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Discussions

Local nutrient regimes determine site-specific environmental triggers of cyanobacterial and microcystin variability in urban lakes

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Abstract

Toxic cyanobacterial blooms in urban lakes present serious health hazards to humans and animals and require effective management strategies. In the management of toxic cyanobacteria blooms, understanding the roles of environmental factors is crucial. To date, a range of environmental factors have been proposed as potential triggers for the spatiotemporal variability of cyanobacterial biomass and microcystins in freshwater systems. However, the environmental triggers of cyanobacteria and microcystin variability remain a subject of debate due to contrasting findings. This issue has raised the question if the environmental triggers are site-specific and unique between water bodies. In this study, we investigated the site-specificity of environmental triggers for cyanobacterial bloom and cyanotoxins dynamics. Our study suggests that cyanobacterial dominance and cyanobacterial microcystin content variability were significantly correlated to phosphorus and iron concentrations. However, the correlations between phosphorus and iron with cyanobacterial biomass and microcystin variability were not consistent between lakes, thus suggesting a site specificity of these environmental factors. The discrepancies in the correlations could be explained by differences in local nutrient concentration and the cyanobacterial community in the systems. The findings of this study suggest that identification of site-specific environmental factors under unique local conditions is an important strategy to enhance positive outcomes in cyanobacterial bloom control measures.

1 Introduction

Urban lakes often serve as recreational spaces for communities and habitats for wildlife (Yan et al., 2012; Liu, 2014). To date, urban lakes continue to deteriorate due to increased anthropogenic activities and often face water quality problems including toxic cyanobacteria bloom (Maria Pineda-Mendoza et al., 2012; Reichwaldt and Ghadouani, 2012; Lei et al., 2014; Sun et al., 2014; Zhang et al., 2014). This issue has received

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great attention from global communities as it presents health hazards to humans and animals who either directly or indirectly received services provided by urban lakes (O'Bannon et al., 2014; Rastogi et al., 2014; Waajen et al., 2014). The management of toxic cyanobacterial blooms is one of the biggest challenges due to the variability cyanobacteria biomass and cyanotoxins (Rolland et al., 2013; Carey et al., 2014).

Cyanobacterial biomass and the amount of microcystins being produced during toxic cyanobacterial blooms can vary significantly on a spatial basis (Reichwaldt et al., 2013; Sinang et al., 2013; Thi Thuy et al., 2014; Waajen et al., 2014). Past studies have found large variations in the percentages of potentially toxic cyanobacteria and microcystin concentration between spatially isolated populations (Sitoki et al., 2012; Li et al., 2014). Furthermore, it was reported that the variability of cyanobacterial biomass in lakes only explained a small fraction of the variability in microcystin concentration (Sinang et al., 2013; Eva and Lindsay, 2014). These findings highlight the importance to fully understand the roles of environmental factors controlling cyanobacteria and microcystin variability.

It has been suggested that cyanobacterial biomass and microcystin variability largely depends upon physical, chemical and biological properties of the water bodies (Engström-Öst et al., 2013; Lehman et al., 2013; Paerl and Otten, 2013; Ruiz et al., 2013). A range of environmental factors, including nitrogen and phosphorus (Schindler, 2012; Srivastava et al., 2012; Chaffin and Bridgeman, 2014; Van de Waal et al., 2014), TN : TP ratio (Smith, 1983; Q. Wang et al., 2010; Van de Waal et al., 2014), temperature (Davis et al., 2009; Rolland et al., 2013), salinity (Tonk et al., 2007), and iron (Ame and Wunderlin, 2005; Nagai et al., 2007; C. Wang et al., 2010) have been shown to have pronounced effects on either cyanobacterial dominance, microcystin production, or both. Nevertheless, the results between studies differ, and there is no clear understanding of the roles of these environmental factors as the triggers of cyanobacterial dominance and microcystin production. It therefore remains as an important challenge for bloom management to fully understand the mechanisms behind toxic cyanobacterial bloom development. For instance, regardless of many studies suggesting the important

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role of phosphorus, reduction of internal and external phosphorus concentration is not always successful in preventing the occurrence of toxic cyanobacterial blooms in water bodies (Lewis and Wurtsbaugh, 2008; Amano et al., 2010; Koreiviene et al., 2014).

By taking into account the contrasting findings of earlier studies including inconsistent outcomes of nutrient reduction strategies, we suggest that the environmental triggers of cyanobacterial and microcystin variability may vary between water bodies. Therefore, the main objective of this study was to investigate the site-specificity of environmental triggers for cyanobacterial biomass and microcystin variability in a local urban lake system. More specifically, the objectives were to (1) determine the variability of cyanobacterial biomass and microcystin concentration in a set of local urban lakes, (2) identify the relationship between environmental factors and cyanobacterial biomass and toxin dynamics bloom in each lake, and (3) investigate the site-specificity of these relationships.

