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Evaluating the contamination of microcystins in Lake Taihu, China: The application of equivalent total MC-LR concentration

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ABSTRACT

The frequent occurrence and accumulation of microcystins (MCs) in freshwater systems pose serious threats to the drinking water safety and health of human beings. However, determining the overall toxicity and environmental risks from MC exposure is complex because of the variety of MC analogues and their respective toxicities. To address this issue, we conducted a survey of particulate (intracellular) and dissolved (extracellular) MC in Lake Taihu from 14 sampling sites in the northern part of the lake, and 32 stations throughout the entire lake, over a 16-month period. We propose a novel indicator, total MC-LR concentration (TLR), defined as the total concentration of MC-LR after transforming other MC variants (i.e., MC-RR and MC-YR) to an equivalent toxicity of MC-LR. Intracellular concentrations of TLR (iTLR) were usually observed, with the maximum values in July and October 2013 corresponding to periods of peaks in phytoplankton biomass. In contrast, extracellular concentrations of TLR (eTLR) were highest in May and June 2014. These differences in temporal patterns exhibited by LR, TLR, and TMC between intracellular and extracellular MC may be attributed to the influence of environmental variables. In addition, the distribution of iTLR and eTLR in the entire lake showed clear spatial heterogeneity. MC concentrations were greatest in the northern part of the lake during warm months, especially in Meiliang Bay. Based on the strong linear relationships between TLR and the concentration of chlorophyll-a (Chl-a), as well as TLR and the cell density of cyanobacteria, we propose not-to-exceed safety thresholds for Chl-a of 21.28 μg/L in the northern lake and 23.26 μg/L in the whole lake, which are paired with safety thresholds for cyanobacterial cell densities of 2.21×10^8 cells/L and 1.15×10^8 cells/L, respectively. The application of this newly proposed indicator, TLR, may contribute to better evaluation of overall MC toxicity and provide guidance on recommended limits for Chl-a concentration and cyanobacterial cell density in other freshwater ecosystems.

1. Introduction

In the past few decades, cyanobacterial blooms have become a major scientific problem in global freshwater systems. Some large freshwater lakes are increasingly experiencing severe cyanobacterial blooms around the world, such as Lake Erie in USA (Rinta-Kanto et al., 2009), Lake Suwa in Japan (Chan et al., 2007), and Lakes Poyang (Zhang et al., 2015), Chaohu (Yu et al., 2014b), Dianchi (Wu et al., 2014), and Erhai (Yu et al., 2014a) in China. Furthermore, global warming and excessive nutrient inputs are simultaneously contributing to the frequency and intensity of large algal blooms (O'Neil et al., 2012). Harmful cyanobacterial blooms can produce diverse kinds of cyanotoxins, such as microcystins (MCs), which are the most common hepatotoxic compounds. MCs are relatively stable in water and are difficult to remove by traditional water treatment techniques (van Apeldoorn et al., 2007). As a consequence, the occurrence of MCs can pose a serious threat to the drinking water supply, affect aquatic organisms by bioaccumulation (Rezaitabar et al., 2017; Xie et al., 2005; Xie et al., 2007), and even endanger mammals and human beings (Dittmann and Wiegand, 2006; Zhao et al., 2015). Despite numerous reports about the presence and distribution of MCs in lakes and reservoirs throughout the world (Otten et al., 2012; Rinta-Kanto et al., 2009; Sinang et al., 2013; Singh et al., 2015; Zhang et al., 2015), it is still difficult to accurately assess MC contamination in different lake systems. Each lake or reservoir has its own limnological and meteorological characteristics, which may lead to differences in the dominant congener and composition of MCs in the water column (Amé et al., 2010; Gurbuz et al., 2009). At present, there is no universal method to

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compare MC contamination within and among freshwater systems. Given these issues, it is important for resource managers to have an index that can identify the severity of environmental risks caused by MC to gain a better understanding of their overall toxicity in freshwater systems (Xie et al., 2016). Unfortunately, such an indictor has not been proposed to date.

In general, MCs stay in the cell while cyanobacterial cells grow and then are released to the water after cell lysis or death. Therefore, MCs in the water column are usually divided into particulate (intracellular) MC and dissolved (extracellular) MC. Most studies have focused on intracellular MC because about 90% of MC is retained in the cyanobacteria cell. However, Sakai et al. (2013) applied the ratio of extracellular to total MC (i.e. E/T) to evaluate the conditions of a bloom cycle; they found the E/T ratio was a better predictor of clarifying the growth stage of algal blooms than MC concentrations alone (Sakai et al., 2013). This suggests that the most robust evaluation of MC in the water column would include consideration of both intracellular and extracellular MC at the same time.

Over ninety MC variants have been identified to date and each MC analogue has a different degree of toxicity (van Apeldoorn et al., 2007). MC-LR, −RR, and −YR are considered the three most common MC variants, with MC-LR regarded as the most toxic one, followed by MC-RR and MC-YR (Gupta et al., 2003). This difference in toxicity must be taken into account when evaluating the potential toxic influence of MC. For example, an indicator of "relative toxic potential", which takes into consideration both MC diversity and their relative toxicity, and toxicity equivalent factor (TEF) (Wolf and Frank, 2002) was proposed to compare the MC risks among different lakes. However, their study didn't distinguish particulate from dissolved MC. We believe an even more robust approach for risk assessment is to include the equivalent MC-LR values instead of the total MC concentration.

Given the World Health Organization's (WHO) guideline of MC-LR in the drinking water, MC concentrations can be used to evaluate potential safe levels of cyanobacteria biomass (Shang et al., 2015) and identify thresholds of total nitrogen and chlorophyll a (Chl-a) concentrations not to be exceeded (Yuan et al., 2014) based on their strong linear relationships with MC. While many relationships between Chl-a and MC concentrations are positive, they also vary considerably depending on the environmental conditions of the lake (Stumpf et al., 2016). Yuan et al. (2014) recommended a national (USA)-scale threshold of Chl-a concentration of 37 μg/L, paired with a total nitrogen concentration of 570 μg/L, based on frequency of occurrence of MC greater than 1 μg/L. Based on a previous study in Lake Taihu, the possible safety value for Chl-a was suggested as 12.43 μg/L (Otten et al., 2012). The inconsistency in findings relating the association between Chl-a (i.e. cyanobacteria) and MC production among and within different lakes highlights the need for further examination of this relationship before it can be applied to other lakes.

Lake Taihu is the third largest freshwater lake in China and it has suffered from harmful cyanobacterial blooms for many years (Xu et al., 2017). Cyanobacterial blooms occurred only in Meiliang Bay in Lake Taihu prior to the 1990s (Ying et al., 2015). However, due to the influence of economic development and human activities, cyanobacterial blooms are now a frequent occurrence in the northern bays and have spread to the center and southern parts of Lake Taihu over the last two decades (Duan et al., 2009). Regularly occurring blooms, dominated by the MC-producing cyanobacterium, *Microcystis* sp., as well as reports about MC concentrations with different MC congeners, have been well documented in Lake Taihu (Otten et al., 2012; Song et al., 2007; Xu et al., 2008; Ye et al., 2009). The current approach for MC monitoring and risk assessment concentrates mainly on the environmental fate of MC in the water, algal cells, sediment, fish and other aquatic organisms (Gurbuz et al., 2016; Li et al., 2017). These studies are not sufficient to provide an overall evaluation of MC risk in the water column. Thus, it has become increasingly important to have a complete and accurate assessment of MC content, toxicity, and environmental risk, as well as potential safety thresholds for key environmental factors in large, shallow, and eutrophic Lake Taihu. From a lake management perspective, it would be desirable to develop an indicator that could provide an accurate assessment of the overall toxicity of MC. Recommended thresholds for Chl-a concentrations and cyanobacterial abundance are also needed to guarantee the drinking water safety of Lake Taihu.

