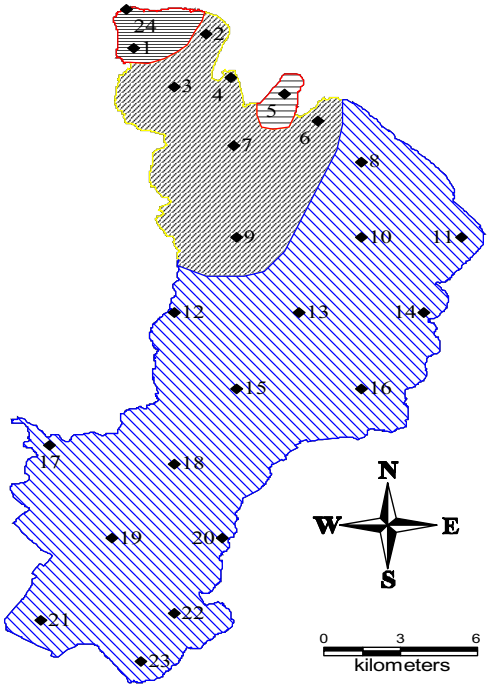


Fig. 1. Sampling sites of Dianchi Lake.

Windows and then analyzed. Detrended correspondence analysis was applied to calculate single-peak response value (*SD*). If $SD > 2$, CCA analysis was performed; otherwise, redundancy analysis (RDA) was applied. Significance was evaluated using Monte Carlo test; CCA or RDA analysis method was used only when $P < 0.05$ (Ter Braak, 1986; Dong *et al.*, 2006).

Laboratory experiments

Based on field results and temperature prediction for the next 100 years in Dianchi Lake (± 5 °C) (unpublished), laboratory experiments were performed to determine the effects of winter warming on phytoplankton functional groups. Currently, the winter temperature around Dianchi Lake is approximately 13 °C. Thus, our experiments were carried out at 13 and 18 °C. The details are presented in the following sections.

Algal cultivation

Toxigenic species *Microcystis aeruginosa* (FACHB-905) and *M.aeruginosa* (PCC7806), and non-toxic species, *Microcystis wesenbergii* (FACHB-908) belonging to group M were selected for the experiments. *M. aeruginosa* (FACHB-905) and *M. aeruginosa* (PCC7806), meant that they are not the same algal strain, separated from different water region. Here *M. aeruginosa* (FACHB-905) was separated from Dianchi Lake, provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences in Wuhan, China. *M. aeruginosa* (PCC7806) was originated from France laboratory (Institute Pasteur). The different strains utilized were to verify the responses of *Microcystis* to warming.

Three representative species belonging to the functional group J were chosen, that was *Pediastrum duplex* (FACHB-DC-BX-1), *Coelastrum microporum* (FACHB-1071) and *Scenedesmus obliquus* (FACHB-416). All of the species were provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences in Wuhan, China.

Before the experiments were conducted, all algae were batch-cultured in Erlenmeyer flasks containing BG_{11} medium (Rippka *et al.*, 1979) at room temperature (25 °C) in a 12:12 h light/dark cycle (25 μmol photons.s^{-1}.m^2). The cultures were manually shaken twice a day to maintain the cells in suspension.

Experimental design

Both types of algal cultures (mixed algal taxa cultures and pure algal taxa cultures) were designed. Mixed algal taxa cultures were conducted to detect the whole functional groups changes under warming; whereas pure algal taxa cultures were meant to further demonstrating the physiological responses of group M (*Microcystis*) to warming, so that speaking more in the prediction of future changes.

Co-cultivation

FACHB-905, PCC7806, FACHB-908, FACHB-DC-BX-1, FACHB-1071 and FACHB-416 with equal optical densities were inoculated in 250 mL Erlenmeyer flasks containing 200 mL of BG_{11} medium. The cultures were cultivated at 13 and 18 °C. Other cultivation conditions were similar to those reported in Section 'Algal cultivation'. The cultivation flasks were then covered with parafilm, and each temperature treatment was performed in triplicate. Regular sampling was conducted at an interval of 5–6 days to determine algal density (cells.L^{-1}). The experiments were conducted for 1 month.

Experimental design: pure culture

FACHB-905, PCC7806 and FACHB-908 were inoculated in 250 mL Erlenmeyer flasks containing 200 mL of BG_{11} medium. The initial optical density was approximately 0.2. The cultivation temperatures were 13 and 18 °C. Other cultivation conditions were similar to those reported in Section 'Algal cultivation'. Regular sampling was conducted at an interval of 3 days for physiological parameter measurement. The experiments were conducted for 36 days.

***Chlorophyll fluorescence*:** Chlorophyll fluorescence parameters, such as maximal PSII quantum yield (F_v/F_m) and maximum electron transport rate of photosynthesis (ETR_{max}), were determined using Phyto-PAM (Walz, Effeltrich, Germany). This device can effectively function at very low biomass densities because of sensitive fluorometric measurements (Körner and Nicklisch, 2002).

***Optical density*:** OD_{665} of *Microcystis* was determined using UV/Vis spectrophotometer (TU-1810; Beijing Purkinje General Instrument Co., Ltd., China) at an interval of 3 days to monitor growth.

