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. Managing such blooms requires a sufficient understanding of the controlling environmental factors. A range of them has been proposed in the literature as potential triggers for cyanobacterial biomass development and cyanotoxin (e.g., microcystin) production 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 relevance of environmental triggers may depend on site-specific combinations of environmental factors. In this study, we investigated the site-specificity of environmental triggers for cyanobacterial bloom and microcystin dynamics in three urban lakes in Western Australia. Our study suggests that cyanobacterial biomass, cyanobacterial dominance and cyanobacterial microcystin content variability were significantly correlated to phosphorus and iron concentrations. However, the correlations were different between lakes, thus suggesting a site specific effect of these environmental factors. The discrepancies in the correlations could be explained by differences in local nutrient concentration. For instance, we found no correlation between cyanobacterial fraction and total phosphorous (TP) in the lake with the highest TP concentration, while correlations were significant and negative in the other two lakes. In addition, our study indicates that the difference of the correlation between TFe and the cyanobacterial fraction between lakes might have been a consequence of differences in the cyanobacterial community structure, specifically the presence or absence of nitrogen-fixing species. In conclusion, our study suggests that identification of significant environmental factors under site-specific conditions is an important strategy to enhance successful outcomes in cyanobacterial bloom control measures.

Keywords: Cyanobacterial variability; Microcystin variability; Environmental triggers; Nutrients; Site-specific; Bloom management.

1 Introduction

Urban lakes often serve as recreational spaces for communities and habitats for wildlife (Yan et al., 2012; Liu, 2014) . To date, many urban lakes continue to deteriorate due to increased anthropogenic activities and often face water quality problems including toxic cyanobacteria blooms (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 great attention from water authorities world-wide 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 often challenging due to the variability in cyanobacteria biomass and microcystins (Rolland et al., 2013; Carey et al., 2014). In addition, microcystin production by cyanobacteria is a complex issue that might depend on their competition with other phytoplankton (e.g., Huisman and Hulot, 2005; Jang et al., 2006). From these earlier studies it can be concluded that the toxin concentration produced by a certain cyanobacterial biomass level might differ, depending on the level of competition (i.e. cyanobacterial fraction) indicating that management should consider biomass and cyanobacterial fractions concurrently.

Cyanobacterial biomass and the amount of microcystins being produced during toxic cyanobacterial blooms can vary significantly on a spatial basis within and between lakes (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 percentage of potentially toxic cyanobacteria and in

the microcystin concentration between spatially isolated phytoplankton communities (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 both, the cyanobacteria and the 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 concentrations (Schindler, 2012; Srivastava et al., 2012; Chaffin and Bridgeman, 2014; Van de Waal et al., 2014), TN:TP ratio (Smith, 1983; Wang et al., 2010b; Van de Waal et al., 2014), temperature (Davis et al., 2009; Rolland et al., 2013), salinity (Tonk et al., 2007), and iron concentration (Ame and Wunderlin, 2005; Nagai et al., 2007; Wang et al., 2010a) have been shown to have pronounced effects on cyanobacterial biomass, cyanobacterial dominance and microcystin production. Nevertheless, the results between studies differ, and there is no clear understanding of the roles of these environmental factors as the triggers of cyanobacterial bloom development and microcystin production. Furthermore, the occurrence of cyanobacterial toxins in a system is the result of a complex interaction between abiotic and biotic factors, including the competition with other phytoplankton. It therefore remains an important challenge for bloom management to fully understand the mechanisms behind toxic cyanobacterial bloom development and the drivers for biomass development, cyanobacterial dominance (fraction) and toxin production. For instance, regardless of the fact that many studies suggesting the important 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 main environmental triggers of cyanobacterial and microcystin variability may vary between water bodies due to the complex, lake specific interplay of environmental conditions. 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 site-specific relationships between environmental factors and cyanobacterial or microcystin dynamics.

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 (Fig. 1). Sampling was carried out between January and March 2010. These lakes are shallow with mean depth of 2.1 m, 1.1 m, and 2.5 m for Jackadder Lake, Bibra Lake and Yangebup Lake, respectively. Jackadder Lake and Yangebup Lake are permanent lakes while Bibra Lake is subjected to seasonal drying due to progressive decline in groundwater levels over the Jandakot Mound. Jackadder Lake has an area of 7.18 ha, is surrounded by 6.6 ha of parkland and is draining a 152 ha catchment area, (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 Lake catchment area and Osborne Park main drain during dry summers (Department of Planning, 2010). Bibra Lake has a size of 135 ha with an open water area of approximately 100 ha (Strategen, 2009) and is located within a 250 ha catchment are. This lake is surrounded by urban areas and a golf course and serves as habitat for many species 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 has a total area of 90.5 ha with an open water area of approximately 68 ha, and is surrounded by residential, agriculture and industrial areas. 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 the Mediterranean climate. The region's mean annual rainfall is reported as 771.5 mm and monthly mean rainfall is 35.1 , 156.3 , 433.3 , and 144.2 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, and the 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 is 24.5 °C and monthly maximum temperature are 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

151 these lakes during summer due to continuous or intermittent wind mixing that creates a
152 homogeneous environment throughout the water column (Davis et al., 1993; Arnold and
153 Oldham, 1997).
154
155 These lakes were selected due to differences reported on physicochemical properties, levels
156 of cyanobacterial biomass and microcystin concentration. Based on an earlier study
157 conducted between November 2008 and July 2009 (Sinang et al., 2013), these lakes represent
158 systems with low, medium and high cyanobacterial biomass and microcystin concentration.
159 In this earlier study, the highest cyanobacterial biomass was reported as 28, 108, and 80 µg
160 chl-*a* L^{-1} in Jackadder, Bibra and Yangebup Lake, respectively. The highest cellular
161 microcystin concentrations (mg g^{-1} cyanobacterial dry mass) was 4.8 mg g^{-1} in Jackadder
162 Lake, 35 mg g^{-1} in Bibra Lake and 1.7 mg g^{-1} in Yangebup Lake (Sinang et al., 2013).
163
164 **2.2 Sampling and analyses**
165 The lakes were sampled twice a month between January and March 2010, leading to 6
166 sampling days. Three samples were collected from the same three points at each lake on
167 every sampling occasion. As Bibra Lake dried up in late February no samples were taken
168 from this lake in March, leading to only 4 sampling days. On-site measurements and samples
169 were taken from shore sites at a water depth of 0.6 to 1 m. Temperature (Temp), pH and
170 Salinity (Sal) were measured on-site with a WP-81 probe (TPS Pty Ltd) at a depth of 0.6 m.
171 Grab water samples for cyanobacteria, microcystin and total phosphorus quantification were
172 taken from approximately 0.15 m below the surface to avoid surface scum. Although there
173 was a slight difference in the depth from which the samples for the physicochemical and
174 water samples were taken, this is not expected to influence the interpretation of the results, as
175 earlier studies in these lakes indicated that the water bodies at these shallow shore sites are

well mixed with respect to physicochemical conditions (Arnold and Oldham, 1997; Song et al., 2015) (Fig. 2). Water samples were stored immediately in glass bottles in the dark on ice. Variables 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 0.45µm syringe filter (Acrodisc, HT Tuffryn) before freezing at -20°C.

Surface water temperatures were between 19.9 and 28.7°C during the study period. However, the onsite measurements of surface water temperatures were dependent on the time of sampling and varied by up to 3.9°C over the course of a day. Therefore, maximum air temperature on each sampling day recorded by weather stations located nearest to the studied lakes was used as a substitute for surface water temperature in all analyses (Yen et al., 2007).

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 used to

calculate total biomass of each phytoplankton group that 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 hours 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.