2 Material and methods

2.1 Study lakes

This study was carried out in Jackadder Lake (31°54′30″ S, 115°47′36″ E), Bibra Lake (32°5′25″ S, 115°49′16″ E) and Yangebup Lake (32°6′56″ S, 115°49′33″ E) located on the Swan Coastal Plain, Western Australia. Sampling was carried out between January and March 2010. These lakes are shallow with mean depth of 2.1, 1.1, and 2.5 m in Jackadder Lake, Bibra Lake and Yangebup Lake, respectively. Jackadder Lake and Yangebup Lake are permanent lake while Bibra Lake is subjected to seasonal drying due to progressive decline in groundwater levels over the Jandakot Mound. Jackadder Lake is located in 152 ha catchment and covers a total area of 7.18 ha and is surrounded by 6.6 ha of parkland (Arnold, 1990; Woodward, 2008). Water levels in Jackadder Lake are maintained by the input of surface runoff via 10 drain inlets (Rajah, 1991, as cited in Kemp, 2009). Jackadder Lake receives water from the Herdsman

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Lake catchment area and Osborne Park main drain during dry summers (Department of Planning, 2010). Bibra Lake is located within 250 ha catchment and covers a total area of 135 ha with open water area of approximately 100 ha (Strategen, 2009). This lake is surrounded by urban areas and a golf course (Davis et al., 1993). Western shore of Bibra Lake serves as habitat for many types of water birds (Kemp, 2009). Water enters Bibra Lake via direct rainfall recharge onto the lake surface or from surface runoff from the surrounding catchment (Strategen, 2009). Yangebup Lake covers a total area of 90.5 ha with open water area of approximately 68 ha, and is surrounded by residential, agriculture and industrial areas (Davis et al., 1993). Yangebup Lake is a groundwater through-flow wetland that accepts groundwater from the east and discharges groundwater to the west (Dunlop, 2008). Yangebup Lake receives urban runoff from three stormwater drains and additionally serves as a compensation basin for the South Jandakot Drainage system with an approximate area of 200 km^2. This includes receiving water from neighbouring Thomson Lake when it reaches its maximum water level. Once Yangebup Lake reaches its maximum allowable water level, water is pumped into nearby Cockburn Sound (Environmental Protection Authority, 1989). The hydrology of Jackadder, Bibra and Yangebup lakes is mainly affected by the strong seasonal rainfall pattern due to Mediterranean climate. The region's mean annual rainfall was 772.2 mm and monthly mean rainfall were 10.9, 55.4, 145.9, and 46.7 mm during summer, autumn, winter and spring, respectively (Bureau of Metereology, 2014). In response, the maximum water levels in all lakes occur in September and October after winter, and minimum water levels occur in March and April at the end of summer months (Davis et al., 1993). The region's mean maximum annual temperature was recorded at 24.5 °C and monthly maximum temperature were 30.9, 25.4, 18.0, and 22.6 °C during summer, autumn, winter and spring, respectively (Bureau of Metereology, 2014). Prolonged stable thermal stratification is usually prevented in these lakes during summer due to continuous or intermittent wind mixing that creates a homogeneous environment throughout the water column (Davis et al., 1993; Arnold and Oldham, 1997).

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These lakes were selected due to differences reported on physicochemical properties, levels of cyanobacterial biomass and microcystin concentration. Based on an earlier study, these lakes represent systems with low, medium and high cyanobacterial biomass and microcystin concentration. Mean cyanobacterial biomass $\mu g\,L^{-1}$ was reported as 28 $\mu g\,L^{-1}$ in Jackadder, 108 $\mu g\,L^{-1}$ in Bibra Lake, and 80 $\mu g\,L^{-1}$ in Yangebup Lake. Mean cellular microcystin concentrations ($mg\,g^{-1}$ cyanobacterial dry mass) was 4.8 $mg\,g^{-1}$ in Jackadder Lake, 35 $mg\,g^{-1}$ in Bibra Lake and 1.7 $mg\,g^{-1}$ in Yangebup Lake (Sinang et al., 2013).

2.2 Sampling and analyses

The lakes were sampled bimonthly between January and March 2010. Three samples were collected from the same three points at each lake on every sampling occasion. Bibra Lake dried up in late February; therefore no samples were taken from this lake in March. On-site measurements and samples were taken from shore sites at a water depth of 0.6 to 1 m. Water temperature (Temp), pH and salinity (Sal) were measured on-site with a WP-81 probe (TPS Pty Ltd) at a depth of 0.6 m. Grab water samples for cyanobacteria, microcystin and total phosphorus quantification were taken from approximately 0.15 m below the surface to avoid surface scum. Although there was a slight difference in the depth from which the samples for the physicochemical and water samples were taken, this is not expected to influence the interpretation of the resuls, as an earlier study in these lakes indicated that the water bodies at these shallow shore sites are well mixed with respect to physicochemical variable (Reichwaldt, unpublished data). Water samples were stored immediately in glass bottles in the dark on ice. Parameters analysed from these samples were total phosphorus (TP), total dissolved phosphorus (TDP), total iron (TFe), total dissolved iron (TDFe), total nitrogen (TN), total dissolved nitrogen (TDN), ammonium (NH_4), cyanobacterial biomass, total phytoplankton biomass, intracellular and extracellular microcystin fractions. Samples for dissolved nutrient analyses were pre-filtered with a 0.45 µm syringe filter (Acrodisc, HT Tuffryn) before freezing at −20 °C.