***Photosynthetic pigments*:** Content of Chl-a and carotenoid was measured to reflect the growth and photosynthetic abilities of the algae. Chl-*a* and carotenoid were extracted in the dark at 4 °C in darkness and then determined by spectrophotometry, as described by Lichtenthaler and Buschmann (2001). The cells were centrifuged at 12000 rpm at 4 °C for 10 min and then extracted with 95% ethanol for 24 h at 4 °C in the dark. Absorbances at 665 (A_{665}), 649 (A_{649}) and 470 nm (A_{470}) were also obtained using a UV/Vis spectrophotometer (TU-1810; Beijing Purkinje General Instrument Co., Ltd., China). Chl-*a* content (C_{Chl-a}, mg.L^{-1}) was calculated using equation (1):

$$C_{Chl-a} = 13.95 \times A_{665} - 6.88 \times A_{649} \quad (1)$$

Carotenoid content ($C_{carotenoid}$, mg.L^{-1}) was calculated using equation (2):

$$C_{Carotenoid}\,(mg.L^{-1}) = (1000 \times A_{470} - 2.05 \times C_{Chl-a})/245 \quad (2)$$

Table 1. Taxonomic and functional groups composition across the Dianchi Lake during the year 2009–2010.

Species	Taxonomic group	Functional groups
Scenedesmus abundans; *Scenedesmus quadricauda*; *Scenedesmus armatus*; *Scenedesmus obliquus*; *Scenedesmus bicaudatus*; *Scenedesmus bijuga*; *Scenedesmus brasiliensis*; *Scenedesmus denticulatus*; *Scenedesmus cavinatus*; *Scenedesmus dimorphus*; *Scenedesmus perforates*; *Pediastrum boryanum*; *Pediastrum integrum*; *Pediastrum duplex*; *Pediastrum duplex var.gracillimum*; *Pediastrum tetras;* *Pediastrum simplex*; *Pediastrum tetras var. tetraodon*; *Coelastrum reticulatum*; *Crucigenia quadrata*; *Crucigenia tetrapedia*; *Crucigenia rectangularis*; *Tetraedron caudatum*; *Tetraedron minimum*; *Tetraedron trigonum*; *Tetraedron trilobulatum*; *Tetrastrum staurogeniaeforme*; *Tetrastrum heterocanthum*	Chlorophyta	J
Staurastrum gracile; *Closterium gracile*	Chlorophyta	P
Melosira granulata var. angustissima	Bacillariophyta	
Ankistrodesmus acicularis; *Ankistrodesmus falcatus*; *Ankistrodesmus angustus*; *Schroederia setigera*; *Chlorella* sp.	Chlorophyta	X1
Oocystis elliptica; *Oocystis borgei; Oocystis lacustis*; *Kirchneriella contorta; Kirchneriella obese*; *Selenastrum westii*; *Selenastrum gracile*	Chlorophyta	F
Dactylococcopsis acicularis	Cyanophyta	
Cryptomonas ovate; *Cryptomonas erosa; Cryptomonas rostrata*	Cryptophyta	Y
Chroomonas acuta	Cryptophyta	X2
Chlamydomonas sp.	Chlorophyta	
Cyclotella meneghiniana; *Stephanodiscus minutulus*	Bacillariophyta	C
Synedra ulna; *Synedra acus*; *Nitzschia palea*; *Nitzschia frustulum; Nitzschia sigmoidea*; *Nitzschia lorenziana*	Bacillariophyta	D
Ulothrix sp.; *Chlorococcum* sp.	Chlorophyta	MP
Navicula viridula; *Navicula halophila*; *Navicula protracta*; *Epithemia* sp.; *Cymbella perpusilla*; *Cymbella affinis*; *Cocconeis placeutula*; *Gomphonema olivaceum*; *Achnanthes* sp.	Bacillariophyta	
Oscillatoria tenuis; *Lyngbya* sp.	Cyanophyta	
Anabaenopsis arnoldii; *Anabaenopsis circinalis*; *Anabaenopsis planctonica*; *Aphanizomenon flos-aquae*	Cyanophyta	H1
Merismopedia glauca; *Merismopedia elegans*; *Chroococcus minutes*	Cyanophyta	Lo
Ceratium hirundinella; *Peridinium pusillum*	Pyrrophyta	
Euglena pisciformis; *Trachelomonas* sp.	Euglenophyta	W2
Microcystis aeruginosa; *Microcystis wesenbergii*; *Microcystis flo-aquae*; *Microcystis incerta*; *Microcystis ichthyoblabe*; *Microcystis viridis*	Cyanophyta	M

***Intracellular soluble carbohydrates*:** Intercellular soluble carbohydrates were measured to detect the responses of *Microcystis* under low temperature and provide certain explanation for the reason of fast growth of algae once the temperature was increasing. The centrifuged cells were suspended in sterile water and boiled for 30 min. The samples were centrifuged again at room temperature (25 °C, 12 000 rpm, 10 min), and soluble carbohydrates were determined using anthrone–sulfuric acid method, as reported in Li (2000).

***TDP*:** The concentration of TDP was determined according to the Protocols for Standard Observation and Measurement in Aquatic Ecosystems of CERN (Huang *et al.*, 2000; Cai, 2007).

Data processing

Mean and standard deviation values for each treatment were calculated from the replicate samples ($n = 3$). One-way analysis of variance according to SPSS 18.0 was used to compare differences between treatments, taking $P < 0.05$ as significant.