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 (μm^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 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-

251 performance liquid chromatography (HPLC) by using the Alliance 2695 (Waters, Australia)
252 with a PDA detector (1.2 nm resolution) and an Atlantis T3 3µm column (4.6 x 150mm i.d).
253 Mobile phases used were acetonitrile + 0.05% v/v TFA and Milli-Q water + 0.05% TFA.
254 Microcystin peaks were separated using a linear gradient as described in Lawton et al., (1994)
255 but with a maximum acetonitrile concentration of 100% and a run time of 37 min. Column
256 temperature was maintained at 37.5 ± 2.5 °C. The limit of detection per microcystin peak was
257 1.12 ng. Microcystin variants were identified based upon their typical absorption spectrum
258 detected by PDA detector at 238 nm (Meriluoto and Codd, 2005). Commercially available
259 microcystin-LR standard (Sapphire Bioscience, Australia; purity ≥ 95 %) was used to
260 quantify microcystin concentrations. Throughout this manuscript we refer to the total
261 concentration of microcystin variants per sample as microcystin concentration.
262
263 In this study, cellular (intracellular) microcystin concentration was expressed as µg
264 microcystin-LR mass equivalents per g cyanobacterial dry mass to illustrate cyanobacterial
265 microcystin content. Extracellular microcystin was expressed as the fraction of extracellular
266 microcystin concentration per total microcystin concentration to allow the quantification of
267 the proportion of microcystin released into the water column in comparison to the total
268 microcystin being produced.
269
270 **2.3 Data processing and statistical analyses**
271 Differences in physicochemical factors, cyanobacterial biomass and microcystin between
272 lakes were analysed with one-way ANOVA (SPSS 17.0) with post hoc test (Least
273 Significance Difference; LSD) as all assumptions for an ANOVA were met (homogeneity of
274 variances, normality). For the descriptive phase, bivariate correlation analysis (Pearson's)
275 was carried out to identify the environmental variables which significantly correlate with

276 cyanobacterial fraction, cyanobacterial biomass, cellular microcystin concentration and
277 extracellular microcystin fraction (SPSS 21.0). We used linear mixed models to identify
278 correlations between environmental variables and cyanobacterial fraction, cyanobacterial
279 biomass, cellular microcystin concentration and extracellular microcystin fraction in each
280 lake using sampling site and sampling date as random factors, and for all lakes combined
281 adding lake as random factor (SPSS 21.0). All dependent variables were ln-transformed. As
282 extracellular microcystins were only detected in five out of twelve samples in Bibra Lake,
283 this resulted in only five data points for this dependent variable in Bibra Lake, making the
284 calculation of linear mixed models for this explanatory variable impossible. Two redundancy
285 analyses (RDA) were calculated to identify the best combination of variables to explain the
286 variability of intracellular microcystin concentration, extracellular microcystin fraction and
287 either cyanobacterial fraction or cyanobacterial biomass, (R version 2.15.1) for each lake.
288 Canonical ordination (999 permutations) with forward selection was computed with
289 standardised explanatory and response variables. All data was ln transformed to meet the
290 assumption of normality. RDA analysis on Bibra Lake was conducted without the inclusion
291 of pH and temperature due to an inadequate number of data points (residual d.f < 0). In all
292 analyses, results were considered significant at $P < 0.05$, unless stated differently.
293
294 **3 Results**
295 **3.1 Physical and chemical characteristics of studied lakes**
296 Mean pH fluctuated between 8.2 and 9.2 (Fig. 3A) and mean air temperature (Fig. 3B) ranged
297 from 27 to 43°C during the study period in all lakes. Salinity in Jackadder and Yangebup was
298 mostly below 1.0 ppk and much lower than in Bibra Lake (Fig. 3C). The sharp increase in
299 salinity in Bibra Lake was probably due to the decreasing water level as the lake dried up by
300 end of February. Nutrient concentrations varied on a temporal basis within lakes and spatially

between lakes. Phosphorus concentrations were higher in Bibra Lake than in Jackadder and Yangebup Lakes throughout the sampling period. Mean TP concentrations (Fig. 3D) ranged from 22 to 92, from 230 to >1000, from and 28 to >150 µg L^{-1} in Jackadder, Bibra and Yangebup Lakes, respectively. Meanwhile, mean TDP concentrations (Fig. 3E) ranged from 12 to 24, from 17 to 142, and from 14 to 37 µg L^{-1} in Jackadder, Bibra and Yangebup Lakes, respectively. Temporal variation of macronutrient concentrations in Yangebup and Jackadder Lakes were much smaller than in Bibra Lake. The large increase of TP, TDP, TN and TDN in Bibra Lake might again have been a concentration effect due to the lake drying up. Mean TFe and TDFe concentrations were higher in Bibra Lake during the earlier three sampling dates. Mean TFe (Fig. 3F) ranged from 77 to 247, from 147 to 220, and from 51 to 110 µg L^{-1} in Jackadder, Bibra and Yangebup Lakes, respectively. Mean TDFe (Fig. 3G) ranged from 24 to 174, from 61 to 117, and from 21 to 89 µg L^{-1} in Jackadder, Bibra and Yangebup Lakes, respectively. TN (Fig. 3H) and TDN (Fig. 3I) concentrations were up to one order of magnitude higher in Bibra Lakes compared to concentrations in Jackadder and Yangebup Lakes. In contrast, mean TN:TP in Bibra Lake were lower than the ratios in Jackadder and Yangebup Lakes (Fig. 3J). Mean TN:TP ranged from 18 to 60, 16 to 38, and 29 to 115 in Jackadder, Bibra and Yangebup Lakes, respectively. NH_4^+ decreased over time in Jackadder and Yangebup Lakes (Fig. 3K) and mean concentrations ranged from 43 to 170, from 157 to 239, and from 40 to 143 µg L^{-1} in Jackadder, Bibra and Yangebup Lakes, respectively.

The three lakes were significantly different in salinity, phosphorus, nitrogen and iron, either as total or dissolved forms (except TDFe) (ANOVA; Table 1), but did not show a significant difference in pH, Air temperature and TDFe. The posthoc tests (LSD) indicated 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 conditions in a lake is usually defined when the TN:TP weight ratios are less than 10 (Graham et al., 2004). As our result indicate 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. 4) with *Microcystis* spp. being the most abundant cyanobacterial genera in all lakes. Mean total cyanobacterial biomass was 5.41 µg L^{-1}, 29.60 µg L^{-1}, 15.14 µg L^{-1} in Jackadder, Bibra and Yangebup Lake, respectively (Fig. 5A). Cyanobacterial biomass varied within an order of magnitude on a temporal basis in Bibra and Jackadder Lake (Jackadder: 1 - 12 µg L^{-1}, Bibra: 5 - 83 µg L^{-1}, Yangebup: 8 - 32 µg $L^{-}1$). 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. 5B). 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. 5C) throughout the sampling events. Mean cellular microcystin concentrations were 0.407 mg g^{-1} in Jackadder Lake, 0.233 mg g^{-1} 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. 5D). 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, cyanobacterial biomass, or microcystin concentration

Most analysed nutrients were weakly, but significantly correlated with cyanobacterial fraction, biomass and microcystin concentrations when data from all lakes were combined (Table 2, 3). The correlations presented in Tables 2 and 3 suggested that, in general, cyanobacterial dominance in the phytoplankton community was favored at relatively lower nutrient concentrations as it was negatively correlated to TP, TDP, TFe, and TDFe. In contrast, cyanobacterial fraction was positively correlated with TN:TP ratio, potentially due to relatively lower TP concentrations in comparison to TN concentrations. Cyanobacterial biomass on the other hand was positively correlated to salinity, TN, TDN and NH_4^+, but negatively correlated with TDFe. Cellular microcystin concentration was positively correlated with phosphorus and iron, but not with nitrogen. 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 (Table 3). In contrast to cellular microcystin, extracellular microcystin fraction was negatively correlated with salinity, TP, TDP, TN, TDN, and positively correlated with TN:TP ratio (Table 3). Correlating environmental variables with cyanobacteria or microcystin for each lake

separately, the correlations that were significant (Pearson's) were different between lakes (Table 2, 3).

Using data from all lakes combined in linear mixed models, cyanobacterial fraction was negatively correlated to TP, TDP, TFe, TDFe (Fig. 6A-D), and positively to TN:TP (Fig. 6 E). However, within each lake, the correlations with cyanobacterial fraction were significant only for TP, TDP and TDFe in Jackadder Lake and TP in Yangebup Lake. Cellular microcystin concentration was on the other hand positively correlated to TP, TDP, TFe and TDFe (Fig. 7A-D) and negatively to TN:TP (Fig. 7E). Within each lake, these correlations were only significant for TP, TFe, TDFe in Jackadder Lake (Fig. 7A, C, D), for TDP in Bibra Lake (Fig. 7B) and for TP in Yangebup Lake (Fig. 7A). When combining all lakes, extracellular microcystin fraction was negatively correlated to salinity (linear mixed model; $p<0.1$), TP and TDP, but positively to TN:TP (Fig. 8A-D). Jackadder Lake was the only lake showing significant correlations between extracellular microcystin fraction and salinity (positive, Fig. 8A) and TP (negative, Fig. 8B). Using linear mixed models, cyanobacterial biomass was only significantly correlated to TDP and TDFe when combining all lakes (Fig. 8E, F), with Bibra Lake showing a significant negative correlation to TDFe (Fig. 8F). The 95% confidence intervals of the slopes of the correlations between TP and cyanobacterial fraction or extracellular microcystin fraction in Jackadder Lake and in all lakes combined (Fig. 6A, 8B) or between salinity and extracellular microcystin fraction in Jackadder Lake and in all lakes combined (Fig. 8A) did not overlap, providing a conservative estimate that the slopes were significantly different (Payton et al., 2003).

