1 Local nutrient regimes determine site-specific environmental triggers of cyanobacterial and
2 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 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. Our study suggests that cyanobacterial dominance and cyanobacterial microcystin content variability were significantly correlated to phosphorus and iron concentrations. However, 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 significant environmental factors under site-specific conditions might be an important strategy to enhance positive outcomes in cyanobacterial bloom control measures.

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

51

52 **1 Introduction**

53 Urban lakes often serve as recreational spaces for communities and habitats for wildlife (Yan
54 et al., 2012; Liu, 2014) . To date, urban lakes continue to deteriorate due to increased
55 anthropogenic activities and often face water quality problems including toxic cyanobacteria
56 blooms (Maria Pineda-Mendoza et al., 2012; Reichwaldt and Ghadouani, 2012; Lei et al.,
57 2014; Sun et al., 2014; Zhang et al., 2014). This issue has received great attention from water
58 authorities world-wide as it presents health hazards to humans and animals who either
59 directly or indirectly received services provided by urban lakes (O'Bannon et al., 2014;
60 Rastogi et al., 2014; Waajen et al., 2014). The management of toxic cyanobacterial blooms is
61 often challenging due to the variability in cyanobacteria biomass and microcystins (Rolland
62 et al., 2013; Carey et al., 2014).

63

64 Cyanobacterial biomass and the amount of microcystins being produced during toxic
65 cyanobacterial blooms can vary significantly on a spatial basis (Reichwaldt et al., 2013;
66 Sinang et al., 2013; Thi Thuy et al., 2014; Waajen et al., 2014). Past studies have found large
67 variations in the percentages of potentially toxic cyanobacteria and microcystin concentration
68 between spatially isolated populations (Sitoki et al., 2012; Li et al., 2014). Furthermore, it
69 was reported that the variability of cyanobacterial biomass in lakes only explained a small
70 fraction of the variability in microcystin concentration (Sinang et al., 2013; Eva and Lindsay,
71 2014). These findings highlight the importance to fully understand the roles of environmental
72 factors controlling cyanobacteria and microcystin variability.

73

74 It has been suggested that cyanobacterial biomass and microcystin variability largely depends
75 upon physical, chemical and biological properties of the water bodies (Engström-Öst et al.,

76 2013; Lehman et al., 2013; Paerl and Otten, 2013; Ruiz et al., 2013). A range of
77 environmental factors, including nitrogen and phosphorus (Schindler, 2012; Srivastava et al.,
78 2012; Chaffin and Bridgeman, 2014; Van de Waal et al., 2014), TN:TP ratio (Smith, 1983;
79 Wang et al., 2010b; Van de Waal et al., 2014), temperature (Davis et al., 2009; Rolland et al.,
80 2013), salinity (Tonk et al., 2007), and iron (Ame and Wunderlin, 2005; Nagai et al., 2007;
81 Wang et al., 2010a) have been shown to have pronounced effects on either cyanobacterial
82 dominance, microcystin production, or both. Nevertheless, the results between studies differ,
83 and there is no clear understanding of the roles of these environmental factors as the triggers
84 of cyanobacterial dominance and microcystin production. It therefore remains an important
85 challenge for bloom management to fully understand the mechanisms behind toxic
86 cyanobacterial bloom development. For instance, regardless of many studies suggesting the
87 important role of phosphorus, reduction of internal and external phosphorus concentration is
88 not always successful in preventing the occurrence of toxic cyanobacterial blooms in water
89 bodies (Lewis and Wurtsbaugh, 2008; Amano et al., 2010; Koreiviene et al., 2014).
90
91 By taking into account the contrasting findings of earlier studies, including inconsistent
92 outcomes of nutrient reduction strategies, we suggest that the environmental triggers of
93 cyanobacterial and microcystin variability may vary between water bodies. Therefore, the
94 main objective of this study was to investigate the site-specificity of environmental triggers
95 for cyanobacterial biomass and microcystin variability in a local urban lake system. More
96 specifically, the objectives were to (1) determine the variability of cyanobacterial biomass
97 and microcystin concentration in a set of local urban lakes, (2) identify the site-specific
98 relationship between environmental factors and cyanobacterial biomass or microcystin
99 dynamics.
100

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

126 receiving water from neighbouring Thomson Lake when it reaches its maximum water level.
127 Once Yangebup Lake reaches its maximum allowable water level, water is pumped into
128 nearby Cockburn Sound (Environmental Protection Authority, 1989). The hydrology of
129 Jackadder, Bibra and Yangebup lakes is mainly affected by the strong seasonal rainfall
130 pattern due to the Mediterranean climate. The region's mean annual rainfall is reported as
131 771.5mm and monthly mean rainfall is 35.1 , 156.3 , 433.3 , and 144.2 mm during summer,
132 autumn, winter and spring, respectively (Bureau of Metereology, 2014). In response, the
133 maximum water levels in all lakes occur in September and October, and the minimum water
134 levels occur in March and April at the end of summer months (Davis et al., 1993). The
135 region's mean maximum annual temperature is 24.5 °C and monthly maximum temperature
136 are 30.9°C, 25.4°C, 18.0°C, and 22.6°C during summer, autumn, winter and spring,
137 respectively (Bureau of Metereology, 2014). Prolonged stable thermal stratification is usually
138 prevented in these lakes during summer due to continuous or intermittent wind mixing that
139 creates a homogeneous environment throughout the water column (Davis et al., 1993; Arnold
140 and Oldham, 1997).
141
142 These lakes were selected due to differences reported on physicochemical properties, levels
143 of cyanobacterial biomass and microcystin concentration. Based on an earlier study, these
144 lakes represent systems with low, medium and high cyanobacterial biomass and microcystin
145 concentration. Mean cyanobacterial biomass was reported as 28, 108, and 80 µg chl-*a* L^{-1} in
146 Jackadder, Bibra and Yangebup Lake, respectively. Mean cellular microcystin concentrations
147 (mg g^{-1} cyanobacterial dry mass) was 4.8 mg g^{-1} in Jackadder Lake, 35 mg g^{-1} in Bibra Lake
148 and 1.7 mg g^{-1} in Yangebup Lake (Sinang et al., 2013).
149
150

151 **2.2 Sampling and analyses**

152 The lakes were sampled twice a month between January and March 2010. Three samples
153 were collected from the same three points at each lake on every sampling occasion. Bibra
154 Lake dried up in late February; therefore no samples were taken from this lake in March. On-
155 site measurements and samples were taken from shore sites at a water depth of 0.6 to 1 m.
156 Temperature (Temp), pH and Salinity (Sal) were measured on-site with a WP-81 probe (TPS
157 Pty Ltd) at a depth of 0.6 m. Grab water samples for cyanobacteria, microcystin and total
158 phosphorus quantification were taken from approximately 0.15 m below the surface to avoid
159 surface scum. Although there was a slight difference in the depth from which the samples for
160 the physicochemical and water samples were taken, this is not expected to influence the
161 interpretation of the results, as an earlier study in these lakes (Arnold and Oldham 1997)
162 indicated that the water bodies at these shallow shore sites are well mixed with respect to
163 physicochemical variable as shown in Fig. 2. Water samples were stored immediately in glass
164 bottles in the dark on ice. Parameters analysed from these samples were total phosphorus
165 (TP), total dissolved phosphorus (TDP), total iron (TFe), total dissolved iron (TDFe), total
166 nitrogen (TN), total dissolved nitrogen (TDN), ammonium (NH_4^+), cyanobacterial biomass,
167 total phytoplankton biomass, intracellular and extracellular microcystin fractions. Samples
168 for dissolved nutrient analyses were pre-filtered with a 0.45µm syringe filter (Acrodisc, HT
169 Tuffryn) before freezing at -20°C.

170

171 **2.2.1 Nutrients and phytoplankton biomass**

172 TP and TDP concentrations were analyzed using the ascorbic acid method, while TFe and
173 TDFe concentrations were analyzed with the Phenanthroline method, according to standard
174 methods (APHA, 1998). TN, TDN, and NH_4^+ were analyzed at the South Coast Nutrients
175 Analysis Laboratory, Albany, Western Australia with the standard colorimetric methods on a

176 segmented flow auto-analyser (Alpkem, Wilsonville, OR, USA). Cyanobacterial and total

177 phytoplankton chlorophyll-*a* were measured with a top-bench version of a Fluoroprobe (bbe

178 Moldaenke, Germany).The FluoroProbe measures chl-*a* fluorescence and differentiates four

179 groups of phytoplankton (chlorophytes, cryptophytes, diatoms, and cyanobacteria) by their

180 specific fluorescence emission spectrum (Beutler et al., 2002). The fluorescence is used to

181 calculate total biomass of each phytoplankton group that is expressed as chl-*a* concentration

182 equivalents (μg chl-*a* L^{-1}) (Beutler et al., 2002; Ghadouani and Smith, 2005). FluoroProbe

183 chl-*a* measurements were validated against chl-*a* data of samples extracted according to

184 standard methods (APHA, 1998) (linear regression analysis: $R^2 = 0.94$, $N = 32$, $P < 0.05$). In

185 our study, chl-*a* fluorescence as measured by FluoroProbe was used as a proxy for

186 cyanobacterial biomass (Geis et al., 2000; Eisentraeger et al., 2003).

187

188 For quantification of cyanobacterial biomass and to separate the intracellular from the

189 dissolved microcystin fraction, water samples were filtered through pre-combusted and pre-

190 weighed 47 mm GF/C filter papers. Filter papers containing particulate organic matter were

191 dried for 24 hours at 60°C and re-weighed to obtain total dry weight (Harada et al., 1999).

