Vertical distribution of *Microcystis* colony size in Lake Taihu: Its role in algal blooms

Wei Zhu^{1,3}, Ming Li^{2,4,*}, Yonggang Luo¹, Xiaoxuan Dai¹, Lili Guo¹, Man Xiao¹,

Jingyu Huang¹, Xiao Tan¹

¹College of Environment, Hohai University, No. 1 Xikang Road, Nanjing 210098, PR China

²College of Resources and Environment, Northwest A & F University, Yangling 712100, PR China

³National Engineering Research Centre of Water Resources Efficient Utilization and Engineering Safety, Hohai University, Nanjing 210098, PR China

⁴Key Laboratory of Plant Nutrition and the Agri-environment in Northwest China, Ministry of Agriculture

Abstract: This study investigated the vertical distributions of *Microcystis* cell density and colony size in Lake Taihu, where algal blooms occur frequently. Measurements were made from April 2011 to January 2012 to gain a seasonal outlook on the role of such distributions in the blooms. It was found that large colonies tended to accumulate on the water surface, but the cell density fluctuated widely. The cell density in the water column increased continuously from spring to summer (i.e., April to October) and decreased after late autumn, showing apparent seasonal variations. The abrupt occurrence and disappearance of *Microcystis* blooms over short periods of time was not caused by the rapid growth of *Microcystis* but by the rise and accumulation of large *Microcystis* colonies at the water surface, both of which are affected by colony size. The ascent velocity of large colonies was higher than that of small ones, which enables large colonies to more easily overcome the stirring effects of water flows, waves and perturbations to rise to the surface. The results of canonical correspondence analysis (CCA) of *Microcystis* vertical distribution in relation to environmental factors suggested that nutrient concentrations and temperature were the main influencing factors related to blooms formation by *Microcystis* in Lake Taihu during our investigation.

Keywords: *Microcystis* bloom, colony, rise and accumulation, vertical distribution

Introduction

Cyanobacterial blooms in lakes and reservoirs, which are caused by eutrophication, bring about a series of environmental and ecological problems and can seriously threaten the safety of regional drinking water (Falconer, 2001; Le, 2010). The cyanobacterium *Microcystis* spp. is responsible for some of the most serious and widespread blooms in freshwaters.

The rise and accumulation of *Microcystis* on the water surface is one of the key mechanisms of bloom formation (Aparicio Medrano et al., 2013; Reynolds and Walsby, 1975; Verspagen et al., 2005). However, the patterns and factors that affect *Microcystis* vertical distributions are still poorly understood. Reynolds et al. (1973) showed that *Microcystis* colonies accumulated mainly in the upper 4 m of water from July to August in Crose Mere, England, and that the vertical distribution of *Microcystis* cell density fluctuates widely. Reynolds and Rogers (1976) also investigated seasonal variations in the vertical distribution and buoyancy of *M. aeruginosa* in Rostherne Mere, England, and found that *Microcystis* colonies stay on the surface of bottom sediments during winter, rise to the epilimnion in summer, and then sink back to the sediments in autumn. Both investigations indicate a seasonal variation in the vertical distribution and buoyancy of *Microcystis*. However, the mechanism of buoyancy regulation alone cannot explain the rapid changes in cell density in surface water layers and the different vertical distributions of *Microcystis* within the same season and environmental conditions. Thus, other factors aside from buoyancy must affect *Microcystis* accumulation on the water surface layer.

Other researchers have focused on the effects of wind on the vertical distribution and accumulation of *Microcystis* on water surface layers. Takamura and Yasuno (1984) investigated the diurnal migration of five species, including *M. aeruginosa*, at the center of Takahamairi Bay in Lake Kasumigaura on a calm summer day and found that *Microcystis* colonies accumulated at the water surface in the early morning and then in the middle of the water column in the afternoon. Their records of the distribution of wind velocity at different times showed it to be high in the afternoon but low early in the morning. Thus, *Microcystis* colonies may be inferred to rise to the water surface under calm conditions and become re-entrained into the surface mixed layer with strong winds. Ha et al. (2000) studied the vertical distribution of *Microcystis* cell density in the Nakdong River (South Korea) during the summer of 1994 and found that *Microcystis* accumulated in the surface water layers during calm nights but was distributed uniformly in the water column during daytime, when strong winds and waves prevailed.