3.4 Multivariate analysis of site-specific environmental factors and the variability of cyanobacteria and microcystin concentration

401 The first RDA analysis showed significant relationships ($P < 0.05$) between the measured
402 environmental factors and the combined variability of cyanobacterial fraction, cellular
403 microcystin concentration and extracellular microcystin fraction for each lake. The canonical
404 ordination indicated that 75% (Jackadder Lake; $R^2_{adj.} = 0.75$; F=5.726), 80% (Bibra Lake; R^2
405 $_{adj.} = 0.80$; F=5.888) and 75% (Yangebup Lake; $R^2_{adj.} = 0.75$; F=5.804) of the combined
406 variability of cyanobacterial fraction, cellular microcystin concentration and extracellular
407 microcystin fraction can be explained by the measured environmental factors (Fig. 9A - C).
408 The second RDA analysis, which sought to find relationships between environmental factors
409 and absolute cyanobacterial biomass, cellular microcystin concentration and extracellular
410 microcystin fraction for each lake found that 71% (Jackadder Lake; $R^2_{adj.} = 0.71$; F=4.725),
411 80% (Bibra Lake; $R^2_{adj.} = 0.80$; F=5.806) and 66% (Yangebup Lake; $R^2_{adj.} = 0.66$; F=3.953)
412 of the combined variability of absolute cyanobacterial biomass, cellular microcystin
413 concentration and extracellular microcystin fraction can be explained by the measured
414 environmental factors (Fig. 10A - C).
415
416 In both sets of analyses, many of the environmental factors that were closely correlated to
417 cyanobacteria and microcystins were slightly different between lakes. TDP was only
418 correlated to either cyanobacteria fraction or cellular microcystin concentration in Bibra and
419 Jackadder Lakes (Fig. 9A, B) but not in Yangebup Lake (Fig. 9C). Additionally, TFe was
420 positively correlated to cyanobacteria only in Bibra Lake (Fig. 9B, 10B) but not in the other
421 two lakes (Fig. 9A, 9C, 10A, 10C). In comparison to the other factors, TDFe was always
422 negatively correlated to cyanobacterial fraction and biomass and positively correlated to
423 cellular microcystin concentration variability (Fig. 9, 10).
424
425 **4 Discussion**

The relationships between the environmental factors and cyanobacterial and microcystin variability were different between lakes. This is an indication that the relevance of factors that drive cyanobacteria and their toxin production depends on their site-specific combinations. Our results suggest that the site-specificity of environmental triggers may be related to spatial heterogeneity of the respective environmental factor, as each factor can be 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 increase from low to high in systems. Our results support these previous findings as the relationships between cyanobacterial fraction, cyanobacterial biomass and cellular microcystin concentration with TFe and TDFe were closely related to the concentration levels of TFe and TDFe in each lake. Mean TFe concentration in Bibra Lake was 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). This could explain why the relationship between cyanobacterial fraction or cellular microcystin and TFe was different for between lakes, while TDFe was not. Further, the correlation between cyanobacterial fraction and TP was only significant in Yangebup and Jackadder Lake, which both had lower TP concentrations than Bibra Lake, in which no significant correlation was found. Meanwhile, the correlation between cellular microcystin concentration and TFe was negative only in Bibra Lake, where TFe was present at significantly higher concentrations compared to the other two lakes. This indicates that the effect of environmental factors on cyanobacterial and microcystin variability may depend on site-specific factors such as concentration regimes, even in non-nutrient limited lakes. Therefore, a generalization by only using concentrations of nutrients might not be sufficient for future management of lakes.

The site-specificity of the environmental triggers of cyanobacterial and microcystin variability may also be a consequence of the variation of cyanobacterial communities between the systems. TFe was negatively correlated to cyanobacterial fraction in Jackadder and Yangebup Lake, and positively in Bibra Lake. The cyanobacterial community in Jackadder Lake was composed of only one nitrogen-fixing cyanobacterial genera (Fig. 4). 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 of the cyanobacterial community in Bibra Lake, in comparison to the cyanobacterial community in Jackadder Lake.

Currently, in the absence of lake-specific information, cyanobacterial management strategies are based on knowledge derived from general trends of the relationship between environmental factors and cyanobacteria or their toxins. Our study clearly indicates that the environmental variables explaining the variability in cyanobacteria and their toxins might be lake-specific and, more importantly, that these lake-specific correlations might also be different to the correlation derived from combining all data (e.g., 6A, 8A, B). This strongly supports the conclusion that site-specific conditions have to be taken into account for managing lakes with cyanobacterial blooms. Due to the site-specific environmental triggers of cyanobacterial and microcystin variability, the results presented in this study are important for the management of these lakes or lakes with similar physical, chemical and biological characteristics. In this study, the cyanobacterial fraction was negatively related with TP, TDP, TFe, TDFe, and positively correlated with TN:TP ratio. These relationships illustrate that in

our study, cyanobacteria may dominate under lower phosphorus availability (Amano et al., 2010). Although the lakes in our study were not limited in phosphorus *per se*, the differences in phosphorus levels could have been responsible for the differences in the phytoplankton communities between lakes. At high concentration, phosphorus had been shown to potentially limit the ability of cyanobacteria to become dominant in the phytoplankton community, even though cyanobacteria as a group can dominate under a wide range of conditions (Chorus and Bartram, 1999; Reynolds et al., 2006). One reason for that is the higher growth rate of other phytoplankton groups compared to cyanobacteria, and, as such, their ability to utilize nutrients faster under high nutrient conditions. This can explain the negative correlation between cyanobacterial fraction and phosphorus concentration found in our study, and, maybe as a consequence of this, a positive correlation with TN:TP. 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, cellular microcystin concentration was positively related to TP, TDP, TFe, TDFe and negatively correlated to TN:TP in all lakes. High availability of phosphorus relative to other nutrients 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 relationship between cellular microcystin and TN:TP ratio, as low microcystin production is expected under conditions where phosphorus is present at lower concentrations in relation to other nutrients. In addition, the 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; Wang et al., 2010a). Our results illustrate that reducing phosphorus and iron concentrations in water bodies could potentially reduce the overall toxicity of cyanobacterial bloom, even though it might not completely prevent the occurrence of cyanobacterial bloom.

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 correlations could be direct or indirect ones. If they are direct, our results suggest that regardless of the potentially low microcystin production, cyanobacteria may release microcystins at lower nitrogen and phosphorus concentrations. This would support by the hypothesis that microcystin is involved in nutrient competition in the phytoplankton community (Huisman and Hulot, 2005).

Based on the RDA results, the measured environmental factors were able to better predict the variability of cyanobacterial fraction than the variability of absolute cyanobacterial biomass in two out of three lakes (Yangebup and Jackadder Lakes). Both descriptors are important indicators for management. The competition with other phytoplankton, described by the cyanobacterial fraction in this study can affect the toxin production within a cell through

allelopathy (Huisman and Hulot, 2005). Therefore, understanding the importance of site-specific drivers of both, biomass and the cyanobacterial fraction is of highest importance to develop successful and sustainable management strategies.

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 physicochemical factors on cyanobacteria and microcystin variability being dependent on the site-specific combination of environmental factors. Our study clearly highlights the importance of taking between-lake heterogeneity in the management of toxic cyanobacterial blooms into account. Site-specific 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 lower phosphorus and iron concentrations in the systems. In contrast, cyanobacteria required higher 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 could 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 (Jan – March 2010), including analysis of differences between lakes (one-way ANOVA).

Factors	Jackadder Lake (N =18)		Bibra Lake (N =12)		Yangebup Lake (N =18)		Differences between lakes (one-way 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	$F_{(2,45)} = 2.16$
Air Temp	33.0 ± 4.9	27.4 - 42.7	35.7 ± 4.7	30.8 - 43.0	34.7 ± 4.1	30.8 - 43.0	$F_{(2,45)} = 1.31$
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	$F_{(2,45)} = 99.08$ *
TP (µg 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 (µg L^{-1})	17.6 ± 4.8	12.0 – 26.7	67.9 ± 51.3	16.0 – 180.0	23.2 ± 7.6	13.3 – 40.7	$F_{(2,45)} = 15.27$ *
TFe (µg 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 (µg 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 (µg 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

* = $P < 0.05$

Table 2: Pearson's correlation coefficients (R) between the environmental factors and cyanobacterial fraction (%) or cyanobacterial biomass (µg chl-a L^{-1}) analysed for each lake and for all lakes combined using bivariate correlation analysis. The dependent variables are ln transformed.