The objectives of this study were to (1) examine the temporal variation and spatial distribution of cyanobacteria in the entire Lake Taihu, (2) develop a new indictor, TLR, to evaluate the contamination of MC and explore its monthly dynamics and spatial distribution, (3) explore the relationship between environmental factors and intracellular TLR as well as with extracellular TLR in the northern lake and the whole lake, and (4) recommend safety thresholds for the concentration of Chl-a and the cell density of cyanobacteria based on TLR concentration in the water column.

2. Materials and methods

2.1. Study area and sample collection

Lake Taihu (30°56′–31°34′ N, 119°54′–120°36′ E) is located in the delta of the Yangtze River (Fig. 1), with a surface area of 2338 km^2 and an average depth of 1.9 m (Qin et al., 2007). Between July 2013 and December 2014, monthly surface (0–0.5 m) water samples were collected from 14 sampling sites (1, 2, 3, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, 32) that were located mostly in the northern areas of Lake Taihu; in addition, seasonal water samples were collected from 32 stations (1–32) that were uniformly distributed through the entire lake (Fig. 1). The four seasonal divisions were February (winter), May (spring), August (summer), and November (autumn). All the water samples were collected with a vertical water sampler and transported to the Taihu Laboratory for Lake Ecosystem Research (TLLER) of the Chinese Academy of Sciences, which is located on the shoreline of Meiliang Bay. For each

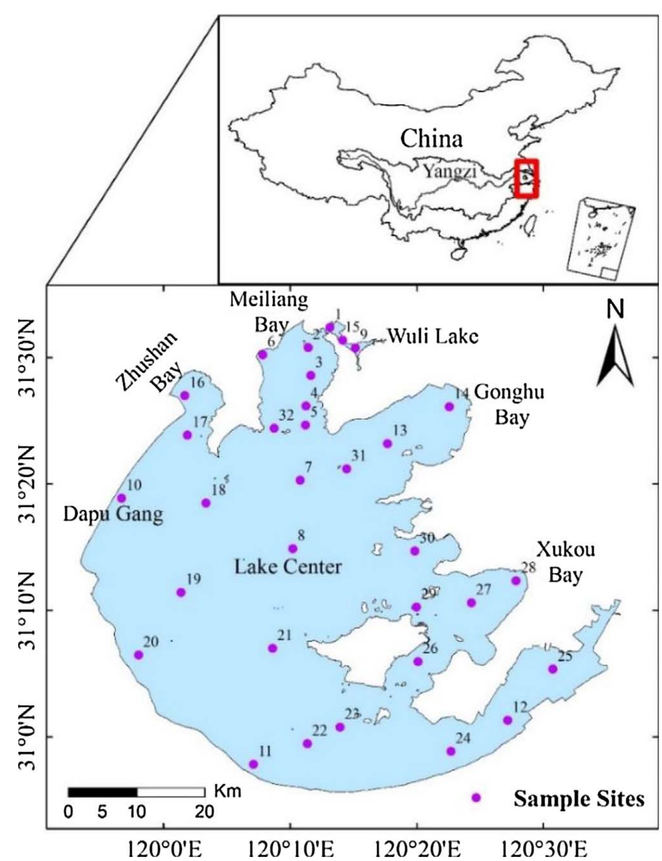

Fig. 1. Map of Lake Taihu and the distribution of sample sites displayed by the dots.

water sample, surface water temperature (WT), pH, conductivity (Cond), and dissolved oxygen (DO) were determined in the field using a YSI 6600 multiparameter sonde (Yellow Springs Instruments Inc., OH, USA). Prior to lab analysis, water samples were stored at 4 °C in the refrigerator. Lake-wide MC concentrations were calculated based on simple arithmetic averages; the spatial distribution of sites was biased with a greater number of sampling locations in the northern part of the lake (14/32). We acknowledge this bias may lead to a small overestimation of lake-wide averages, but it also reflects the greater population base and societal value of this part of the lake.

2.2. Phytoplankton analysis

For phytoplankton analysis, each of the water samples (1 L) was preserved with 2% acid Lugol's solution immediately after sampling and was stored in cool and dark conditions. Before counting, the samples were kept undisturbed for at least 48 h and then concentrated to 30–50 mL by siphoning off the supernatant. Phytoplankton species were identified to the lowest possible taxonomic level (Hu and Wei, 2006), and cell densities were measured with a Sedgwick-Rafter counting chamber at 200×–400× magnification with an inverted microscope. At least 300–500 individual cells or filaments were enumerated in an algal counting chamber for each sample. Conversions to biomass assumed that 1 mm^3 of volume was equivalent to 1 mg of fresh weight biomass. The relative abundance of the taxa was calculated from the cell density of each taxon relative to total cell density of phytoplankton.

2.3. Environmental analysis

Total nitrogen (TN), total dissolved nitrogen (TDN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), total phosphorus (TP), total dissolved phosphorus (TDP) and orthophosphate (PO_4^{3-}-P) were determined according to standard methods (Jin and Tu, 1990). For Chl-a analysis, 200–500 mL of water was filtered through a GF/F glass fiber filter (Whatman, UK). The concentration of Chl-a was determined spectrophotometrically at 665 and 750 nm after extraction in 90% hot (80 °C) ethanol (Papista et al., 2002). Dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) were measured with a TOC analyzer (TOC-V CPN, Japan) on filtrate passed through pre-ashed GF/F filters. All analyses were performed at TLLER.

2.4. MC analysis

MC analysis of water samples was performed on particulate MC and dissolved MC. Lake water was filtered with a GF/C glass fiber filter (47 mm diameter) to separate toxins dissolved in the water (extracellular MC) and particulate toxins (intracellular MC). A 100–1000 mL water sample (according to the amount of algae) was filtered on a GF/C filter and stored at −40 °C until further analysis. The freeze-dried filter was cut into pieces and extracted with 15 mL aqueous acetic acid (5%, v/v) before sonication and centrifugation at 10,000 r/min. This operation was repeated three times and the supernatant was pooled. For each sample, a 1 L filtrate was collected for the determination of dissolved MC.

Solid phase extraction was conducted as follows: a 0.2 g HLB cartridge (Oasis®, Waters, USA) was previously activated with 5 mL methanol and washed with 5 mL distilled water; then the supernatant was concentrated on the cartridge at a flow rate of 1 mL/min. Afterward, the cartridge was washed with 15 mL methanol (5%, v/v) to remove impurities; finally, the MC was eluted with 10 mL methanol. The eluent was blown to dryness under N_2 gas at 40 °C and reconstituted in 1 mL methanol. A subsample of 0.5 mL supernatant was prepared after centrifugation at 5000 r/min for 5 min and quantified by high performance liquid chromatography (HPLC) with a DAD detector (Agilent 1200, USA) according to previous descriptions (Su et al., 2015). The identification of each MC variant was dependent on their retention time and the concentrations of MC variants in each sample was calculated based on the absorbance peak area and corresponding standard curves. MC standards (MC-LR, −RR and −YR) for the three variants were obtained from Sigma-Aldrich (München, Germany).