192 These filter papers were then moistened with Milli-Q water and kept frozen (at -20°C) until

193 intracellular microcystin extraction. As we were interested in the microcystin concentration

194 per unit cyanobacterial dry mass, cyanobacterial dry mass was calculated from the total dry

195 mass (from the filters) by adjusting it to the percentage of cyanobacteria measured with the

196 FluoroProbe. Cyanobacterial dry mass was only used for microcystin quantification.

197

198 Water samples collected for cyanobacterial identification and enumeration were preserved

199 with acidic Lugol's iodine solution (5 g I_2+10 g KI, 20 ml distilled water and 50 ml of 10%

200 acetic acid) and cyanobacteria were identified to the genus level using phytoplankton

201 taxonomic guideline (Komarek and Hauer, 2011). The relative abundance of each
202 cyanobacterial genera (cells or colonies ml^{-1}) was determined from 10-50 ml of sample using
203 an inverse microscope (Utermöhl, 1958) and converted into biovolume per ml (μm^3 ml^{-1}) by
204 multiplying the mean cell or colony biovolume (μm^3) with the total cells or colonies per ml
205 (cells or colonies ml^{-1}). Mean cell or colony biovolume for each cyanobacterial genus was
206 calculated by finding the geometric figure that best approximated the shape of each genera,
207 and by measuring the dimension of 20 individual cells or colonies (Hillebrand et al., 1999). A
208 minimum of 200 cells or colonies of the most abundant cyanobacteria were counted for each
209 sample. Different cyanobacterial species within each genus can vary in size by several orders
210 of magnitude. However, as we measured the mean biovolume of each cyanobacterial genus,
211 differences in sizes between species are evened out as a larger mean is expected, if larger
212 species are more abundant and vice versa. The calculated mean biovolume of each
213 cyanobacterial genus was used to compute the dominant cyanobacteria genera in the studied
214 lakes.
215
216 **2.2.2 Microcystin extraction and quantification**
217 Filters were freeze-thawed twice to break the cells prior to methanol extraction (Lawton et
218 al., 1994). Filters were placed into centrifuge tubes and 5 ml of 75% methanol-water (v/v)
219 was added. Filters were sonicated on ice for 25 min, followed by gentle shaking for another
220 25 min. The extracts were then centrifuged at 3273*g* (Beckman and Coulter, Allegra X-12
221 Series) for 10 min at room temperature. Extracts were carefully transferred into conical
222 flasks, and two more extractions were done per filter. All three extracts were pooled and
223 diluted with Milli-Q to 20% methanol (v/v).
224

225 Intracellular microcystin extracts and the pre-filtered water containing dissolved
226 (extracellular) microcystin were subjected to solid-phase extraction (SPE) (Waters Oasis
227 HLB) for clean-up and concentration with a loading speed of < 10 ml min^{-1}. SPE cartridges
228 were then rinsed with 10 ml of 10, 20 and 30% methanol-water (v/v), before microcystin was
229 eluted with 100% methanol + 0.1% trifluoroacetic acid (TFA) and evaporated with nitrogen
230 gas at 40°C. Finally, samples were re-dissolved in 30% acetonitrile and analysed with high-
231 performance liquid chromatography (HPLC) by using the Alliance 2695 (Waters, Australia)
232 with a PDA detector (1.2 nm resolution) and an Atlantis T3 3µm column (4.6 x 150mm i.d).
233 Mobile phases used were acetonitrile + 0.05% v/v TFA and Milli-Q water + 0.05% TFA.
234 Microcystin peaks were separated using a linear gradient as described in Lawton et al., (1994)
235 but with a maximum acetonitrile concentration of 100% and a run time of 37 min. Column
236 temperature was maintained at 37.5 ± 2.5 °C. The limit of detection per microcystin peak was
237 1.12 ng. Microcystin variants were identified based upon their typical absorption spectrum
238 detected by PDA detector at 238 nm (Meriluoto and Codd, 2005). Commercially available
239 microcystin-LR standard (Sapphire Bioscience, Australia; purity ≥ 95 %) was used to
240 quantify microcystin concentrations. Throughout this manuscript we refer to the total
241 concentration of microcystin variants per sample as microcystin concentration.
242
243 **2.3 Data processing and statistical analyses**
244 Surface water temperatures were between 19.9 and 28.7°C during the study period. However,
245 the onsite measurements of surface water temperatures were dependent on the time of
246 sampling and varied by up to 3.9°C over the course of a day. Therefore, maximum air
247 temperature on each sampling day recorded by weather stations located nearest to the studied
248 lakes was used as a substitute for surface water temperature in all analysis. Autocorrelation
249 Function (ACF) and Partial Autocorrelation Function (PACF) were calculated (SPSS 17.0) in

250 order to verify if autocorrelation exists between the data points. The analyses revealed that
251 autocorrelation coefficients for all parameters were within the upper and lower confidence
252 limits, thus suggest independency between data from each sampling date. In this study,
253 cellular (intracellular) microcystin concentration was expressed as µg (microcystin-LR mass
254 equivalents) per g cyanobacterial dry mass to illustrate cyanobacterial microcystin content.
255 Extracellular microcystin was expressed as the fraction of extracellular microcystin
256 concentration per total microcystin concentration to allow the quantification of the proportion
257 of microcystin released into the water column in comparison to the total microcystin being
258 produced. For all variables, no significant differences (ANOVA, $P > 0.05$) were detected
259 between three samples collected from three different points in each lake per sampling date.
260 Therefore, average values of all variables per sampling date were calculated from the three
261 samples. Between lakes variability of physicochemical factors, cyanobacterial biomass and
262 microcystin were analysed with one-way ANOVA (SPSS 17.0) with post hoc test (Least
263 Significance Difference; LSD) as all assumptions for an ANOVA were met (homogeneity of
264 variances, normality). Bivariate correlation analysis was carried out to identify the
265 environmental variables which significantly correlate with cyanobacterial fraction, cellular
266 microcystin concentration and extracellular microcystin fraction (SPSS 17.0). Site-specificity
267 analysis was performed with a General Linear Model (SPSS 17.0) to identify if the
268 correlation between environmental variables and cyanobacterial biomass and microcystin
269 concentration was different between lakes. The site-specificity was determined by the
270 significant interaction between lake and environmental variable in predicting the variability
271 of cyanobacteria biomass or microcystin concentration. Redundancy analysis (RDA) was
272 used to identify the best combination of explanatory variables to explain the variability of
273 cyanobacterial biomass and microcystin concentration (R version 2.15.1) for each lake.
274 Canonical ordination (999 permutations) was computed with standardised explanatory and

275 response variables. All data was log transformed to meet the assumption of normality. RDA
276 analysis on Bibra Lake was conducted without the inclusion of pH and temperature due to an
277 inadequate number of data points (residual d.f < 0). In all analyses, results were considered
278 significant at $P < 0.05$.
279

280 **3 Results**

281 **3.1 Physical and chemical characteristics of studied lakes**

282 The three lakes were significantly different in most physicochemical factors except for pH,
283 Temp and TDFe (Table 1). Salinity, phosphorus, nitrogen and iron, either as total or
284 dissolved forms (except TDFe), were significantly different between all lakes (one-way
285 ANOVA). The posthoc tests (LSD) indicates that Jackadder and Yangebup Lake did not
286 differ in TP, TDP, and NH_4^+, however, both lakes were different to Bibra Lake. Furthermore,
287 all lakes were different in salinity, TN, TDN, and TFe. Jackadder and Yangebup Lakes can
288 be classified as eutrophic, while Bibra Lake can be classified as hypereutrophic, based on the
289 mean TP concentrations (Carlson, 1977). Nitrogen limited condition in a lake is usually
290 defined when TN:TP weight ratio is less than 10 (Graham et al., 2004). As our result showed
291 that TN:TP ratios below 10 were rare, the studied lakes were not associated with persistent
292 nitrogen limitation.
293

294 **3.2 Variability of cyanobacterial biomass and microcystin concentration**

295 Cyanobacterial communities in all lakes contained potentially toxin-producing cyanobacteria
296 including *Microcystis* spp., *Planktothrix* spp., *Anabaenopsis* spp., *Anabaena* spp and
297 *Nodularia* spp. (Fig. 3) with *Microcystis* spp. being the most abundant cyanobacterial genera
298 in all lakes. Mean total cyanobacterial biomass was 5.41 µg L^{-1}, 29.60 µg L^{-1}, 15.14 µg L^{-1} in
299 Jackadder, Bibra and Yangebup Lake, respectively (Fig. 4A). Cyanobacterial biomass varied

300 within an order of magnitude on a temporal basis in Bibra and Jackadder Lake (Jackadder: 1 -
301 12 μg L^{-1}, Bibra: 5 - 83 μg L^{-1}, Yangebup: 8 - 32 μg $L^{-}1$) (Fig. 4A). Although cyanobacterial
302 biomass was significantly higher in Bibra Lake compared to the other two lakes ($F_{(2,45)}$= 7.62,
303 $P < 0.05$), the cyanobacterial fraction (the ratio of cyanobacterial chlorophyll-*a* to total
304 phytoplankton chlorophyll-*a*) in this lake was significantly lower than in Jackadder and
305 Yangebup Lake ($F_{(2,45)}$= 3.59, $P < 0.05$) (Fig. 4B). Cyanobacterial fraction ranged between
306 0.05 to 0.71 in Jackadder Lake, 0.16 to 0.68 in Yangebup Lake, and 0.11 to 0.51 in Bibra
307 Lake. The post hoc tests indicated that Jackadder and Yangebup Lakes did not differ in
308 cyanobacterial biomass and cyanobacterial fraction, but both lakes were different to Bibra
309 Lake.
310
311 Cellular microcystin concentration (mg g^{-1} cyanobacterial dry mass) varied over three orders
312 of magnitude in Jackadder Lake, and two orders of magnitude in both Bibra Lake and
313 Yangebup Lake (Fig. 4C) throughout the sampling events. Mean cellular microcystin
314 concentrations were 0.407 mg g^{-1} in Jackadder Lake, 0.233 mg g^{-1} in Bibra Lake, and 0.150
315 mg g^{-1} in Yangebup Lake. Cellular microcystin concentration was not significantly different
316 between lakes ($F_{(2,45)}$= 2.07, P >0.05). Mean extracellular microcystin fraction was 0.18 in
317 Jackadder Lake, 0.04 in Bibra Lake, and 0.26 in Yangebup Lake (Fig. 4D). The post hoc tests
318 indicated that Bibra Lake was the only lake that had a significantly different extracellular
319 microcystin fraction when compared to other lakes ($F_{(2,45)}$= 6.49, P <0.05).
320
321 **3.3 Relationship between environmental factors and cyanobacterial fraction or**
322 **microcystin concentration**
323 Most environmental factors were weakly, but significantly correlated with cyanobacterial
324 fraction and microcystin concentrations when data from all lakes were combined (Table 2).

