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Temporal and spatial distribution of *Microcystis* biomass and genotype in bloom areas of Lake Taihu

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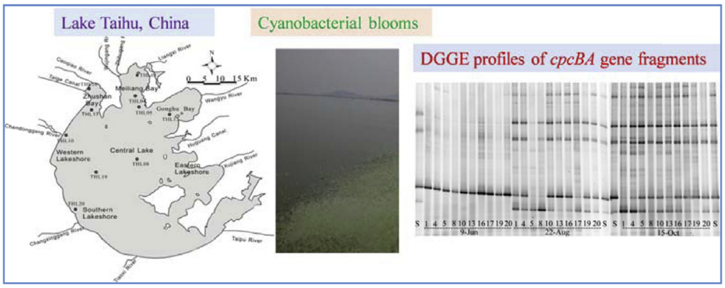
HIGHLIGHTS

- The horizontal distribution of toxic *Microcystis* in Lake Taihu was investigated.
- The spatial variations in chl-a and *mcyJ*/*cpcBA* ratio correlated with N and P.
- The temporal variation in *cpcBA* genotype was significantly correlated with N and P.
- Horizontal transport determined spatial distribution of potentially toxic *Microcystis*.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyanobacterial blooms as a global environmental issue are of public health concern. In this study, we investigated the spatial (10 sites) and temporal (June, August and October) variations in: 1) their biomass based on chlorophyll-a (chl-a) concentration, 2) their toxic genotype based on gene copy ratio of *mcyJ* to *cpcBA*, and 3) their *cpcBA* genotype composition of *Microcystis* during cyanobacterial bloom in Lake Taihu. While spatial-temporal variations were found in chl-a and *mcyJ*/*cpcBA* ratio, only spatial variation was observed in *cpcBA* genotype composition. Samples from northwestern part had a higher chl-a, but *mcyJ*/*cpcBA* ratio didn't vary among the sites. High chl-a was observed in August, while *mcyJ*/*cpcBA* ratio and genotypic richness increased with time. The spatial variations in chl-a and *mcyJ*/*cpcBA* ratio and temporal variation in *cpcBA* genotype were correlated negatively with dissolved N and positively with dissolved P. Spatial distribution of *Microcystis* biomass was positively correlated with nitrite and P excluding October, but no correlation was found for spatial distribution of *mcyJ*/*cpcBA* ratio and *cpcBA* genotype. Spatial distribution of toxic and *cpcBA* genotypes may result from horizontal transport of *Microcystis* colonies, while spatial variation in *Microcystis* biomass was probably controlled by both nutrient-mediated growth and horizontal transport of *Microcystis*. The temporal variation in *Microcystis* biomass, toxic genotype and

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cpcBA genotype composition were related to nutrient levels, but cause-and-effect relationships require further study.

1. Introduction

Both climate change and eutrophication contribute to cyanobacterial blooms in freshwater ecosystems (Reichwaldt and Ghadouani, 2012; Pace et al., 2017). The outbreak of cyanobacterial blooms is a global environmental issue, resulting in water quality deterioration, loss of habitat and natural resources, and oxygen depletion (Paerl et al., 2001; Xu et al., 2016). The toxins and taste-and-odor compounds synthesized by cyanobacteria are released into the water phase, causing health risks to humans and limiting the utilization of recreational and drinking water (Briand et al., 2003; Graham et al., 2010; Xu et al., 2013). Among the bloom-forming species, *Microcystis* spp. are the most common species responsible for toxic cyanobacterial bloom worldwide (Fastner et al., 2001; Wang et al., 2012).

In natural water bodies, *Microcystis* blooms contain multiple genotypes, including toxic and non-toxic (Otten and Paerl, 2011; Otten et al., 2012; Berry et al., 2017). Toxic *Microcystis* genotypes contain a gene cluster for biosynthesis of hepatotoxic microcystins spanning 55 kb, composed of 10 bidirectionally transcribed open reading frames arranged in two putative operons, i.e., *mcy*(microcystin synthetase)*A–C* and *mcyD–J* (Tillett et al., 2000). Microcystins synthesized by these genes are a family of more than 100 structurally similar hepatotoxins, inhibiting serine/threonine protein phosphatase 1 and 2A (Qi et al., 2015). The presence of *mcy* genes is necessary to synthesize microcystins, therefore many of the *mcy* genes have been used for detection and quantitative analysis of microcystin-producing toxic cyanobacteria genotypes (Rinta-Kanto et al., 2005; Kim et al., 2010; Otten et al., 2017).