	Cyanobacterial fraction (%)				Cyanobacterial biomass (µg chl-a L^{-1})			
Factor	All lakes N = 48	Jackadder N = 18	Bibra N = 12	Yangebup N = 18	All lakes N = 48	Jackadder N = 18	Bibra N = 12	Yangebup N = 18
pH	-0.108	-0.363	**-0.653**	0.225	0.087	-0.181	**-0.671**	0.287
Air Temp	0.018	0.119	-0.112	0.016	0.138	0.002	0.080	0.043
Salinity	-0.250	-0.423	-0.204	-0.460	**0.454**	-0.063	-0.038	-0.236
TP	**-0.337**	**-0.873**	-0.272	**-0.742**	0.282	**-0.808**	-0.090	0.092
TDP	**-0.357**	-0397	**-0.641**	0.147	0.060	-0.320	-0.574	0.406
TFe	**-0.570**	**-0.789**	0.389	-0.304	-0.040	**-0.577**	0.340	-0.326
TDFe	**-0.777**	**-0.903**	-0.355	-0.432	**-0.339**	**-0.727**	-0.424	-0.113
NH_4	0.105	0.375	0.576	**0.543**	**0.345**	0.042	**0.721**	0.222
TN	-0.236	**-0.487**	0.035	**-0.628**	**0.477**	-0.185	0.197	0.025
TDN	-0.265	**-0.534**	-0.219	-0.305	**0.430**	-0.314	-0.078	-0.084
TN:TP	**0.423**	**0.570**	0.299	0.264	0.164	**0.741**	0.145	-0.339

Significant (P<0.05) correlations are highlighted in bold.

778 Table 3: Pearson's correlation coefficients (R) between the environmental variables and cellular microcystin concentration (µg g^{-1}) or
779 extracellular microcystin fraction (%) analysed for each lake and for all lakes combined using bivariate correlation analysis. The dependent
780 variables are ln transformed. Extracellular microcystin fraction was zero in seven cases, leading to an N = 5 only.

	Cellular microcystin concentration (µg g^{-1})				Extracellular microcystin fraction (%)			
Factor	All lakes N = 48	Jackadder N = 18	Bibra N = 12	Yangebup N = 18	All lakes N = 38	Jackadder N = 18	Bibra N = 5	Yangebup N = 18
pH	0.227	0.426	**0.762**	0.190	-0.297	0.155	-0.714	-0.360
Air Temp	-0.246	-0.288	-0.185	-0.160	0.077	0.138	-0.686	0.130
Salinity	0.067	0.330	0.448	**0.587**	**-0.375**	**0.570**	-0.775	**-0.659**
TP	**0.399**	**0.826**	0.489	**0.696**	**-0.392**	-0.303	-0.441	-0.295
TDP	**0.296**	**0.553**	**0.764**	0.225	**-0.428**	-0.088	-0.498	**-0.587**
TFe	**0.343**	**0.715**	**-0.605**	0.230	-0.037	0.380	0.499	-0.245
TDFe	**0.590**	**0.811**	0.135	0.400	-0.063	0.166	0.162	-0.252
NH_4	-0.267	-0.433	-0.338	**-0.579**	-0.115	-0.382	0.013	**0.530**
TN	0.085	0.441	0.268	**0.613**	**-0.376**	0.420	-0.633	-0.417
TDN	0.095	**0.482**	0.533	**0.479**	**-0.400**	0.324	**-0.921**	**-0.633**
TN:TP	**-0.446**	**-0.593**	-0.257	-0.382	**0.386**	0.492	0.514	0.239

781 Significant (P<0.05) correlations are highlighted in bold.

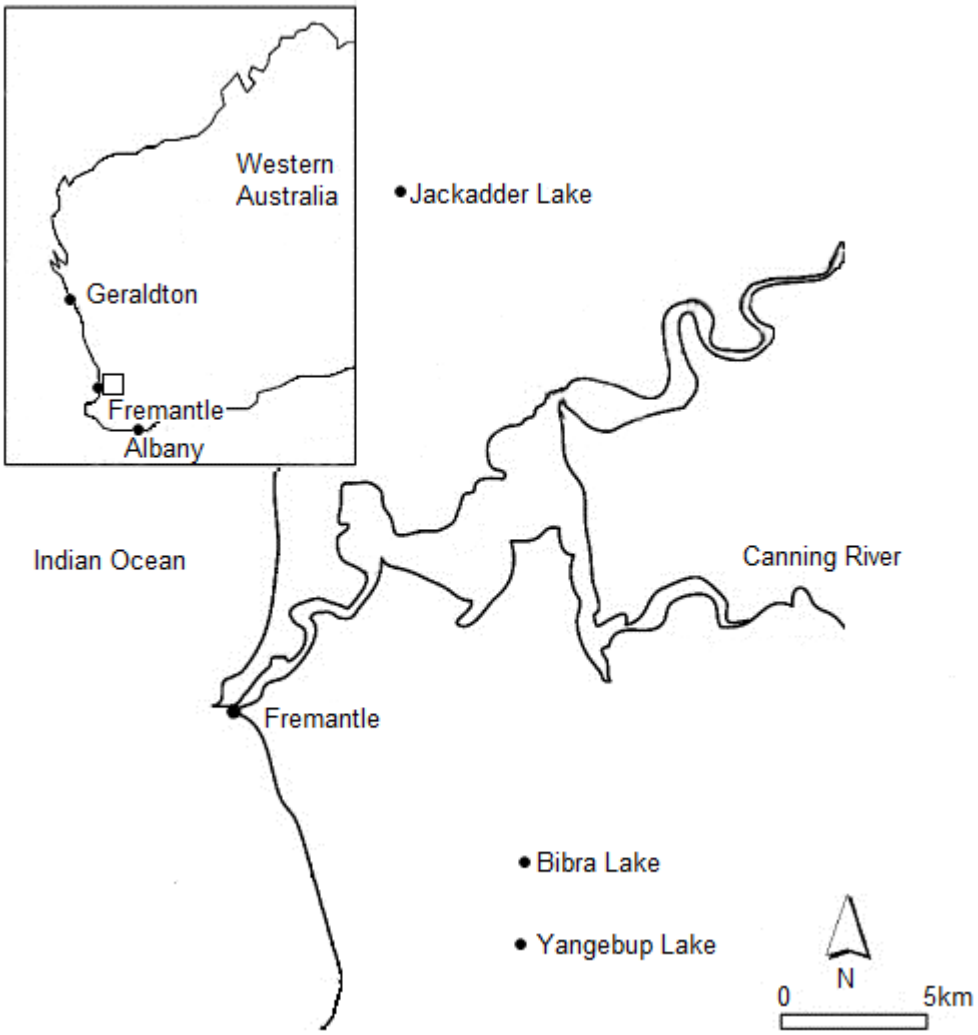


Fig. 1. The locations of three studied lakes on Swan Coastal Plain.