The effect of colony size on ascent velocities has also been studied. Kromkamp and Walsby (1990) investigated the buoyant density change rates of cyanobacteria in laboratory cultures and established a model to simulate diurnal changes in the buoyancy and vertical migration. This model suggested that large colonies experience more rapid vertical migrations. In addition, Wallace et al. (2000) investigated the vertical distribution of *M. aeruginosa* and established a model of buoyancy regulation. They found that the vertical distribution of *M. aeruginosa* depends on its carbohydrate content and colony size. Moreover, Wu and Kong (2009) investigated the vertical

distribution of different colony sizes of *M. aeruginosa* in Lake Taihu and found that smaller colonies were more easily affected by wind-induced mixing than larger ones.

The studies discussed thus far confirm that vertical distribution of *Microcystis* is affected not only by the regulating mechanism of buoyancy but also by winds and waves. Colony size may influence the effect that wind has on the vertical distribution of *Microcystis*. As such, the relationship between the vertical distribution of cell density and colony size of *Microcystis* requires investigation to explain the mechanisms of vertical migration and bloom formation by *Microcystis*.

In this study, we investigated the vertical distribution of *Microcystis* cell density and colony size in Meiliang and Gonghu Bays in Lake Taihu, where *Microcystis* blooms occur frequently. We also investigated the relationships between *Microcystis* blooms, colony size and environmental factors. The findings obtained helped increase our understanding of mechanisms controlling *Microcystis* blooms.

Materials and Methods

Study site description

Sampling was carried out in two bays within the northern region of Lake Taihu, namely, Meiliang Bay and Gonghu Bay. Six sampling sites were established in the middle of the two bays (Figure 1).

[Insert Fig. 1 here]

Sampling and analytical methods

Sampling

The study period was from April 2011 to January 2012. Sampling was carried out twice a month from April 2011 to October 2011 and then once a month for the rest of the study period. Since the depths of sites were about 2 m, 100 mL of water was collected from the bottom to the water surface at 0.5 m intervals using a polymethyl methacrylate sampler (Finucane and May, 1961) for *Microcystis* cell density and colony size analysis. Formalin (2% (v/v)) was immediately added to the samples for subsequent cell counting and colony size analysis. At each site, water temperature and pH was measured in the field using an electronic thermometer (Mettler SG7) and compact pH meter (pH Testr30), respectively. Equal volumes of water collected at both the surface and a depth of 1.0 m below the surface were mixed in a 500 mL plastic bottle. It was kept refrigerated and carried to the laboratory for chemical analysis. The information of wind speed and direction was collected from a meteorological station (see Fig. 1; Ding et al. 2012).

Chemical analysis: Water samples for chemical analysis were filtered through a 0.45 μm pore size membrane and the filtrate was used to analyze for total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) concentrations. The TDN and TDP concentrations were analyzed by colorimetry after digestion with $\text{K}_2\text{S}_2\text{O}_8 + \text{NaOH}$ (Ebina et al., 1983).

Cell counting

A sample of 3 mL was placed into a centrifuge tube (10 mL). The centrifuge tube was

then placed in a 100 °C water-bath oscillator and shaken at 120 rpm for 5 min (Joung et al., 2006). Finally, cells were counted three times in a blood cell counting chamber using an optical microscope (Olympus CX31; Olympus Corporation) at ×400 magnification, after which the cell density was calculated. If the difference between three calculated results was less than 10%, the average value of these results was used as the final estimate of the cell density; otherwise an additional count was carried out.