325 The correlations presented in Table 2 suggested that, in general, cyanobacterial dominance in
326 the phytoplankton community was favored at relatively lower nutrient concentrations as it
327 was negatively correlated to TP, TDP, TFe, and TDFe. In contrast, cyanobacterial dominance
328 was positively correlated with TN:TP ratio. Cellular microcystin concentration was positively
329 correlated with phosphorus and iron, but not nitrogen. TDFe showed the strongest positive
330 correlation with cellular microcystin concentration, followed by TP, TFe, and TDP. Cellular
331 microcystin was also negatively correlated with TN:TP ratio. In contrast to cellular
332 microcystin, extracellular microcystin fraction was negatively correlated with salinity, TP,
333 TDP, TN, TDN, and positively correlated with TN:TP ratio.
334
335 **3.4 Site-specific relationship between environmental factors and cyanobacterial fraction**
336 **or microcystin concentration**
337 Most of the significant correlations between environmental factors and cyanobacterial
338 fraction, cellular microcystin concentration or extracellular microcystin fraction were
339 different between lakes (Table 2). The correlations between cyanobacterial fraction and TP,
340 TDP, TFe and TN:TP ratios were significantly different between lakes, while the correlation
341 with TDFe was consistent between lakes. In terms of cellular microcystin concentration, the
342 correlations with TP, TDP, and TFe were significantly different between lakes, while the
343 correlations with TDFe and TN:TP ratio were consistent between lakes. The correlations
344 between extracellular microcystin fraction and salinity, TDP, and TDN were significantly
345 different between lakes, while the correlation with TP, TN and TN:TP ratio was consistent
346 between lakes.
347
348 The differences in the correlations between environmental factors and cyanobacterial
349 fraction, cellular microcystin concentration or extracellular microcystin fraction between

350 lakes are shown in Fig. 5, Fig. 6, Fig. 7 and Table 3. In Bibra Lake, no significant correlation
351 between cyanobacterial fraction and TP (Fig. 5A) or TN:TP (Fig. 5E) were found.
352 Additionally, the correlations between cyanobacterial fraction and TDP (Fig. 5B) and TFE
353 (Fig. 5C) were positive in Yangebup and Bibra lakes. Meanwhile, the correlation between
354 cyanobacterial fraction and TDFe was negative in all lakes (Fig. 5D). In terms of cellular
355 microcystin concentration, its correlation with TP was weak in Bibra Lake (Fig. 6A). The
356 correlation between cellular microcystin concentration and TDP (Fig. 6B) was not significant
357 in Yangebup Lake. The correlation between cellular microcystin concentration and TFe (Fig.
358 6C) was negative only in Bibra Lake. Meanwhile, the correlations between cellular
359 microcystin concentration and TDFe (Fig. 6D) and TN:TP (Fig. 6E) were positive and
360 negative in all lakes, respectively. In terms of extracellular microcystin fraction, the
361 correlation with salinity (Fig. 7A) was not significnt in Bibra Lake. In addition, its correlation
362 with TDP (Fig. 7C) was significant only in Yangebup Lake. The correlation between
363 extracellular microcystin fraction and TDN (Fig. 7E) was positive in Jackadder Lake. The
364 correlations between extracellular microcystin fraction and TP (Fig. 7B) and TN:TP (Fig. 7F)
365 were negative and positive in all lakes, respectively.
366

367 **3.5 Multivariate analysis of environmental factors and the variability of cyanobacterial**
368 **fraction and microcystin concentration**

369 RDA analyses performed with forward selection by permutation (nperm = 999) showed
370 significant relationships ($P < 0.05$) between the measured environmental factors and the
371 combined variability of cyanobacterial fraction, cellular microcystin concentration and
372 extracellular microcystin fraction for each lake. The canonical ordination showed that 75%
373 (Jackadder Lake; $R^2_{adj.} = 0.75$; F=5.726), 80% (Bibra Lake; $R^2_{adj.} = 0.80$; F=5.888) and 75%
374 (Yangebup Lake; $R^2_{adj.} = 0.75$; F=5.804) of the combined variability of cyanobacterial

375 fraction, cellular microcystin concentration and extracellular microcystin fraction can be
376 explained by the measured environmental factors (Fig. 8A - C). The environmental factors
377 that were closely correlated to the cyanobacterial fraction, cellular microcystin concentration
378 and extracellular microcystin fraction were not always the same between lakes. TDP was
379 only correlated to either cyanobacterial fraction or cellular microcystin concentration in Bibra
380 and Jackadder Lakes (Fig. 8A, 8B) but not in Yangebup Lake (Fig. 8C). Additionally, TFe
381 was positively correlated to cyanobacterial fraction only in Bibra Lake (Fig. 8B) but not in
382 the other two lakes (Fig. 8A, 7C). In comparison to the other factors, TDFe was always
383 consistently correlated to cyanobacterial fraction and cellular microcystin concentration
384 variability.
385

386 **4 Discussion**

387 The correlation between the environmental factors and cyanobacterial and microcystin
388 variability were different between lakes. This is a strong indication that the relevance of
389 environmental triggers of cyanobacterial fraction, cellular microcystin concentration, and
390 extracellular microcystin fraction may depend on site-specific combinations of environmental
391 factors. Our results suggest that the site-specificity of environmental triggers may be related
392 to spatial heterogeneity of the respective environmental factor, as each factor could present at
393 different concentration regimes in each lake. Graham et al., (2004) and Dolman et al., (2012)
394 have suggested that the correlations between the environmental factors and cyanobacterial
395 biomass and microcystin concentration could change when the concentrations of the
396 respective environmental factors increased from low to high in systems. Our results support
397 these previous findings as the between lakes consistencies in the correlations between
398 cyanobacterial fraction and cellular microcystin variability with TP, TFe and TDFe were
399 closely related to the levels of TP, TFe and TDFe concentrations in each lake. Mean TP and

400 TFe concentrations in Bibra Lake were one order of magnitude higher than in Jackadder and
401 Yangebup Lakes, while mean TDFe concentrations in all lakes ranged within the same order
402 of magnitude (Table 1). This could explain why the correlations between cyanobacterial
403 fraction and cellular microcystin variability with TP and TFe were significantly different
404 across lakes, while TDFe was not (Table 2). Further, the correlation between cyanobacterial
405 fraction and TP was only significant in Yangebup and Jackadder Lake, which both had lower
406 TP concentrations than Bibra Lake, in which no significant correlation was found.
407 Meanwhile, the correlation between cellular microcystin concentration and TFe was negative
408 only in Bibra Lake, whereTFe was present at significantly higher concentrations compared to
409 the other two lakes. This indicates that the effect of environmental factors on cyanobacterial
410 and microcystin variability may depend on site-specific factors such as concentration
411 regimes, even in non-nutrient limited lakes. Therefore, a generalization by only using
412 concentrations of nutrients might not be sufficient for future management of lakes.
413
414 The site-specificity of the environmental triggers of cyanobacterial and microcystin
415 variability may also be a consequence of the variation of cyanobacterial communities
416 between the systems. TFe was negatively correlated to cyanobacterial fraction in Jackadder
417 and Yangebup Lake, while in Bibra Lake, a positive correlation (even not may not
418 significant) was observed between the two (Fig. 8A, B). The cyanobacterial community in
419 Jackadder Lake was composed of only one nitrogen-fixing cyanobacterial genera (Fig. 3). In
420 contrast, multiple nitrogen-fixing cyanobacterial genera were present in Bibra Lake.
421 Nitrogen-fixing cyanobacteria are known to utilize more iron in comparison to non nitrogen-
422 fixers (Wilhelm, 1995). Therefore, the site-specific correlation between TFe and
423 cyanobacterial fraction may be explained through a greater iron requirement of the

424 cyanobacterial community in Bibra Lake, in comparison to the cyanobacterial community in
425 Jackadder Lake.
426
427 Due to the potentially site-specific environmental triggers of cyanobacterial and microcystin
428 variability, the results presented in this study are important for the management of these lakes
429 or lakes with similar physical, chemical and biological characteristics. In this study, the
430 variability of cyanobacterial fraction was negatively correlated with TP, TDP, TFe, TDFe;
431 positively correlated with TN:TP ratio. These correlations illustrate that in our study,
432 cyanobacteria dominated under lower phosphorus availability (Amano et al., 2010). Although
433 cyanobacteria as a group can dominate under a wide range of conditions, high phosphorus
434 concentrations have been shown to potentially limit the ability of cyanobacteria to become
435 dominant in the phytoplankton community (Chorus and Bartram, 1999; Reynolds et al.,
436 2006). One reason for that is the higher growth rate of other phytoplankton groups compared
437 to cyanobacteria, and, as such, their ability to utilize nutrients faster under high nutrient
438 conditions. This can explain the negative correlation between cyanobacterial fraction and
439 phosphorus concentration found in our study, and, maybe as a consequence of this, a positive
440 correlation with TN:TP. In terms of iron, low availability was correlated to high
441 cyanobacterial fraction in these lakes. This result indicated that cyanobacteria pose a
442 competitive advantage to dominate the phytoplankton community under low iron availability.
443 Cyanobacteria are capable to alter their cellular iron requirements, and increase the ability to
444 utilize iron at a low concentration, through the present of siderophores (Boyer et al., 1987;
445 Lee et al., 2011). As reported in the Nagai et al., (2007), cyanobacteria including *Microcystis*
446 spp. and *Planktothrix* spp. can produce siderophores and become a superior competitor under
447 iron limited conditions. These results indicate that phosphorus and iron reduction in water