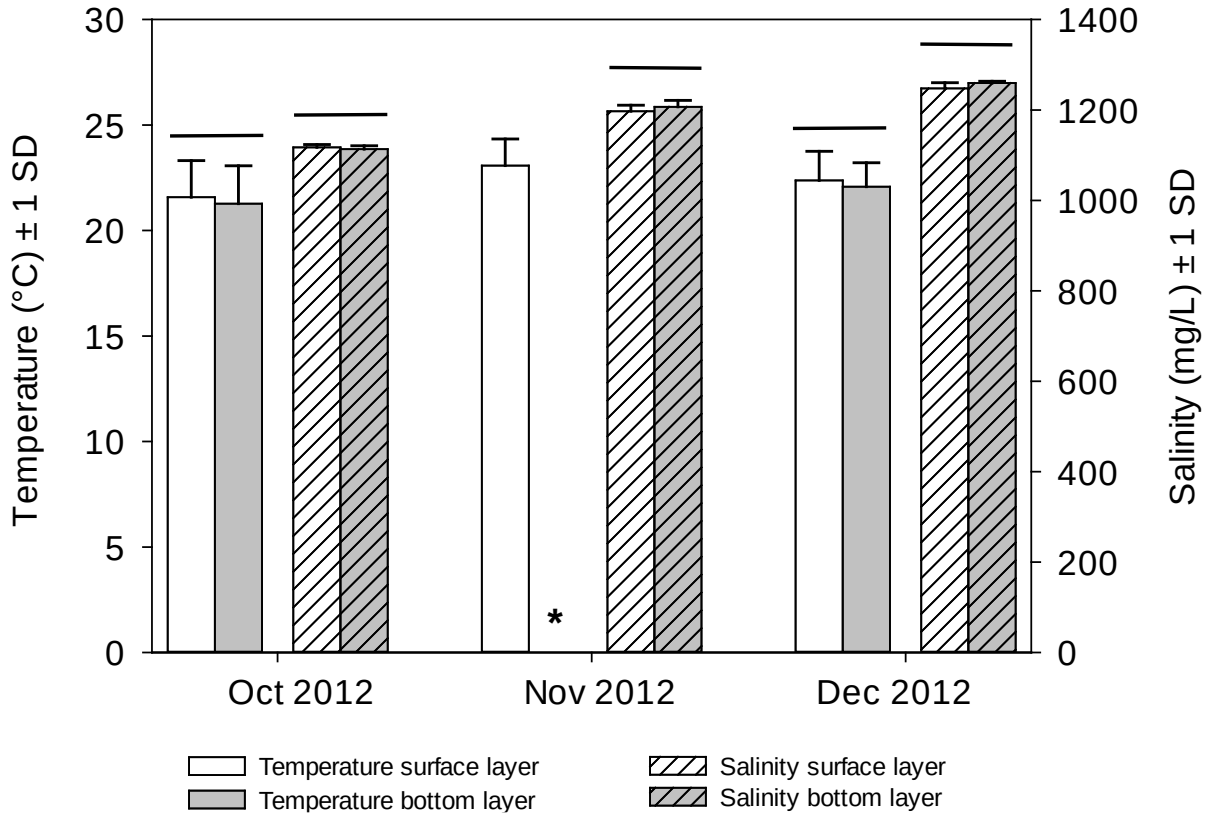
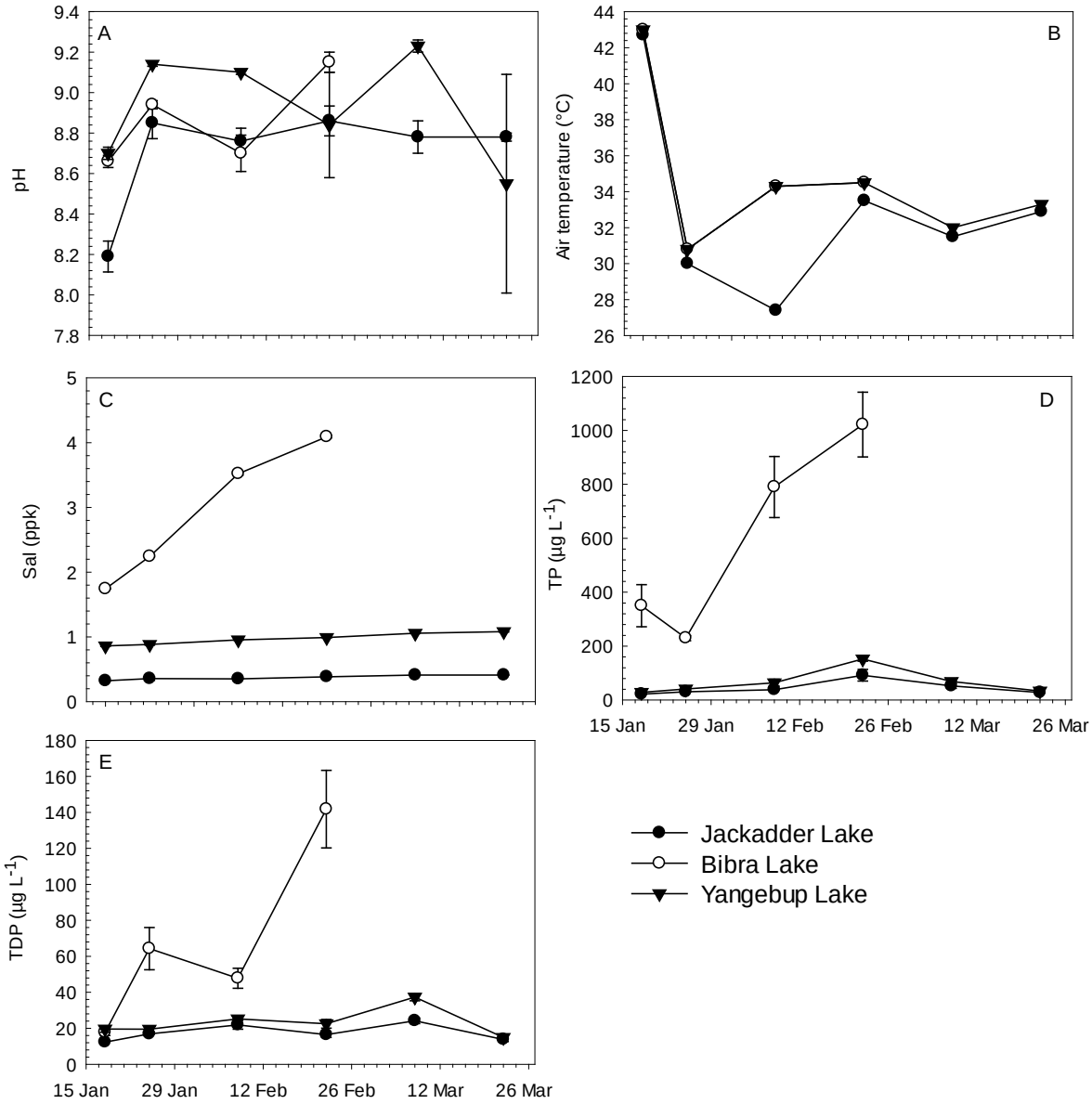


Fig. 2. Temperature (°C) and salinity (ppm) in the surface and bottom layers measured at 7 sites over three months in Lake Yangebup during a previous study in 2012. * = missing data; horizontal line indicates that no significant difference between layers were detected (t-test) (from Song et al., 2015).



796

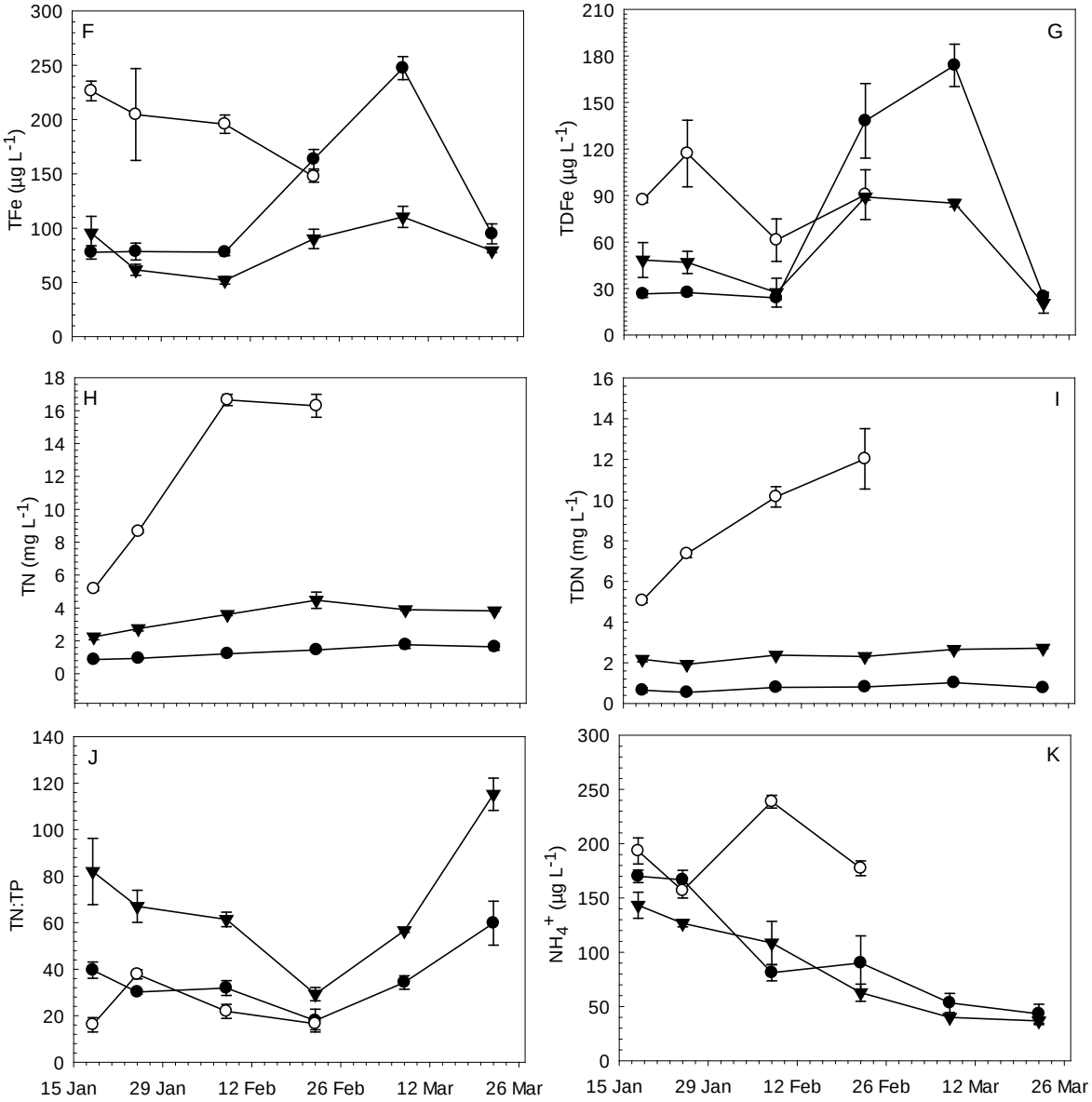
797 **Fig. 3.** Mean values (± one standard error) of physicochemical variables over time

798 (A = pH; B = Air Temp; C = Sal; D = TP; E = TDP; F = TFe; G = TDFe; H = TN;

799 I = TDN; J = TN:TP; K = NH_4^+) in Jackadder, Bibra and Yangebup Lakes from January

800 to March 2010. The mean is calculated from the three locations per lakes.