Colony size analysis

Sample remaining was shaken and examined using the optical microscope (Olympus CX31) at ×100 magnification. Photomicrographs were taken using an optical C-5050 digital camera, and the colony size of *Microcystis* was directly analyzed using the UTHSCSA ImageTool program version 3 (Wilcox et al., 2002). A minimum of 200 colonies per sample was counted to calculate the D₅₀ (signifying where 50% of the particles are smaller than the value of D₅₀; D is particle diameter) of the *Microcystis* colony size; the D₅₀ was used to indicate the average size of algal colonies.

Statistical analyses

The average cell density in the water column was calculated as follows:

$$C_{\text{Average}} = (C_{0\text{ m}} \times h_1 + C_{0.5\text{ m}} \times h_2 + C_{1.0\text{ m}} \times h_3 + C_{1.5\text{ m}} \times h_4 + C_{2.0\text{ m}} \times h_5) / 2$$

where C_{Average} is the average cell density of *Microcystis* in the water column, C_i is the cell density of *Microcystis* at the depth i , and h_i is the height of the water column at depth i (h_1 and h_5 were both 0.25 m; h_2 , h_3 and h_4 were all 0.5 m).

Canonical correspondence analysis (CCA) was applied to reveal relationships between environmental factors and $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$. The CCA was performed with the software CANOCO 4.5 (SCIENTIA Software).

Results

Environmental parameters

Water temperature increased gradually from April to August and then decreased (Fig. 2). The maximum and minimum of water temperature during the investigation was 31.0 °C in mid-August and 4.9 °C in January. The pH values fluctuated in the range of 7.5 to 8.5 and were low (below 8) from August to October.

[Insert Fig. 2 here]

The maximum TDN concentration of 4.56 mg/L was measured in August whereas the maximum TDP concentration (0.371 mg/L) was measured in October (Fig. 3). The minimum values of TDN and TDP were 0.15 mg/L and 0.001 mg/L, respectively. TDP was sufficient in summer but nearly depleted during fall.

[Insert Fig. 3 here]

Table 1 shows wind speed and direction obtained from the meteorological station from April to October 2011. The average wind speed was in the range of 1.7 to 2.9 m/s and the wind direction was primarily eastern. The maximum wind speed was 6.3 m/s on 1st May.

[Insert Table 1 here]

Vertical distribution of *Microcystis* cell density

The cell density at the water surface was generally higher than that below the surface at all sampling sites and 2 to 3 times higher than the average value in the water column (Fig. 4). From August to October, when *Microcystis* blooms occur frequently, the cell density at the surface was over 5×10^8 cells/L, reflecting the intense accumulation of biomass at the surface during the bloom. Instances when the cell density near the bottom was comparable to that at the water surface occurred occasionally. For example, all cell densities at different depths in site 1 on August 11 were in the range 5×10^8 to 7×10^8 cells/L; only minor fluctuations in cell density were observed at this time.

The cell density at the water surface was observed to fluctuate strongly whereas the cell density below the surface showed only slight fluctuations. The cell density in the top 0.5 m was higher than the average value in the water column, whereas the cell density at depths greater than 1.0 m was generally lower than the average value.

The average cell density in the water column in Meiliang Bay was about 4×10^8 cells/L while that in Gonghu Bay was only about 3×10^8 cells/L. In addition, the maximum cell density in the water surface was 8×10^8 to 10×10^8 cells/L in Meiliang Bay whereas it was 7×10^8 to 9×10^8 cells/L in Gonghu Bay.

[Insert Fig. 4 here]

The average cell density in the water column showed obvious seasonal variations (solid line in Fig. 4). The average cell density in the water column increased

continuously from April, showed maximum values from September to October, and then decreased from November onwards.

Vertical distribution of *Microcystis* colony size

The size of *Microcystis* colonies increased from April to October and then decreased thereafter (Fig. 5). The D₅₀ of *Microcystis* colony size was about 20 µm to 50 µm in spring, increased to > 200 µm by October, and then showed a gradual decrease from November onwards.