Results

Field results

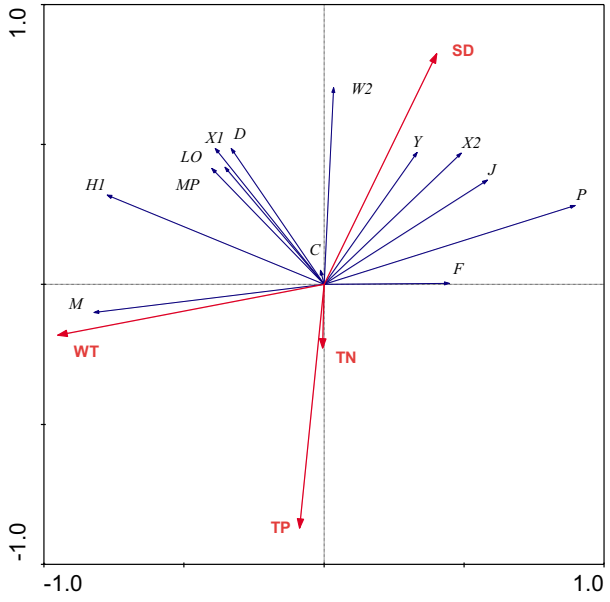
Phytoplankton composition in Dianchi Lake

In Dianchi Lake, 87 different algae species (genera) were identified in samples from October 2009 to September 2010. Then, the identified algae were classified into functional groups according to Reynolds *et al.* (2002) and Padisák *et al.* (2009). A total of 13 phytoplankton functional groups with these groups included **M**(mainly consisted of *M. aeruginosa*, *M. wesenbergii*, *Microcystis flo-aquae*), **J**(*Scenedesmus*, *Pediastrum*, *Coelastrum*), **H1**(*Aphanizomenon flos-aquae*), **P**(*Melosira*, *Staurastrum*), **F**(*Oocystis*, *Kirchneriella*), **X1**(*Ankistrodesmus*), **Y** (*Cryptomonas*), **X2**(*Chroomonas acuta*, *Chlamydomonas* sp.), **C**(*Cyclotella meneghiniana*, *Stephanodiscus minutulus*), **D**(*Synedra ulna*, *Synedra acus*, *Nitzschia*), **MP**(*Navicula*, *Cymbella*, *Cocconeis*, *Gomphonema*, *Achnanthes*, *Oscillatoria*), $\mathbf{L_O}$(*Merismopedia glauca*, *Merismopedia elegans*, *Chroococcus minutes*) and **W2** (*Euglena pisciformis*, *Trachelomonas* sp.) were identified (Table 1).

Relationship between environmental variables and phytoplankton functional groups in Dianchi Lake

This study mainly focused on TN concentration, TP concentration, WT and SD. The relationship between environmental variables and 13 phytoplankton functional groups were also examined.

After the experiment was conducted, an RDA model (Redundancy analysis: detrended correspondence analysis was applied to calculate single-peak response value-SD. SD < 2, RDA analysis was applied) was fit to analyze the relationship between environmental variables and species ($P < 0.01$). J, P, F, Y and X_2 assemblages were significantly and negatively correlated with WT; by contrast, M and H1 groups were significantly and positively correlated with WT. Group M was significantly negatively correlated with SD (Fig. 2). These results suggested that the high biomass of group M increased as temperature increased and lower temperature was favorable for group J development.



Note: TN = total nitrogen content, TP = total nitrogen phosphorous content, WT = water temperature, SD = water transparency

Fig. 2. Functional groups-environment biplot CCA of Dianchi Lake.

Seasonal changes in the dominant phytoplankton functional groups in Dianchi Lake

Functional groups that contributed to $>5\%$ of the total phytoplankton biomass for at least 1 month are classified as dominant groups (Xiao *et al.*, 2011). Five dominant groups, including M, H1, J, F and P, were recognized. Groups M and J were prevalent throughout the year and contributed to $>90\%$ of the total biomass. The development of groups M and J was remarkably seasonal. Group M was prevalent at relatively high temperature in summer and autumn; group J was dominant at low temperature in winter and early spring (Figs. 3 and 4).

Laboratory experimental results

Density and biomass dynamics of the co-cultivated groups M and J at 13 and 18 °C

Groups M and J demonstrated different responses to temperatures (Fig. 5). In the co-cultivated groups M and J, the density and biomass of group M were significantly lower at 13 °C than at 18 °C ($P < 0.05$). By contrast, the density and biomass of group J were higher at 13 °C than at 18 °C ($P < 0.05$). These results suggested that warming

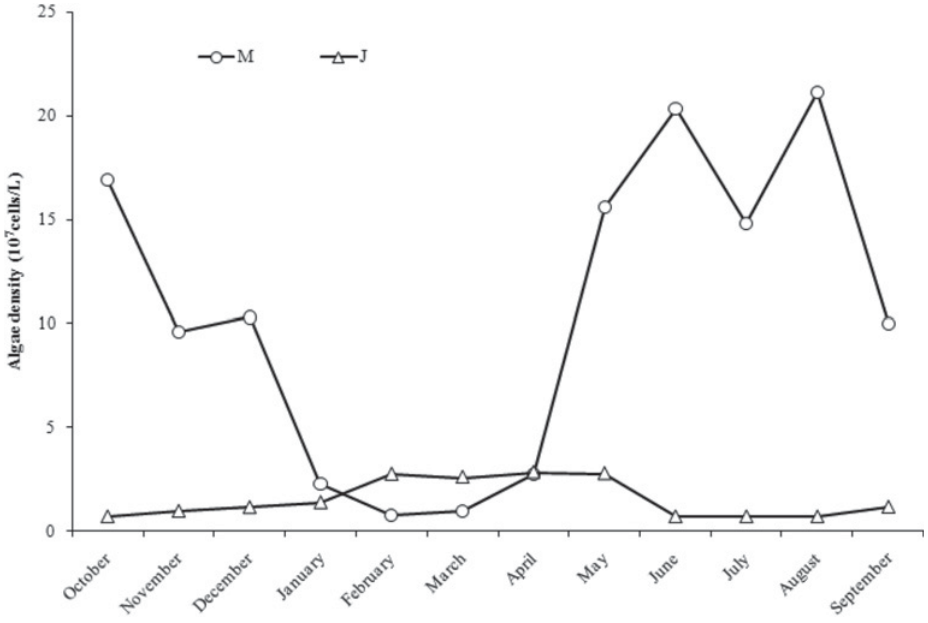


Fig. 3. Seasonal dynamics of functional groups M and J density (10^7 cells.L^{-1}) in Dianchi Lake during 2009–2010.

