LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 00, 2015, 00–00

doi: 10.1002/lno.10246

The persistence of cyanobacterial (*Microcystis* spp.) blooms throughout winter in Lake Taihu, China

Jianrong Ma,[1,2] Boqiang Qin,*[1,2] Hans W. Paerl,[3] Justin D. Brookes,[4] Nathan S. Hall,[3] Kun Shi,[2] Yongqiang Zhou,[2] Jinsong Guo,[1] Zhe Li,[1] Hai Xu,[2] Tingfeng Wu,[2] Shengxing Long[5]

[1]Key Laboratory of Reservoir Aquatic Environment, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing, P. R. China
[2]State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, P. R. China
[3]Institute of Marine Sciences, The University of North Carolina at Chapel Hill, Morehead City, North Carolina
[4]Water Research Centre, Environment Institute, School of Biological, University of Adelaide, Adelaide, Australia
[5]College of Life Science and Technology, Jinan University, Guangzhou, P. R. China

Abstract

Temperature is generally considered as a key factor controlling algal bloom formation. Previous studies have indicated that the bloom-forming cyanobacteria *Microcystis* spp. overwinters near the sediment surface and does not actively grow below 15°C. However, satellite images and field collections from Lake Taihu, China have shown that *Microcystis* spp. blooms persisted when water temperatures were below 10°C during winter, although their magnitudes were smaller than during periods of higher temperature. Winter *Microcystis* cells maintained low activity and were able to grow again when exposed to elevated temperatures (≥12.5°C). Hence, cyanobacterial blooms may appear year-round in eutrophic lakes. Temperature increases coupled with nutrient enrichment promoted the growth of cyanobacteria, while low temperature decreased the loss rate of *Microcystis*, allowing winter blooms to persist. High concentrations of overwintering vegetative cells may provide a large inoculum for blooms during warmer seasons. Controlling winter blooms may reduce their magnitude during the warmer seasons.

Over-enrichment of nitrogen and phosphorus has accelerated eutrophication in lakes globally and promoted algal blooms that are often dominated by cyanobacteria (Schindler and Hecky 2009; Paerl et al. 2011b; Heather et al. 2015; Taranu et al. 2015). Bloom-forming cyanobacteria can produce toxins, taste, and odor compounds which threaten drinking water supplies and recreational use of affected waters (Carmichael 1997; Chorus and Bartram 1999). Cyanobacterial blooms can lead to hypoxia and adversely affect food webs. Consequently, they are a public health threat and cause negative ecological and economic water resource impacts (Brookes and Carey 2011; Paerl et al. 2011a). Some of the largest freshwater ecosystems in the world are increasingly experiencing severe cyanobacteria blooms (Paerl et al. 2011a).

Cyanobacteria often exhibit optimal growth when water temperatures exceed 20°C (Reynolds 2006; Jöhnk et al. 2008; Carey et al. 2012). A review of temperature-dependent growth rates (Paerl and Huisman 2008) concluded that growth rates of eukaryotic algae tend to be either maximized or decline at temperatures exceeding 20°C, while the growth rate of many cyanobacteria species increases. Buoyant cyanobacteria accumulate in dense surface blooms when mixing is weak (Reynolds 2006), which can shade nonbuoyant underlying phytoplankton groups (Jöhnk et al. 2008). Cyanobacterial blooms may also increase surface water temperatures and strengthen stratification by increasing light absorption and heat trapping within the surface layers (Kumagai et al. 2000; Ibelings et al. 2003), thus, providing an additional competitive advantage to buoyant cyanobacteria over nonbuoyant algae. There is a range of physiological adaptations that allow cyanobacteria to thrive in warmer climates (Carey et al. 2012). Elevated temperatures and heat waves often promote cyanobacterial blooms in summer and autumn (Jöhnk et al. 2008).

Concerns about global warming have prompted considerable research into how cyanobacterial blooms will respond to both direct and indirect temperature effects (Paerl and Huisman 2008; Wagner and Adrian 2009; Paerl et al. 2011a;

Additional Supporting Information may be found in the online version of this article.

*Correspondence: qinbq@niglas.ac.cn

Elliott 2012; Zhang et al. 2012). However, the response of cyanobacterial blooms to cold conditions has received much less attention. Generally, it is thought that *Microcystis* spp. reduce their metabolic rates and cannot grow when water temperatures are below 10–15°C (Takamura et al. 1984; Robarts and Zohary 1987). *Microcystis* species overwinter by sinking to the sediment surface (Reynolds 1973; Reynolds and Rogers 1976; Preston et al. 1980; Fallon and Brock 1981), while other genera have specialized resting cells/spores/akinetes to survive under adverse conditions (Livingstone and Jaworski 1980; Wiedner et al. 2007).

As the third largest freshwater lake in China, Lake Taihu (see Supporting Information) has suffered from annual cyanobacterial blooms dominated by *Microcystis aeruginosa*, *Microcystis flos-aquae*, and *Microcystis wesenbergii* which have contributed more than 40% and up to 98% of total algal biovolumes between May and Oct (Chen et al. 2003) over the past several decades. To expand the knowledge of cyanobacterial blooms over a range of temperatures, we present long-term satellite-based monitoring and field observations from Lake Taihu that show cyanobacterial blooms dominated by *Microcystis* spp. persist through winter at temperatures consistently below 10°C. In addition, we incubated *Microcystis* spp. from winter blooms to reveal the effects of low temperatures on its growth and biomass maintenance.

Materials and procedures

Defining a cyanobacterial bloom: It is generally agreed that a cyanobacterial bloom is defined by a blue-green colored surface layer and/or chlorophyll *a* (Chl *a*) concentrations in excess of 10 μg L^{-1} and/or algal cell densities that exceed 1.5×10^7 cells L^{-1} (Australian and New Zealand Environment and Conservation Council, Canberra 1992). A visible surface cyanobacterial bloom in highly eutrophic Lake Taihu was defined as a Chl *a* concentration of 20μg L^{-1} which is equivalent to a cell density of 5.0×10^7 cells L^{-1} (Qin et al. 2015). Estimates of areal coverage of algal blooms from Moderate-resolution imaging spectroradiometer (MODIS) imagery have been consistent with Chl *a* more than 30 μg L^{-1} from field sampling (Supporting Information Fig. S1) (Duan et al. 2009; Huang et al. 2012). In this research, winter cyanobacterial blooms dominated by *Microcystis* were Chl *a* more than 30 μg L^{-1}.