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2.2.1 Nutrients and phytoplankton biomass

TP and TDP concentrations were analyzed using the ascorbic acid method, while TFe and TDFe concentrations were analyzed with the Phenanthroline method, according to standard methods (APHA, 1998). TN, TDN, and NH_4 were analyzed at the South Coast Nutrients Analysis Laboratory, Albany, Western Australia with the standard colorimetric methods on a segmented flow auto-analyser (Alpkem, Wilsonville, OR, USA). Cyanobacterial and total phytoplankton chlorophyll *a* were measured with a top-bench version of a Fluoroprobe (bbe Moldaenke, Germany).The FluoroProbe measures chl *a* fluorescence and differentiates four groups of phytoplankton (chlorophytes, cryptophytes, diatoms, and cyanobacteria) by their specific fluorescence emission spectrum (Beutler et al., 2002). The fluorescence is then transformed through an algorithm and total biomass of each phytoplankton group is expressed as chl *a* concentration equivalents (µg chl $a\,L^{-1}$) (Beutler et al., 2002; Ghadouani and Smith, 2005). FluoroProbe chl *a* measurements were validated against chl *a* data of samples extracted according to standard methods (APHA, 1998) (linear regression analysis: $R^2 = 0.94$, $N = 32$, $P < 0.05$). In our study, chl *a* fluorescence as measured by FluoroProbe was used as a proxy for cyanobacterial biomass (Geis et al., 2000; Eisentraeger et al., 2003).

For quantification of cyanobacterial biomass and to separate the intracellular from the dissolved microcystin fraction, water samples were filtered through pre-combusted and pre-weighed 47 mm GF/C filter papers. Filter papers containing particulate organic matter were dried for 24 h at 60 °C and re-weighed to obtain total dry weight (Harada et al., 1999). These filter papers were then moistened with Milli-Q water and kept frozen (at −20 °C) until intracellular microcystin extraction. As we were interested in the microcystin concentration per unit cyanobacterial dry mass, cyanobacterial dry mass was calculated from the total dry mass (from the filters) by adjusting it to the percentage of cyanobacteria measured with the FluoroProbe. Cyanobacterial dry mass was only used for microcystin quantification.

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Water samples collected for cyanobacterial identification and enumeration were preserved with acidic Lugol's iodine solution (5 g I_2 + 10 g KI, 20 mL distilled water and 50 mL of 10 % acetic acid) and cyanobacteria were identified to the genus level using phytoplankton taxonomic guideline (Komarek and Hauer, 2011). The relative abundance of each cyanobacterial genera (cells or colonies mL^{-1}) was determined from 10–50 mL of sample using an inverse microscope (Utermöhl, 1958) and converted into biovolume per mL ($\mu m^3\ mL^{-1}$) by multiplying the mean cell or colony biovolume (μm^3) with the total cells or colonies per mL (cells or colonies mL^{-1}). Mean cell or colony biovolume for each cyanobacterial genus was calculated by finding the geometric figure that best approximated the shape of each genera, and by measuring the dimension of 20 individual cells or colonies (Hillebrand et al., 1999). A minimum of 200 cells or colonies of the most abundant cyanobacteria were counted for each sample. Different cyanobacterial species within each genus can vary in size by several orders of magnitude. However, as we measured the mean biovolume of each cyanobacterial genus, differences in sizes between species are evened out as a larger mean is expected, if larger species are more abundant and vice versa. The calculated mean biovolume of each cyanobacterial genus was used to compute the dominant cyanobacteria genera in the studied lakes.

2.2.2 Microcystin extraction and quantification

Filters were freeze–thawed twice to break the cells prior to methanol extraction (Lawton et al., 1994). Filters were placed into centrifuge tubes and 5 mL of 75 % methanol-water (v / v) was added. Filters were sonicated on ice for 25 min, followed by gentle shaking for another 25 min. The extracts were then centrifuged at 3273*g* (Beckman and Coulter, Allegra X-12 Series) for 10 min at room temperature. Extracts were carefully transferred into conical flasks, and two more extractions were done per filter. All three extracts were pooled and diluted with Milli-Q to 20 % methanol (v / v).

Intracellular microcystin extracts and the pre-filtered water containing dissolved (extracellular) microcystin were subjected to solid-phase extraction (SPE) (Waters Oasis

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HLB) for clean-up and concentration with a loading speed of < 10 mL min^{-1}. SPE cartridges were then rinsed with 10 mL of 10, 20 and 30 % methanol-water (v / v), before microcystin was eluted with 100 % methanol + 0.1 % trifluoroacetic acid (TFA) and evaporated with nitrogen gas at 40 °C. Finally, samples were re-dissolved in 30 % acetonitrile and analysed with high-performance liquid chromatography (HPLC) by using the Alliance 2695 (Waters, Australia) with a PDA detector (1.2 nm resolution) and an Atlantis T3 3 µm column (4.6 × 150 mm i.d.). Mobile phases used were acetonitrile + 0.05 % v / v TFA and Milli-Q water + 0.05 % TFA. Microcystin peaks were separated using a linear gradient as described in Lawton et al. (1994) but with a maximum acetonitrile concentration of 100 % and a run time of 37 min. Column temperature was maintained at 37.5 ± 2.5 °C. The limit of detection per microcystin peak was 1.12 ng. Microcystin variants were identified based upon their typical absorption spectrum detected by PDA detector at 238 nm (Meriluoto and Codd, 2005). Commercially available microcystin-LR standard (Sapphire Bioscience, Australia; purity ≥ 95 %) was used to quantify microcystin concentrations. Throughout this manuscript we refer to the total concentration of microcystin variants per sample as microcystin concentration.