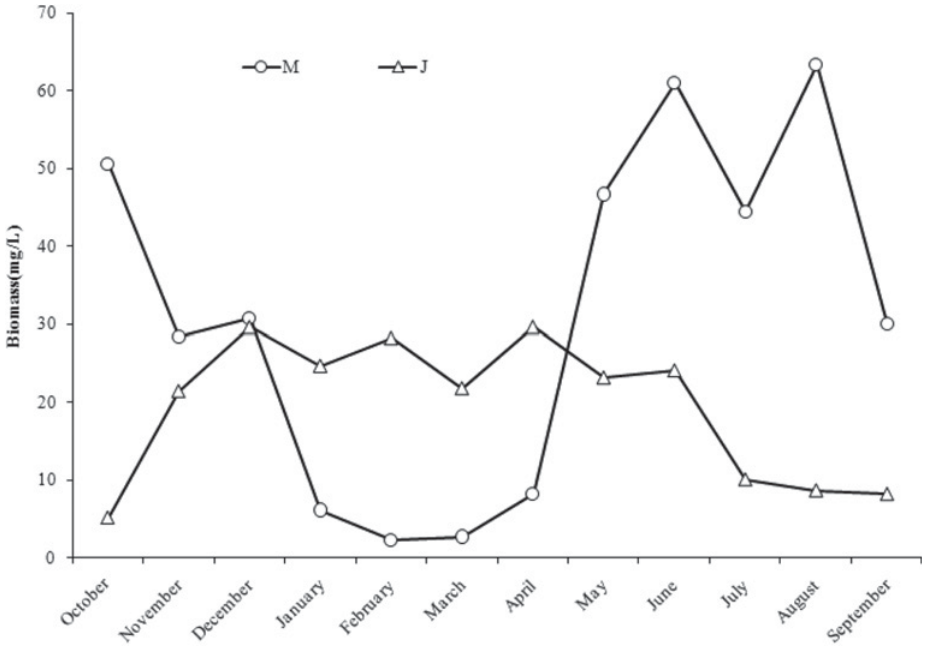


Fig. 4. Seasonal dynamics of functional groups M and J biomass (mg.L^{-1}) in Dianchi Lake during 2009–2010.

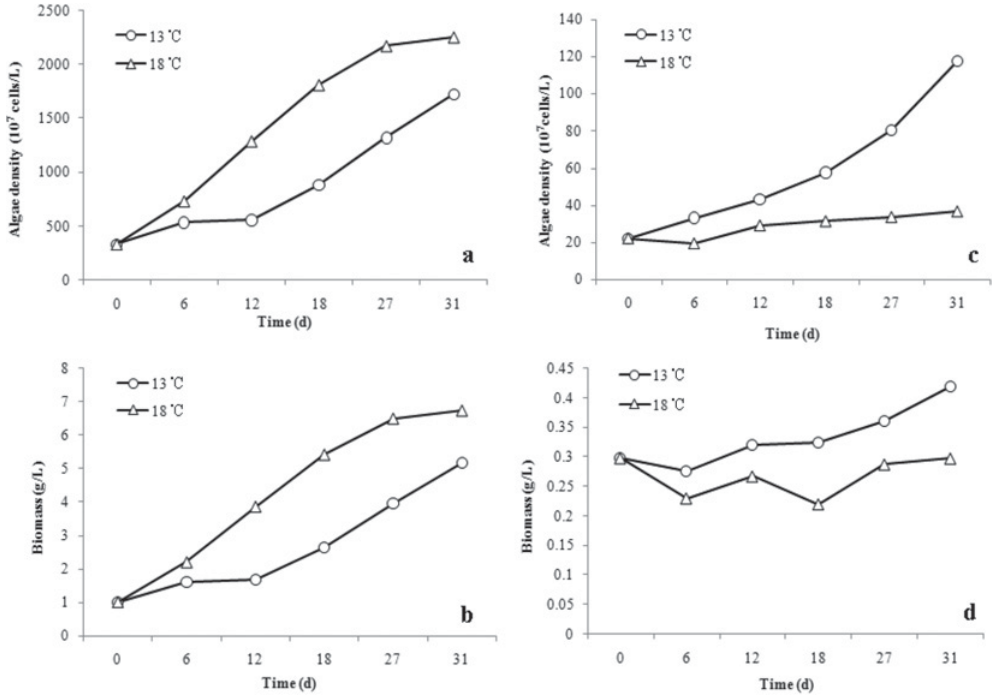


Fig. 5. The changes in the algae density (10^7 cells.L^{-1}) and biomass (g.L^{-1}) of phytoplankton functional groups M (a, b) and J (c, d) at 13 and 18 °C.

favored the growth and development of the species in group M but inhibited the growth and development of the species in the co-cultivated group J.

Dynamics of species in the pure culture of group M

Chlorophyll fluorescence

Photosynthetic activities, including F_v/F_m (Fig. 6(a) and (c)) and ETR_{max} (Fig. 6(d) and (f)), of FACHB-905 and PCC7806 were significantly inhibited at 13 °C ($P < 0.05$). At 3 days, F_v/F_m decreased to 0.05 and ETR_{max} ranged from 4 to 5. These photosynthetic activities were enhanced when the two species were cultivated at 18 °C ($P < 0.05$). F_v/F_m was approximately 0.4, and ETR_{max} ranged from 70 to 80. For non-toxic FACHB-908, F_v/F_m varied between 0.35 and 0.4 at 13 °C. In later cultivation period, F_v/F_m was higher at 13 °C than at 18 °C (Fig. 6(b) and (e)).