448 bodies might not be a sufficient remedial strategy against the occurrence of toxic
449 cyanobacterial bloom.
450
451 In contrast to cyanobacterial fraction, the variability of cellular microcystin concentration was
452 positively correlated to TP, TDP, TFe, TDFe and negatively correlated with TN:TP and
453 NH_4^+. High availability of phosphorus relative to other nutrients is required for energy and
454 material supply in microcystin biosynthesis as microcystin production in cyanobacterial cells
455 is an energy intensive process (Vezie et al., 2002). This is further supported through the
456 observed negative correlation between cellular microcystin and TN:TP ratio, as low
457 microcystin production is expected under conditions where phosphorus is present at lower
458 concentrations in relation to other nutrients. In addition, the positive correlation between iron
459 and cellular microcystin concentration is in agreement with earlier studies which suggested
460 that iron plays an essential role in many metabolic pathways including microcystin
461 biosynthesis in cyanobacteria (Jiang et al., 2008; Wang et al., 2010a).
462
463 These results illustrate that reducing phosphorus and iron concentrations in water bodies
464 could potentially reduce the overall toxicity of cyanobacterial bloom, even though it might
465 not completely prevent the occurrence of cyanobacterial bloom. In terms of NH_4^+, our results
466 suggest that reducing NH_4^+ concentrations may be associated with higher microcystin
467 concentration. This is possible as toxic cyanobacterial genotypes are known to be favored
468 under low inorganic nitrogen conditions (Ame et al., 2003).
469
470 Environmental conditions influencing the release of microcystin into the environment,
471 besides cells lyses, are-not well understood (Rohrlack and Hyenstrand, 2007; Barrington et
472 al., 2013). Our results showed that correlations exist between extracellular microcystin

473 fraction and nutrients, however, the correlations could be direct or indirect ones. If they are
474 direct, our results suggest that regardless of the potentially low microcystin production,
475 cyanobacteria may release microcystins at lower nitrogen and phosphorus concentrations. Th
476 would support by the hypothesis that microcystin is involved in nutrient competition in the
477 phytoplankton community (Huisman and Hulot, 2005).
478
479 **5 Conclusions**
480 The current approach to water body restoration and the prevention of toxic cyanobacterial
481 blooms relies on reducing nutrient loading into water bodies and limiting the availability of
482 nutrients in the water column. This approach might not always be successful in preventing
483 the occurrence of cyanobacterial blooms, due to the roles of physicochemical factors on
484 cyanobacteria and microcystin variability being dependent on the site-specific combination of
485 environmental factors. Thus, it is important to take into account the effect of spatial
486 heterogeneity in the management of toxic cyanobacterial blooms. Site-specific studies may be
487 required to determine the factors causing cyanobacterial dominance and microcystin
488 production in different systems with different characteristics such as the hydrology, land use
489 and water chemistry.
490
491 In our study, the dominance of cyanobacteria in the phytoplankton community is correlated to
492 lower phosphorus and iron concentrations in the systems. In contrast, cyanobacteria required
493 higher phosphorus and iron concentrations in the water column to produce a high amount of
494 microcystin. Therefore, reducing phosphorus and iron concentration in the water column
495 might not be a sufficient remedial strategy against the occurrence of toxic cyanobacterial
496 bloom, if these nutrients are still available in sufficient amount to support the growth of

497 highly competitive cyanobacteria. However, reducing phosphorus and iron could reduce the
498 amount of microcystin being produced within cyanobacterial cells.
499

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507

508 **7 References**

509 Amano, Y., Sakai, Y., Sekiya, T., Takeya, K., Taki, K., and Machida, M.: Effect of
510 phosphorus fluctuation caused by river water dilution in eutrophic lake on
511 competition between blue-green alga *Microcystis aeruginosa* and diatom *Cyclotella*
512 *sp*, J Environ Sci-China, 22, 1666-1673, 2010.
513 Ame, M. V., Díaz, M. d. P., and Wunderlin, D. A.: Occurrence of toxic cyanobacterial
514 blooms in San Roque Reservoir (Córdoba, Argentina): A field and chemometric
515 study, Environ Toxicol, 18, 192-201, 2003.
516 Ame, M. V., and Wunderlin, D. A.: Effects of iron, ammonium and temperature on
517 microcystin content by a natural concentrated *Microcystis aeruginosa* population,
518 Water Air Soil Poll, 168, 235-248, 2005.
519 APHA: Standard methods for the examination of water and wastewater 20th edition, 20th
520 ed., edited by: Clesceri, L. S., Greenberg, A. E., and Eaton, A. D.,Washington, D.C.,
521 1998.
522 Arnold, J.: Perth Wetlands Resource Book, Environmental Protection Authority, Perth,
523 1990.
524 Arnold, T. N., and Oldham, C. E.: Trace-element contamination of a shallow wetland in
525 Western Australia, Mar Freshwater Res 48, 531-539, 1997.

526 Barrington, D. J., Ghadouani, A., and Ivey, G. N.: Cyanobacterial and microcystins
527 dynamics following the application of hydrogen peroxide to waste stabilisation
528 ponds, Hydrol Earth Syst Sci, 17, 2097-2105, 10.5194/hess-17-2097-2013, 2013.
529 Beutler, M., Wiltshire, K. H., Meyer, B., Moldaenke, C., Luring, C., Meyerhofer, M.,
530 Hansen, U. P., and Dau, H.: A fluorometric method for the differentiation of algal
531 populations in vivo and in situ, Photosynth. Res., 72, 39-53, 2002.
532 Boyer, G. L., Gillam, A. H., and Trick, C.: Iron chelation and uptake, in: The Cyanobacteria,
533 edited by: Fay, P., and Baalen, C. V., Elsevier Science Publishers, Amsterdam,
534 Netherlands, 415-431, 1987.
535 Bureau of Metereology: Climate Data Online, Western Australia, 2014.
536 Carey, C. C., Weathers, K. C., Ewing, H. A., Greer, M. L., and Cottingham, K. L.: Spatial
537 and temporal variability in recruitment of the cyanobacterium *Gloeotrichia*
538 *echinulata* in an oligotrophic lake, Freshwater Science, 33, 577-592,
539 10.1086/675734, 2014.
540 Carlson, R. E.: A Trophic State Index for Lakes, Limnol. Oceanogr, 22, 361- 369, 1977.
541 Chaffin, J. D., and Bridgeman, T. B.: Organic and inorganic nitrogen utilization by nitrogen-
542 stressed cyanobacteria during bloom conditions, J Appl Phycol, 26, 299-309,
543 10.1007/s10811-013-0118-0, 2014.
544 Chorus, I., and Bartram, J.: Toxic cyanobacteria in water: A guide to their public health
545 consequences, monitoring and management, E & FN Spon, London and New York,
546 1999.
547 Davis, J. A., Rosich, R. S., Bradley, J. S., Growns, J. E., Schmidt, L. G., and Cheal, F.:
548 Wetland classification on the basis of water quality and invertebrate community
549 data, R/N:0730952487, Water Authority of Western Australia, Perth, 242pp, 1993.
550 Davis, T. W., Berry, D. L., Boyer, G. L., and Gobler, C. J.: The effects of temperature and
551 nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis*
552 during cyanobacteria blooms, Harmful Algae, 8, 715-725, 2009.
553 Department of Planning: Stirling City Centre District Water Management Strategy, Perth,
554 2010.
555 Dolman, A., Rucker, J., Pick, F., Fastner, J., Rohrlack, T., Mischke, U., and Wiedner, C.:
556 Cyanobacteria and cyanotoxins: The influence of nitrogen versus phosphorus, PLoS
557 ONE, 7, e38757, doi:10.1371/journal.pone.0038757, 2012.
558 Dunlop, M.: Yangebup lake environmental management study, Prepared for City of
559 Cockburn, ENV Australia Pty Ltd, Perth, 2008.

560 Eisentraeger, A., Dott, W., Klein, J., and Hahn, S.: Comparative studies on algal toxicity
561 testing using fluorometric microplate and Erlenmeyer flask growth-inhibition assays,
562 Ecotox Environ Safe, 54, 346-354, 2003.
563 Engström-Öst, J., Repka, S., Brutemark, A., and Nieminen, A.: Clay- and algae-induced
564 effects on biomass, cell size and toxin concentration of a brackish-water
565 cyanobacterium, Hydrobiologia, 714, 85-92, 10.1007/s10750-013-1523-8, 2013.
566 Environmental Protection Authority: Drainage Management in South Jandakot and Beeliar
567 Weflands (EPA Bulletin 371), Perth, 1989.
568 Eva, P., and Lindsay, B.: Microcystin and algal chlorophyll in relation to nearshore nutrient
569 concentrations in Lake Winnipeg, Canada, Environment & Pollution, 3, 36-47, 2014.
570 Geis, S. W., Fleming, K. L., Korthals, E. T., Searle, G., Reynolds, L., and Karner, D. A.:
571 Modifications to the algal growth inhibition test for use as a regulatory assay,
572 Environ Toxicol Chem, 19, 36-41, 2000.
573 Ghadouani, A., and Smith, R. E. H.: Phytoplankton distribution in Lake Erie as assessed by
574 a new in situ spectrofluorometric technique, J. Great. Lakes. Res., 31, 154-167,
575 2005.
576 Graham, J. L., Jones, J. R., Jones, S. B., Downing, J. A., and Clevenger, T. E.:
577 Environmental factors influencing microcystin distribution and concentration in the
578 Midwestern United States, Water Res, 38, 4395-4404, 2004.
579 Harada, K., Kondo, F., and Lawton, L. A.: Laboratory analysis of cyanotoxins, in: Toxic
580 cyanobacteria in water: A guide to their public health consequences,monitoring and
581 management, edited by: Chorus, I., and Bartram, J., E & FN Spon on behalf of the
582 World Health Organization., London and New York, 363-367, 1999.
583 Hillebrand, H., Durselen, C., Kirschtel, D., Pollingher, U., and Zohary, T.: Biovolume
584 calculation for pelagic and benthic microalgae, J. Phycol., 35, 403-424, 1999.
585 Huisman, J., and Hulot, F. D.: Population dynamic of harmful cyanobacteria, in: *Harmful
586 cyanobacteria*, edited by: Huisman, J., Matthijs, H. C. P., and Visser, P. M.,
587 springer, The Netherlands, 143-176, 2005.
588 Jiang, Y., Ji, B., Wong, R. N. S., and Wong, M. H.: Statistical study on the effects of
589 environmental factors on the growth and microcystins production of bloom-forming
590 cyanobacterium-*Microcystis aeruginosa*, Harmful Algae, 7, 127-136, 2008.
591 Kemp, A. S.: Freshwater cyanoprokaryota blooms in the Swan Coastal plain wetlands:
592 Ecology, taxonomy and toxicology, Department of environmental biology, Curtin
593 University of Technology, Perth, 2009.