The colony size of *Microcystis* at the water surface fluctuated widely, and the D₅₀ of *Microcystis* colony size ranged from 50 µm to 700 µm. The colony size of *Microcystis* at depths of 0.5 m and 1.0 m also fluctuated, but the D₅₀ of *Microcystis* colony size at these depths was below 200 µm. The colony size of *Microcystis* at depths greater than 1.0 m fluctuated slightly, and its D₅₀ was mostly below 100 µm.

[Insert Fig. 5 here]

Discussion

Vertical distribution patterns of *Microcystis* cell density and colony size

Cell density

The vertical distribution of *Microcystis* cell density can be concluded to show four patterns (Fig. 6a). In the growth and decline phases, cell densities were uniformly low throughout the water column (Fig. 6a, pattern I). Three patterns can be found in the bloom phase (Fig. 6a, patterns II, III, and IV). In pattern II, most of the *Microcystis*

colonies accumulate at the surface, causing the cell density at other depths to decrease. In pattern III, high cell densities of *Microcystis* are distributed uniformly through the water column whereas in pattern IV, cell density declines gradually from the surface.

Colony size

Four patterns of vertical distribution of *Microcystis* colony size corresponding to the four patterns of *Microcystis* vertical distribution of cell density are shown in Fig. 6b. The D_{50} of colony size distributes uniformly in the water column in patterns A and C, with a colony size threshold of 100 μm distinguishing between the two patterns. In pattern B, the D_{50} at the water surface is high (about 500 μm), whereas the values at other depths are low and distribute uniformly through the water column. Pattern D shows the same regularity of distribution as in pattern B, but its D_{50} is about 200 μm at the water surface.

[Insert Fig. 6 here]

Relationship between the vertical distribution of cell density and colony size of *Microcystis*

Our investigation showed that a water column profile of uniformly low *Microcystis* cell density (pattern I) corresponded to pattern A in terms of colony size distribution. Under this condition, the cell density and colony size at different depths showed no discernible differences. In the growth phase (before May), the small *Microcystis* colonies that form cannot readily rise and accumulate on the water surface.

From May to October, the cell densities at different depths increased noticeably and

the colony size in the water column also increased. Patterns II, III, and IV of the vertical distribution of cell density corresponded to patterns B, C, and D of the vertical distribution of colony size, respectively. In the decline phase, slight changes were observed.

Roles of growth and colony rise and accumulation on the water surface in *Microcystis* blooms

The average *Microcystis* cell density in the unit water column is used here to show the growth in the water column. The average water column cell density of *Microcystis* in September was ten times higher than that in April. Moreover, the cell density at the water surface was ten times higher than that below the water in the bloom phase. The increase in total cell number is due to the growth of the *Microcystis* population. However, our study indicated that growth alone cannot bring about rapid bloom formation. The cell density at the water surface rises and falls rapidly because of vertical migration of *Microcystis* colonies. Accumulation of most of the colonies at the water surface could lead to *Microcystis* blooms even if the total cell number in the water column is not high (Site 6 on July 4). Sometimes (Site 3 on August 11), the cell density at the water surface was similar to the average value in the water column from the surface to a depth of 0.25 m but it was observed (data are not shown in the current study) that most of the colonies would accumulate as a very thin layer (only several millimeters) on the water surface. When this occurs (similar to telescoping defined by Reynolds and Walsby (1975)), the cell density in the thin layer may be hundreds of times larger than that observed below the layer. Bloom formation can thus be

concluded to be directly caused by the rapid rise and accumulation of *Microcystis* colonies, not by their growth.

Our results also showed that the cell density distribution is related to the colony size of *Microcystis*. Nakamura et al. (1993) determined the rising velocity of *Microcystis* colonies of different sizes using a microscopic video camera and recorder and found that the rising velocity of colonies 100 μm in size is 30 $\mu\text{m/s}$ whereas that of colonies 400 μm in size is 300 $\mu\text{m/s}$. Stokes' law describes the vertical migration velocity as follows: $v = \frac{kgD^2(\rho - \rho_{\text{water}})}{18\mu}$ where v is the vertical migration velocity, k is fractal coefficient, g is acceleration due to gravity, D is particle diameter, ρ is particle density, ρ_{water} is density of water, μ is viscosity of surrounding liquid. If we assume ρ of *Microcystis* colonies to be constant and smaller than ρ_{water} , the floating velocity of a 200 μm colony would be 100 times higher than that of a 20 μm colony. In calm weather, when colonies have adequate buoyancy, large colonies may rise swiftly to the water surface and form blooms.