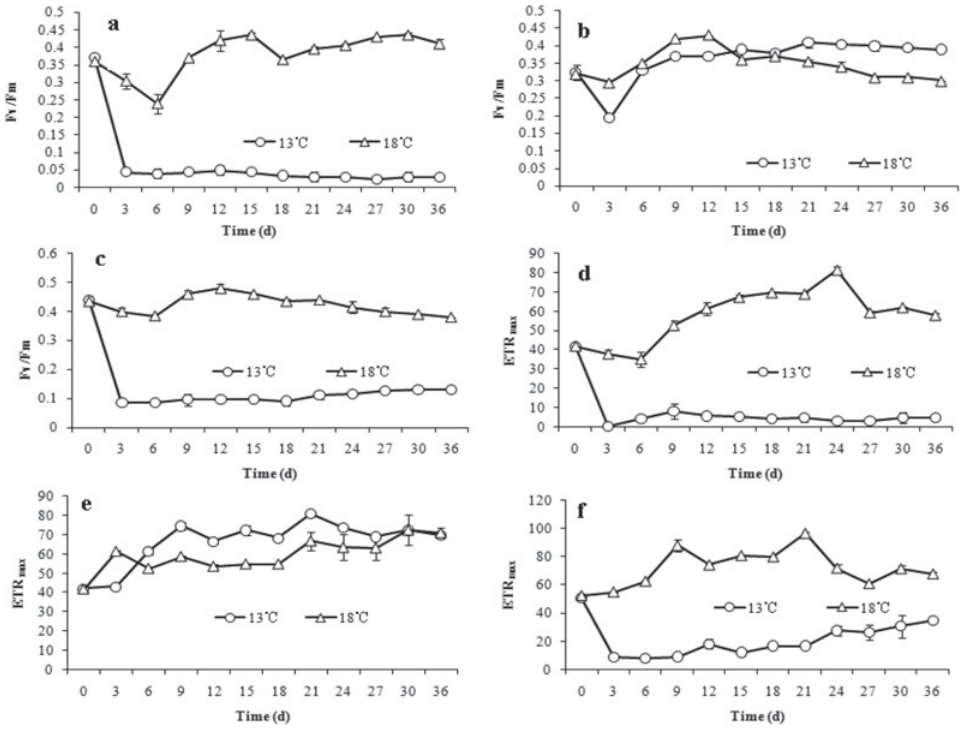


Fig. 6. The F_v/F_m (a, b, c) and ETR_{max} (d, e, f) of *Microcystis aeruginosa* (FACHB-905), *Microcystis wesenbergii* (FACHB-908), *Microcystis aeruginosa* (FACHB-7806) at 13 and 18 °C.

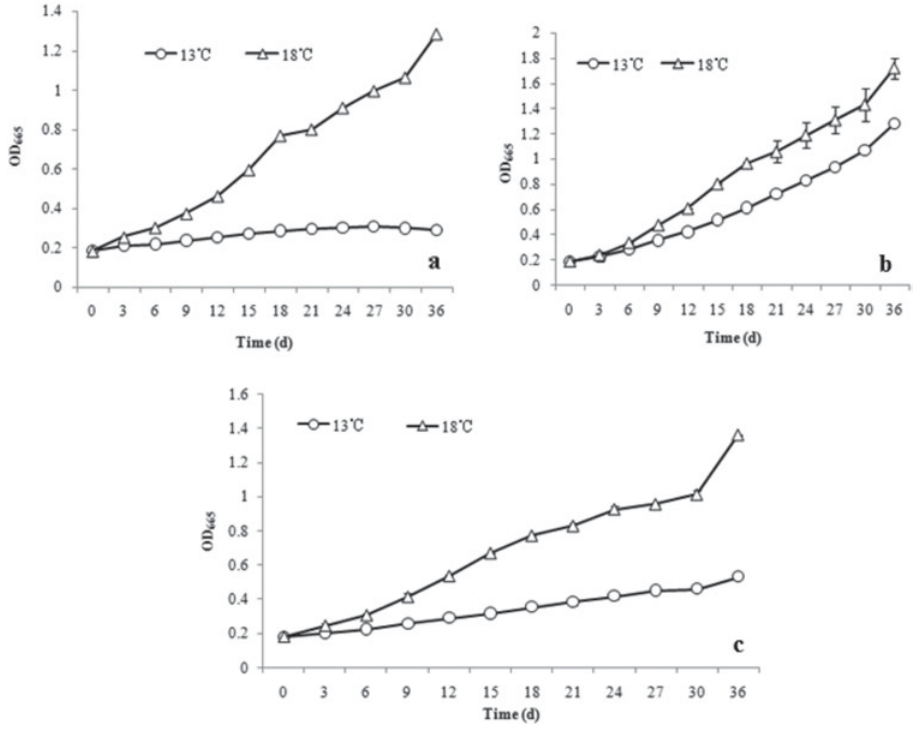


Fig. 7. The growth of *Microcystis aeruginosa* (FACHB-905) (a), *Microcystis wesenbergii* (FACHB-908) (b), *Microcystis aeruginosa* (FACHB-7806) (c) at 13 and 18 °C.