2.3 Data processing and statistical analyses

Cellular (intracellular) microcystin concentration was expressed as µg (microcystin-LR mass equivalents) per g cyanobacterial dry mass to illustrate cyanobacterial microcystin content. Extracellular microcystin was expressed as the fraction of extracellular microcystin concentration per total microcystin concentration to allow the quantification of the proportion of microcystin released into the water column in comparison to the total microcystin being produced. For all variables, no significant differences (ANOVA, $P > 0.05$) were detected between three samples collected from three different points in each lake per sampling date. Therefore, average values of all variables per sampling date were calculated from the three samples. Between lakes variability of physico-chemical factors, cyanobacterial biomass and microcystin were analysed with one-way

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ANOVA (SPSS 17.0) with post hoc test (Least Significance Difference; LSD) as all assumptions for an ANOVA were met (homogeneity of variances, normality). Bivariate correlation analysis was carried out to identify the environmental variables which significantly correlate with cyanobacterial fraction, cellular microcystin concentration and extracellular microcystin fraction (SPSS 17.0). Site-specificity analysis was performed to analyse if the correlation between environmental variables and cyanobacterial biomass and toxin concentration was similar in all lakes. This was done by comparing the slopes of two linear models (model A and B) (R version 2.15.1). For a particular environmental variable, model A was run to predict dependent variable (Y) (cyanobacterial fraction, cellular microcystin concentration, or extracellular microcystin fraction) from an environmental variable (X). Model B was run to predict the dependent variable from an environmental variable with the inclusion of an interaction between environmental factor and lake (coded as factor). It was then tested, whether the slopes of the regressions between the environmental and dependent variables were significantly different between the two models (A and B), which would indicate lake-specific correlations (ANOVA within linear regression function). Redundancy analysis (RDA) was used to identify the best combination of explanatory variables to explain the variability of cyanobacterial biomass and microcystin concentration (R version 2.15.1) for each lake. Canonical ordination (999 permutations) was computed with standardised explanatory and response variables. All data was log transformed to meet the assumption of normality. In all analyses, results were considered significant at $P < 0.05$.

3 Results

3.1 Physical and chemical characteristics of studied lakes

The three lakes were significantly different in most physicochemical factors (Table 1). Salinity, phosphorus, nitrogen and iron, either as total or dissolved forms (except TDFe), were significantly different between all lakes (one-way ANOVA). The posthoc

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tests (LSD) indicates that Jackadder and Yangebup Lake did not differ in TP, TDP, and NH_4, however, both lakes were different to Bibra Lake. Furthermore, all lakes were different in salinity, TN, TDN, and TFe. Jackadder and Yangebup Lakes can be classified as eutrophic, while Bibra Lake can be classified as hypereutrophic, based on the mean TP concentrations (Carlson, 1977). Nitrogen limited condition in a lake is usually defined when TN : TP weight ratio is less than 10 (Graham et al., 2004). As our result showed that TN : TP ratios below 10 were rare, the studied lakes were not associated with persistent nitrogen limitation.

3.2 Variability of cyanobacterial biomass and microcystin concentration

Cyanobacterial communities in all lakes contained potentially toxin-producing cyanobacteria including *Microcystis* spp., *Planktothrix* spp., *Anabaenopsis* spp., *Anabaena* spp and *Nodularia* spp. (Fig. 1) with *Microcystis* spp. being the most abundant cyanobacterial genera in all lakes. Mean total cyanobacterial biomass was 5.41, 29.60, 15.14 $\mu g\,L^{-1}$ in Jackadder, Bibra and Yangebup Lake, respectively (Fig. 2a). Cyanobacterial biomass varied within an order of magnitude on a temporal basis in Bibra and Jackadder Lake (Jackadder: 1–12 $\mu g\,L^{-1}$, Bibra: 5–83 $\mu g\,L^{-1}$, Yangebup: 8–32 $\mu g\,L^{-1}$) (Fig. 2a). Although cyanobacterial biomass was significantly higher in Bibra Lake compared to the other two lakes ($F_{(2,45)} = 7.62$, $P < 0.05$), the cyanobacterial fraction (the ratio of cyanobacterial chlorophyll *a* to total phytoplankton chlorophyll *a*) in this lake was significantly lower than in Jackadder and Yangebup Lake ($F_{(2,45)} = 3.59$, $P < 0.05$) (Fig. 2b). Cyanobacterial fraction ranged between 0.05 to 0.71 in Jackadder Lake, 0.16 to 0.68 in Yangebup Lake, and 0.11 to 0.51 in Bibra Lake. The post hoc tests indicated that Jackadder and Yangebup Lakes did not differ in cyanobacterial biomass and cyanobacterial fraction, but both lakes were different to Bibra Lake.