594 Komarek, J., and Hauer, T.: CyanoDB.cz - On-line database of cyanobacterial genera. -
595 Word-wide electronic publication, http://www.cyanodb.cz, last access: 14 August
596 2011.
597 Koreiviene, J., Anne, O., Kasperoviciene, J., and Burskyte, V.: Cyanotoxin management and
598 human health risk mitigation in recreational waters, Environ Monit Assess, 186,
599 4443-4459, 10.1007/s10661-014-3710-0, 2014.
600 Lawton, L. A., Edwards, C., and Codd, G. A.: Extraction and high-performance liquid
601 chromatographic method for the determination of microcystins in raw and treated
602 waters, Analyst, 119, 1525-1530, 1994.
603 Lee, W., van Baalen, M., and Jansen, V. A. A.: An evolutionary mechanism for diversity in
604 siderophore-producing bacteria, Ecology Letters, 15, 119-125, 2011.
605 Lehman, P. W., Marr, K., Boyer, G. L., Acuna, S., and Teh, S. J.: Long-term trends and
606 causal factors associated with *Microcystis* abundance and toxicity in San Francisco
607 Estuary and implications for climate change impacts, Hydrobiologia, 718, 141-158,
608 10.1007/s10750-013-1612-8, 2013.
609 Lei, L., Peng, L., Huang, X., and Han, B.-P.: Occurrence and dominance of
610 *Cylindrospermopsis raciborskii* and dissolved cylindrospermopsin in urban
611 reservoirs used for drinking water supply, South China, Environ Monit Assess, 186,
612 3079-3090, 10.1007/s10661-013-3602-8, 2014.
613 Lewis, W. M., and Wurtsbaugh, W. A.: Control of lacustrine phytoplankton by nutrients:
614 Erosion of the phosphorus paradigm, Int Rev Hydrobiol, 93, 446-465,
615 10.1002/iroh.200811065, 2008.
616 Li, D., Yu, Y., Yang, Z., Kong, F., Zhang, T., and Tang, S.: The dynamics of toxic and
617 nontoxic *Microcystis* during bloom in the large shallow lake, Lake Taihu, China,
618 Environ Monit Assess, 186, 3053-3062, 10.1007/s10661-013-3600-x, 2014.
619 Liu, Y.: Dynamic evaluation on ecosystem service values of urban rivers and lakes: A case
620 study of Nanchang City, China, Aquat Ecosyst Health, 17, 161-170,
621 doi:10.1080/14634988.2014.907223, 2014.
622 Maria Pineda-Mendoza, R., Olvera-Ramirez, R., and Martinez-Jeronimo, F.: Microcystins
623 produced by filamentous cyanobacteria in urban lakes. A case study in Mexico City,
624 Hidrobiologica, 22, 290-298, 2012.
625 Meriluoto, J., and Codd, G.: Toxic - cyanobacterial monitoring and cyanotoxin analysis,
626 Acta Academiae Aboensis Ser. B, Mathematica et physica, edited by: Högnäs, G.,
627 Åbo Akademi University Press, Åbo, 2005.

628 Nagai, T., Imai, A., Matsushige, K., and Fukushima, T.: Growth characteristics and growth
629 modeling of *Microcystis aeruginosa* and *Planktothrix agardhii* under iron limitation,
630 Limnology, 8, 261-270, 2007.
631 O'Bannon, C., Carr, J., Seekell, D. A., and D'Odorico, P.: Globalization of agricultural
632 pollution due to international trade, Hydrol Earth Syst Sc, 18, 503-510,
633 10.5194/hess-18-503-2014, 2014.
634 Paerl, H. W., and Otten, T. G.: Harmful cyanobacterial blooms: Causes, consequences, and
635 controls, Microb Ecol, 65, 995-1010, 10.1007/s00248-012-0159-y, 2013.
636 Rastogi, R. P., Sinha, R. P., and Incharoensakdi, A.: The cyanotoxin-microcystins: current
637 overview, Reviews in Environmental Science and Bio-Technology, 13, 215-249,
638 10.1007/s11157-014-9334-6, 2014.
639 Reichwaldt, E., Song, H., and Ghadouani, A.: Effects of the distribution of a toxic
640 *Microcystis* bloom on the small scale patchiness of zooplankton, PLoS ONE, 8,
641 e66674, doi:10.1371/journal.pone.0066674,, 2013.
642 Reichwaldt, E. S., and Ghadouani, A.: Effects of rainfall patterns on toxic cyanobacterial
643 blooms in a changing climate: Between simplistic scenarios and complex dynamics,
644 Water Res, 46, 1372-1393, 10.1016/j.watres.2011.11.052, 2012.
645 Reynolds, C. S., Usher, M., Saunders, D., Dobson, A., Peet, R., Adam, P., Birks, H. J. B.,
646 Gustafssor, L., McNelly, J., Paine, R. T., and Richardson, D.: Growth and replication
647 of phytoplankton, in: *The ecology of phytoplankton*, Cambridge University Press,
648 178-238, 2006.
649 Rohrlack, T., and Hyenstrand, P.: Fate of intracellular microcystins in the cyanobacterium
650 *Microcystis aeruginosa* (Chroococcales, Cyanophyceae), Phycologia, 46, 277-283,
651 2007.
652 Rolland, D. C., Bourget, S., Warren, A., Laurion, I., and Vincent, W. F.: Extreme variability
653 of cyanobacterial blooms in an urban drinking water supply, J Plankton Res, 35,
654 744-758, 10.1093/plankt/fbt042, 2013.
655 Ruiz, M., Galanti, L., Laura Ruibal, A., Ines Rodriguez, M., and Alberto Wunderlin, D.:
656 First report of microcystins and anatoxin-a co-occurrence in San Roque Reservoir
657 (Cordoba, Argentina), Water Air Soil Poll, 224, 1593-1593, 2013.
658 Schindler, D.: The dilemma of controlling cultural eutrophication of lakes, Proceedings -
659 Royal Society. Biological sciences, 279, 4322-4333, 2012.

660 Sinang, S., Reichwaldt, E., and Ghadouani, A.: Spatial and temporal variability in the
661 relationship between cyanobacterial biomass and microcystins, Environ. Monit.
662 Assess., 185, 6379-6395, 2013.
663 Sitoki, L., Kurmayer, R., and Rott, E.: Spatial variation of phytoplankton composition,
664 biovolume, and resulting microcystin concentrations in the Nyanza Gulf (Lake
665 Victoria, Kenya), Hydrobiologia, 691, 109-122, 2012.
666 Smith, V. H.: Low nitrogen to phosphorus ratios favor dominace by blue-green algae in lake
667 phytoplankton, Science, 221, 669-671, 1983.
668 Srivastava, A., Choi, G.-G., Ahn, C.-Y., Oh, H.-M., Ravi, A., and Asthana, R.: Dynamics of
669 microcystin production and quantification of potentially toxigenic *Microcystis* sp.
670 using real-time PCR, Water Res, 46, 817-827, 2012.
671 Strategen: Bibra Lake: Landscape, recreational and environmental management plan, Perth,
672 Prepared for City of Cockburn, 2009.
673 Sun, F., Yang, Z., and Huang, Z.: Challenges and solutions of urban hydrology in Beijing,
674 Water Resour Manage, 28, 3377–3389, DOI 10.1007/s11269-014-0697-9, 2014.
675 Thi Thuy, D., Jaehnichen, S., Thi Phuong Quynh, L., Cuong Tu, H., Trung Kien, H., Trung
676 Kien, N., Thi Nguyet, V., and Dinh Kim, D.: The occurrence of cyanobacteria and
677 microcystins in the Hoan Kiem Lake and the Nui Coc reservoir (North Vietnam),
678 Environmental Earth Sciences, 71, 2419-2427, 10.1007/s12665-013-2642-2, 2014.
679 Tonk, L., Bosch, K., Visser, P. M., and Huisman, J.: Salt tolerance of the harmful
680 cyanobacterium *Microcystis aeruginosa*, Aquat Microb Ecol, 46, 117-123, 2007.
681 Utermöhl, H.: Zur vervollkommnung der quantitativen phytoplankton-methodik, Mitt. int.
682 Ver. theor. angew. Limnol., 9, 1-38, 1958.
683 Van de Waal, D. B., Smith, V. H., Declerck, S. A. J., Stam, E. C. M., and Elser, J. J.:
684 Stoichiometric regulation of phytoplankton toxins, Ecol Lett, 17, 736-742,
685 10.1111/ele.12280, 2014.
686 Vezie, C., Rapala, J., Vaitomaa, J., Seitsonen, J., and Sivonen, K.: Effect of nitrogen and
687 phosphorus on growth of toxic and nontoxic *Microcystis* strains and on intracellular
688 microcystin concentrations, Microb. Ecol., 43, 443-454, 2002.
689 Waajen, G. W. A. M., Faassen, E. J., and Lürling, M.: Eutrophic urban ponds suffer from
690 cyanobacterial blooms:Dutch examples, Environ Sci Pollut Res, DOI
691 10.1007/s11356-014-2948-y, 2014.