2.5. Data analysis

The acute (24 h) toxicity of MC in mice was evaluated with three common MC variants (MC-LR, −RR, and −YR) and the intraperitoneal medium lethal dose (LD_{50}) was determined as 43, 235.4 and 110.6 μg/kg body weight, respectively (Gupta et al., 2003). According to comparative toxicity evaluation of MC variants (Gupta et al., 2003), the LD_{50} in mice for MC-RR and MC-YR is about 5- and 2.5-fold that for MC-LR, corresponding to 0.2 and 0.4 MC-LR equivalents, respectively. All the MC parameters in this study were calculated according to the formulas below.

$$iTMC = iLR + iRR + iYR; \tag{1}$$

$$iTLR = iLR + 0.2 \times iRR + 0.4 \times iYR; \tag{2}$$

$$eTMC = eLR + eRR + eYR; \tag{3}$$

$$eTLR = eLR + 0.2 \times eRR + 0.4 \times eYR; \tag{4}$$

$$TMC = iTMC + eTMC; \tag{5}$$

$$TLR = iTLR + eTLR; \tag{6}$$

where iLR, iRR, and iYR represent intracellular MC-LR, −RR, and −YR, respectively; eLR, eRR, and eYR represent extracellular MC-LR, −RR, and −YR, respectively; iTMC and eTMC represent the sum of the three congeners of intracellular and extracellular MC, respectively; iTLR and eTLR represent the sum of intracellular and extracellular MC-LR, respectively, after transforming MC-RR and MC-YR into the same toxic MC-LR by the toxicity coefficient; and TMC and TLR represent the total MC in the water column before and after transformation, respectively.

The relationship between intracellular and extracellular TLR, as well as with environmental variables, was assessed by Spearman correlation. Spearman's rank correlation coefficient is a nonparametric (distribution-free) rank statistic that measures the strength of an association between two variables. Statistical significance was set at P values less than 0.05. We performed linear regressions between TLR and Chl-a concentration, as well as with cyanobacterial cell density. All statistical analyses were conducted in SPSS 22.0 software (Statistical Product and Service Solutions). The interpolation map was generated by ArcGIS 10.1 software with the Inverse Distance Weighting method.

3. Results

3.1. Seasonal and spatial distribution of cyanobacteria in Lake Taihu

The spatial distribution of cyanobacterial cell density over the entire lake showed significant heterogeneity throughout the lake in all seasons except winter, over the past ten years (Fig. 2). In the spring, cyanobacteria densities were highest in Zhushan Bay (site 17), with a maximum cell density of 3.79×10^8 cells/L. In the summer, cyanobacteria began to increase in quantity and expand to other lake areas. As a result, the northern region of the lake was densely covered by cyanobacteria, with the maximum density in Meiliang Bay, which reached up to 7.43×10^8 cells/L (site 1; Fig. 2). The autumn distribution of cyanobacteria was similar to that in the summer, but the maximum was recorded at site 18 with a value of 6.27×10^8 cells/L. Cyanobacterial cell density declined in the winter, when average cell density was around 4.74×10^6 cells/L (Fig. 2). The northern region of the lake, including Zhushan Bay, Meiliang Bay, and Gonghu Bay, was characterized by greater cyanobacterial cell densities than the other lake

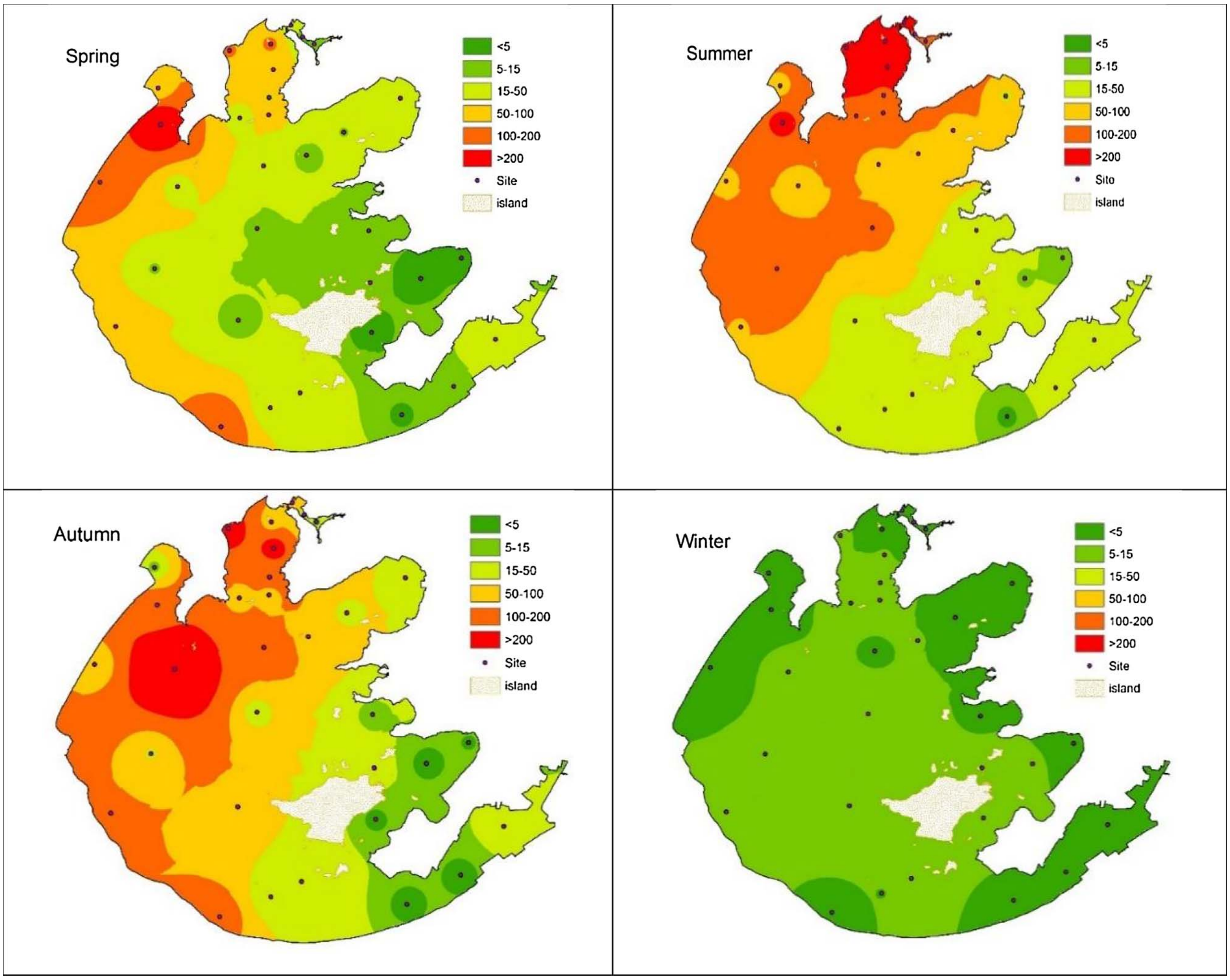

Fig. 2. Spatial distribution of cyanobacterial cell density (unit: 10^6 cells/L) for each season in Lake Taihu. Data are averages from 2005 to 2014 based on thirty-two sites throughout the lake. The interpolation map was generated using ArcGIS 10.1 software with the Inverse Distance Weighting method.

areas, especially the eastern region of the lake.