801



802

803 **Fig. 3. continued**

804

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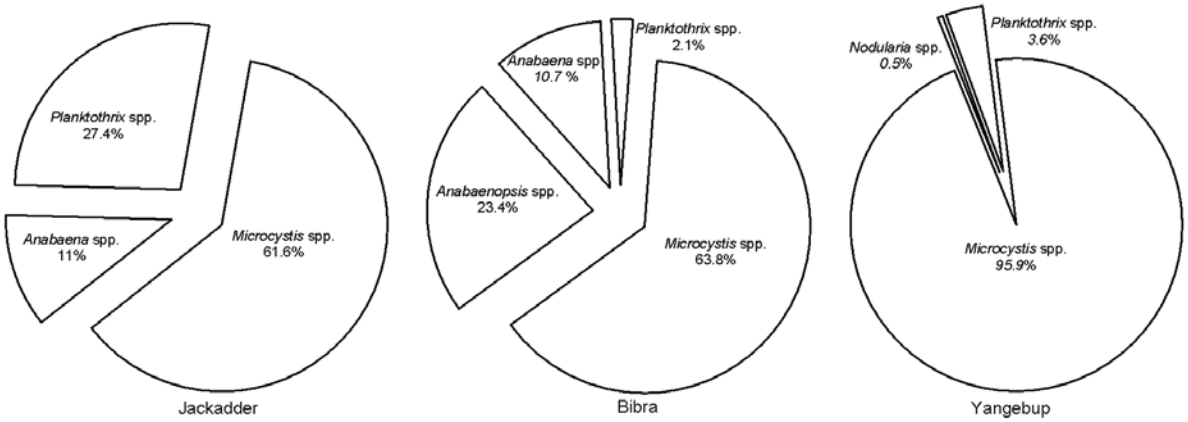


806

807 **Fig. 4.** Mean biomass (μm^3 mL^{-1}) proportions of potentially toxic cyanobacterial genera in

808 Jackadder, Bibra and Yangebup lakes during the study period.

809

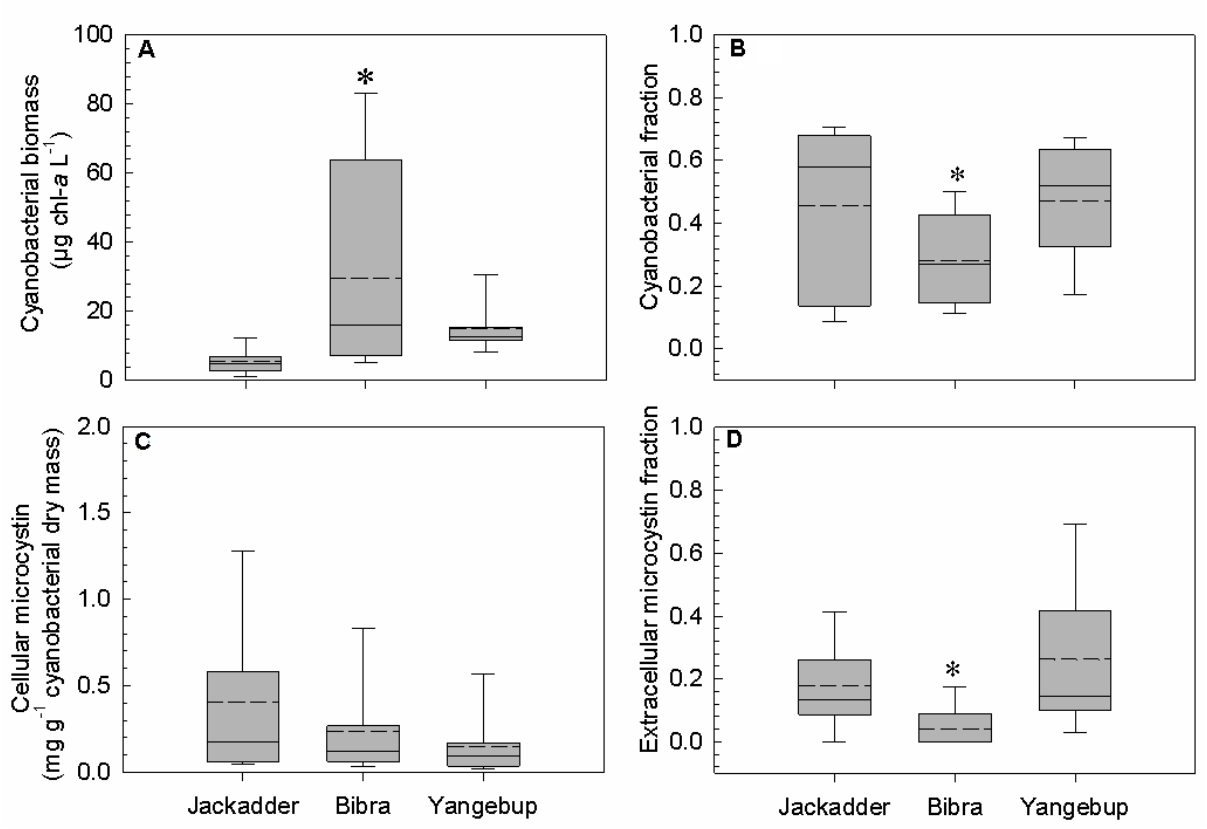
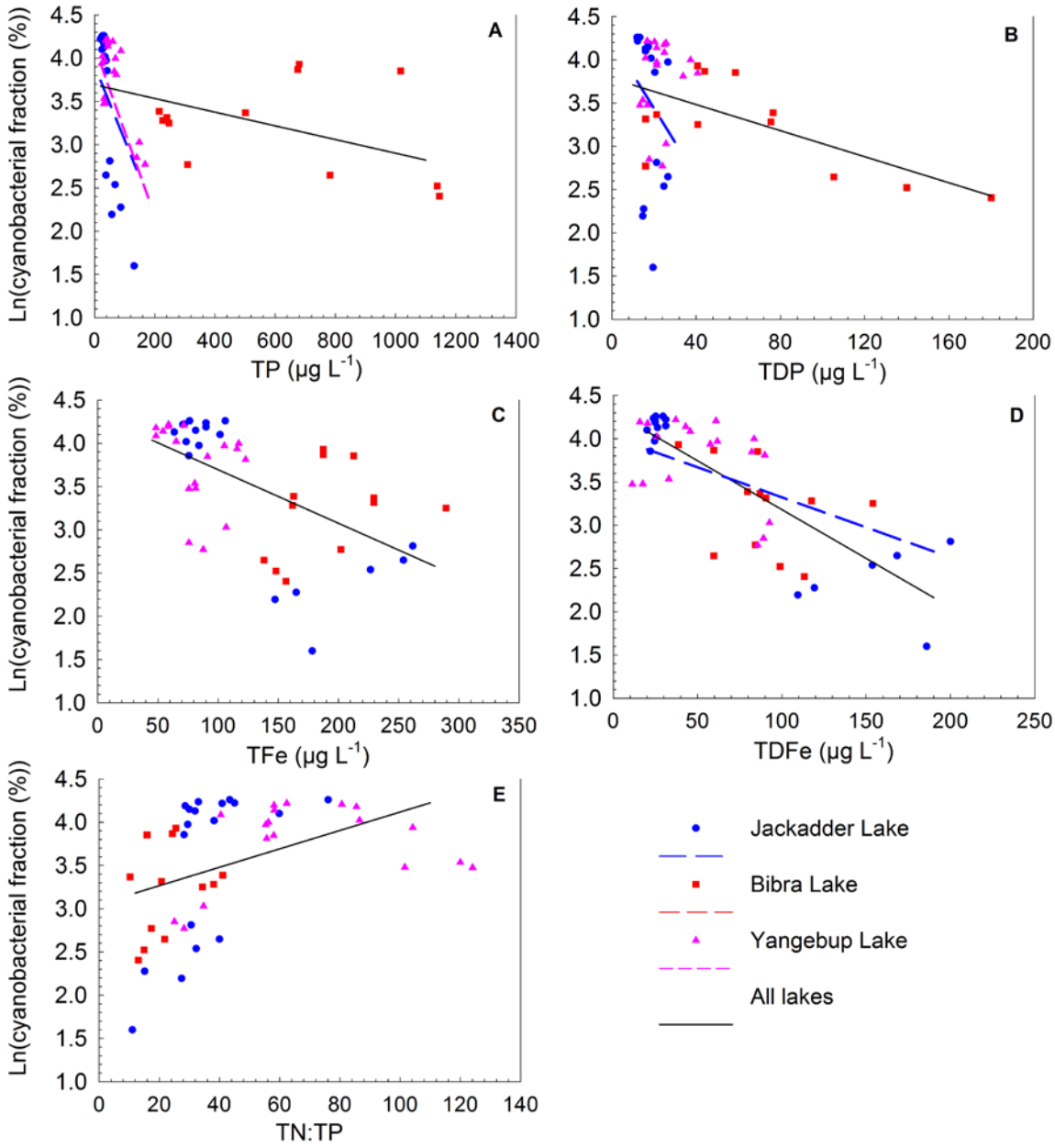