692 Wang, C., Kong, H.-N., Wang, X.-Z., Wu, H.-D., Lin, Y., and He, S.-B.: Effects of iron on
693 growth and intracellular chemical contents of *Microcystis aeruginosa*, Biomed
694 Environ Sci, 23, 48-52, 2010a.
695 Wang, Q., Niu, Y. A., Xie, P., Chen, J., Ma, Z. M., Tao, M., Qi, M., Wu, L. Y., and Guo, L.
696 G.: Factors affecting temporal and spatial variations of microcystins in Gonghu Bay
697 of Lake Taihu, with potential risk of microcystin contamination to human health,
698 TheScientificWorldJo, 10, 1795-1809, 2010b.
699 Wilhelm, S.: Ecology of iron-limited cyanobacteria: A review of physiological responses
700 and implications for aquatic systems, Aquat Microb Ecol, 9, 295-303, 1995.
701 Woodward, B.: Literature and Interview Project: Constructed Lakes in the Perth
702 Metropolitan and South West Region, Perth, Prepared for Department of Water,
703 Western Australian Local Government Association, 2008.
704 Yan, D. H., Wang, G., Wang, H., and Qin, T. L.: Assessing ecological land use and water
705 demand of river systems: a case study in Luanhe River, North China, Hydrol Earth
706 Syst Sc, 16, 2469-2483, 10.5194/hess-16-2469-2012, 2012.
707 Zhang, T., Zeng, W. H., Wang, S. R., and Ni, Z. K.: Temporal and spatial changes of water
708 quality and management strategies of Dianchi Lake in southwest China, Hydrol
709 Earth Syst Sc, 18, 1493-1502, 10.5194/hess-18-1493-2014, 2014.

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Table 1. Physical and chemical properties of the three lakes throughout the sample period (Jan – 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	$F_{(2,45)} = 2.16$
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. 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 (µg g^{-1})	Extracellular microcystin fraction (%)
pH	-0.108	0.227	-0.297
Temp	0.018	-0.246	0.078
Sal (ppk)	-0.250	0.067	**-0.374***
TP (µg L^{-1})	**-0.337***	**0.399***	**-0.392**
TDP (µg L^{-1})	**-0.357***	**0.296***	**-0.427***
TFe (µg L^{-1})	**-0.570***	**0.343***	-0.037
TDFe (µg L^{-1})	**-0.777**	**0.590**	-0.064
NH_4 (µg 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 site-specific correlation of the respective environmental variables. Site-specific correlation was determined through a significant interaction between lake and environmental variable when analyzed in General Linear Model ($P<0.05$);

N = number of samples.

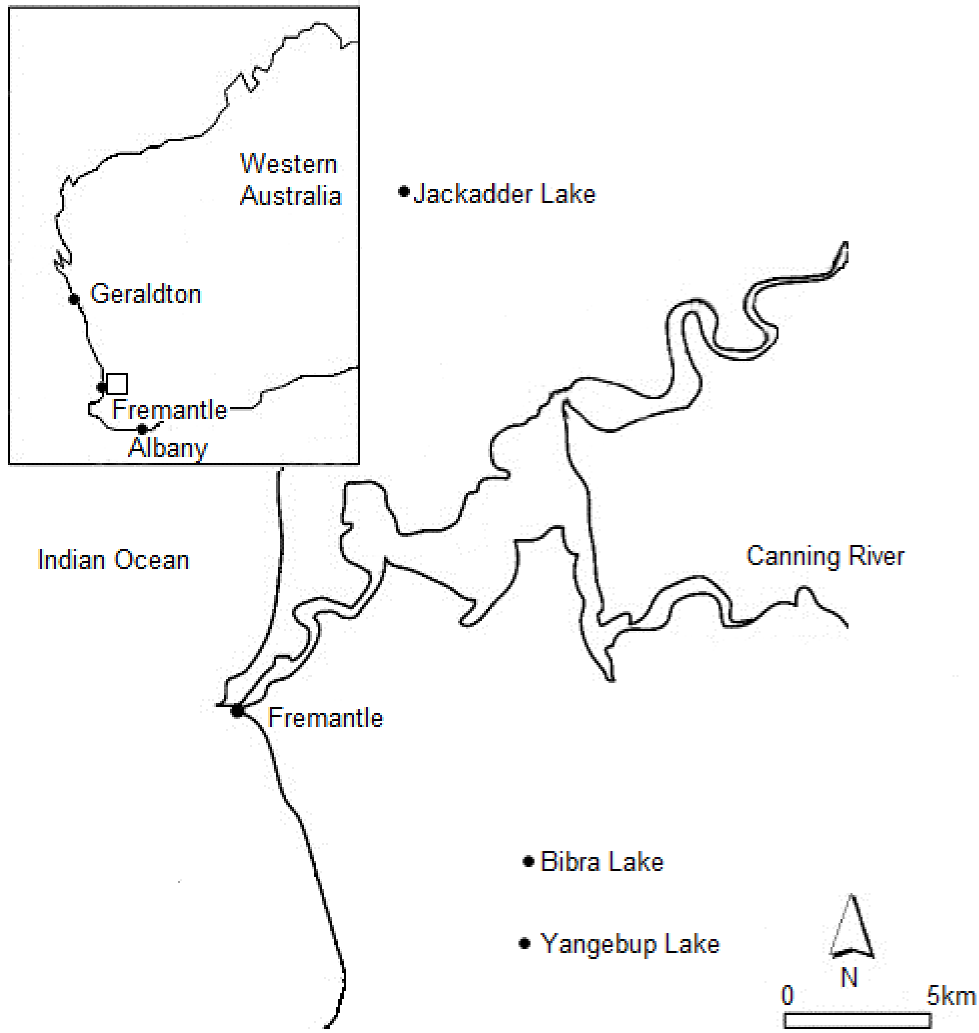
Table 3: Correlation coefficients (R) between the environmental factors and cyanobacterial fraction, cellular microcystin concentration and extracellular microcystin fraction analyzed for each lake using bivariate correlation analysis.

Factor N=48	Cyanobacterial fraction (%)			Cellular microcystin concentration (μg g^{-1})			Extracellular microcystin fraction (%)		
	Jackadder	Bibra	Yangebup	Jackadder	Bibra	Yangebup	Jackadder	Bibra	Yangebup
pH	-0.363	**-0.653**	0.225	0.426	**0.762**	0.190	0.155	-0.714	-0.360
Temp	0.119	-0.112	0.016	-0.288	-0.185	-0.160	0.138	-0.686	0.130
Sal (ppk)	-0.423	-0.204	-0.460	0.330	0.448	**0.587**	**0.570**	-0.775	**-0.659**
TP (μg L^{-1})	**-0.873**	-0.272	**-0.742**	**0.826**	0.489	**0.696**	-0.303	-0.441	-0.295
TDP(μg L^{-1})	-0397	**-0.641**	0.147	**0.553**	**0.764**	0.225	-0.088	-0.498	**-0.587**
TFe (μg L^{-1})	**-0.789**	0.389	-0.304	**0.715**	**-0.605**	0.230	0.380	0.499	-0.245
TDFe (μg L^{-1})	**-0.903**	-0.355	-0.432	**0.811**	0.135	0.400	0.166	0.162	-0.252
NH_4 (μg L^{-1})	0.375	0.576	**0.543**	-0.433	-0.338	**-0.579**	-0.382	0.013	**0.530**
TN (mg L^{-1})	**-0.487**	0.035	**-0.628**	0.441	0.268	**0.613**	0.420	-0.633	-0.417
TDN (mg L^{-1})	**-0.534**	-0.219	-0.305	**0.482**	0.533	**0.479**	0.324	**-0.921**	**-0.633**
TN:TP	**0.570**	0.299	**0.464**	**-0.593**	-0.257	-0.382	0.492	0.514	0.239

Significant (*P*<0.05) factors are highlighted in bold;

N = number of samples.

19



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

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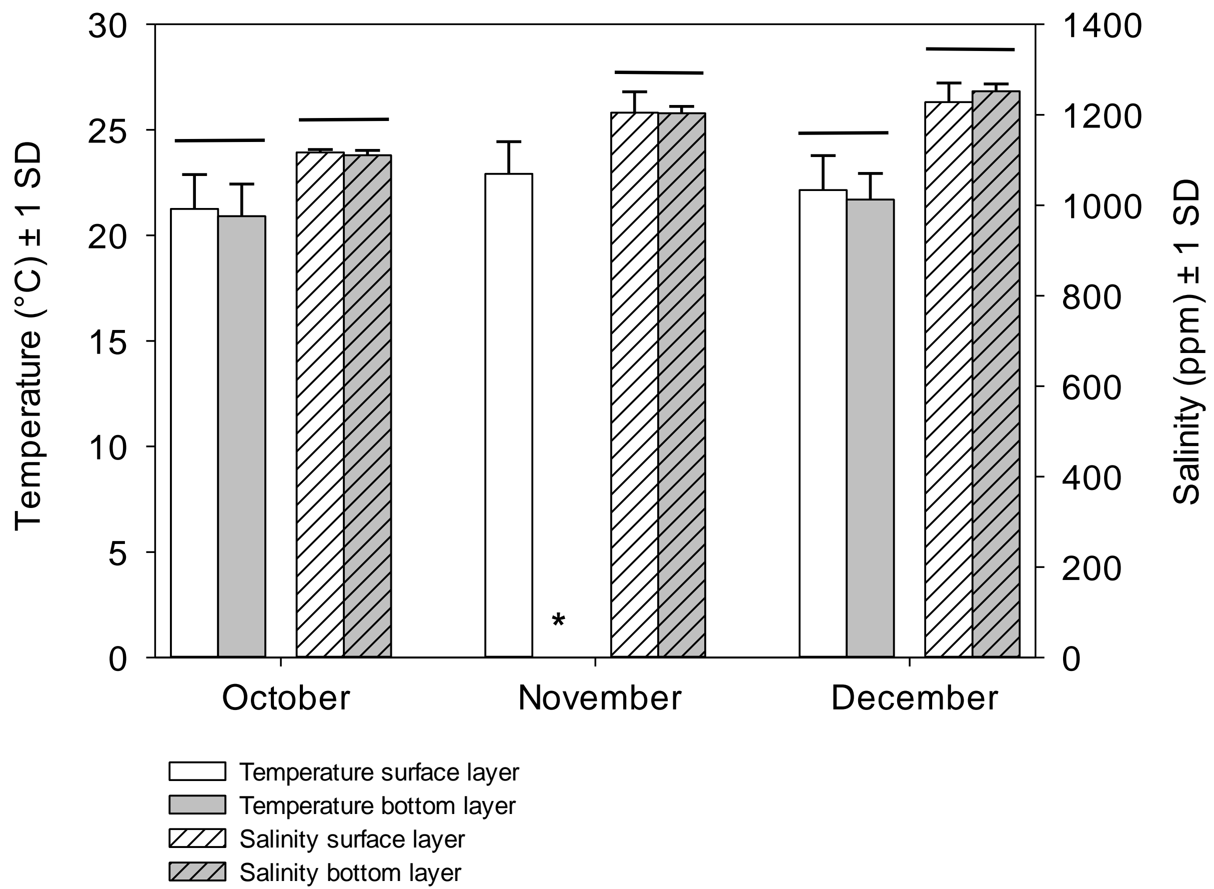

25

26 **Fig. 2.** Temperature (°C) and salinity (ppm) in the surface and bottom layers

27 measured at 7 sites over three months in Lake Yangebup during a previous study in

28 2012. * = missing data; horizontal line indicates that no significant difference between

29 data were detected (t-test).