3.2. Phytoplankton composition and biomass

During the study period, the phytoplankton community at the phylum level from the northern lake region was dominated by Cyanobacteria from July to December 2013 and from May to December 2014, with an average relative abundance of 98.38% (Fig. 3). Even though the relative abundance of Cyanobacteria declined from January to April 2014, it was still the most abundant phytoplankton phylum, with proportions ranging from 48.98% (March 2014) to 59.56% (January 2014). During the period of lower Cyanobacteria dominance, Chlorophyta increased its relative abundance from 3.81% to 15.18% of the total phytoplankton. Bacillariophyta were relatively more abundant in January, February, and March 2014 than in other months, accounting for 22.56%, 15.31%, and 18.64%, respectively. The relative abundance of Cryptophyta was relatively high in January and April 2014, with values of 11.81% and 20.99%, respectively. Chrysophyta showed a high relative abundance in February (17.25%) and March (11.26%) 2014. All other algal phyla accounted for less than 1% of total phytoplankton.

In addition, there was temporal variation in the phytoplankton biomass (Fig. 3). The maximum phytoplankton biomass occurred in July 2013 (15.39 mg/L), dropped sharply in August 2013 and peaked in October 2013, then declined in December 2013 and peaked again in March 2014. A similar temporal pattern was also found in 2014, when high biomass formed in July 2014, declined in August 2014 and then peaked in September 2014.

3.3. Intracellular and extracellular TLR in Lake Taihu

The percentage of each congener (LR, RR, and YR) varied from month to month at the 14 sites in the northern part of the lake (Fig. 4). For intracellular MC, iRR was the primary congener overall with a relative abundance of 40.37%, although its relative abundance was greater in the warmer than colder months (Fig. 4a). iLR and iYR were less abundant overall, with relative abundances of 31.53% and 28.10%, respectively (Fig. 4a). In contrast, for extracellular MC, eLR was the most dominant overall with a proportion of 64.47% and greater relative abundance in warmer months (Fig. 4b). eYR and eRR, with proportions of 19.56% and 15.97%, respectively, were much less abundant (Fig. 4b).

The intracellular concentrations of LR, TLR, and TMC have a similar temporal pattern with a maximum in October 2013 and a secondary maximum in July 2013, low values from December 2013 to May 2014, and rising concentrations starting in June 2014 (Fig. 5a). Extracellular LR, TLR, and TMC concentrations followed a different temporal pattern, with low levels from October 2013 to April 2014, highest values in May and June 2014 and a slight decline in July 2014 (Fig. 5b). During the study period, the average concentrations of iLR and eLR were 1.72 and

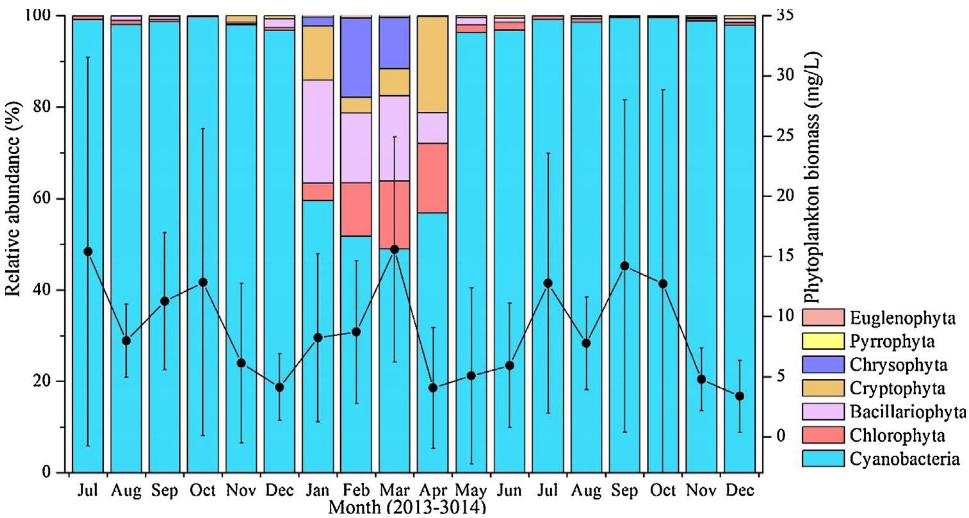

Fig. 3. Relative abundance of phytoplankton at the phylum level (calculated from cell density of each taxa with respect to total cell density of phytoplankton) and mean (±SD) phytoplankton biomass (solid line) from July 2013 to December 2014 in Lake Taihu. The data were obtained from fourteen sites in the northern part of the lake.

1.12 μg/L, respectively. The highest concentrations of iLR, iTLR, and iTMC were 13.07, 16.84, and 29.60 μg/L, respectively, compared to maximum concentrations of 3.96, 4.08, and 4.40 μg/L for eLR, eTLR, and eTMC, respectively.

3.4. Seasonal and spatial distribution of TLR in Lake Taihu

In general, there was a distinct temporal variation in the concentration of intracellular TLR, with the highest values in August 2013, followed by November 2013 and August 2014 based on the data from thirty-two stations throughout the lake (Fig. 6). Very low iTLR concentrations (< 0.75 μg/L) were measured in February and May 2014. The distribution of iTLR within the lake was heterogeneous, with high levels mainly concentrated in the northern part of Lake Taihu in August and November 2013, especially in Zhushan Bay, Meiliang Bay and Gonghu Bay. In August 2014, the highest values of iTLR were recorded in the western part of Lake Taihu (Fig. 6).

The spatial distribution of extracellular TLR (eTLR) was very different from that of iTLR (Fig. 6 and 7). The highest concentrations of eTLR were measured in May 2014, a period when iTLR was very low. The lake-wide average concentrations of eTLR were 0.48, 0.49, and 0.66 μg/L in November 2013, February 2014, and November 2014, respectively. The concentration of eTLR was less than 0.75 μg/L at almost all sampling stations; site 6 (1.13 μg/L) in February 2014 and site 5 (3.83 μg/L) in November 2014 at Meiliang Bay were the exceptions. The highest values of eTLR were observed in May 2014 with a lake-wide average of 3.34 μg/L and a maximum of 11.66 μg/L at site 3 located in Meiliang Bay. As with iTLR, there was considerable spatial heterogeneity of eTLR in Lake Taihu when concentrations were high (Fig. 7). For example, in May 2014, the concentrations of eTLR were greater than 5.0 μg/L in all sampling sites of Meiliang Bay, but declined at stations further south and east.