Fig. 5. The variability of (A) cyanobacterial biomass (µg chl-*a* L^{-1}), (B) cyanobacterial fraction (cyanobacterial biomass to total biomass), (C) cellular microcystin concentration (mg g^{-1} cyanobacterial dry mass) and (D) extracellular microcystin fraction over time for each lake. Boxes represent 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.



819

820 **Fig. 6.** The correlations between cyanobacterial fraction and (A) TP, (B) TDP, (C) TFe, (D)

821 TDFe, (E) TN:TP in Jackadder, Bibra and Yangebup lakes during the study period.

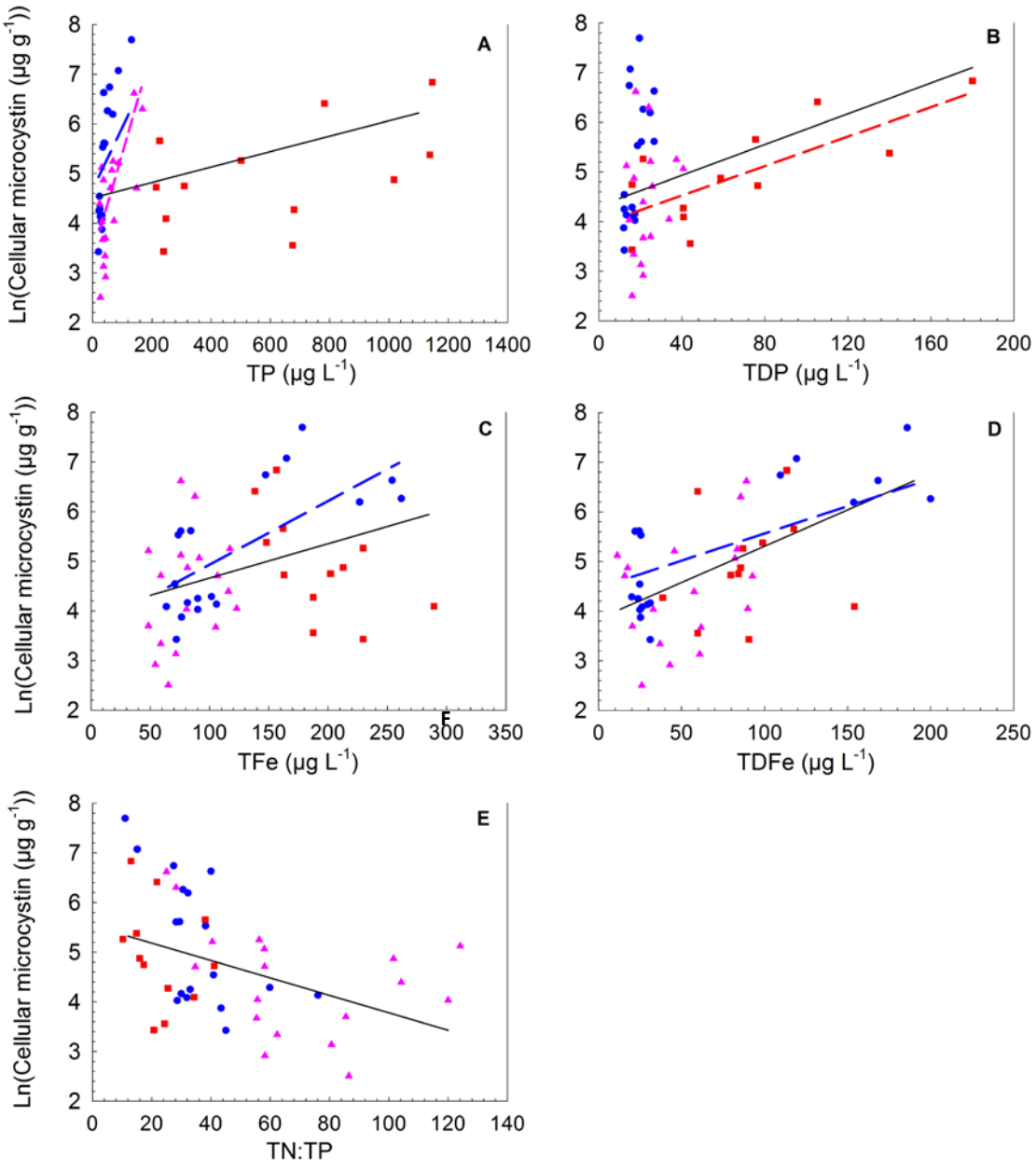
822 Regression curves for each individual lake were calculated by linear mixed models with site

823 and date as random factors on data from each lake (broken lines) while all data points were

824 combined for the overall regression using a linear mixed model adding lake as random factor

825 (solid line). Only significant ($p<0.05$) regressions are shown.

826



827

828 **Fig. 7.** The correlations between cellular microcystin concentration and (A) TP, (B) TDP, (C)

829 TFe, (D) TDFe, (E) TN:TP in Jackadder, Bibra and Yangebup lakes during the study period.

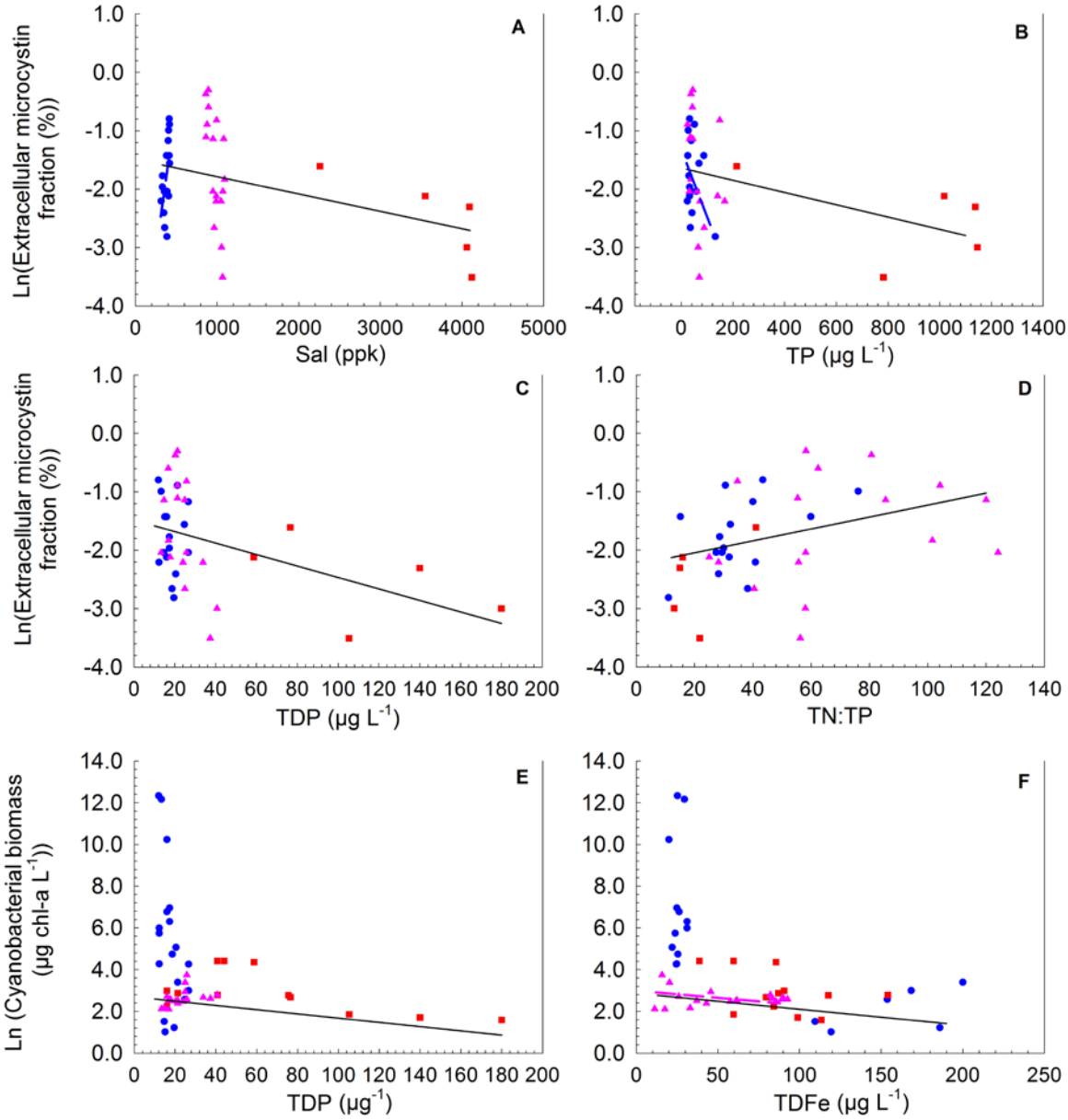
830 Regression curves for each individual lake were calculated by linear mixed models with site