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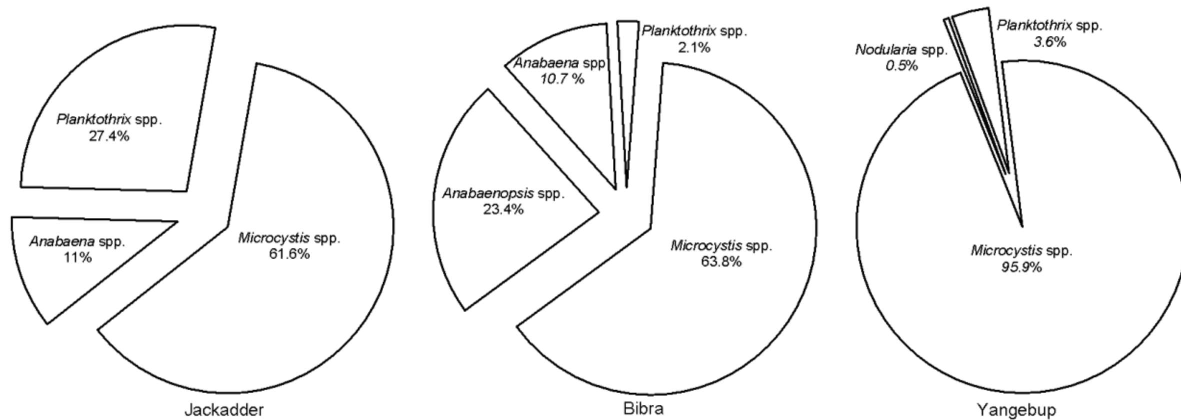

31

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

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

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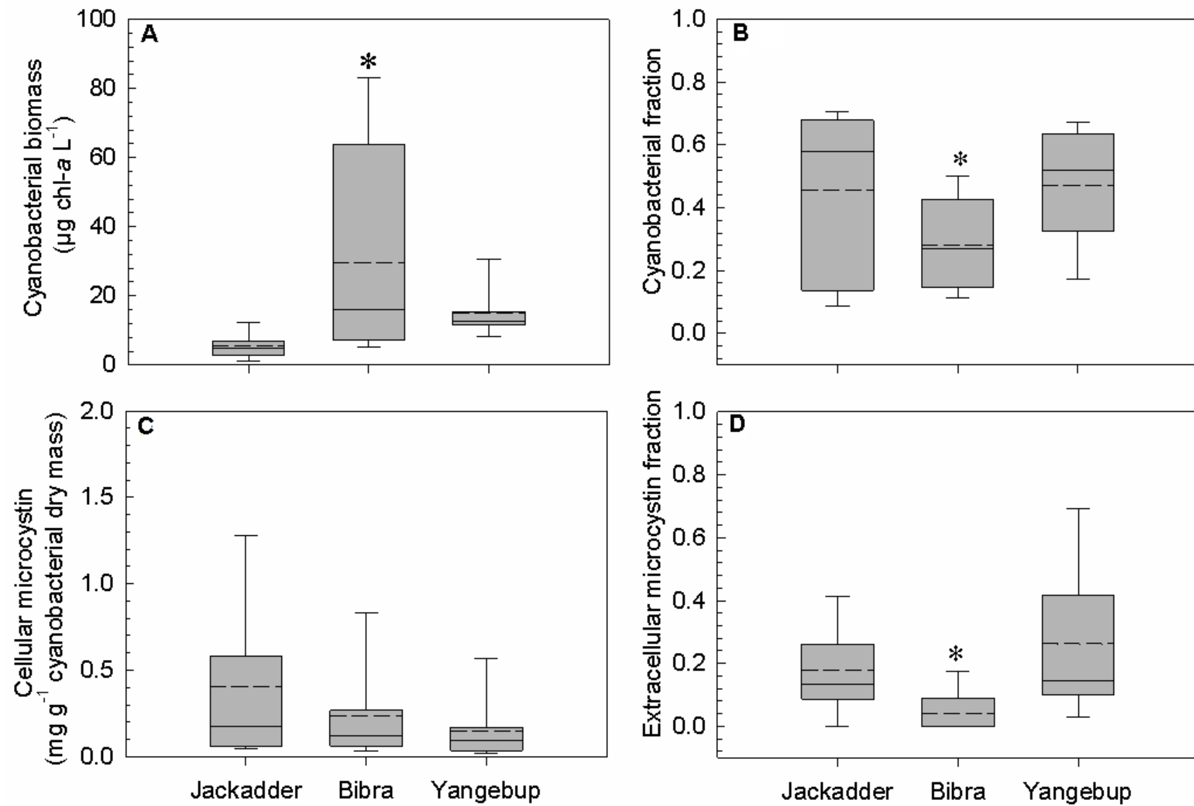

36 **Fig. 4.** The variability of (A) cyanobacterial biomass (µg chl-*a* L^{-1}), (B) cyanobacterial

37 fraction (cyanobacterial biomass to total biomass), (C) cellular microcystin concentration (mg

38 g^{-1} cyanobacterial dry mass) and (D) extracellular microcystin fraction over time for each

39 lake. Boxes represent 25th to 75th percentiles; straight lines within the boxes mark the median

40 short dashed lines the mean; whiskers below and above the boxes indicate 10th and 90th

41 percentiles. Asterisks (*) indicated lakes that are significantly (P<0.05) different from other

42 lakes.

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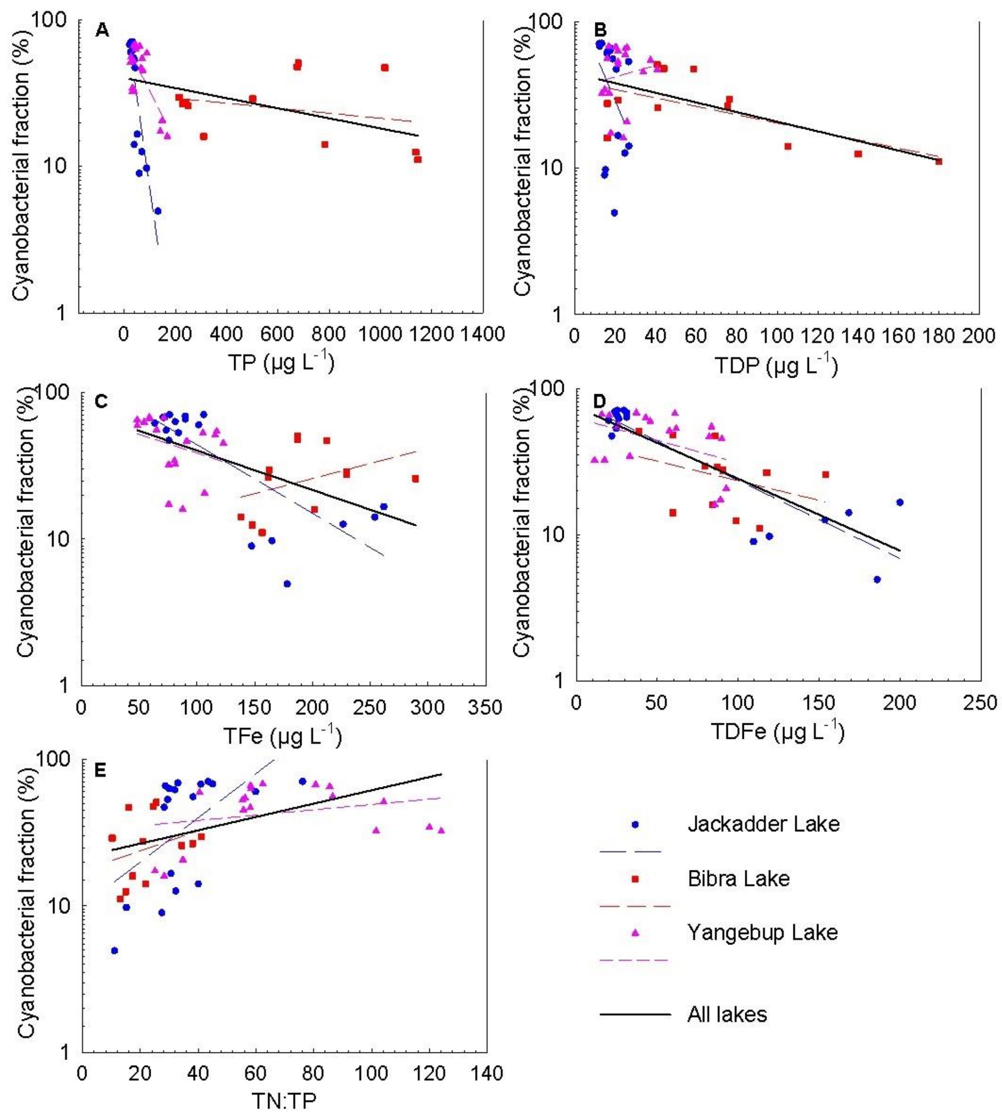