3.5. Relationship between TLR and environmental variables

Spearman correlation was conducted to explain the relationship between intracellular and extracellular TLR, as well as with the environmental variables (Table 1). Environmental conditions in Lake

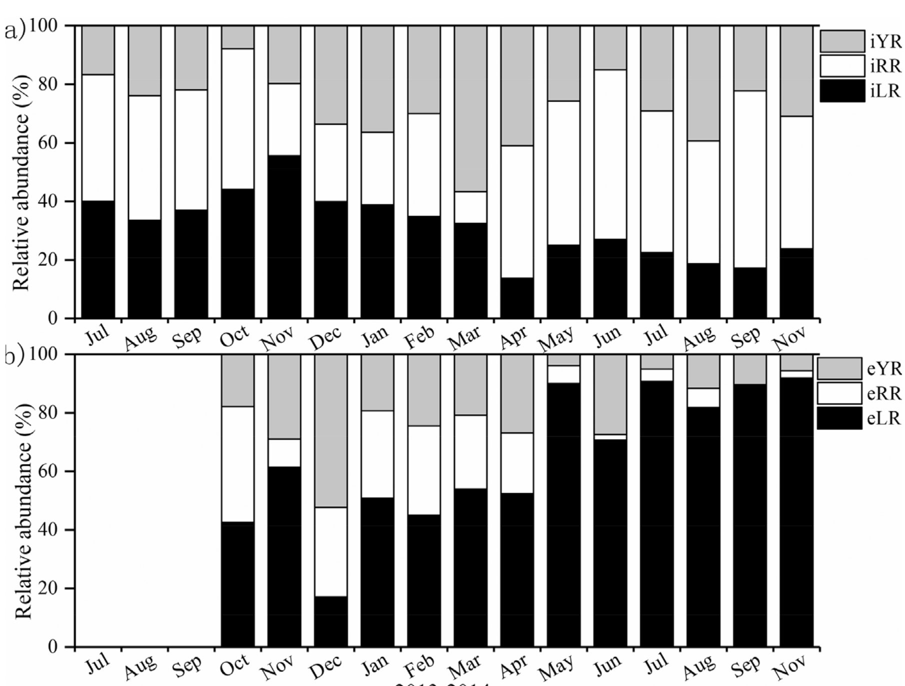

Fig. 4. Relative abundance of three MC congeners (LR, RR, and YR) for intracellular (a) and extracellular (b) MC by month based on the data from fourteen sites in the northern part of Lake Taihu. Extracellular MC data were not collected from July to September 2013.

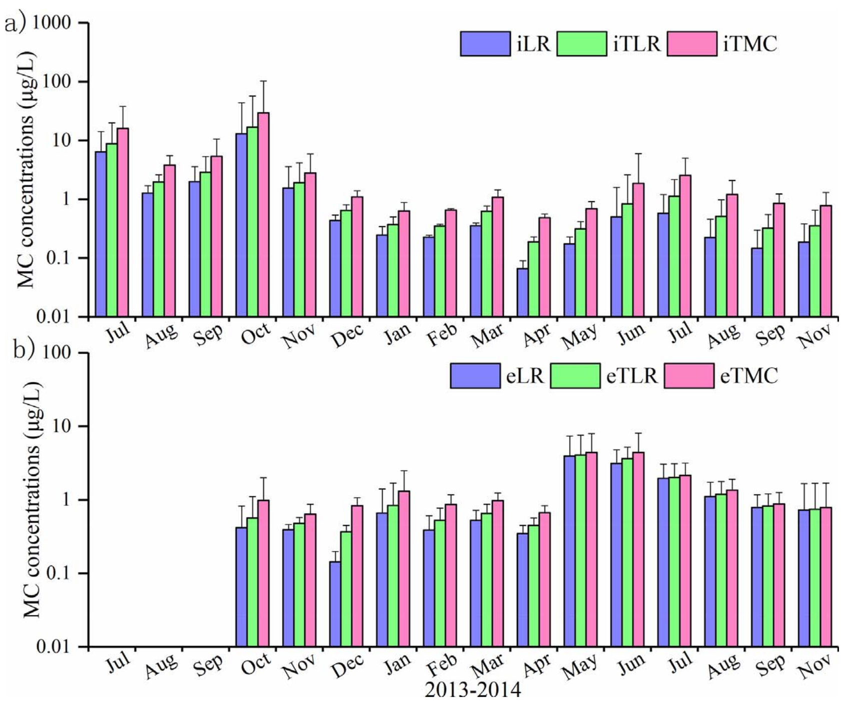

Fig. 5. Concentrations of intracellular (a) and extracellular (b) LR, TLR, and TMC by month based on the data from fourteen sites in the northern part of Lake Taihu. TLR represents the total MC-LR concentration expressed as LR equivalents; TMC represents the total concentration of three MC congeners. Extracellular MC data were not collected from July to September 2013. Note the y-axis scale is logarithmic.

Taihu are shown in Table S1. Significant relationships were found between intracellular and extracellular TLR and LR as well as with TMC ($P < 0.01$). Both iTLR and eTLR in the northern lake and the whole lake showed significant and positive correlations with water temperature ($P < 0.01$, Table 1). iTLR was more strongly related to Chl-a ($P < 0.01$) and cyanobacteria ($P < 0.01$) than eTLR for both the whole lake and northern lake (Table 1). In addition, iTLR was significantly and positively correlated with TP for both the whole lake and northern lake