831 and date as random factors on data from each lake (broken lines) while all data points were

832 combined for the overall regression using a linear mixed model adding lake as random factor

833 (solid line). All regression shown are $p<0.05$, except for the regression calculated for all lakes

834 combined in panel A, which is $p<0.1$. Symbols and lines are explained in Fig. 6.



835

836 **Fig. 8.** The correlations between extracellular microcystin fraction and (A) Sal, (B) TP, (C)

837 TDP, (D) TN:TP, and between cyanobacterial biomass and (E) TDP, (F) TDFe in Jackadder,

838 Bibra and Yangebup lakes during the study period. Regression curves for each individual

839 lake were calculated by linear mixed models with site and date as random factors on data

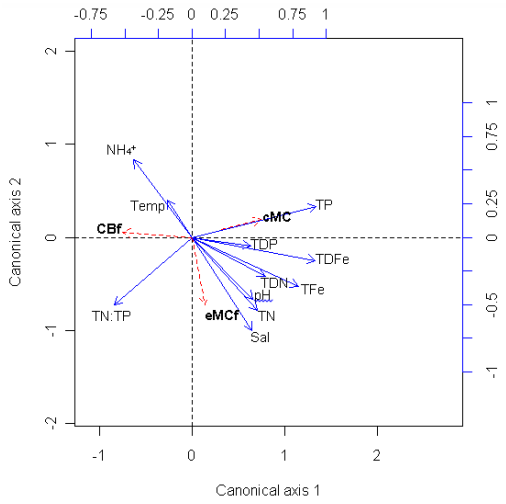
840 from each lake (broken lines) while all data points were combined for the overall regression

841 using a linear mixed model adding lake as random factor (solid line). All regression shown

842 are $p<0.05$, except for the regression calculated for all lakes combined in panel A, which is

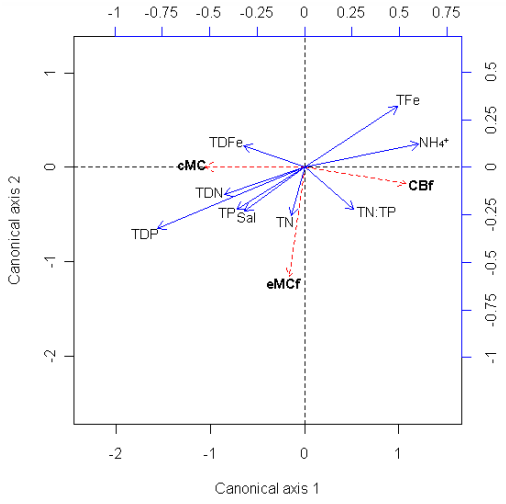
843 p<0.1. Symbols and lines are explained in Fig. 6.

844 **A**



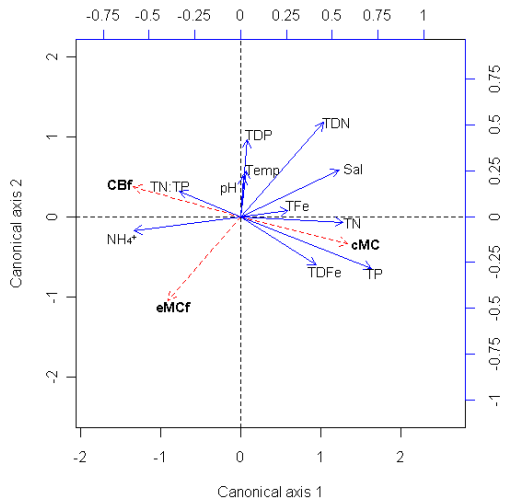
845

846 B



847

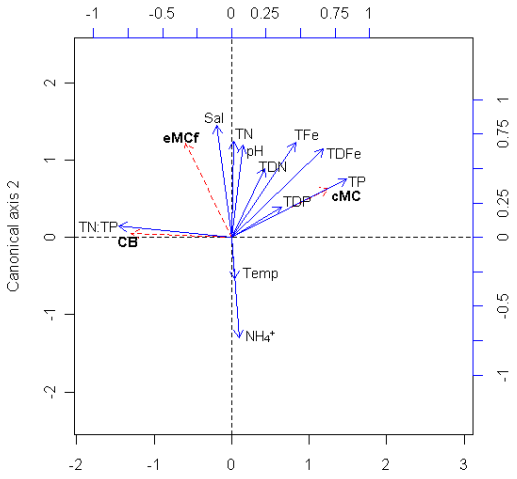
848 C



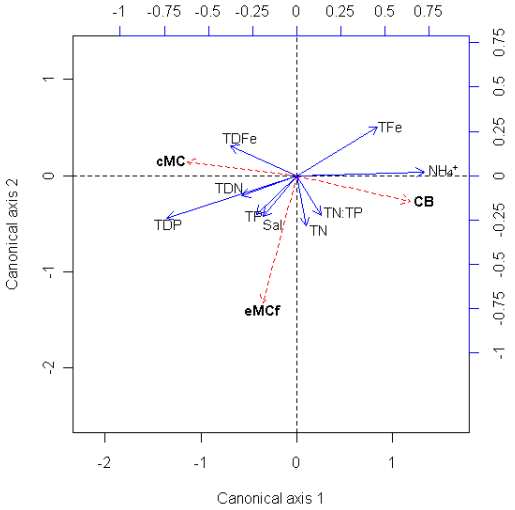
849

Fig. 9. RDA biplots for the environmental variables and the 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.

862 A

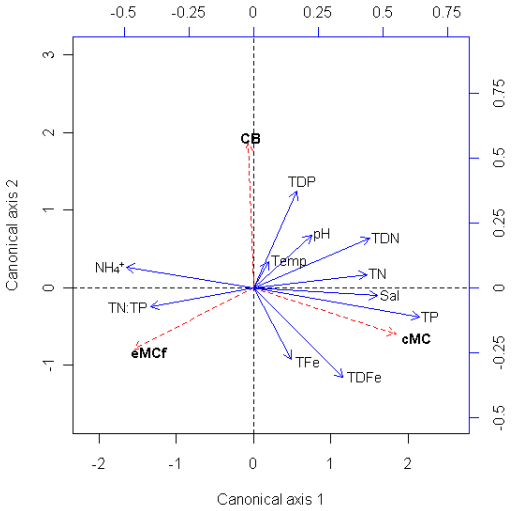


863 B



864

865 C



866

867 **Fig. 10.** RDA biplots for the of environmental variables and the absolute cyanobacteria
868 biomass (CB), cellular microcystin (cMC) and extracellular microcystin fraction (eMCf) in
869 (A) Jackadder Lake, (B) Bibra Lake, (C) Yangebup Lake; solid arrows = environmental
870 variables; short dashed arrows = response variables. Canonical axis 1 and 2 represents a
871 linear combination of the environmental variables, and axes are scaled by the square root of
872 their eigenvalues.
873