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Seasonal dynamics of photosynthetic activity, *Microcystis* genotypes and microcystin production in Lake Taihu, China

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ABSTRACT

In recent years, toxic *Microcystis* blooms have occurred annually in Lake Taihu, China. In order to elucidate the relationships between photosynthetic activity of *Microcystis*, *Microcystis* genotypic composition, microcystin (MC) production and environmental factors, water samples and associated environmental data were collected from January to December 2014 in Lake Taihu. Seasonal variations in photosynthetic activity were measured using a Phyto-PAM Analyzer, abundances of total and toxic *Microcystis* genotypes were determined by quantitative real-time PCR (qPCR), and MC concentrations were quantified by HPLC. The maximum quantum yield of photosystem II (F_v/F_m) of *Microcystis* cells changed on a seasonal basis. F_v/F_m was not detectable in the winter, but increased from spring to early summer, after which, it gradually decreased until the winter. The level of non-photochemical quenching (*NPQ*) increased from March to August and then decreased until December. qPCR data showed that the abundances of total *Microcystis* genotypes ranged from 1.91×10^5 to 8.64×10^7 copies/mL and toxic *Microcystis* genotypes ranged from 2.38×10^4 to 5.67×10^7 copies/mL. The toxic proportion varied from 12.5 to 65.6%, with an average value of 27.9%. Correlation analysis revealed that there was a positive correlation between photosynthetic activity, genotypic composition and MC production. Water temperature was the only environmental factor that was positively correlated with F_v/F_m, *NPQ*, total and toxic *Microcystis* and intracellular MC. Additionally, total phosphorus was also significantly correlated with intracellular MC. These results indicate that future global warming, in addition to eutrophication, could promote *Microcystis* blooms in Lake Taihu and that blooms may increase in intensity and toxicity.

Introduction

In recent decades, toxic cyanobacterial blooms have become one of the most serious environmental problems in eutrophic freshwater bodies globally. Many genera of cyanobacteria are known to produce a wide variety of toxins that pose potential risks to animal and human health, caused by drinking water or recreational activity on water bodies (Dow and Swoboda, 2000; Falconer, 1999). Microcystins (MCs) are the most common and among the most potent toxins found in freshwater systems and are mainly produced by *Microcystis* spp., which is one of the most pervasive bloom-forming cyanobacteria (Otten et al., 2012). To date, more than 90 structural variants of MCs, with variable toxicity, have been reported (Neilan et al., 2012; Singh et al., 2012). The World Health Organization (WHO) has proposed a guideline value of 1 μg/L as a maximum concentration of total microcystin-LR in drinking water (WHO, 1998). In order to safeguard water resources and prevent health risks, it is necessary to understand the dynamics of toxic cyanobacterial blooms and MC production in freshwater ecosystems.

Chlorophyll *a* (Chl-*a*) is the main photosynthetic pigment in algae. Chl-*a* concentration is widely used as an indicator of phytoplankton biomass and is commonly used to monitor cyanobacterial bloom dynamics. Photosynthesis is a basic, but fundamental metabolic process for algae, and photosynthetic activity has been widely used for assessing changes in the physiological state of cyanobacteria during bloom formation (Kong and Gao, 2005). However, it is often logistically difficult to determine the photosynthetic capacity of algae using traditional measurement methods (^{14}C-uptake or O_2 evolution). Chl-*a* fluorescence has been used as a rapid non-invasive probe to monitor the photosynthetic properties of cyanobacteria in recent decades (Goto et al., 2008). Additionally, recent improvements in pulse-amplitude modulation (PAM) measurement techniques have made the fluorescence method an important tool in basic and applied algal physiology research (Schreiber et al., 1986, 1995). One of the main advantages of the PAM fluorescence technique is its ability to provide rapid, real-time information on the photosynthetic properties of cyanobacteria (Krause and Weis, 1991).