Cellular microcystin concentration ($mg\,g^{-1}$ cyanobacterial dry mass) varied over three orders of magnitude in Jackadder Lake, and two orders of magnitude in both Bibra Lake and Yangebup Lake (Fig. 2c) throughout the sampling events. Mean cellular microcystin concentrations were 0.407 $mg\,g^{-1}$ in Jackadder Lake, 0.233 $mg\,g^{-1}$

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in Bibra Lake, and 0.150 mg g^{-1} in Yangebup Lake. Cellular microcystin concentration was not significantly different between lakes ($F_{(2,45)} = 2.07$, $P > 0.05$). Mean extracellular microcystin fraction was 0.18 in Jackadder Lake, 0.04 in Bibra Lake, and 0.26 in Yangebup Lake (Fig. 2d). The post hoc tests indicated that Bibra Lake was the only lake that had a significantly different extracellular microcystin fraction when compared to other lakes ($F_{(2,45)} = 6.49$, $P < 0.05$).

3.3 Relationship between environmental factors and cyanobacterial fraction or microcystin concentration

Most environmental factors were weakly, but significantly correlated with cyanobacterial fraction and microcystin concentrations when data from all lakes were combined (Table 2). Cyanobacterial dominance in the phytoplankton community was favored under low nutrient concentrations as it was negatively correlated to TP, TDP, TFe, and TDFe. In contrast, cyanobacterial dominance was positively correlated with TN : TP ratio. Cellular microcystin concentration was positively correlated with most nutrients, except TN and TDN. TDFe showed the strongest positive correlation with cellular microcystin concentration, followed by TP, TFe, and TDP. Cellular microcystin was also negatively correlated with TN : TP ratio and NH_4. In contrast to cellular microcystin, extracellular microcystin fraction was negatively correlated with salinity, TP, TDP, TN, TDN, and positively correlated with TN : TP ratio.

3.4 Site-specific relationship between environmental factors and cyanobacterial fraction or microcystin concentration

Most correlations between environmental factors and cyanobacterial fraction, cellular microcystin concentration or extracellular microcystin fraction were different for each lake (Table 2). The correlations between cyanobacterial fraction and TP, TDP, TFe and TN : TP ratios were significantly different between lakes, while the correlation with TDFe was consistent between lakes. In terms of cellular microcystin concentration, the

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correlations with TP, TDP, TFe and NH_4 were significantly different between lakes, while the correlations with TDFe and TN : TP ratio were consistent between lakes. The correlations between extracellular microcystin fraction and salinity, TP, TDP, TN and TDN were significantly different between lakes, while the correlation with TN : TP ratio was consistent between lakes.

3.5 Multivariate analysis of environmental factors and the variability of cyanobacterial fraction and microcystin concentration

RDA analysis showed significant relationships between the measured environmental factors and the combined variability of cyanobacterial fraction, cellular microcystin concentration and extracellular microcystin fraction for each lake. The canonical ordination showed that 72, 80 and 70 % (Jackadder, Bibra and Yangebup Lakes, respectively) of the combined variability of cyanobacterial fraction, cellular microcystin concentration and extracellular microcystin fraction can be explained by the measured environmental factors (Fig. 3a–c). The environmental factors that were closely correlated to the cyanobacterial fraction, cellular microcystin concentration and extracellular microcystin fraction were not always the same between lakes. TDP was only correlated to either cyanobacterial fraction or cellular microcystin concentration in Bibra and Jackadder Lakes (Fig. 3a and b) but not in Yangebup Lake (Fig. 3c). Additionally, TFe was positively correlated to cyanobacterial fraction only in Bibra Lake (Fig. 3b) but not in the other two lakes (Fig. 3a and c). In comparison to the other factors, TDFe was always consistently correlated to cyanobacterial and microcystin variability.

4 Discussion

The correlation between the environmental factors and cyanobacterial and microcystin variability were different between lakes. This is a strong indication that the environmental triggers of cyanobacterial fraction, cellular microcystin concentration, and

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extracellular microcystin fraction are site-specific and unique among lakes. Our results suggest that the site-specificity of environmental triggers may be related to spatial heterogeneity of the respective environmental factor, as each factor could present at different concentration regimes in each lake. Graham et al. (2004) and Dolman et al. (2012) have suggested that the correlations between the environmental factors and cyanobacterial biomass and microcystin concentration could change when the concentrations of the respective environmental factors increased from low to high in systems. Our results support these previous findings as the between lakes consistencies in the correlations between cyanobacterial and microcystin variability with TP, TFe and TDFe were closely related to the levels of TP, TFe and TDFe concentrations in each lake. Mean TP and TFe concentrations in Bibra Lake were one order of magnitude higher than in Jackadder and Yangebup Lakes, while mean TDFe concentrations in all lakes ranged within the same order of magnitude (Table 1). Consequently, the correlations between cyanobacterial and microcystin variability with TP and TFe were significantly different across lakes, while TDFe was not (Table 2).