Roles of colony size in *Microcystis* blooms

The $D_{50(0\text{ m})}/D_{50(1.0\text{ m})}$ can reflect the separation of colony size in the water column, where $D_{50(0\text{ m})}$ is the D_{50} of *Microcystis* colony size at the water surface and $D_{50(1.0\text{ m})}$ is the D_{50} of *Microcystis* colony size at 1.0 m below the water surface. In this study, $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ was used to indicate the separation of cell number in the water column; $C_{0-0.25\text{ m}}$ is the cell number in the column from the surface to 0.25 m below the water surface and $C_{0-2.0\text{ m}}$ is the total cell number in the whole water column. If *Microcystis*

is distributed uniformly in the water column, $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ would be about 12.5%. Fig. 7a shows data obtained for the six sites in this study; $D_{50(0\text{ m})}$ in different ranges (100 μm intervals) is also marked with different graphic symbols. When $D_{50(0\text{ m})}/D_{50(1.0\text{ m})}$ was less than 1, $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ was generally below 12.5% and $D_{50(0\text{ m})}$ was less than 100 μm . Excluding $D_{50(0\text{ m})} < 100\text{ }\mu\text{m}$, $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ increased linearly with increasing $D_{50(0\text{ m})}/D_{50(1.0\text{ m})}$. When $D_{50(0\text{ m})}/D_{50(1.0\text{ m})}$ was 10, $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ reached nearly 50%. Moreover, when $D_{50(0\text{ m})}/D_{50(1.0\text{ m})}$ was 100, $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ reached nearly 80%, suggesting that accumulation of *Microcystis* on the water surface is related to the separation of the colony size in the water column. When obvious separation of colony size in the water column and accumulation of *Microcystis* at the surface occurred, $D_{50(0\text{ m})}$ was higher than 100 μm (mainly ranging from 200 μm to 400 μm). Therefore, we can conclude that colonies larger than 100 μm rise rapidly to the water surface and form blooms more easily than small ones.

[Insert Fig. 7 here]

Fig. 7b shows that when $D_{50(0\text{ m})}/D_{50(1.0\text{ m})}$ ranged from 1 to 10, the cell density at the surface was about 5×10^8 to 10×10^8 cells/L, indicating that *Microcystis* blooms occurred at the surface.

The discussion above strongly suggests that the rise and accumulation of *Microcystis* colonies on the water surface is the main mechanism of *Microcystis* bloom formation. Accumulation is affected by the colony size of *Microcystis*; large colonies easily rise and accumulate on the water surface, whereas small colonies encounter more

difficulty rising to the water surface due to the effects of winds and waves. Water in lakes is affected by the wind and tides, and flows, waves, and disturbances are ever-present in the lake, stirring colonies continuously. The rising velocity of large colonies is larger than that of small ones; this velocity helps large colonies overcome the stirring effects of flows, waves, and disturbances. Small colonies cannot overcome these effects; thus, they cannot rise to the water surface.

Effects of environmental factors on vertical distribution of *Microcystis* colonies

Figure 8 shows canonical correspondence analysis (CCA) ordination diagram of $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ in relation to environmental factors. The two axes accounted for 79.9% of observed variation of the species-environment relationship (the first and second axis explained 47.8% and 32.0%, respectively). It was also obvious that $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ was negatively related to wind speed, TDN, temperature, pH and TDN: TDP ratio when D_{50} of *Microcystis* at the water surface was in range of 100 to 400 μm . However, when D_{50} of *Microcystis* at the water surface was larger than 400 μm , $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ was positively related to TDP. Nevertheless, $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ positively related to all the environmental factors while D_{50} of *Microcystis* at water surface was smaller than 100 μm . Regardless of size, no significant difference was found between $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ and environmental factors.