Growth

The growth of FACHB-905 and PCC7806 cultivated at 13 °C was significantly inhibited; the corresponding growth rates were lower at 13 °C than at 18 °C ($P < 0.05$). The optical density of FACHB-905 and PCC7806 varied between 0.2 and 0.4 (Fig. 7(a) and (c)). Furthermore, the optical density of FACHB-905 and PCC7806 increased from 0.2 to 1.4 at the end of the

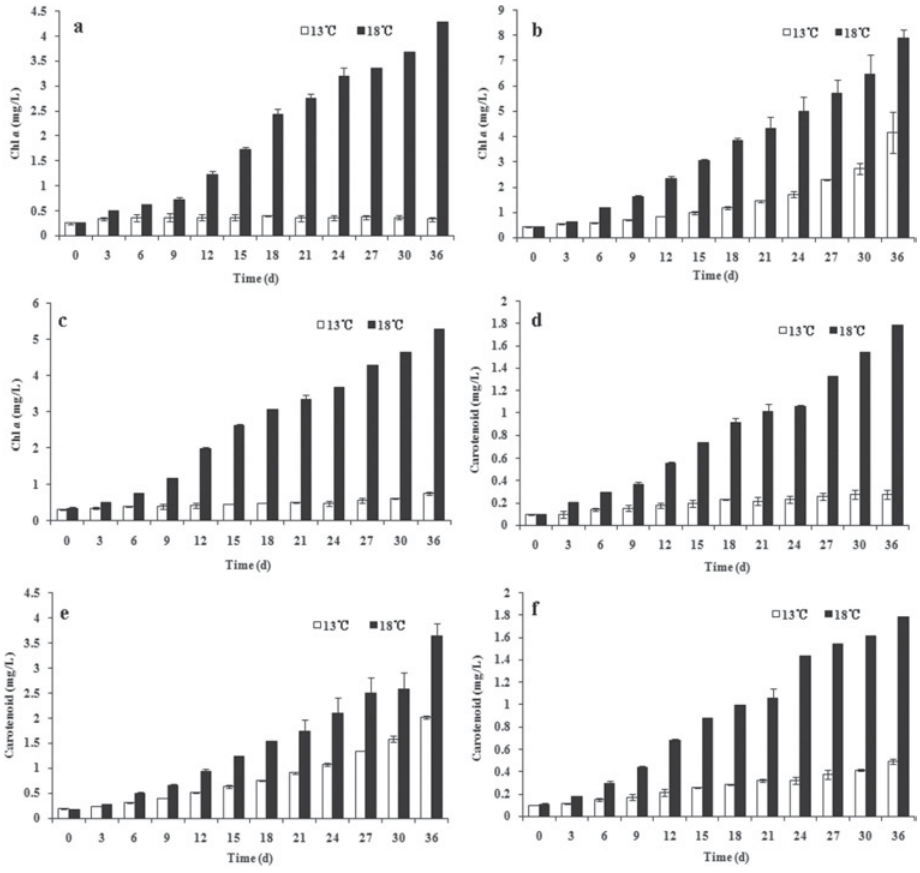


Fig. 8. The content of Chl *a* (mg.L^{-1}) and carotenoid (mg.L^{-1}) in *Microcystis aeruginosa* (FACHB-905) (a, d), *Microcystis wesenbergii* (FACHB-908) (b, e), *Microcystis aeruginosa* (FACHB-7806) (c, f), significantly different at 13 and 18 °C ($P < 0.05$).

experiment at 18 °C. The growth of FACHB-908 was partially inhibited at 13 °C, and the corresponding optical density increased from 0.2 to 1.4 at the end of the experiment. At 18 °C, the optical density of FACHB-908 increased from 0.2 to 1.9 (Fig. 7(b)).

Photosynthetic pigments

Chl-*a* and carotenoid concentrations of FACHB-905 and PCC7806 did not significantly increase when these species were cultivated at 13 °C. However, these pigments significantly increased when the two species were cultivated at 18 °C ($P < 0.05$). Chl-*a* concentrations of FACHB-905 and PCC7806 increased from 0.25 to 4.5 mg.L^{-1} and from 0.3 to 5.3 mg.L^{-1}, respectively (Fig. 8(a) and (c)); their carotenoid contents increased from 0.1 to 1.8 mg.L^{-1} (Fig. 8(d) and (f)). These photosynthetic pigments also accumulated when FACHB-908 was cultivated at 13 °C. Likewise, Chl-*a* and carotenoid contents increased from 0.4 to 4 mg.L^{-1} and from 0.2 to 2 mg.L^{-1}, respectively. This increase was significantly lower at 13 °C than at 18 °C. Chl-*a* and carotenoid concentrations also increased from 0.4 to 7.9 mg.L^{-1} and from 0.2 to 3.6 mg.L^{-1}, respectively, when the species were cultivated at 18 °C (Fig. 8(b) and (e)).

Intracellular soluble carbohydrates

Intracellular soluble carbohydrate contents in FACHB-905, PCC7806 and FACHB-908 were significantly higher at 13 °C than at 18 °C ($P < 0.05$). This finding indicated that *Microcystis* could tolerate and adapt to low temperatures (Fig. 9).

TDP

The TDP content in the medium cultivated with FACHB-905 and PCC7806 did not change at 13 °C ($P > 0.05$). Conversely, the TDP concentration decreased from the initial 4 to 2 mg.L^{-1} at 6 days to 0.6 mg.L^{-1} at the end of the experiment when these species were cultivated at 18 °C (Fig. 10(a) and (c)). The TDP concentration of FACHB-908 also decreased from 5 mg.L^{-1} to 2.8 and to 0.96 mg.L^{-1} at 6 days at 13 and 18 °C, respectively; this parameter further decreased to 0.06 mg.L^{-1} at the end of the experiments (Fig. 10(b)).