The site-specificity of the environmental triggers of cyanobacterial and microcystin variability may be also subjected to variation of cyanobacterial communities between the systems. TFe was negatively correlated to cyanobacterial fraction in Jackadder Lake, while in Bibra Lake, a positive correlation was shown between the two (Fig. 3a and b). Cyanobacterial community in Jackadder Lake was composed of only one nitrogen-fixing cyanobacterial genera (Fig. 1). In contrast, multiple nitrogen-fixing cyanobacterial genera were present in Bibra Lake. Nitrogen-fixing cyanobacteria are known to utilize more iron in comparison to non nitrogen-fixers (Wilhelm, 1995). Therefore, the site-specific correlation between TFe and cyanobacterial fraction may be explained through a greater iron requirement in cyanobacterial community in Bibra Lake, in comparison to the cyanobacterial community in Jackadder Lake.

Due to the potentially site-specific environmental triggers of cyanobacterial and microcystin variability, the results presented in this study are important to the local lakes or lakes with similar physical, chemical and biological characteristics. In this study, the

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variability of cyanobacterial fraction was negatively correlated with TP, TDP, TFe, TDFe; positively correlated with TN : TP ratio. These correlations illustrate the cyanobacterial ability to dominate under low phosphorus availability (Briand et al., 2008; Amano et al., 2010).The storage adaptation strategy to phosphorus deficiency in cyanobacteria suggests that cyanobacteria can store a substantial amount of internal phosphorus and thereby gain a competitive advantage (Pettersson et al., 1993, as cited in Hyenstrand et al., 1998). On the other hand, phosphorus rich conditions are potentially limiting the ability of cyanobacteria to become dominant in the phytoplankton community (Chorus and Bartram, 1999; Reynolds et al., 2006). This is due to the ability of other phytoplankton groups to dominate under nutrient rich conditions in systems as they can grow faster than cyanobacteria. Therefore, phosphorus storage strategy coupled with slower growth rate in cyanobacteria supports the negative correlation between cyanobacterial fraction and phosphorus concentration found in our study. In terms of iron, low availability was correlated to high cyanobacterial fraction in these lakes. This result indicated that cyanobacteria pose a competitive advantage to dominate the phytoplankton community under low iron availability. Cyanobacteria are capable to alter their cellular iron requirements, and increase the ability to utilize iron at a low concentration, through the present of siderophores (Boyer et al., 1987; Lee et al., 2011). As reported in the Nagai et al. (2007), cyanobacteria including *Microcystis* spp. and *Planktothrix* spp. can produce siderophores and become a superior competitor under iron limited conditions. These results indicate that phosphorus and iron reduction in water bodies might not be a sufficient remedial strategy against the occurrence of toxic cyanobacterial bloom.

In contrast to cyanobacterial fraction, the variability of microcystin concentration was positively correlated to TP, TDP, TFe, TDFe and negatively correlated with TN : TP and NH_4. High availability of phosphorus is required for energy and material supply in microcystin biosynthesis as microcystin production in cyanobacterial cells is an energy intensive process (Vezie et al., 2002). This is further supported through the observed negative correlation between cellular microcystin and TN : TP ratio, as low microcystin production is expected under phosphorus limited conditions. In addition, the

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positive correlation between iron and cellular microcystin concentration is in agreement with earlier studies which suggested that iron plays an essential role in many metabolic pathways including microcystin biosynthesis in cyanobacteria (Jiang et al., 2008; C. Wang et al., 2010).

These results illustrate that reducing phosphorus and iron concentrations in water bodies will potentially reduce the overall toxicity of cyanobacterial bloom, even though it might not completely prevent from the occurrence of cyanobacterial bloom. In terms of NH_4, our results suggest that reducing NH_4 concentrations may be associated with higher microcystin concentration. This is possible as toxic cyanobacterial genotypes are known to be favored under low inorganic nitrogen conditions (Ame et al., 2003).

Environmental conditions influencing the release of microcystin into the environment, besides cells lyses, are-not well understood (Rohrlack and Hyenstrand, 2007; Barrington et al., 2013). Our results showed that correlations exist between extracellular microcystin fraction and nutrients, however, the correlation could be direct or indirect ones. If they are direct ones, our results suggest that cyanobacteria may release more microcystin into the surrounding water, under low nitrogen and phosphorus concentrations. These observations are potentially supported by the hypothesis that microcystin is involved in nutrient competition in the phytoplankton community (Huisman and Hulot, 2005).

5 Conclusions

The current approach to water body restoration and the prevention of toxic cyanobacterial blooms relies on reducing nutrient loading into water bodies and limiting the availability of nutrients in the water column. This approach might not always be successful in preventing the occurrence of cyanobacterial blooms, due to the roles of physico-chemical factors on cyanobacteria and microcystin variability being site-specific and subject to unique local conditions. Thus, it is important to take into account the effect of spatial heterogeneity in the management of toxic cyanobacterial blooms. Site-specific

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studies may be required to determine the factors causing cyanobacterial dominance and microcystin production in different systems with different characteristics such as the hydrology, land use and water chemistry.