High wind speed is expected to promote mixing making *Microcystis* colonies distribute uniformly in the water column (Wu et al., 2013). The phenomenon that $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ was negatively related to wind speed was a good interpretation of the

effects of wind speed.

The buoyancy of *Microcystis* colonies is dependent upon their gas vesicle volume and carbohydrate metabolism (Thomas and Walsby, 1985). High light intensity would induce collapse of gas vesicles and increase carbohydrate ballast, both of which promote sinking of *Microcystis* colonies (Ibelings et al., 1991). However, massive *Microcystis* blooms occurred during our investigation in summer and autumn when the light intensity was high (although light intensity was not measured in 2011, instantaneous light intensity was recorded as 400 to 1000 $\mu\text{mol}\cdot\text{photons}/(\text{m}^2\cdot\text{s})$ during the same sampling process in 2012; Li et al., 2014). Moreover, most colonies collected at different depths were floating at the water surface in the bottles when they were taken back to the laboratory. This suggests that the buoyancy of *Microcystis* colonies was sufficient to float to the water surface. Therefore, light intensity was not the main influencing factor related to blooms formation of *Microcystis* in Lake Taihu during our investigation.

Laboratory experiments demonstrated that gas vesicle volume per cell of *Microcystis* decreased significantly during nitrogen-limited growth but decreased only slightly during phosphorus-limited growth (Chu et al., 2007). However, Brooks and Ganf (2001) illustrated that *Microcystis* cells growing in 1.2 mg/L N and 0.31 mg/L P increased their gas vesicle volume and retained positive buoyancy. Fig. 3 showed that TDN concentrations in Lake Taihu were more than 1.2 mg/L most of the time, but TDP concentrations were always below than 0.31 mg/L. Thus, neither N nor P could be the limiting factors influencing formation of gas vesicle of *Microcystis* colonies in

Lake Taihu in the current study. However, those results were obtained from laboratory experiments in which unicellular or small colonial *Microcystis* was used (Brooks and Ganf, 2001) and we cannot make assumptions on the nutrient status of the cell based on measured nutrient levels without knowledge of cellular nutrient stoichiometry. Fig. 8 showed that $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ positively related to TDP while D_{50} of *Microcystis* at the water surface was larger than 400 μm and positively related to TDN while D_{50} of *Microcystis* at the water surface was smaller than 100 μm . It was also suggested that the decreasing P concentration resulted in a rise of *Microcystis* colonies smaller than 400 μm and decreasing N concentration caused a rise of *Microcystis* colonies larger than 100 μm . Our recent study also showed that the effects of nutrient concentrations on the growth of *Microcystis* in Lake Taihu was size-dependent. Thus, the effects of nutrient concentrations on changes in buoyancy of different sized *Microcystis* colonies should be studied in the future.

$C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ positively related to temperature while D_{50} of *Microcystis* at the water surface was smaller than 200 μm (Fig. 8). However, the relationship between $C_{0-0.25\text{ m}}/C_{0-2.0\text{ m}}$ and D_{50} of *Microcystis* was negative while D_{50} of *Microcystis* at water surface was larger than 200 μm . These results showed that high temperature was in favour of positive buoyancy of small *Microcystis* colonies. This could be because carbohydrate ballast of *Microcystis* colonies decreased at high temperature (Thomas and Walsby, 1986; Visser et al., 1995).

This study found that the vertical distribution of cell density was closely correlated with colony size, with the bloom mainly composed of large colonies. This suggests a

different explanation of bloom formation from the previous buoyancy regulation by cell metabolism. This could certainly be a future research topic to elucidate what controls the colony size so as to affect the accumulation. Future investigations will be aimed toward identifying factors that control colony size in bloom-forming *Microcystis*.