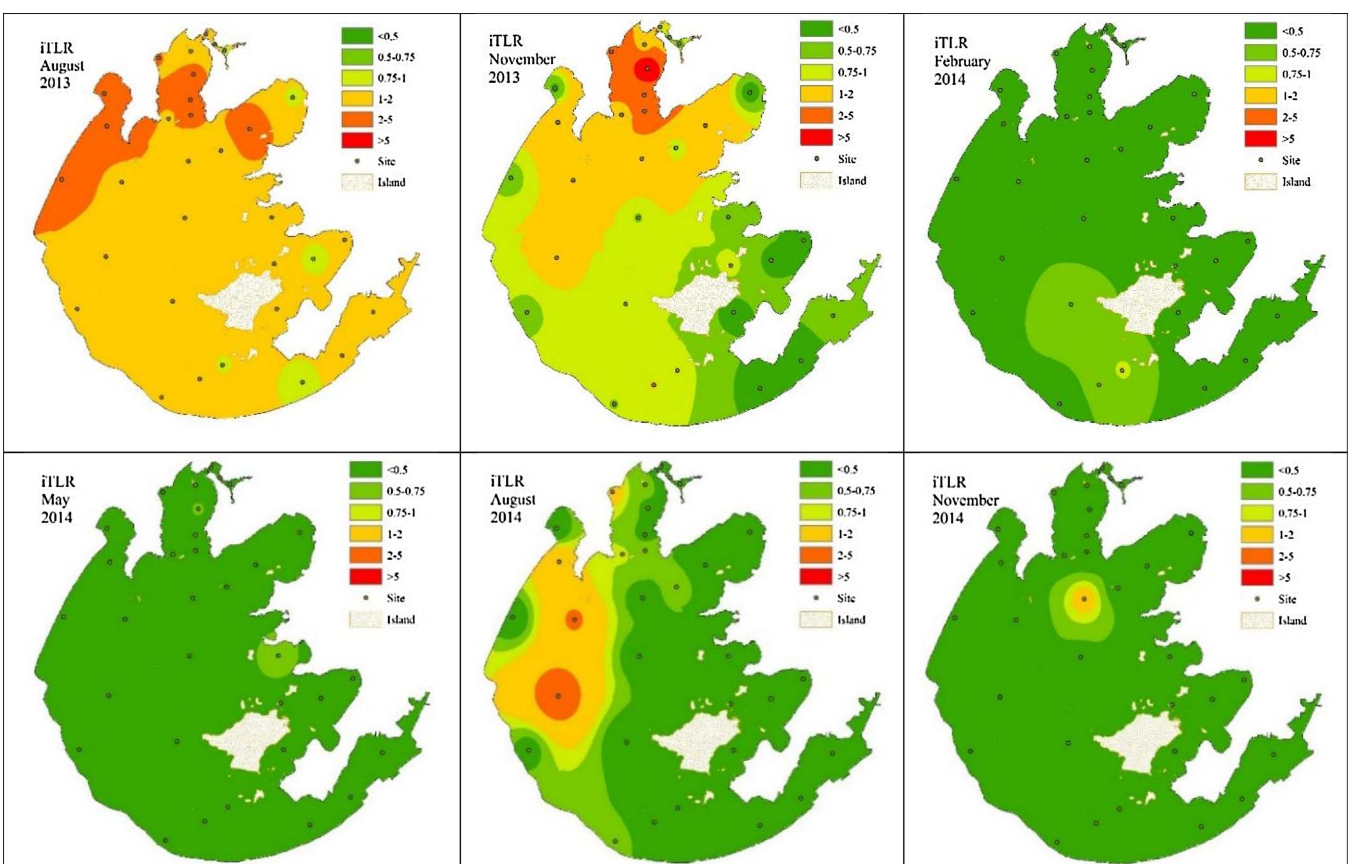

Fig. 6. Spatial distribution of total intracellular LR (iTLR) by season after transforming other MC variants to equal toxicity MC-LR. The data were obtained from thirty-two sites throughout the lake. The interpolation map was generated using ArcGIS 10.1 software with the Inverse Distance Weighting method.

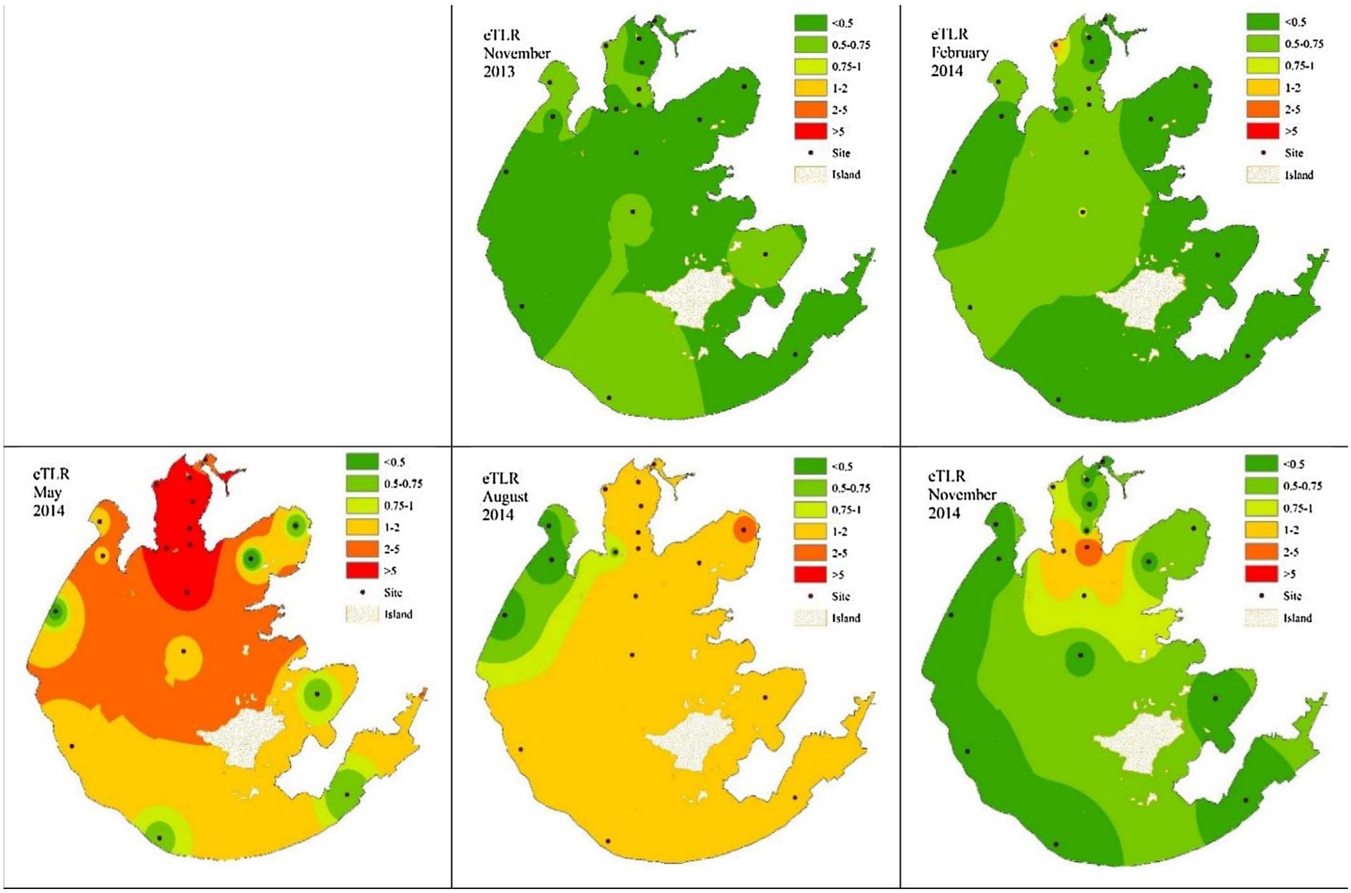

Fig. 7. Spatial distribution of total extracellular LR (eTLR) by season after transforming other MC variants to equal toxicity MC-LR. The data were obtained from eighteen sites (1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 16, 17, 20, 27, 32) throughout the lake. Data were not collected in August 2013. The interpolation map was generated using ArcGIS 10.1 software with the Inverse Distance Weighting method.

Table 1
Spearman correlation analysis between iTLR and eTLR as well as with the environmental variables (including cyanobacterial cell density) both in the northern lake and the whole lake. Significant values are in bold type. ** and * indicate significance at the 0.01 level and the 0.05 level (2-tailed), respectively.

Variables	Northern lake		Whole lake	
	iTLR (n = 224)	eTLR (n = 182)	iTLR (n = 192)	eTLR (n = 90)
LR	**0.987****	**0.937****	**0.972****	**0.971****
TMC	**0.966****	**0.937****	**0.959****	**0.924****
WT	**0.331****	**0.428****	**0.333****	**0.484****
pH	**0.197****	**0.219****	−0.084	**0.348****
DO	−0.098	**−0.151***	**−0.221****	−0.164
Cond	−0.036	**0.589****	**0.162***	**0.609****
TN	−0.085	−0.026	0.124	0.000
TDN	**−0.294****	−0.057	−0.141	0.025
NO_3^--N	**−0.295****	−0.002	0.063	0.063
NO_2^--N	**−0.133***	0.008	**−0.152***	0.047
NH_4^+-N	0.042	**−0.208****	0.109	**−0.275****
TP	**0.351****	**−0.151***	**0.374****	−0.144
TDP	**0.173****	**−0.156***	**0.234****	−0.161
PO_4^{3-}-P	**0.301****	−0.135	**0.346****	−0.198
DIC	**−0.446****	**−0.193****	**−0.322****	−0.030
DOC	**0.449****	**−0.261****	**0.572****	**−0.280****
Chl-a	**0.499****	**0.152***	**0.445****	**0.310****
Cyanobacteria	**0.499****	**0.246****	**0.477****	**0.365****

(both $P < 0.01$), while no such relationship was observed between eTLR and TP. DIC showed a significant and negative correlation with iTLR ($P < 0.01$). In contrast, DOC was significantly and positively correlated with iTLR ($P < 0.01$) both in the northern lake and the whole lake.