Satellite images and field monitoring data

The buoyancy of *Microcystis* (Supporting Information Fig. S2) leads to surface blooms in Lake Taihu, which can be readily observed by satellite imagery (MODIS images, resolution 250 m) of Lake Taihu were downloaded from the NASA EOS Data Gateway. The difference in the reflectance signal at 900 nm between surface cyanobacterial blooms and clear water can be used to delineate the areal coverage of cyanobacterial blooms with ERDAS9.1 software (Duan et al. 2009). Unfortunately, cloud cover obfuscates the bloom signal, so the bloom area could only be reliably retrieved from MODIS imagery on days when there were few or no clouds.

Daily water temperature data were obtained from three depths (0.2 m, 0.7 m, and 1.7 m) using a YSI 6600 multiparameter sonde (Yellow Springs, Yellow Springs, Ohio) permanently deployed at the end of the pier at the Taihu Laboratory for Lake Ecosystem Research (TLLER), Chinese Academy of Sciences, located on Meiliang Bay of Lake Taihu (Supporting Information Fig. S3). Values given are averages of measurements made throughout the water column.

Lake wide routine monitoring of phytoplankton species and biomass, nutrients, and water temperature was conducted monthly at 32 representative sampling sites by TLLER staff (Supporting Information Fig. S3). Water temperatures were measured using a YSI 6600 sonde after water from the surface (within 0.5 m), middle (1 m), and bottom (1.7–2.2 m) was pumped into a bucket.

Photosynthetic active radiation was recorded at an automatic weather station at TLLER. All experiments and laboratory analyses were conducted at TLLER.

Determination of low temperature and nutrient impacts on photosynthetic activity and growth of *Microcystis*

Microcystis colonies were collected from the winter bloom in Taihu and incubated in the laboratory at different temperatures to determine if the cells were moribund or had low activity because of the cold temperatures. Samples were collected on 06 Jan 2013 and 08 Jan 2014 at the shore of TLLER. Water samples (1 L) were collected initially from within the bloom area to determine biological parameters, including phytoplankton community composition, abundance as cell density, the frequency of dividing cell (FDC), photosynthetic activity, and Chl *a* concentrations.

To determine the potential photosynthetic activity of these winter *Microcystis* blooms, 0.5 L triplicate subsamples of lake water containing cyanobacteria were incubated in 1 L Erlenmeyer flasks for 24 h under light conditions (55 μmol photons m^{-2} s^{-1}) in an illuminated incubator (Xinmiao GZX-250BS–III, Shanghai Xinmiao Medical Devices Emerging, Ltd.) with a 12 h : 12 h light : dark cycle. All subsequent experiments used the same light regime. Photosynthetic activity was measured with a PAM fluorometer (described below) prior to and following 24 h of incubation.

The samples of *Microcystis* were collected from blooms at Lake Taihu (Supporting Information Table S1) and incubated in the laboratory at different temperatures to determine temperature-dependent growth rates. Samples were collected on 14 Feb (winter), 08 Mar (early spring), 16 Apr (spring), 22 Aug (summer), and 30 Nov (fall) 2014 followed by immediate measurement of photosynthetic activity at in situ light/temperature conditions. Subsamples of 0.5 L were then incubated as before but under a range of different temperature conditions, as described below.

On 14 Feb, samples were incubated in 1 L glass bottles (0.5 L water sample including *Microcystis*) for 22 d in illuminated incubators at 10°C, 15°C, 20°C, and 25°C (55 μmol photons m^{-2} s^{-1}; 12 h : 12 h light : dark cycle) and in a dark 2°C refrigerator. The 08 Mar samples were incubated for 13 d in an illuminated incubator at 7.5°C, 12.5°C, 17.5°C, and 22.5°C (55 μmol photons m^{-2} s^{-1}; 12 h : 12 h light: dark cycle) and in a refrigerator (2°C, dark).

The samples from 16 Apr were incubated in six illuminated incubators at 6°C, 12°C, 18°C, 24°C, 30°C, and 36°C (55 μmol photons m^{-2} s^{-1}; 12 h : 12 h light : dark cycle). In each illuminated incubator, half of Erlenmeyer flasks were kept in dark by wrapped with aluminum foil. In both light and dark, half of the Erlenmeyer flasks had nitrogen and phosphorus added as $NaNO_3$ and KH_2PO_4 (6 mg L^{-1} TN and 0.3 mg L^{-1} TP). Each treatment was triplicated and flasks were sampled in the morning (9:00–10:00 h) on days 3, 6, 9, 12, 15, 18, 23.

The samples from 22 Aug (summer) and 30 Nov (fall) only measured photosynthetic activity.

Analysis of biomass, growth, and photosynthetic potential

Phytoplankton samples were preserved with Lugol's iodine solution (2% final conc.) and sedimented for 48 h. Phytoplankton species were identified according to Hu et al. (Hu et al. 1980), and cell densities were measured with a Sedgwick–Rafter counting chamber at 200X–400X magnification.

The FDC of *Microcystis* was calculated by dividing the density of doublets by total cell density (Latour et al. 2004). Three to five *Microcystis* colonies from each sample were selected randomly and placed on a glass slide. After gently pressing the coverslip, *Microcystis* cells in colonies were dispersed. They were then photographed and counted as solitary or dividing cells. This process was repeated three times for each sample and then the three FDCs averaged to get a mean value for the sample.

Photo-physiological parameters, including maximum quantum yield (Fv : Fm), apparent quantum yield (Fv′ : Fm′), and light utilization efficiency of *Microcystis* were measured from each treatment or the initial "bloom" by PHYTO-PAM (Walz, Effeltrich, Germany) after 15 min dark exposure (Brookes et al. 2002; Cosgrove and Borowitzka 2006; Zhang et al. 2008).