In our study, the dominance of cyanobacteria in the phytoplankton community is correlated to low phosphorus and iron concentrations in the systems. In contrast, cyanobacteria require high phosphorus and iron concentrations in the water column to produce a high amount of microcystin. Therefore, reducing phosphorus and iron concentration in the water column might not be a sufficient remedial strategy against the occurrence of toxic cyanobacterial bloom, if these nutrients are still available in sufficient amount to support the growth of highly competitive cyanobacteria. However, reducing phosphorus and iron is likely to reduce the amount of microcystin being produced within cyanobacterial cells.

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Table 1. Physical and chemical properties of the three lakes throughout the sample period (January–March 2010).

Factors	Jackadder Lake (N = 18)		Bibra Lake (N = 12)		Yangebup Lake (N = 18)		ANOVA
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
pH	8.7 ± 0.3	8.1–9.0	8.9 ± 0.2	8.5–9.2	8.9 ± 0.4	7.5–9.3	$F_{(2,45)} = 2.16$
Sal (ppk)	0.4 ± 0.04	0.3–0.4	2.9 ± 1.0	1.7–4.1	0.9 ± 0.1	0.8–1.1	$F_{(2,45)} = 99.08^{*}$
DO (ppm)	8.3 ± 2.3	6.1–15.3	8.8 ± 3.8	5.6–15.2	9.1 ± 1.7	7.5–14.3	$F_{(2,45)} = 0.42$
TP ($\mu g\,L^{-1}$)	44.0 ± 28.0	20.0–131.6	598.1 ± 362.0	214.7–1145.9	64.8 ± 44.2	24.0–168.0	$F_{(2,45)} = 40.28^{*}$
TDP ($\mu g\,L^{-1}$)	17.6 ± 4.8	12.0–26.7	67.9 ± 51.3	16.0–18.0	23.2 ± 7.6	13.3–40.7	$F_{(2,45)} = 15.27^{*}$
TFe ($\mu g\,L^{-1}$)	123.3 ± 66.2	63.6–261.8	192.1 ± 43.4	138.2–289.3	81.5 ± 24.1	48.4 –122.9	$F_{(2,45)} = 18.91^{*}$
TDFe ($\mu g\,L^{-1}$)	69.2 ± 66.3	20.0–200.0	89.1 ± 30.4	38.6–154.1	52.9 ± 28.9	11.2–92.6	$F_{(2,45)} = 2.15$
NH_4 ($\mu g\,L^{-1}$)	100.8 ± 54.9	30.0 –180.0	191.5 ± 33.8	150.0–250.3	86.3 ± 45.6	30.0–160.0	$F_{(2,45)} = 20.04^{*}$
TN ($mg\,L^{-1}$)	1.3 ± 0.4	0.7–2.2	11.7 ± 5.2	4.9–17.3	3.5 ± 0.8	1.9–5.2	$F_{(2,45)} = 59.38^{*}$
TDN($mg\,L^{-1}$)	0.8 ± 0.2	0.4–1.1	8.7 ± 3.0	4.9–14.0	2.4 ± 0.3	1.9–2.8	$F_{(2,45)} = 104.98^{*}$
TN : TP	35.6 ± 14.9	11.1–76.1	23.1 ± 10.0	10.3–41.1	68.6 ± 29.9	25.0–124.1	$F_{(2,45)} = 19.51^{*}$

N = number of samples.
SD = standard deviation.
* = $P < 0.05$.

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Table 2. Correlation coefficients (R) between the environmental factors and cyanobacterial fraction, cellular microcystin concentration and extracellular microcystin fraction analyzed from combined data from all lakes using bivariate correlation analysis.

Factor $N = 48$	Cyanobacterial Fraction (%)	Cellular microcystin concentration ($\mu g\,g^{-1}$)	Extracellular microcystin fraction (%)
Sal (ppk)	−0.250	0.067	**−0.374***
TP ($\mu g\,L^{-1}$)	**−0.337***	**0.399***	**−0.392***
TDP($\mu g\,L^{-1}$)	**−0.357***	**0.296***	**−0.427***
TFe ($\mu g\,L^{-1}$)	**−0.570***	**0.343***	−0.037
TDFe ($\mu g\,L^{-1}$)	**−0.777**	**0.590**	−0.064
NH_4 ($\mu g\,L^{-1}$)	0.105	**−0.267***	−0.114
TN ($mg\,L^{-1}$)	−0.236	0.085	**−0.375***
TDN ($mg\,L^{-1}$)	−0.265	0.095	**−0.400***
TN : TP	**0.423***	**−0.446**	**0.386**

Significant ($P < 0.05$) factors are highlighted in bold.
* Indicates a significant differences between the slopes of the regressions lines between lakes when tested with ANOVA within linear regression function.
N = number of samples.