The genetic structure of *Microcystis* population is also important for *Microcystis* to survive in the changing environment. A study showed that mean colony size, toxicity and growth rate of *M. aeruginosa* vary intra-specifically (Wilson et al., 2006). High genetic variability in photosynthetic pigment concentration and chemical quenching are also found among *M. aeruginosa* strains (Bañares-España et al., 2007). The heterogeneity observed in quantum yield, respiration, toxin production, and cell size of *Microcystis* is mainly attributed to genetic factors (López-Rodas et al., 2006; Rico et al., 2006). All these results demonstrate that both interspecific and intraspecific differences in physiobiochemical and morphological properties exist in the genus of *Microcystis*, which are largely determined by genetic factors. Therefore, the genetic structure of *Microcystis* populations is important for the growth and toxicity of *Microcystis* blooms.

Lake Taihu is the third largest freshwater lake in China, with an area of 2250 square kilometers and mean depth of 1.9 m (Qin et al., 2007). Unfortunately, because of pollution and resultant eutrophication, toxic *Microcystis* blooms in Lake Taihu have caused massive problems for industry, recreation, tourism and local drinking water supplies. Seasonal dynamics of toxic *Microcystis* blooms was observed in the northern part (Otten et al., 2012) or Meiliang Bay (Li et al., 2017) of Lake Taihu. But the temporal distribution of *Microcystis* biomass and genotype during cyanobacterial blooms in the whole bloom areas of the lake requires further research. A recent study revealed that the exchange of *Microcystis* blooms occurs between hypereutrophic Meiliang Bay and the adjacent open water of the lake (Wu et al., 2010). Therefore, we proposed that during cyanobacterial blooms (June to October) the exchange of *Microcystis* cells between adjacent water areas impact the distribution of *Microcystis* biomass and genotype in the entire bloom area of Lake Taihu. To confirm this hypothesis, a spatial-temporal investigation was conducted in northern, western and central parts of Lake Taihu during *Microcystis* blooms in 2011. The specific objectives of this study were to investigate the spatial (10 sites) and temporal (June, August and October) variations in: 1) their biomass based on chlorophyll-a (chl-a) concentration, 2) their toxic genotype based on gene copy ratio of *mcyJ* (microcystin synthetase J) to *cpcBA* (intergenic spacer of c-phycocyanin genes cpcB and cpcA) (do Carmo Bittencourt-Oliveira et al., 2001), and 3) *cpcBA* genotype composition of *Microcystis* in cyanobacterial bloom areas of Lake Taihu. Statistical analysis was further conducted to assess the relationship between *Microcystis* variables (chl-a *mcyJ*/*cpcBA*, and *cpcBA* genotype composition) and hydrochemical parameters (total and dissolved N and P, and chemical oxygen demand-COD). This study will not only provide valuable/historical data about the spatial-temporal distribution of *Microcystis* biomass and genotype during cyanobacterial blooms in the whole bloom areas of a large shallow lake, but also contribute to the overall understanding of the relationship between dynamics of toxic *Microcystis* blooms and environmental factors in Lake Taihu.

2. Material and methods

2.1. Water sampling

Water samples were collected at 10 sites (Fig. 1) on three dates: June 9 (early summer when cyanobacterial blooms start to occur) and August 22 (summer) and October 15 (autumn) in 2011 during cyanobacterial bloom. Samples (S01, S04, S05, S08, S10, S13, S16, S17, S19 and S20) are distributed in northern, western and central areas of Lake Taihu, where cyanobacterial blooms take place frequently. In each site, 10 L water sample was collected from the water column using a 2-m-long sampler, which was transported to the lab within several hours. Subsamples (1 L) were filtered through 0.45 μm filters for physiochemical parameter analysis (Xu and Guo, 2017). The remaining water samples were filtered through 1.2 μm GF/C filters (Whatman, UK), with the resulting filters being freeze-dried and stored at −20 °C before *Microcystis* DNA extraction and chl-a determination.

2.2. Hydrochemical parameters and chlorophyll a analyses

Ammonia (NH_4^+-N), nitrate (NO_3^--N), nitrite (NO_2^--N), total dissolved N (TDN), total N (TN), dissolved inorganic P (DIP), total dissolved P (TDP), total P (TP), COD and chl-a were determined (Huang et al., 1999). Analyses were performed in Taihu Laboratory for Lake Ecosystem Research at the northeast bank of Lake Taihu. According to Sun et al. (2012), *Microcystis* constituted over 90% of the phytoplankton in the heavy bloom areas from May to December, which was confirmed in our study. Recently, chl-a was identified as a robust and useful metric for predicting microcystin variance and *Microcystis* cell equivalents (Otten et al., 2012). Therefore, chl-a was used to represent *Microcystis* biomass.