Chlorophyll *a* (Chl *a*) concentrations were determined spectrophotometrically after extraction in 90% hot ethanol (Pápista et al. 2002). Total phosphorus (TP) and total nitrogen (TN) were analyzed using a combined persulfate digestion (Ebina et al. 1983).

Data analysis

Data are presented as means ± standard deviation (SD). Significant differences between control and treated samples were determined by ANOVA followed by Tukey post-hoc tests.

The growth rate (μ) under each set of treatment conditions was calculated according to the exponential growth equation:

$$\mu = \ln\ (X_2/X_1)/(t_2 - t_1)$$

where X_1 is the initial concentration of *Microcystis* cells at the beginning of the incubation (t_1), and X_2 and t_2 are the concentration and time when cell densities reached their maximum value.

Results

Cyanobacterial bloom dynamics in Lake Taihu monitored by MODIS imagery

Cyanobacterial bloom dynamics in Lake Taihu were determined from 588 clear-weather satellite images obtained from 2009 to 2011. Among these, 143 images showed that cyanobacterial blooms occurred when water temperatures were below 10°C (mostly from Dec to Feb). The areal coverage of these blooms ranged from 0.1 km^2 to 870.8 km^2, with an average of 71.4 ± 116.3 km^2. From 2009 to 2011, water temperatures in Lake Taihu ranged from ~0°C to 36°C (Supporting Information Fig. S4), reaching annual maxima in Jul and Aug and minima in Dec and Jan. The largest cyanobacterial bloom areas that were observed when water temperature below 10°C was 870.8 km^2 on 23 Dec 2010 and 79.8 km^2 on 08 Jan 2011 (Supporting Information Fig. S5).

Another group of 158 satellite images was recorded at water temperatures between 10°C and 20°C showing blooms ranging in size from 0.1 km^2 to 553.5 km^2, with an average of 123.4 ± 132.2 km^2. The remaining 287 satellite images were taken at water temperatures exceeding 20°C (primarily from May to Oct). Bloom areas ranged from 4.1 km^2 to 1226.2 km^2 with an average of 258.0 ± 230.4 km^2. The bloom area at temperatures above 20°C was significantly larger than at water temperatures between 10°C and 20°C, and also significantly larger than when water temperature was below 10°C ($p < 0.0001$). From 2009 to 2011, cyanobacterial bloom area derived from the satellite images showed a significant, positive relationship with water temperature in total (Fig. 1), but within each season there was no significant relationship between bloom area and temperature ($p > 0.05$).

The dynamics of cyanobacteria bloom area in 2009–2011 showed that blooms in Lake Taihu occurred in each week of the year (Fig. 2). The magnitude of summer bloom was highest and the followed fall blooms was lower. The areas of winter blooms were significantly smaller than that of fall and spring ($p < 0.0001$).

Field monitoring of winter cyanobacterial blooms in Lake Taihu

Routine monitoring (2009–2014) and cell counts demonstrated frequent cyanobacterial blooms in Lake Taihu during winter (Dec–Feb when water temperature was less than

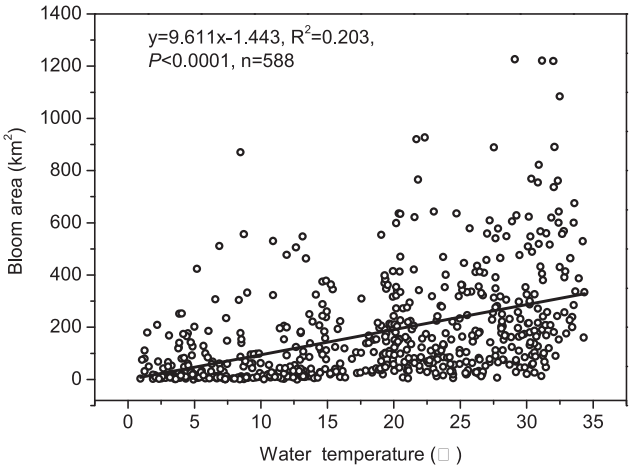

Fig. 1. Relationship between water temperature and areal extent of cyanobacterial blooms from 2009 to 2011. Water temperature data were obtained from YSI permanently deployed at TLLER. The data are averages of measurements made across the water column.

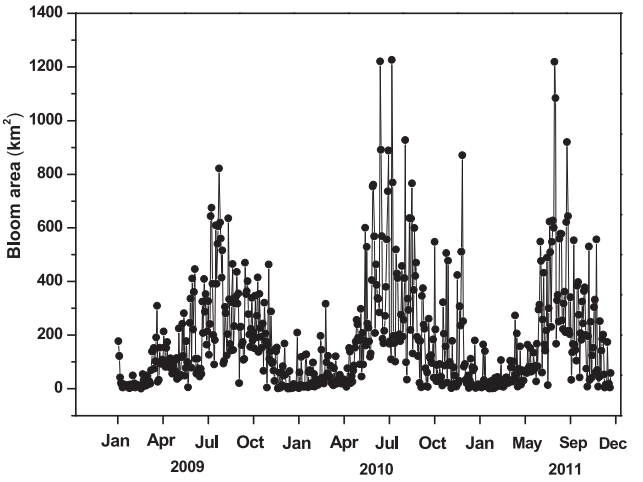

Fig. 2. The dynamic of cyanobacterial blooms observed by MODIS image in Lake Taihu from 2009 to 2011.

10°C), in Meiliang Bay and the lake center (Fig. 3a). This matched the distribution of Chl *a* (Fig. 3b), but differed from TP (Fig. 3c) and TN (Fig. 3d) distributions in Lake Taihu. In addition, visual inspection of Meiliang Bay adjacent to the field station identified 93 d when cyanobacterial blooms (all were more than Chl *a* concentration of 30 μg L^{-1}) were present during the winter period from 2011 to 2014 (Fig. 4). *Microcystis* cell density was strongly correlated with total phytoplankton density ($R^2 = 0.98$, $p < 0.0001$, $n = 93$), and *Microcystis* contributed 58.5–99.9% of total phytoplankton abundance. Chl *a* of bloom samples showed a significant relationship with cell density of total phytoplankton ($R^2 = 0.772$, $p < 0.0001$, $n = 93$) and *Microcystis* ($R^2 = 0.766$, $p < 0.0001$). Supporting Information Figs. S6, S7 shows the examples of a near-shore bloom accumulation.