44

45 **Fig. 5.** The correlations between cyanobacterial fraction and (A) TP, (B) TDP, (C) TFe, (D)

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

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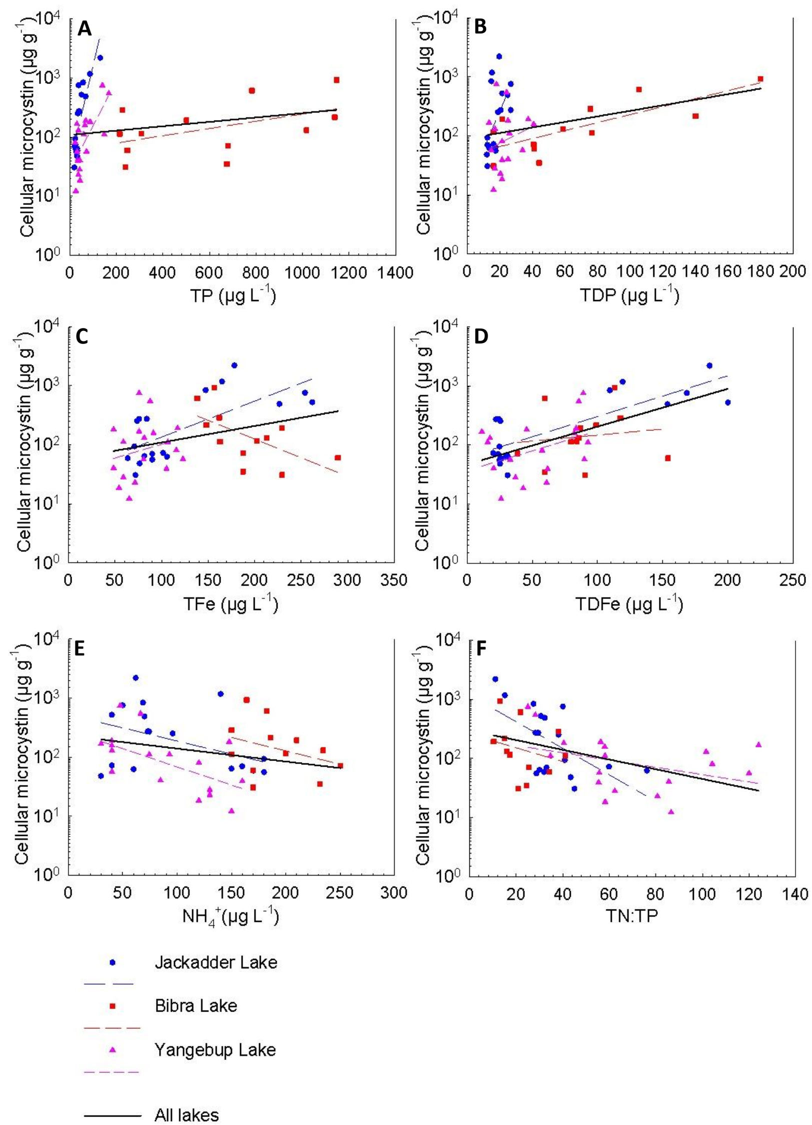

48

49 **Fig. 6.** The correlations between cellular microcystin concentration and (A) TP, (B) TDP, (C)

50 TFe, (D) TDFe, (E) NH_4^+, (F) TN:TP in Jackadder, Bibra and Yangebup lakes during the

51 study period.

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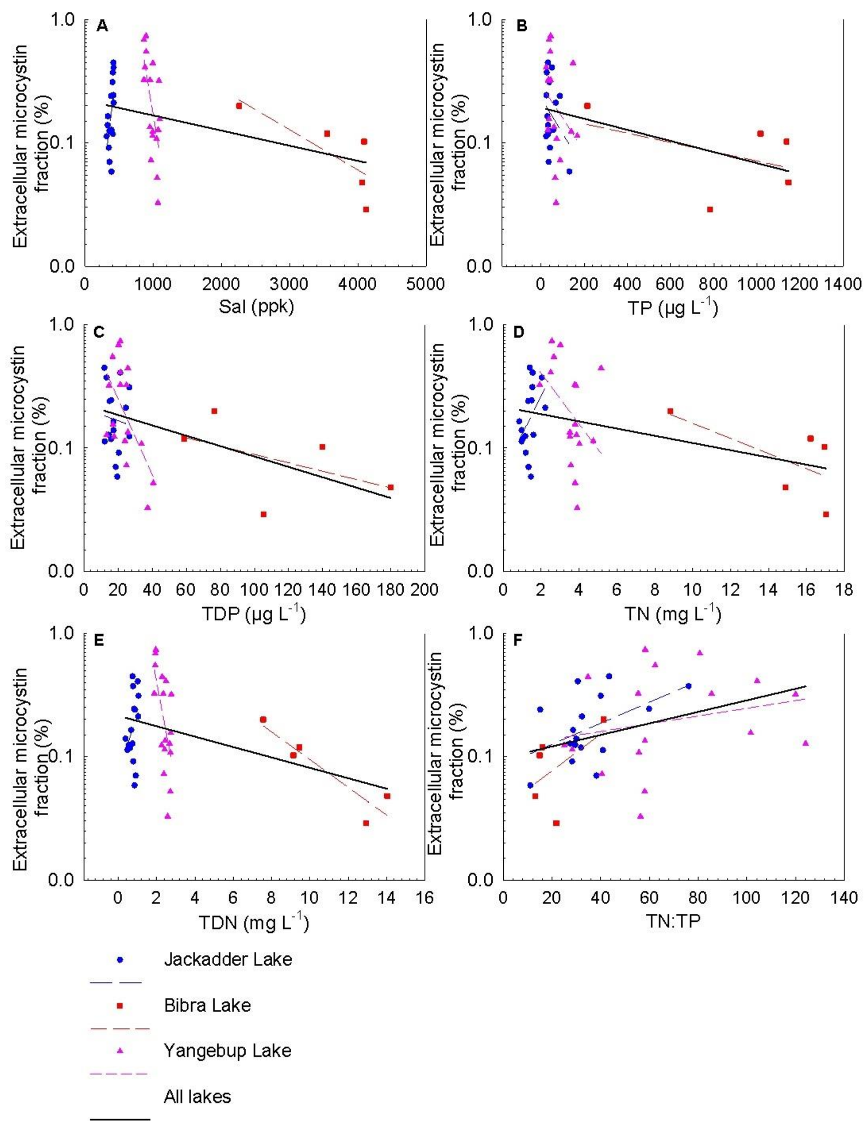

53

54 **Fig. 7.** The correlations between extracellular microcystin fraction and (A) Sal, (B) TP, (C)

55 TDP, (D) TN, (E) TDN, (F) TN:TP in Jackadder, Bibra and Yangebup lakes during the study

56 period.

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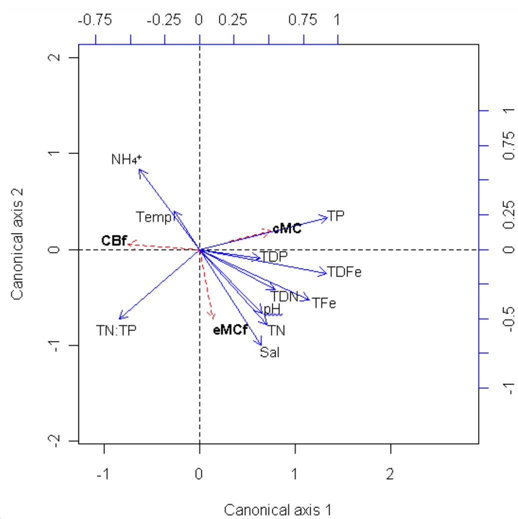

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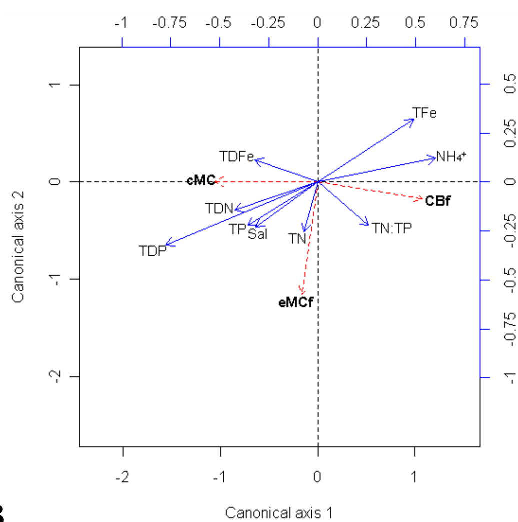

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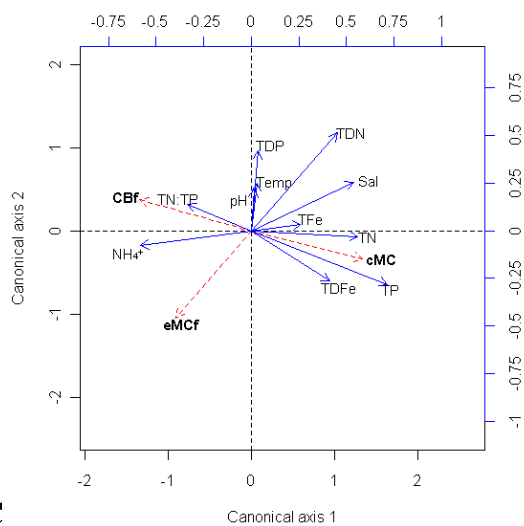

C

59

60 **Fig. 8.** RDA biplots of environmental variables with cyanobacterial fraction (CBf), cellular
61 microcystin (cMC) and extracellular microcystin fraction (eMCf) in (A) Jackadder Lake, (B)
62 Bibra Lake, (C) Yangebup Lake; solid arrows = environmental variables; short dashed arrows
63 = response variables. Canonical axis 1 and 2 represents a linear combination of the
64 environmental variables, and axes are scaled by the square root of their eigenvalues.
65
66