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High nutrient concentration and temperature alleviated formation of large colonies of *Microcystis*: Evidence from field investigations and laboratory experiments

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ABSTRACT

Correlations between *Microcystis* colony size and environmental factors were investigated in Meiliang Bay and Gonghu Bay of Lake Taihu (China) from 2011 to 2013. Compared with Gonghu Bay, both nutrient concentrations and *Microcystis* colony sizes were greater in Meiliang Bay. The median colony size (D_{50}: 50% of the total mass of particles smaller than this size) increased from April to August and then decreased until November. In both bays, the average D_{50} of *Microcystis* colonies were <100 μm in spring, but colonies within moderate-size (100–500 μm) dominated in summer. The differences in colony size in Meiliang Bay and Gonghu Bay were probably due to horizontal drift driven by the prevailing south wind in summer. Redundancy analysis (RDA) of field data indicated that colony size was negatively related to nutrient concentrations but positively related to air temperature, suggesting that low nutrient concentrations and high air temperature promoted formation of large colonies. To validate the field survey, *Microcystis* colonies collected from Lake Taihu were cultured at different temperatures (15, 20, 25 and 30 °C) under high and low nutrient concentrations for 9 days. The size of *Microcystis* colonies significantly decreased when temperature was above 20 °C but had no significant change at 15 °C. The differences in temperature effects on colony formation shown from field and laboratory suggested that the larger colonies in summer were probably due to the longer growth period rather than the higher air temperature and light intensity. In addition, colony size decreased more significantly at high nutrient levels. Therefore, it could be concluded that high nutrient concentration and temperature may alleviate formation of large colonies of *Microcystis*.

1. Introduction

Formation of large colonies plays an important role in the occurrence of *Microcystis* blooms in freshwater lakes and ponds (Cao and Yang, 2010; Yamamoto et al., 2011; Zhu et al., 2014). Large colonies have faster vertical migration (Nakamura et al., 1993) and mainly concentrate at the water surface to form blooms (Wu and Kong, 2009). Colony formation is also considered a strategy for resisting predators (Cyr and Curtis, 1999; Yang et al., 2009) and for reducing damage from high light intensities (Wu et al., 2011; Zhang et al., 2011). However, *Microcystis* colonies collected from the field normally disaggregate to single-cell or paired-cell morphology following long-term cultivation (Yang et al., 2006). Thus, studies on the effects of various environmental factors on colony formation and colony size of *Microcystis* can provide a better understanding of *Microcystis* bloom formation. It can also bring benefits in developing measurements to control blooms by preventing the formation of large colonies and reducing the size of *Microcystis* colonies.

Zooplankton grazing was the first factor reported to induce colony formation of *Microcystis* in laboratory culture conditions (Burkert et al., 2001; Jang et al., 2003). Subsequently, the effects of zooplankton species and their secretions on colony formation and physiological changes in *Microcystis* have been well-studied (Yang et al., 2005, 2006; Yang and Kong, 2012). Size of induced *Microcystis* colony can be as large as 180 μm (Yang and Kong, 2012). Other

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biotic factors such as microcystins (Gan et al., 2012), heterotrophic bacteria (Shen et al., 2011), and other cyanobacteria, for instance, *Cylindrospermopsis raciborskii* (e Mello et al., 2013) also have induced colony formation under laboratory culture conditions. Additionally, some abiotic factors including temperature, light intensity, nutrient concentration and metal ions (Li et al., 2013a; Bi et al., 2013; Xu et al., 2015; Yang et al., 2012) are also likely to affect the colony formation and size of *Microcystis*.

The above studies were all performed under laboratory culture conditions. There is only one field investigation that has been published (Ma et al., 2014). Ma et al. (2014) pumped lake water containing *Microcystis* colonies into buckets and exposed the colonies to varying nitrogen and phosphorus concentrations. The buckets were then incubated in Lake Taihu. It was found that higher nutrient concentrations promoted the formation of single cells (Ma et al., 2014). However, this investigation only lasted for 18 days. To improve our knowledge about mechanisms of colony formation, as well as the factors affecting these mechanisms, a long-term investigation on the relationship between colony size and environmental factors would be highly desired.

This study aimed to reveal the relationship between colony size and environmental factors via a long-term field investigation. Laboratory experiments were carried out to verify the results obtained from field investigation. Lake Taihu, the third largest freshwater lake in China (2338 km^2), was selected as representative. This shallow, eutrophic lake has a mean depth of 2 m. In recent years, massive, frequent *Microcystis* blooms occurred from May to November (Duan et al., 2009). Water quality variables and the distribution and population of *Microcystis* species have already been well-reported (Li et al., 2013b; Xu et al., 2010). Therefore, sufficient references are available to understand the factors that affect colony size variation in this lake.