Cell division was occurring as some cells in the *Microcystis* colonies were doublets or dividing cells when water temperatures near 0°C on 06 Jan 2013 (Supporting Information Fig. S6). However, the FDC was only $1.7 \pm 0.6\%$ for the entire *Microcystis* population. The average Fv : Fm was 0.182 ± 0.043 and the Fv′ : Fm′ was 0.148 ± 0.040. Fv: Fm increased to 0.413 ± 0.049 and FDC up to $13.7 \pm 2.1\%$ after 24 h of incubation at 25°C, 55 μmol photons m^{-2} s^{-1}. Results from winter bloom samples collected on 08 Jan 2014 were similar to that of 06 Jan 2013 (Supporting Information Fig. S7).

Growth and photosynthetic activity of *Microcystis* at low temperatures

Blooms were mostly green from May to Oct and only occasionally yellow-green or yellow. The color of *Microcystis* was yellow in winter (Dec–Feb). In early spring (08 Mar) when the water temperature was ~ 10°C, their color turned yellow-green. Their color was green in spring (16 Apr, ~18°C) samples (Supporting Information Table S1).

At the onset of the experiments from waters collected on 14 Feb 2014 and 08 Mar 2014, *Microcystis* incubated at 7.5°C and 10°C showed low but measureable growth and growth rates approximately tripled at 12.5°C (Table 1). Decreasing temperatures led to a reduction in Fv : Fm but cells of *Microcystis* maintained low photosynthetic activity, even at 2°C incubation (Fig. 5).

Cell density and Fv: Fm dynamics of *Microcystis* sampled on 16 Apr incubations were shown in Fig. 6. Decreasing temperature reduced the growth and Fv : Fm. The cell density and Fv : Fm of *Microcystis* at 6°C was significantly ($p < 0.01$) lower than at 12°C and 18°C. In nutrient addition treatments, cell density and Fv : Fm showed no change ($p > 0.05$) at 6°C but cell densities increased significantly at 12°C and 18°C ($p < 0.01$).

Most colonies of *Microcystis* in all experiments were float in water surface in all time.

Discussion

Microcystis winter blooms

MODIS images, field observations and low temperature incubations showed that buoyant *Microcystis* blooms persisted throughout cold winter temperatures in Lake Taihu. However, growth rates and photosynthetic activities remained low. Fallon and Brock (1981) found that *Microcystis* collected from winter sediments incubated in sediment under in situ conditions in the laboratory maintained photosynthetic activity for at least four months. Cyanobacteria are broadly distributed organisms (Quesada and Vincent 2012) and can be found in freshwater, terrestrial and marine ecosystems across a broad temperature range from near the poles to the equator. The scum-forming cyanobacteria are generally found in freshwaters with high temperatures and