Discussion

Previous studies focused on phytoplankton phenology and biomass responses to climate warming (McKnight *et al.*, 1996; Elliott *et al.*, 2006; Staehr and Sand-Jensen,

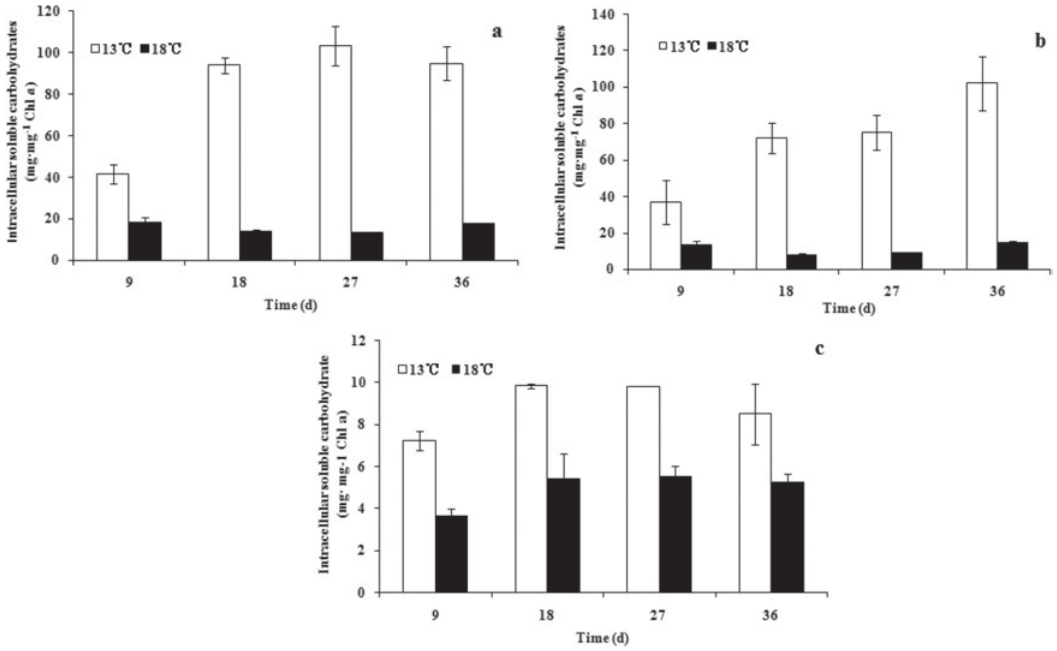


Fig. 9. The content of total intracellular soluble carbohydrate (mg.mg^{-1} Chl-*a*) in *Microcystis aeruginosa* (FACHB-905) (a), *Microcystis wesenbergii* (FACHB-908) (b), *Microcystis aeruginosa* (FACHB-7806) (c), significantly different at 13 and 18 °C ($P < 0.05$).

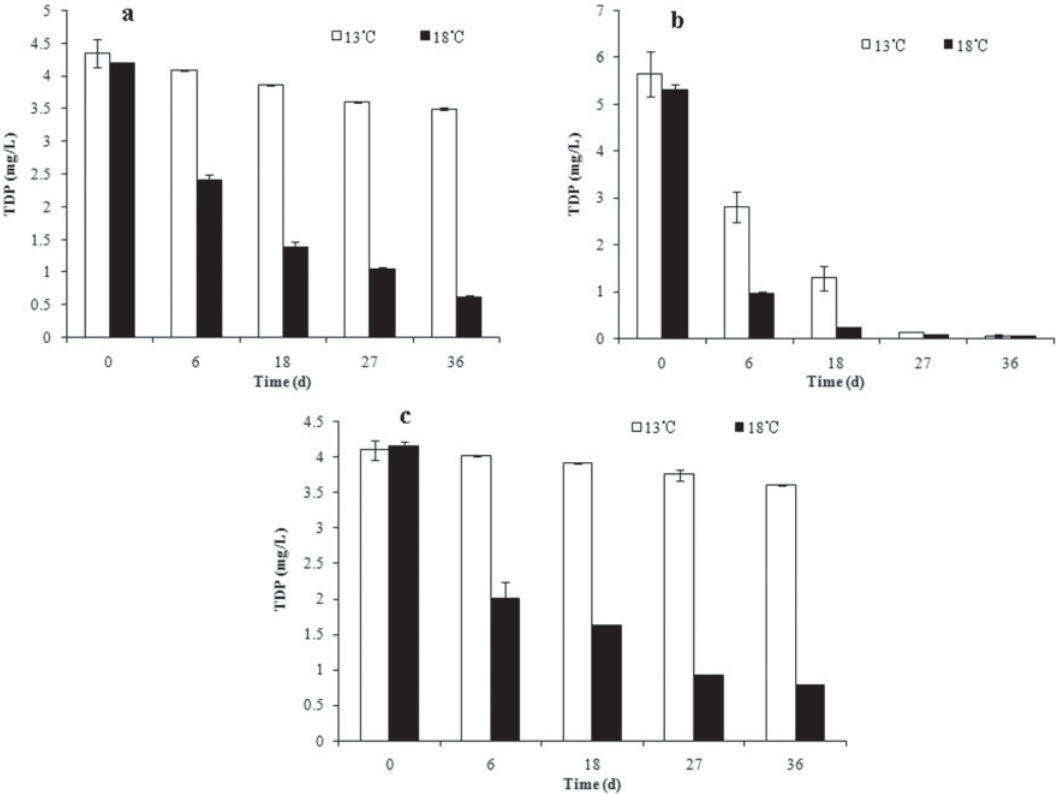


Fig. 10. The content of TDP (mg.L^{-1}) in *Microcystis aeruginosa* (FACHB-905) (a), *Microcystis wesenbergii* (FACHB-908) (b), *Microcystis aeruginosa* (PCC7806) (c) cultivation, significantly different at 13 and 18 °C ($P < 0.05$).