2. Materials and methods

2.1. Study sites description

Six sampling sites were chosen in the pelagic areas of Meiliang Bay and Gonghu Bay within the northern part of Lake Taihu, China (Fig. 1). The sites were uniformly distributed in each of the areas.

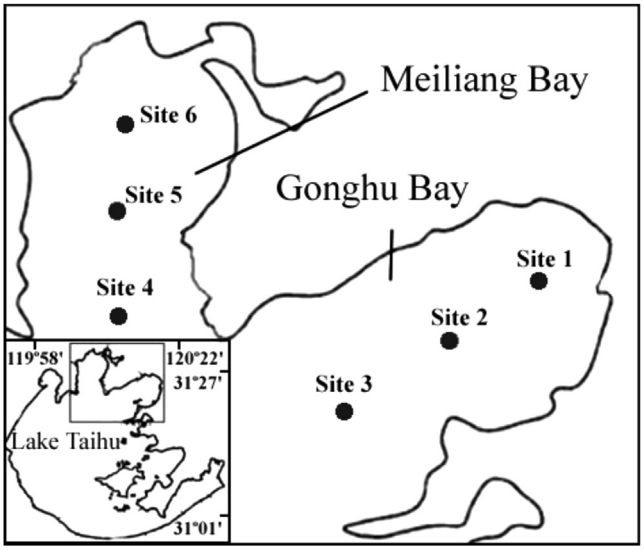

Fig. 1. Locations of sampling sites in Meiliang Bay and Gonghu Bay, Lake Taihu.

2.2. Field sampling

Investigation was conducted from April 2011 to December 2013. Sampling was performed fortnightly from April to October and monthly for the remaining period due to low algal biomass. At each site, 100 mL water samples were collected at 50-cm intervals from the lake surface to the lake bottom using a polymethyl methacrylate sampler (Finucane and May 1961). The samples were then mixed and recorded as algal samples. Formalin (2% (v/v)) was immediately added to the mixed water sample for subsequent measurements of *Microcystis* cell density and colony size. An additional 200-mL water samples were collected at depths of 0 m (i.e., lake surface) and 1 m at each site via the polymethyl methacrylate sampler, the mixture of which was recorded as water sample and was used for nutrient concentration analysis.

2.3. Analyses of environmental factors

Daily average values of air temperature, wind speed and sunshine duration for Wuxi City (approximately less than 10 km from Meiliang Bay) were obtained from a network database (http://data.cma.cn/). Daily global radiation was calculated using SWAT according to data of temperature, sunshine duration, longitude and latitude of Wuxi City. The average values of weeks before and after sampling day were used in the current study. Total nitrogen (TN) and total phosphorus (TP) concentrations of the water samples were measured using colorimetry after digestion with $K_2S_2O_8$ + NaOH (Ebina et al., 1983). After filtration through a 0.45-μm pore size membrane, each filtrate was assayed for total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) analysis using colorimetry (similar to the methods for TN and TP analysis).

2.4. Cell counting and colony size measurement

The photomicrographs of the algal samples were taken using an Olympus C-5050 digital camera (Olympus, Tokyo, Japan) coupled to an Olympus CX31 optical microscope and were analyzed using UTHSCSA ImageTool v3.00 software (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA). The size of each *Microcystis* colony was calculated as diameter = (length × width)$^{1/2}$ (Li et al., 2014a). The median colony diameter, D_{50} (50% of the total mass of particles smaller than this size), was used to estimate colony size. A minimum of 200 colonies per sample were measured to calculate D_{50}.

To estimate the cell density, 3 mL of each algal sample was placed in a 10 mL centrifuge tube. The centrifuge tube was then placed in a 100 °C oscillating water bath and shaken at 120 rpm for 5 min (Joung et al., 2006). Cells were counted 3 times in a hemocytometer using an optical microscope (Olympus CX31; Olympus Corporation) at ×400 magnification. Cell density was calculated as the mean value of the 3 counts.

2.5. Laboratory experiments

Microcystis colonies were collected by a phytoplankton net (64 μm mesh size) at a depth of 30 cm below the lake surface at site 6 in July 2015. Stored in a 5000-mL plastic bottle, these colonies were immediately taken back to laboratory for culture experiment to study the effects of nutrient concentration and air temperature on colony size. Poured gently through sieves of 500 and 250 μm, the colonies on the 250-μm sieve were washed 3 times and the residual colonies were re-suspended in 300 mL BG-11 medium (Stanier et al., 1971). D_{50} of the re-suspended colonies