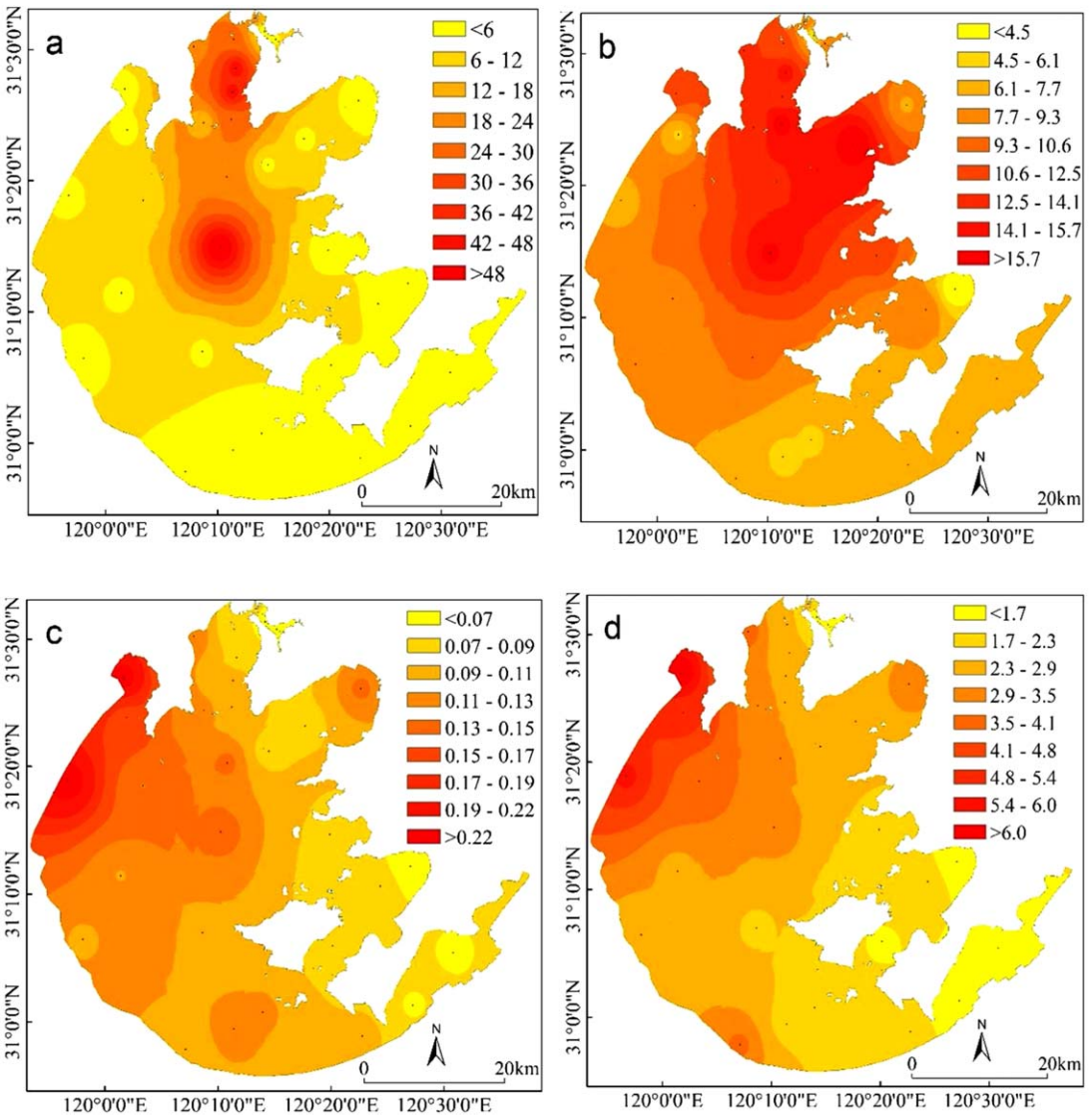

Fig. 3. Winter time cell density of *Microcystis* (a, 10^6 cells L^{-1}), Chl *a* (b, $\mu g\ L^{-1}$), TP (c, mg L^{-1}), and TN (d, mg L^{-1}) distribution on Lake Taihu (mean value from 2009 to 2014). Winter time was Dec to Feb when water temperatures were less than 10°C in Lake Taihu. The distribution of *Microcystis* was related to Chl *a* ($R^2 = 0.41$, $p < 0.01$, $n = 192$). However, *Microcystis* and Chl *a* have no significant relationship with nutrient (TP and TN, $p > 0.05$). TP and TN showed they come from the same pollution source ($R^2 = 0.83$, $p < 0.01$, $n = 192$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

high nutrient conditions. The fact that cyanobacteria are favored by warm temperatures and thermal stratification has prompted many investigations to explore how cyanobacterial bloom expansion may change with global warming (Jöhnk et al. 2008; Paerl et al. 2011a; Carey et al. 2012; Rigosi et al. 2014; Heather et al. 2015).

Even though bloom area and density are strongly dependent on factors such as water depth, hydrologic cycle, meteorological condition and so forth, the relationship between water temperature and bloom area was significant; bloom area expanded as water temperature increased ($R^2 = 0.203$, $p < 0.0001$, $n = 588$; Fig. 1). The monitoring data from 1992 to 2002 also showed that *Microcystis* biomass increased with temperature significantly in Lake Taihu (Liu et al. 2011). However, temperature did not necessarily determine the presence or absence of cyanobacterial blooms in Lake Taihu, and blooms occurred even under very cold temperatures (Figs. 2–5). Persistent blooms of *M. aeruginosa* during winter also occurred in Hartbeesport Dam, South Africa (Zohary 1985), where they formed massive accumulations termed hyperscums. Cells from the hyperscum in Hartbeesport Dam were photosynthetically active for 2 month but moribund after 3 month in low temperatures (Zohary 1985). Similarly, Lake Dianchi in China supports overwintering vegetative blooms of *Microcystis* at the water surface (Supporting Information Fig. S8).

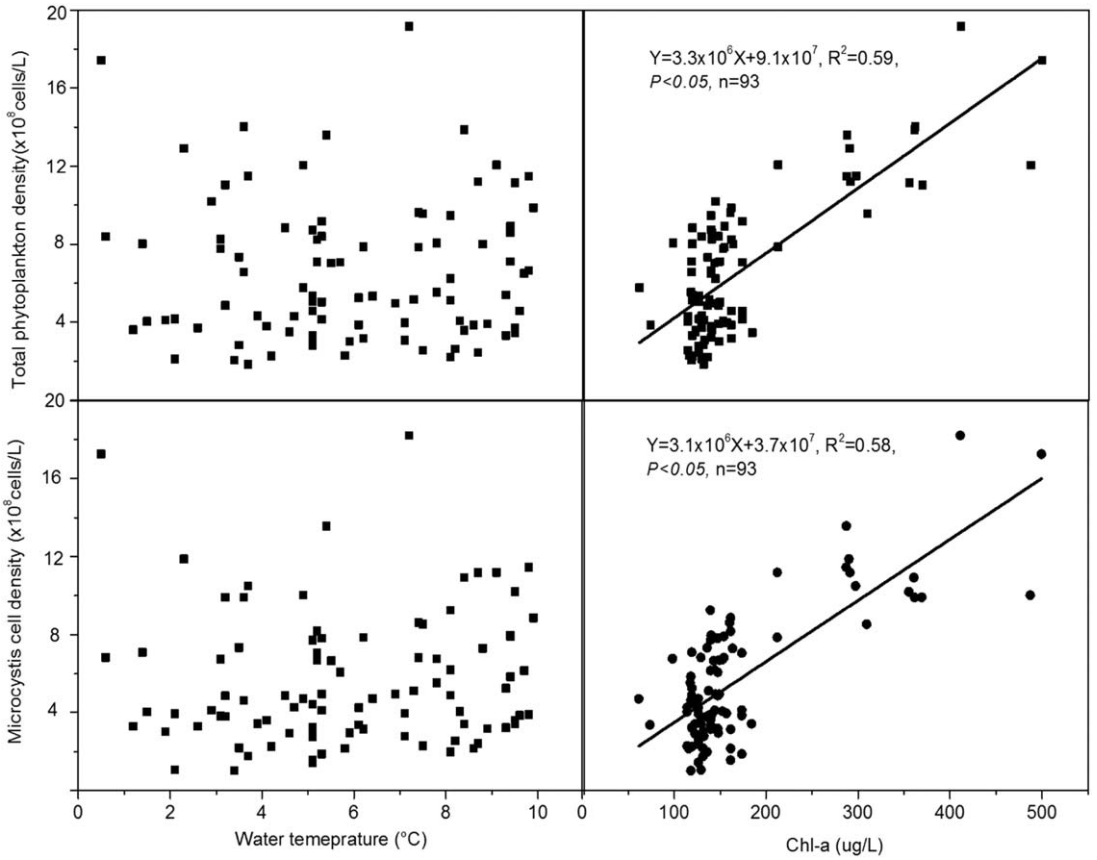

Fig. 4. Cyanobacteria *Microcystis* formed blooms in winter at Meiliang Bay adjacent to the field station (TLLER). There were 93 d (times) in winter have been observed from 2011 to 2014. Water temperature data were obtained from YSI permanently deployed at TLLER. The data are averages of measurements made across the water column. *Microcystis* cell density was strongly correlated with total phytoplankton density ($R^2 = 0.98$, $p < 0.0001$, $n = 93$).