Acknowledgments: This study was sponsored by the National Program on Key Basic Research Project of China (No. 2012CB719804), the Natural Science Foundation of Jiangsu Province (No. SBK2011025) and the Hydraulic Science & Technology Project of Jiangsu Province (2012012).

References

- Aparicio Medrano, E., Uittenbogaard, R., Dionisio Pires, L., van de Wiel, B., Clercx, H. 2013, Coupling hydrodynamics and buoyancy regulation in *Microcystis aeruginosa* for its vertical distribution in lakes. Ecol. Model. 248: 41-56.
- Brookes, J.D., Ganf, G.G., 2001, Variations in the buoyancy response of *Microcystis aeruginosa* to nitrogen, phosphorus and light. J. Plankton Res. 23, 1399-1411.
- Chu, Z., Jin, X., Yang, B., Zeng, Q., 2007, Buoyancy regulation of *Microcystis flos-aquae* during phosphorus-limited and nitrogen-limited growth. J. Plankton Res. 29, 739-745.
- Ding Y, Qin B, Zhu G, Wu T, Wang Y, Luo L. 2012. Effects of typhoon Morakot on a large shallow lake ecosystem, Lake Taihu, China. Ecohydrology 5, 798-807.
- Ebina J, Tsutsui T, Shirai T. 1983. Simultaneous determination of total nitrogen and

total phosphorus in water using peroxodisulfate oxidation. Water Res. 17: 1721-1726.

Falconer, I.R., 2001, Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. Phycologia 40, 228-233.

Finucane, J.H., May, B.Z., 1961, Modified van Dorn water sampler. Limnol. Oceanogr. 6, 85-87.

Ha, K., Kim, H.W., Jeong, K.S., Joo, G.J., 2000, Vertical distribution of *Microcystis* population in the regulated Nakdong River, Korea. Limnology 1, 225-230.

Ibelings, B.W., Mur, L.R., Walsby, A.E., 1991, Diurnal changes in buoyancy and vertical distribution in populations of *Microcystis* in two shallow lakes. J. Plankton Res. 13, 419-436.

Joung, S.H., Kim, C.J., Ahn, C.Y., Jang, K.Y., Boo, S.M., Oh, H.M., 2006, Simple method for a cell count of the colonial cyanobacterium, *Microcystis* sp. J. Microbiol. 44, 562-565.

Kromkamp, J., Walsby, A.E., 1990, A computer model of buoyancy and vertical migration in cyanobacteria. J. Plankton Res. 12, 161-183.

Le, C., Zha, Y., Li, Y., Sun, D., Lu, H., Yin, B., 2010, Eutrophication of Lake Waters in China: Cost, Causes, and Control. Environ. Manage. 45, 662-668.

Li, M., Zhu, W., Dai, X., Xiao, M., Appiah-Sefah, G., Nkrumah, P.N., 2014, Size-dependent growth of *Microcystis* colonies in a shallow, hypertrophic lake: use of the RNA-to-total organic carbon ratio. Aquat. Ecol. 48, 207-217.

- Nakamura, T., Adachi, Y., Suzuki, M., 1993, Flotation and sedimentation of a single *Microcystis* floc collected from surface bloom. Water Res. 27, 979-983.
- Reynolds, C., 1973, Growth and buoyancy of *Microcystis aeruginosa* Kütz. emend. Elenkin in a shallow eutrophic lake. P. Roy. Soc. B – Biol. Sci. 184, 29-50.
- Reynolds, C.S., Walsby, A., 1975, Water-blooms. Biol. Rev. 50, 437-481.
- Reynolds, C., Rogers, D., 1976, Seasonal variations in the vertical distribution and buoyancy of *Microcystis aeruginosa* Kütz. emend. Elenkin in Rostherne Mere, England. Hydrobiologia 48, 17-23.
- Takamura, N., Yasuno, M., 1984, Diurnal changes in the vertical distribution of phytoplankton in hypertrophic Lake Kasumigaura, Japan. Hydrobiologia 112, 53-60.
- Thomas, R., Walsby, A., 1985, Buoyancy regulation in a strain of *Microcystis*. J. Gen. Microbiol. 131, 799-809.
- Visser, P.M., Ibelings, B.W., Mur, L.R., 1995, Autumnal sedimentation of *Microcystis* spp. as result of an increase in carbohydrate ballast at reduced temperature. J. Plankton Res. 17, 919-933.
- Verspagen, J.M.H., Snelder, E.O.F., Visser, P.M., Jöhnk, K.D., Ibelings, B.W., Mur, L.R., Huisman, J., 2005, Benthic-pelagic coupling in the population dynamics of the harmful cyanobacterium *Microcystis*: Freshwater Biol. 50, 854-867.
- Wallace, B.B., Bailey, M.C., Hamilton, D.P., 2000, Simulation of vertical position of buoyancy regulating *Microcystis aeruginosa* in a shallow eutrophic lake. Aquat. Sci. 62, 320-333.