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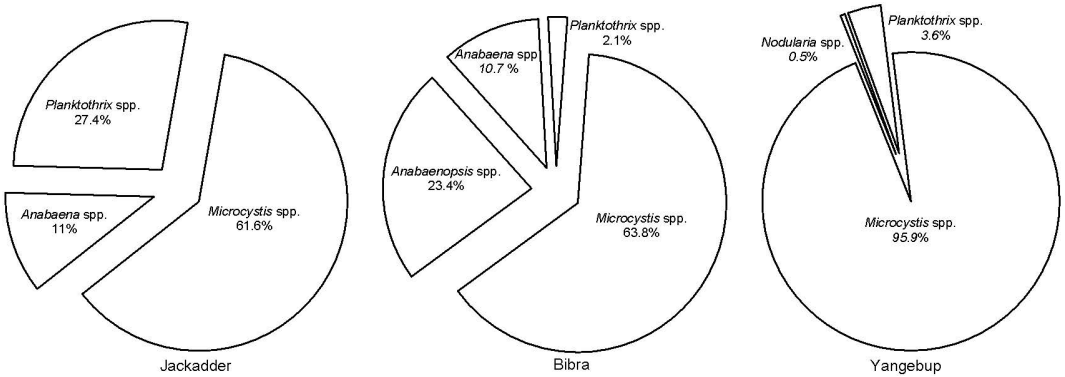

Figure 1. Average biomass ($\mu m^3\,m\,L^{-1}$) proportions of different cyanobacterial genera in Jackadder, Bibra and Yangebup lakes during the study period.

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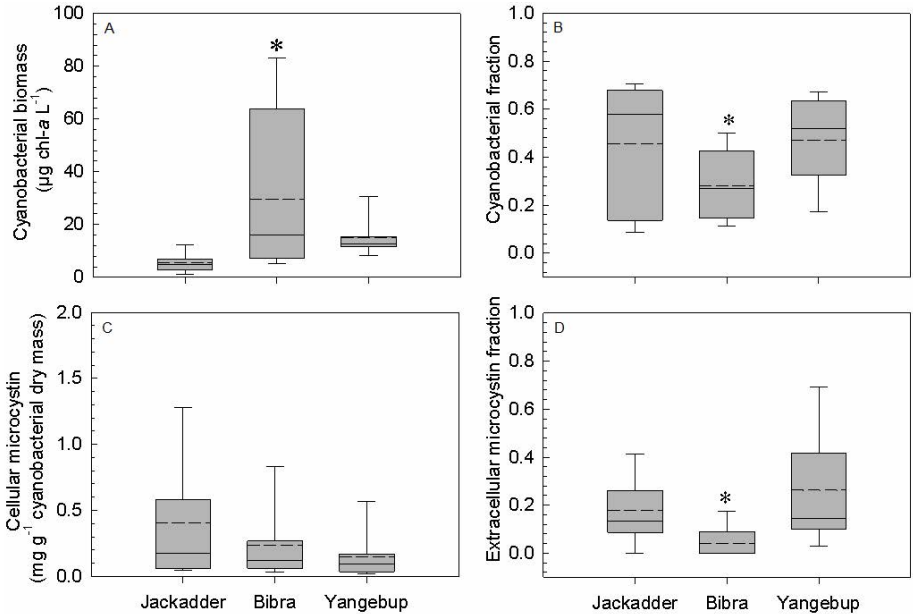

Figure 2. The variability of **(a)** cyanobacterial biomass (µg chl *a* L^{-1}), **(b)** cyanobacterial fraction (cyanobacterial biomass to total biomass), **(c)** cellular microcystin concentration (mg g^{-1} cyanobacterial dry mass) and **(d)** extracellular microcystin fraction over time for each lake. Boxes represent 25th to 75th percentiles; straight lines within the boxes mark the median short dashed lines the mean; whiskers below and above the boxes indicate 10th and 90th percentiles. Asterisks (*) indicated lakes that are significantly ($P < 0.05$) different from other lakes.

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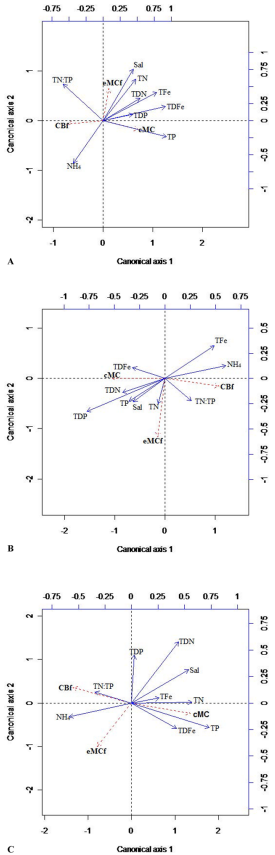

Figure 3. RDA biplots of environmental variables with cyanobacterial fraction (CBf), cellular microcystin (cMC) and extracellular microcystin fraction (eMCf) in **(a)** Jackadder Lake, **(b)** Bibra Lake, **(c)** Yangebup Lake; solid arrows = environmental variables; short dashed arrows = response variables. Canonical axis 1 and 2 represents a linear combination of the environmental variables, and axes are scaled by the square root of their eigenvalues.

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