The low frequency (1.7%) of dividing cells within the *Microcystis* population at the time of sampling indicates that the populations we observed in wintertime may have simply remained in a state of "suspended animation" (Supporting Information Figs. S6, S7). When cells were exposed to 25°C and 55 μmol photons m^{-2} s^{-1} the proportion of population in active cell division increased up to $13.7 \pm 2.1\%$, similar to values (5–20%) reported during summer (Wu and Kong 2008). The exposure to warmer temperatures in this experiment also increased the quantum yield, up to 0.41 which is similar to quantum yield measured in summer (Zhang et al. 2008). These two lines of evidence suggest that during winter in Lake Taihu, the metabolism of *Microcystis* was limited by low temperatures, but that cells were still active. Previous studies have also shown that cyanobacteria embedded in lake ice with liquid water rapidly resumed metabolism and exhibited increasing photosynthetic rates when the ice melted (Pinckney and Paerl 1996; Paerl and Priscu 1998). This indicates that *Microcystis* may grow quickly and form blooms during sunny weather conditions in the coming spring, once temperature limitation is alleviated.

The bloom-forming species in winter included *M. aeruginosa*, *M. flos-aquae*, *M. wesenbergii, Microcystis ichthyoblabe*, and *Microcystis firma*, similar to species that have dominated the annual summer and autumn cyanobacterial blooms in Lake Taihu for decades (Chen et al. 2003). Some studies suggest that *Microcystis* genera cannot grow at water temperatures below 10–15°C (Takamura et al. 1984; Robarts and Zohary 1987). They overwinter by sinking to sediment surface (Preston et al. 1980; Reynolds et al. 1981), although settling and recruitment from the sediment can occur all the year round (Verspagen et al. 2005). However, in Lake Taihu, many *Microcystis* colonies existed in the water surface or column and formed blooms at temperatures below 10°C (Figs. 2–5). Simulation experiments and field observation suggest that the vegetative cells of *Microcystis* in Lake Taihu have the ability to adapt to low temperatures and, more importantly, can grow again at higher temperatures with suitable light conditions (Supporting Information Fig. S7).

The mechanism of *Microcystis* bloom formation in winter

The correlation between bloom area and temperature in winter is not significant ($p > 0.05$, $n = 143$). The key factors

for algae growth, such as temperature, nutrients (TP and TN) and light have no significant relationship with *Microcystis* biomass in winter ($p > 0.05$, $n = 192$). Moreover, the experiments also indicated that the growth of *Microcystis* was very slow under 10°C, as has been previously shown (Takamura et al. 1984; Robarts and Zohary 1987). Therefore, in situ growth is likely not the major source of the biomass in these observed winter *Microcystis* blooms. The *Microcystis* spp. in Lake Taihu is a true cold-tolerant strain, but cannot grow quickly enough to form the large standing biomass observed in winter. Hence, the winter population is likely a remnant from autumn blooms. The dynamics of cyanobacteria bloom area as a function of Julian date also showed that winter blooms always followed fall blooms (Fig. 2). The huge biomass of cyanobacteria following blooms in autumn declined slowly, due to the low rate of death and decomposition at low temperatures. These cyanobacteria may accumulate to high, bloom-level concentrations under appropriate hydrological and meteorological conditions during winter (Supporting Information Figs. S5–S8). However, the possibility cannot be excluded that during sunny winter days, adequate light supply and increasing water temperatures may foster significant growth of the cyanobacteria. With warmer winter water temperature predicted in the future (Qin et al. 2010) the over-wintering pelagic populations may have greater opportunities to grow.

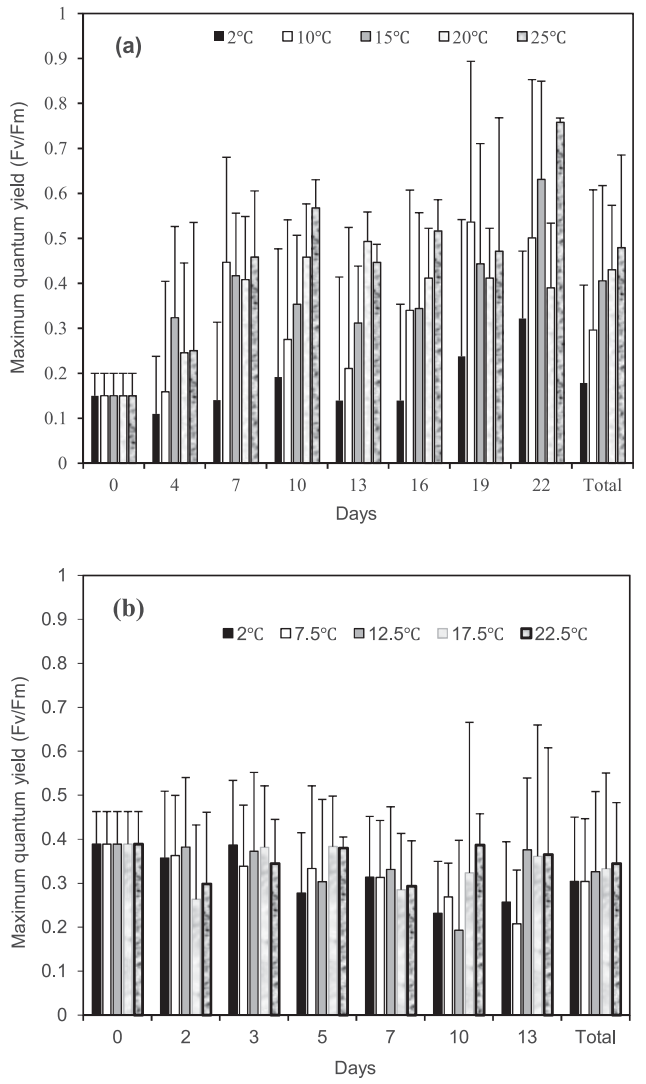

Fig. 5. The dynamics of maximum quantum yield of *Microcystis* incubated at different temperatures. The *Microcystis* sampling from blooms in Lake Taihu on 14 Feb (a) and 8 Mar (b), 2014.