2006; Huber *et al.*, 2008; Sommer and Lengfellner, 2008; Elliott, 2012; Lv *et al.*, 2014). However, it was controversial to indicate the relative importance of warming and eutrophication influences on lake phytoplankton. Anneville *et al.* (2005) indicted that phosphorous concentration was the main factor in driving phytoplankton composition changes of European peri-alpine lakes. Jeppesen *et al.* (2005) also showed that phytoplankton composition in 35 lakes expanding from north America to Europe, subtropical to temperate region was dependent on nutrition. However, Paerl and Huisman (2008), Kosten *et al.* (2012) and Posch *et al.* (2012) suggested that warming was more important in promoting cyanobacterial blooms, in comparison with nutrition. Despite this controversy, a consensus has been established that the relative importance of eutrophication and climate warming was with regard to the phytoplankton composition.

On the basis of morphological, physiological and ecological characteristics, Reynolds *et al.* (2002) proposed the concept of phytoplankton functional groups, in which assemblages with common environmental requirements and simultaneous occurrence are classified in one functional group. The importance and the usefulness of functional groups in predicting species existence and describing conditions are emphasized compared with phylogenetic representatives (Huszar *et al.*, 2000; Kruk *et al.*, 2002; Salmaso and Padisák, 2007). Rigosi *et al.* (2014) also indicated that different functional assemblages of cyanobacteria exhibit varied responses to warming and eutrophication. Those authors were the first to apply functional groups to evaluate the effects of climate warming and eutrophication on cyanobacteria, and the responses of functional assemblages of cyanobacteria were also investigated. The results showed that phytoplankton composition, in addition to phenology characteristics (the timing and lasting time of algae peak biomass occurrence could alter zooplankton community composition, playing important roles in the whole food web structure dynamics, thus affecting the whole ecosystem structure and functions) and algae standing crops, is also important when the effects of climate warming are evaluated. In the present study, population composition and dominant species responses to warming in Dianchi Lake were mainly investigated.

In the field survey, group M dominated most of the time in Dianchi Lake and WT might play important roles in deciding the relative dominance of assemblage M and J (Figs. 2–4). In the other study by us, it was suggested that the temperature in Dianchi Lake has continuously increased over the recent decades; furthermore, the temperature in this area will likely increase by 5 °C in the next 100 years (unpublished). Thus, the evidence that occurrence of *Microcystis* bloom in Dianchi Lake is longer than before (Wu *et al.*, 2010) might due to warming. Deng *et al.* (2014) also demonstrated that the *Microcystis* growing season has advanced by approximately 20 days over the last two decades in Lake Taihu, China. Huang *et al* (2014) showed that warming played roles in extending the duration of algal blooms in Lake Taihu, China.

Except for group M, it was indicated that the functional group J occupied a specific position in the phytoplankton community in winter (Figs. 3 and 4). To detect changes or relative dominance of these assemblages confronted with warming, we conducted indoor simulated winter warming experiments. A 5 °C temperature gradient was selected to predict the responses of phytoplankton in Dianchi Lake to winter warming. The co-cultivation experimental results suggested that high winter warming (18 °C) favored the outbreak of group M but adversely affected the development of group J (Fig. 5). In pure culture experiments, photosynthetic activities (maximal PSII quantum yield, F_v/F_m and maximum electron transport rate of photosynthesis, ETR_{max}), growth rate and pigment accumulation (Photosynthetic pigment-based growth) of *Microcystis*, which is the representative species of the group M, were significantly inhibited at 13 °C; conversely, intracellular soluble carbohydrate content was significantly increased. This result is consistent with that of Aaronson (1973), who demonstrated that low temperature can increase the amount of carbohydrates per cell. Tan *et al.* (2009) further reported that growth rate and Chl-*a* accumulation of *Microcystis* are promoted during warming and grow faster than those of green algae and diatoms. The capability of accumulating sufficient soluble carbohydrates under cold condition likely provides adequate energy for further growth until such condition becomes favorable. The physiological characteristics of *M. wesenbergii* slightly differed from those of *M. aeruginosa*; their photosynthetic activities were not inhibited in cold climate. However, the growth of *M. wesenbergii* was inhibited because phosphorous in the medium was depleted (Fig. 10(b)). All of the tested *Microcystis* species, particularly *M. aeruginosa*, demonstrated significantly faster growth and higher pigment accumulation at 18 °C than at 13 °C ($P < 0.05$). Therefore, our indoor-simulated experiments also indicated that the dominance of group M can considerably increase in future winter warming.

The succession and composition of phytoplankton are affected by multiple factors, such as climate change and eutrophication. Combined the indoor experiments and field survey in the present study, it was suggested that future winter warming in Dianchi Lake may reduce the dominance of group J and increase the dominance of group M in winter; as a result, *Microcystis* outbreak likely occurs in winter. The results are consistent with that of Adrian *et al.* (1995), who demonstrated that average winter warming from 1988 to 1992 resulted in an increase in Chl-*a* concentration from 5–12 to 9–63 $\mu g.L^{-1}$ and cyanobacterial dominance. Other studies have also reported that climate warming can increase the occurrence of blue-green algae (Figueiredo *et al.*, 2004; Paerl and Huisman, 2009; De Senerpont Domis *et al.*, 2013).

The increased cyanobacterial bloom intensity would further reduce the SD, dissolved oxygen and biodiversity in lake ecosystems. Slim *et al.* (2014) suggested that blooms of cyanobacteria, specifically *M. aeruginosa* and *Aphanizomenon ovalisporum* caused by warming could also

disturb the ecosystem and the functioning of the Lake Karaoun, Lebanon. Furthermore, Joung *et al.* (2011) and Gkelis *et al.* (2014) suggested that high temperature would promote the growth of MC-producing genotypes and Li *et al.* (2014) also indicated that global warming could promote more frequent toxic blooms in Lake Taihu, China. The increased microcystin produced by *Microcystis* would have accelerating negative effects on lake ecosystem structure and ecosystem.

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