Linear regressions were performed between TLR and Chl-a, as well as with cyanobacteria (Fig. 8), given their significant relationships with each other. We calculated the potential safety thresholds for Chl-a concentration and cyanobacterial cell density based on the guideline value for TLR. Due to the different sampling sites in monthly and seasonal sampling, we analyzed the northern lake (n = 182) and the whole lake (n = 90) separately. Thus, the four regression equations derived to estimate the thresholds of Chl-a and cyanobacteria were as follows:

$$TLR = 0.047\ (\text{Chl-a}); \quad (7)$$

$$TLR = 4.52 \times 10^{-9}\ (\text{Cyanobacteria}); \quad (8)$$

$$TLR = 0.043\ (\text{Chl-a}); \quad (9)$$

$$TLR = 8.70 \times 10^{-9}\ (\text{Cyanobacteria}); \quad (10)$$

Where: TLR = total MC-LR (μg/L), Chl-a = chlorophyll a (μg/L), and Cyanobacteria = cyanobacterial cell density (cells/L). (7) and (8) are for the northern lake; (9) and (10) are for the whole lake.

Using a maximum allowable concentration of TLR of 1.0 μg/L, the possible thresholds for Chl-a concentration in the northern lake and the whole lake were 21.28 and 23.26 μg/L, respectively. In addition, the potential thresholds for cyanobacterial cell density in the northern lake and the whole lake were 2.21×10^8 and 1.15×10^8 cells/L, respectively.

4. Discussion

In the last few years, severe water eutrophication and dense cyanobacterial blooms have been common in Lake Taihu, causing major

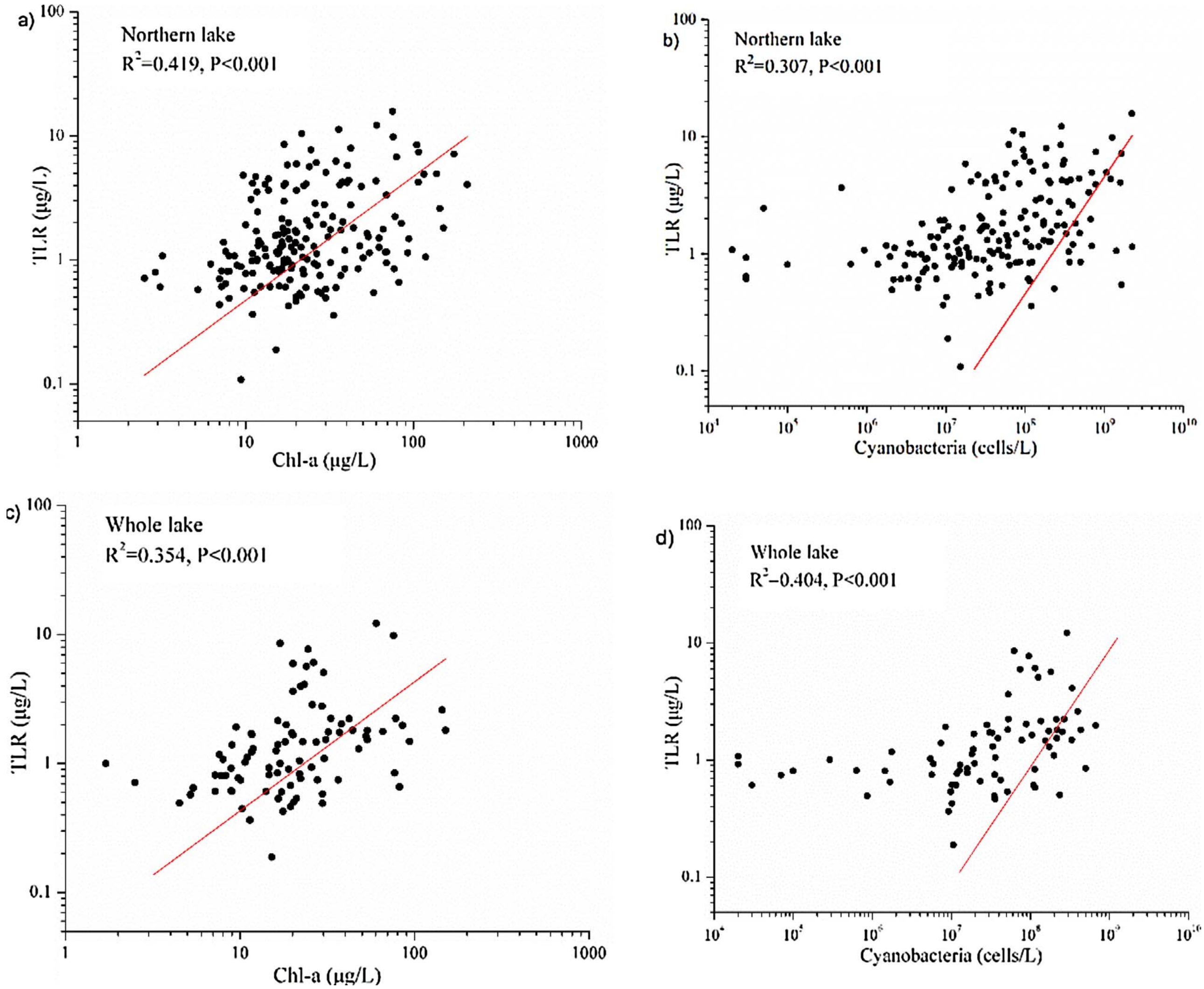

Fig. 8. Linear regression analysis between TLR concentration and both Chl-a concentration and cyanobacterial cell density in the northern lake (a, b) and the whole lake (c, d) of Lake Taihu. Note the axis scales are logarithmic.

ecological problems. Our results indicate that the cyanobacterial densities are temporally and spatially heterogeneous in this large, shallow lake (Fig. 2). The temporal heterogeneity is most likely caused by differences in water temperature among different seasons. Previous studies have shown a positive relationship between water temperature and cyanobacterial dominance, both in the laboratory and in the field for freshwater lakes (Deng et al., 2016; Imai et al., 2009; Kosten et al., 2012; Paerl and Paul, 2012). The average temperatures in the summer (August) in our study were 30.2 °C and 24.6 °C in 2013 and 2014, respectively. The optimal temperature for the growth of cyanobacteria, mainly *M. aeruginosa*, ranges from 25 to 30 °C (Imai et al., 2009), indicating that summer water temperatures in Lake Taihu are favorable for the formation and development of large algae blooms (Deng et al., 2014; Paerl and Huisman, 2008). In addition, we found that the cyanobacteria tended to concentrate mainly in the northern lake, especially in Meiliang Bay, Zhushan Bay, Dapu kou and some open areas close to the lake center. These bays are well-known for their high nutrient concentrations, including all forms of nitrogen and phosphorus, which are the primary limiting factors influencing the occurrence of cyanobacterial blooms (Conley et al., 2009; O'Neil et al., 2012; Xu et al., 2017). Cyanobacteria were dominant over a broad region and for many months (Fig. 3), indicative of strong dispersal and survival capabilities. Although their densities declined in winter, *Microcystis* cells can persist through the winter in Lake Taihu (Ma et al., 2015). In contrast, the eastern lake was characterized by macrophyte dominance with relatively good water quality (Liu et al., 2016), allowing for a geographic separation of the lake into a phytoplankton-dominated region and a macrophyte-dominated region (Qin et al., 2015).