A mechanism for promotion of settling at cooling temperatures was proposed by Visser et al. (Visser et al. 1995) who found that at lower temperatures cellular respiration rates decrease and glycogen concentration increased, thereby increasing the density of cells. This mechanism may have taken place in Lake Taihu, but at least a proportion of the population was persistently buoyant. Persistent buoyancy has been observed in *Anabaena circinalis* (Brookes et al. 1999) and *M. aeruginosa* (Brookes et al. 2000). Persistent buoyancy is attributable to gas vesicles providing more buoyancy than can be overcome by an increase in cell constituents (Brookes et al. 2000). Given the low rate of photosynthesis, as evidenced by the low Fv: Fm, cells may not accumulate sufficient carbohydrate to negate the buoyancy provided by the gas vesicles.

The low growth rate and loss rate of the *Microcystis* in Lake Taihu suggests that there is also an apparent lack of viral lysis or grazing control of the population. Although phages capable of lysing phytoplankton have been observed at high abundances at very low temperatures (Yager et al. 2001), lower winter temperatures may lessen mortality due to viral infections. In the subtropical Gulf of Mexico, cyanophages were found to be responsible for 5–15% mortality of the cyanobacteria population per day in the subtropical Gulf

Table 1. Growth rate (day^{-1}) of *Microcystis* from Lake Taihu in winter incubated at different temperatures.

14 Feb	Temperature (°C)	2	7.5	12.5	17.5	22.5
	Growth rate	0 ± 0	0.03 ± 0.02	0.13 ± 0.03	0.21 ± 0.01	0.23 ± 0.02
8 Mar	Temperature (°C)	2	10	15	20	25
	Growth rate	0 ± 0	0.05 ± 0.02	0.14 ± 0.04	0.22 ± 0.03	0.26 ± 0.04

Experiment in 14 Feb and 8 Mar was conducted for 22 d and 13 d, respectively. The growth rates were calculated with initial and the highest biomass in each treatment.

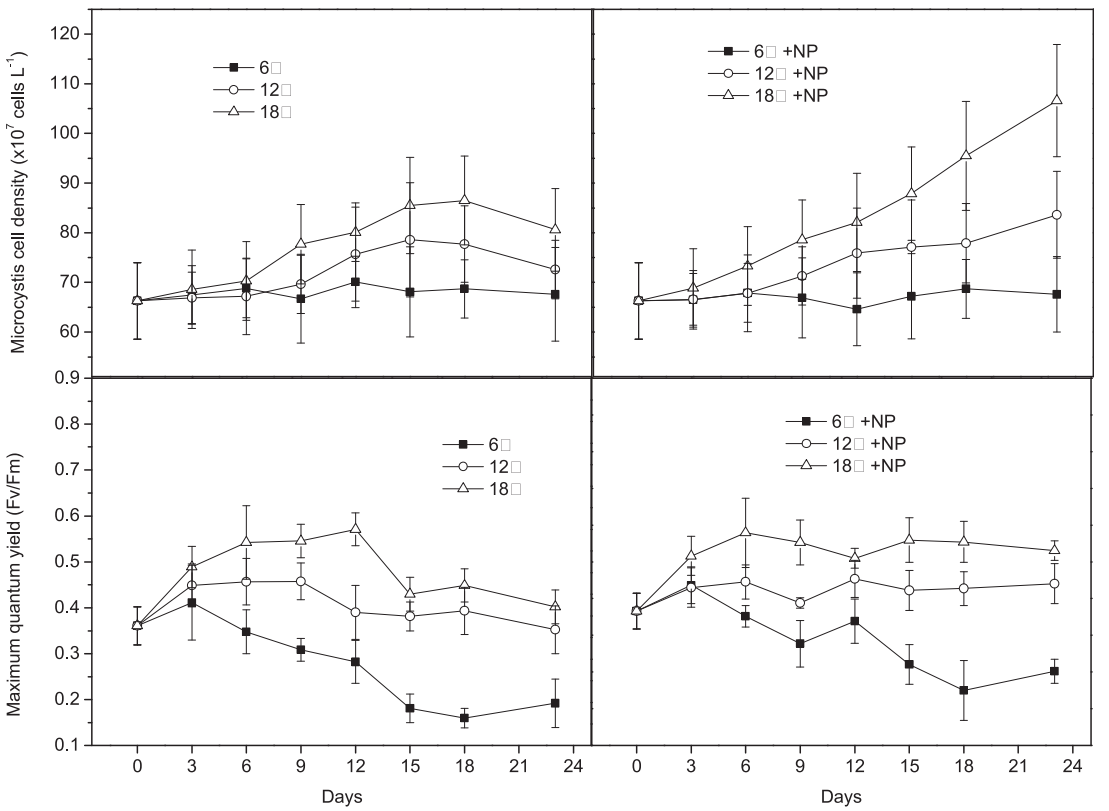

Fig. 6. Cell density and maximum quantum yield dynamics of *Microcystis* from Lake Taihu incubate in lake water, lake water +NP treatments (added 6 mg L^{-1} TN, 0.3 mg L^{-1} TP) at 6°C, 12°C, and 18°C. Light: dark = 12 h: 12 h. The *Microcystis* sampling from blooms in Lake Taihu on 16 Apr (spring), 2014 and the incubation experiment started on the same day.

of Mexico but only 3% per day in temperate waters of the Atlantic Ocean (Fuhrman 1999). In German Lake Müggelsee, increasing temperatures have altered phytoplankton-grazer interaction, decoupling the peaks of cladocerans and diatoms (Shatwell et al. 2008). While *M. aeruginosa* is typically considered a poor food item (Ghadouani et al. 2004), the temperature control of zooplankton grazing may be a factor contributing to the persistence of this species during the winter. Sun et al. (2012) found that *Microcystis* was the most important factor controlling the spatial dynamics of zooplankton and enhanced the small-sized cladocerans in Lake Taihu. *Microcystis* was shown to be a carbon source for zooplankton in eutrophic Lake Taihu, determined by ^{13}C labeling and fatty acid biomarkers (de Kluijver et al. 2012). Yang et al. (2012) found that total zooplankton abundance in Lake Taihu during winter was significantly lower than during warmer seasons. Low zooplankton abundance may contribute to reduced mortality of *Microcystis* during winter.