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The potential maximum quantum yield (F_v/F_m) is one of the parameters most often used in the study of aquatic photosynthesis and it indicates the degree of potential photosynthetic competence of algae (Genty et al., 1989). Non-photochemical quenching (*NPQ*) is another important and useful fluorescence parameter, which indicates photoprotection activity by dissipating excess light energy via thermal processes (Olaizola et al., 1994). These two fluorescence parameters play an important role in understanding the mechanisms of cyanobacterial bloom formation (Zhang et al., 2008). Zhang et al. (2008) investigated the diurnal changes of F_v/F_m and *NPQ* of three phytoplankton groups (cyanobacteria, chlorophytes, and diatoms/dinoflagellates) at different depths in Lake Taihu. Their results indicated that cyanobacteria had a greater ability to photoacclimate in comparison to chlorophytes and diatoms/dinoflagellates at the surface at midday. Wu et al. (2011) compared diurnal changes of F_v/F_m and the effective quantum yield ($\Delta F/F_m'$) of colonial and unicellular cells of *Microcystis* during a summer bloom in Lake Taihu. The results indicate that colony formation has a protective effect on *Microcystis* cells by reducing the occurrence of photoinhibition under high light intensities. The photosynthetic activity of cyanobacteria in winter and spring has also been measured in Lake Taihu (Wu et al., 2007; Zhang et al., 2012). However, to our knowledge, the seasonal dynamics of photosynthetic activity of cyanobacteria in Lake Taihu remains poorly understood.

It is well known that blooms of MC-producing cyanobacteria in natural freshwater ecosystems are usually comprised of toxic and non-toxic strains. Changes in the composition of toxic and non-toxic genotypes within a cyanobacterial bloom have been suggested to be one of the most important factors affecting MC levels in water bodies (Kurmayer and Kutzenberger, 2003; Sivonen and Jones, 1999). Therefore, it is important to understand the dynamics of the abundance of toxic and non-toxic genotypes and their relationship with environmental factors. However, it is not possible to distinguish toxic species from non-toxic species using traditional light microscopy identification, as many strains of cyanobacteria are very similar in appearance. MCs are nonribosomally synthesized via mixed polyketide synthase/nonribosomal peptide synthetase complexes, which are encoded by the MC synthetase (*mcy*) gene (Tillett et al., 2000). Advancement in the identification and sequencing of the *mcy* gene cluster, which involves MC biosynthesis (Tillett et al., 2000), has led to the development of molecular methods to directly detect MC-producing strains in water samples (Dittmann and Börner, 2005). In particular, quantitative real-time PCR (qPCR) with primers targeting *mcy* genes has been successfully applied to quantify MC-producing genotypes in a diverse range of aquatic environments worldwide (Conradie and Barnard, 2012; Ha et al., 2009; Hotto et al., 2008; Joung et al., 2011; Kurmayer and Kutzenberger, 2003; Rinta-Kanto et al., 2005, 2009; Singh et al., 2015; Tea and Gin, 2011; Vaitomaa et al., 2003; Yoshida et al., 2007). However, there have been relatively few studies carried out in China, despite frequent occurrences of toxic cyanobacterial blooms with high MC concentrations being reported in recent decades.

In the past 30 years, water pollution has become an increasingly serious problem in China due to rapid economic development, population growth and excessive exploitation of the environment. Lake Taihu is the third largest freshwater lake in China and is situated in the Yangtze delta, one of the most developed areas in China. It has an area of 2338 km^2, a mean depth of 1.9 m and is an important freshwater resource for several cities, including Shanghai, Suzhou and Wuxi (Qin et al., 2007). Toxic cyanobacterial blooms consisting mainly of *Microcystis* have been observed in Lake Taihu since the 1950s; however, blooms became more extensive in the 1980s (Xie, 2008). The toxic *Microcystis* blooms in the lake cause great public concern as they contaminate both the drinking water and the products of fisheries through the production of MCs (Shen et al., 2003; Chen and Xie, 2005; Chen et al., 2009a). It is common for the MC concentration in Lake Taihu to exceed the provisional guideline of 1 μg/L set by the WHO (Shen et al., 2003; Xu et al., 2005; Song et al., 2007). Previous studies indicated that variations in MC concentrations were associated with the frequent alteration of three *Microcystis* colonial types (*Microcystis flos-aquae*, *Microcystis aeruginosa* and *Microcystis wesenbergii*) in Lake Taihu (Chen et al., 2009b). However, the relationship between MC concentrations and *Microcystis* genotype composition in the lake is unclear and requires further research.