The cyanobacterial blooms during our study period favored the production of massive MC. The frequent occurrence of MC in lakes is a major environmental concern for both drinking water supplies and recreational water use (Paerl and Paul, 2012). Even though there are continuous reports about the occurrence and distribution of MC in Lake Taihu (Table S2), none of them has addressed the spatial distribution of MC in the entire lake. The World Health Organization (WHO) established a provisional guideline of 1 μg/L for MC-LR in drinking water (Falconer, 1999). This recommended guideline applies to dissolved plus the cell-bound MC-LR in the drinking water (Rezaitabar et al., 2017). We propose a new indicator of TLR (total MC-LR concentration that accounts for the toxicity of all three common MC congeners) to reflect the overall toxicity level and evaluate the environmental risks posed by MC in Lake Taihu. There are several reasons for using this TLR index to assess MC contamination in Lake Taihu. First, TLR takes into account different toxicities of MC analogues. Thus, TLR has more direct application than TMC, which merely sums MC analogues and doesn't consider the differences in toxicity and environmental risks posed by different MC analogues. Second, unlike the use of MC-LR, which considers only one (albeit the most toxic) of the MC analogues, TLR integrates the composition and percentage of the three most common MC congeners, facilitating comparisons of MC over spatial and temporal scales. Finally, the TLR index has real-world applicability in terms of drinking water safety. According to the requirements by WHO, both intracellular and extracellular MC are needed to be considered to evaluate the exact concentration of MC in the water column. Intracellular MC will be released to the water when the cyanobacterial cells break down or die, ultimately contributing to extracellular MC. Since assigning the relative toxicity of MC variants is based on clinical trials in mammals (mice), this proposed TLR is aimed at protecting human health. With appropriate validation, TLR may be applied in other lakes or reservoirs when conducting risk assessments about the contamination of MC.

In this study, we also evaluated the temporal pattern of MCs, including intracellular and extracellular MCs both in the northern lake and the whole lake. The temporal dynamics of iTLR and eTLR were similar to the patterns exhibited by MC-LR and TMC. The average concentrations of iLR and eLR both exceeded the WHO's provisional guideline value (1.0 μg/L) for MC-LR in drinking water, albeit by a small absolute amount. The differences in the monthly variation of iTLR and eTLR can be attributed to the intracellular MC production resulting from the abundance of cyanobacteria, specifically toxic *Microcystis* spp.; in contrast, extracellular MC is influenced mostly by natural UV degradation and the presence of bacteria capable of degrading MC (Dziga et al., 2013). Therefore, the concentrations of total MC are dependent on different environmental factors. Because MCs may accumulate in water over time, their concentrations are not necessarily based solely on densities of MC-producers. Our study showed that the concentrations of TLR can reach up to 20.92 μg/L, which is 20 times greater than the drinking water guideline value recommended by WHO; clearly, more studies are warranted on the distribution of MC and its producers. High TLR values were concentrated in the northern lake, similar to the distribution of cyanobacteria, indicating that contamination by MC was most serious in the northern lake. These areas are mostly bays with less advection and long water retention times (Zhang et al., 2016). Once the cyanobacteria are advected or dispersed north by strong winds, they are more likely to form blooms because of nutrient-replete and calmer waters in these bays (Deng et al., 2016). The significant relationship between iTLR and TP suggested that the nutrients promote the production of MC. Therefore, reducing the external and internal loading of nutrients may be helpful to control contamination by MC in Lake Taihu.

Evidence for a strong relationship between Chl-a concentrations and MC production has been confirmed in many field observations, such as in Lake Erie (Yuan et al., 2014) and in Lake Taihu (Otten et al., 2012). In our opinion, the use of the TLR index might be helpful in determining the appropriate safe threshold for Chl-a concentration and cyanobacteria abundance. In this study, we established thresholds for Chl-a and cyanobacteria cell density based on their significant relationships with TLR concentrations. These threshold values of 21.28 μg/L in the northern lake and 23.26 μg/L in the whole lake for Chl-a are less restrictive than WHO's threshold of 10 μg/L (WHO, 2003). A previous study in Lake Taihu established a safe threshold value of Chl-a at 12.42 μg/L based on the correlation coefficient between MC and Chl-a, without consideration of the transformation of other MC variants (Otten et al., 2012). The threshold value in our study is likely greater because we considered MC both in the cell and dissolved in the water. It has been suggested that when Chl-a concentrations in the water exceed 20 μg/L, *Microcystis* begins to form cyanobacterial blooms in Lake Taihu (Xu et al., 2014; Xu et al., 2017).

We also found obvious differences among the possible Chl-a values depending on the selection of MC concentrations (Table S3), indicating the development of TLR will help provide a more accurate evaluation of MC risk and establish desirable safety thresholds of Chl-a concentrations. Given that Chl-a is easier to measure than MC, and reported more frequently, establishing a robust relationship between these two variables is important for lake management purposes. Our results also identified a recommended safe value for cell density of cyanobacteria at 2.21×10^8 cells/L in the northern lake and 1.15×10^8 cells/L in the whole lake. These densities are almost ten times greater than the recommendation by WHO. A similar investigation was conducted in Lake Chaohu, China, where the preliminary safety threshold density for cyanobacteria was 1×10^8 cells/L based on periodic variations in different MC congeners (Shang et al., 2015), which is very close to our proposed threshold. These density thresholds may not apply universally, but they can serve as an early warning system for toxic blooms leading to potential human and ecological impairments (Gee and Stirling, 2003).

5. Conclusion

In summary, we analyzed the long-term seasonal variation and spatial distribution of cyanobacterial abundance in the entirety of Lake Taihu and our results show that cyanobacterial dominance is a continuing problem, greatly contributing to MC accumulation, especially in the northern portion of the lake. We also proposed a new indicator (TLR) to both evaluate contamination by MC and represent the environmental risk of MC in Lake Taihu. The index takes into account the different toxicities of MC variants and both intra- and extracellular forms of MC in the water column. Intracellular and extracellular TLR concentrations were influenced by a number of environmental factors and exhibited different temporal dynamics. In addition, we identified potential thresholds for Chl-a concentration and cyanobacterial cell density for both the northern lake and the whole lake, based on their significantly positive relationships with TLR. Our proposed index, TLR (including iTLR and eTLR), was developed for Lake Taihu, but may be applicable in other lakes, and has lake management implications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ecolind.2017.11.042.

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