Wilcox, D., Dove, B., McDavid, D., Greer, D., 2002, UTHSCSA image tool for windows v 3.00. The University of Texas Health Science Centre, San Antonio.

Wu, T., Qine B., Zhu, G., Luo, L., Ding, Y., Bian, G., 2013, Dynamics of cyanobacterial bloom formation during short-term hydrodynamic fluctuation in a large shallow, eutrophic, and wind-exposed Lake Taihu, China. Environ. Sci. Pollut. R. 20, 8546-8556.

Wu, X., Kong, F., 2009, Effects of light and wind speed on the vertical distribution of *Microcystis aeruginosa* colonies of different sizes during a summer bloom. Int. Rev. Hydrobiol. 94, 258-266.

Table 1 Wind speed and direction in Lake Taihu from April to October 2011

	Wind speed (m/s)		Wind Direction
	Average	Maximum	
17-Apr	2.30	5.40	NE
1-May	2.50	6.30	E
17-May	1.70	4.90	S
4-Jun	2.10	3.80	SE
14-Jun	2.60	5.80	E
4-Jul	2.30	5.00	N
18-Jul	2.30	4.00	E
29-Jul	2.60	4.70	S
11-Aug	1.80	5.80	NE
1-Sep	2.60	4.30	NE
17-Sep	2.00	6.10	N
1-Oct	2.90	5.40	NE

1 **Figure captions:**

2 Fig. 1 Sampling sites in Meiliang Bay and Gonghu Bay in Lake Taihu, China. The
3 insert in the figure describes the location of the sampling area in Lake Taihu.

4 Fig. 2 Seasonal variation of average water temperature and pH of all the six sites in
5 Lake Taihu.

6 Fig. 3 The TDN and TDP concentrations at different sites in different months.

7 Fig. 4 Seasonal variation in *Microcystis* cell density at different depths.

8 Fig. 5 Seasonal variation in the D_{50} of *Microcystis* colony sizes at different depths.

9 Fig. 6 Vertical distribution patterns of *Microcystis* (a) cell density and (b) colony
10 size. I: growth and decline phase; II, III and IV: different patterns of bloom phase.

11 Fig. 7 Relationships between colony size separation, cell density separation, and
12 cell density in the water surface: (a) Relationship between colony size separation and
13 cell density separation and (b) relationship between colony size separation and cell
14 density in the water surface. The dashed line shows the linear regression trendline of
15 the data of which $D_{50(0\text{ m})}$ was larger than $100\text{ }\mu\text{m}$.

16 Fig. 8 Canonical correspondence analysis (CCA) ordination diagram of $C_{0-0.25}$
17 $\text{m}/C_{0-2.0\text{ m}}$ in relation to environmental factors

[Fig. 1]

[Fig. 2]

[Fig. 3]

[Fig. 4]

[Fig. 5]

57

58

59

[Fig. 6a]

60

61

[Fig. 6b]

62

63

64

65

66

67

68

[Fig. 7a]

69

70

[Fig. 7b]

[Fig. 8]