The aims of the present study were (1) to describe the seasonal patterns of F_v/F_m and *NPQ* of *Microcystis* blooms in Lake Taihu, (2) to investigate the seasonal dynamics of *Microcystis* genotypic composition and MC concentrations, and (3) to illustrate correlations among environmental variables, photosynthetic activity, abundances of total and toxic *Microcystis* genotypes and MC concentrations. The data collected in this study will also contribute to the overall understanding of the relationship between dynamics of toxic *Microcystis* blooms, MC concentrations and environmental factors in Lake Taihu.

Materials and methods

Study area and water sample collection

Lake Taihu (119°54′–120°36′ E, 30°56′–31°33′ N) is the third largest shallow freshwater lake in China (2338 km^2, maximum depth = 2.6 m, mean depth = 1.9 m). It is located in the highly developed and densely populated Yangtze Delta, and the lake has a mean water residence time of approximately 264 days. Meiliang Bay is located in the hypereutrophic northern part of the lake and cyanobacterial blooms have usually occurred here for six to seven months annually in recent years (Xu et al., 2005). Water samples were collected on a monthly basis in Meiliang Bay (31°24′ N, 120°18′ E) from January to December 2014. Water samples were taken at a depth of 0.5 m using a Plexiglas water sampler. Three replicate samples were taken on each sampling occasion. Each water sample was stored in a sterile glass bottle, which was then transported to the laboratory for further analysis.

Environmental factors

During the sampling period, water quality parameters (i.e. water temperature, pH, dissolved oxygen (DO) and turbidity) were measured in-situ using a multiparameter water quality sonde (YSI 6600, Yellow Spring Instruments, USA). To determine the concentrations of dissolved inorganic nutrients (PO_4^{3-}, NO_3^- and NH_4^+), 50 mL of each water sample was filtered through a glass fiber filter (GF/F, Whatman, UK) and analyzed using a continuous flow system (San^{plus} analyzer, Skalar, Netherlands) following the manufacturer's instructions. Chl-*a* was measured spectrophotometrically by the acetone method (Huang et al., 1999). Total phophorus (TP) (GB 11893-89) (EPA of China,1989a) and total nitrogen (TN) (GB 11894-89) (EPA of China, 1989b) were determined according to the Chinese national standard methods for water quality analysis.

Fluorescence measurements

Chl-*a* fluorescence was determined by the pulse amplitude modulation (PAM) method, with a Phyto-PAM fluorometer (Heinz Walz; Effeltrich, Germany) run by the software Phyto-Win 2.10. Water samples measuring 3 mL were taken for chlorophyll fluorescence analysis. Prior to measurement, *Microcystis* cells were acclimatized in conditions of total darkness for 15 min. The maximum quantum yield $F_v/F_m = F_m - F_o/F_m$ was calculated according to Genty et al. (1989 F_o is the minimum fluorescence which was determined by measuring light (32 μmol/m^2 s^1) after the acclimatization period; F_m is the maximum fluorescence that was stimulated using a saturating actinic light (up to 4000 μmol/m^2 s^1) pulse following the closure of all reaction centers. The $NPQ = (F_m - F_m')/F_m'$ were calculated according to Krause and Weis (1991). F_m', the maximum fluorescence signal in the light adapted state, was determined by a 600-ms pulse of saturating irradiance.

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