It is intriguing to see that *Microcystis* density showed no apparent relationship with nutrient (N or P) concentrations (in Fig. 3). This may reflect a strong reduction in nutrient requirements due to strong temperature-limited growth. Hydrodynamics may also be a factor promoting the movement of buoyant *Microcystis* colonies in winter, when calm conditions enable blooms to accumulate at the surface and light winds transport them laterally increasing the density on the leeward direction (Wu et al. 2015). In addition, Lake Taihu is a large shallow and eutrophic lake (see Supporting Information). Wind influences hydrodynamics strongly throughout the year. Wind-induced waves may maintain *Microcystis* in suspension and prevent settlement on the sediment surface. Most likely, constant re-entrainment in shallow, Lake Taihu plays a role in *Microcystis* overwintering in the water column.

The significance of this research

It is imperative to understand the mechanisms underlying the persistence of cyanobacteria in winter as this has implications for lake management and for the accuracy of models attempting to predict cyanobacteria development in response to climate and water pollution. Most of these models include empirically based regulation of the temperature-dependent growth rates of diatoms, chlorophytes, and cyanobacteria, which along with light and nutrient conditions determine which species will dominate under various scenarios. The persistence of *Microcystis* blooms in winter challenges paradigms that we use to model the relationship between the ambient environmental factors and

phytoplankton biomass. Furthermore, controls on population size by grazing or lysis are usually captured in a single loss or respiration term and so they do not explicitly model the actual mechanisms at play. Until the mechanisms responsible for the persistence of *Microcystis* during cold months are better known, the models cannot progress to simulate these dynamics. Neural networks (Maier et al. 1998), fuzzy logic (Ibelings et al. 2003), or Bayesian networks (Rigosi et al. 2015), which are trained or incorporate past observations in making future predictions, may better predict these winter blooms; however, the models may not be widely applicable to other lakes.

Considerable research attention is focused on cyanobacteria risk and bloom expansion with climate change and global warming (Paerl and Huisman 2008; Wagner and Adrian 2009; Paerl et al. 2011a; Carey et al. 2012; Elliott 2012; Zhang et al. 2012). The persistence of *Microcystis* in Lake Taihu during the cold winter months highlights the apparent plasticity of this species or a breakdown in the processes that normally disrupt blooms and support other phytoplankton taxa. The patterns in Fv : Fm as a function of temperature between the two sampling dates where *Microcystis* collected in Feb show greater variability than that of the Mar samples (Fig. 5). This illustrates the plasticity of this genus. Although the growth of *Microcystis* was likely slow in winter, the cells were active and showed reasonably high growth rates when transferred to warmer temperatures in the laboratory. This suggests that overwintering populations at the water surface may act as a massive seed source for bloom expansion with warming temperatures in the lake. All *Microcystis* samples from winter time incubated in laboratory have shown that they can regrow at elevated temperatures. Additionally, the dynamics of cyanobacteria bloom area in 2009–2011 also showed that spring blooms were always followed winter blooms (Fig. 2). Previous studies suggested that *Microcystis* recruited from sediments in spring were the main source of blooms in warmer season. However, Cao et al. (2005) found that *Microcystis* recruitment from sediments only contributed a small portion of the pelagic growth (<2.5%) in Lake Taihu. Hence, the major portion of pelagic growth was contributed by *Microcystis* that overwintered in the cold water column. Models such as PROTECH have shown the importance of over-wintering seed sources for producing high biomass under favorable, summertime conditions (Elliott 2010, 2012). Hence, controlling blooms in winter time will effectively help to reduce the magnitude of blooms during warmer seasons and controlling autumn blooms may help to decrease the biomass of *Microcystis* in winter. Reducing nutrients inputs and maximizing removal of internally-stored nutrients are primary objectives of environmental policy and management to control water blooms (Smith and Schindler 2009). Direct removal of *Microcystis* from the water column by net or machinery is an emergency method at bloom time in Lake Taihu (Supporting Information Fig. S9), which may be a good choice to decrease the biomass of *Microcystis* in winter.

The *Microcystis* persistence throughout winter becomes a serious problem seen within the context of the future climate changed induced increases in winter temperatures, which may exacerbate the frequency and intensity of cyanobacterial bloom during the warmer seasons. Cyanobacterial blooms have occurred earlier and lasted longer with the increases of temperature over the past 20 yr (Duan et al. 2009; Zhang et al. 2012). However, with year-round blooms, control of nutrient inputs is the only viable long-term strategy to manage cyanobacteria (Brookes and Carey 2011; Paerl et al. 2011b).

Conclusions

Winter time cyanobacterial blooms dominated by *Microcystis* spp. were commonly observed when water temperatures were less than 10°C in Lake Taihu. Winter *Microcystis* maintained low activity and could resume high rates of photosynthesis and growth when temperatures increased. Thus, cyanobacterial blooms may appear year-round in eutrophic lakes. Controlling *Microcystis* during the winter will help to reduce the magnitude of blooms during warmer seasons.

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Acknowledgments

We appreciate Erik Jeppesen's help with revisions of this article. We are grateful to all staff in Taihu Laboratory for Lake Ecosystem Research (TLLER). We are also grateful for useful comments and suggestions made by two anonymous reviewers. This work was supported by the National Natural Science Foundation of China (41230744, 41471446, and 41471021), The Major Projects on Control and Rectification of Water Body Pollution (2014ZX07104-006) and The US National Science Foundation CBET projects 0826819, 1230543, and Dimensions in Biodiversity project 1240851.

Submitted 12 May 2015
Revised 29 October 2015; 18 November 2015
Accepted 19 November 2015

Associate editor: Yong Liu