1 Local nutrient regimes determine site-specific environmental triggers of cyanobacterial and
2 microcystin variability in urban lakes
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26 **Abstract**

27 Toxic cyanobacterial blooms in urban lakes present serious health hazards to humans and
28 animals and require effective management strategies. Managing such blooms requires a
29 sufficient understanding of the controlling environmental factors. A range of them has been
30 proposed in the literature as potential triggers for cyanobacterial biomass development and
31 cyanotoxin (e.g., microcystin) production in freshwater systems. However, the environmental
32 triggers of cyanobacteria and microcystin variability remain a subject of debate due to
33 contrasting findings. This issue has raised the question, if the relevance of environmental
34 triggers may depend on site-specific combinations of environmental factors. In this study, we
35 investigated the site-specificity of environmental triggers for cyanobacterial bloom and
36 microcystin dynamics in three urban lakes in Western Australia. Our study suggests that
37 cyanobacterial biomass, cyanobacterial dominance and cyanobacterial microcystin content
38 variability were significantly correlated to phosphorus and iron concentrations. However, the
39 correlations were different between lakes, thus suggesting a site specific effect of these
40 environmental factors. The discrepancies in the correlations could be explained by
41 differences in local nutrient concentration. For instance, we found no correlation between
42 cyanobacterial fraction and total phosphorous (TP) in the lake with the highest TP
43 concentration, while correlations were significant and negative in the other two lakes. In
44 addition, our study indicates that the difference of the correlation between total iron (TFe)
45 and the cyanobacterial fraction between lakes might have been a consequence of differences
46 in the cyanobacterial community structure, specifically the presence or absence of nitrogen-
47 fixing species. In conclusion, our study suggests that identification of significant
48 environmental factors under site-specific conditions is an important strategy to enhance
49 successful outcomes in cyanobacterial bloom control measures.

50

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

53

54

55 1 Introduction

56 Urban lakes often serve as recreational spaces for communities and habitats for wildlife (Yan
57 et al., 2012; Liu, 2014) . To date, many urban lakes continue to deteriorate due to increased
58 anthropogenic activities and often face water quality problems including toxic cyanobacteria
59 blooms (Pineda-Mendoza et al., 2012; Reichwaldt and Ghadouani, 2012; Lei et al., 2014; Sun
60 et al., 2014; Zhang et al., 2014). This issue has received great attention from water authorities
61 world-wide as it presents health hazards to humans and animals who either directly or
62 indirectly received services provided by urban lakes (O'Bannon et al., 2014; Rastogi et al.,
63 2014; Waajen et al., 2014). The management of toxic cyanobacterial blooms is often
64 challenging due to the variability in cyanobacteria biomass and microcystins (Rolland et al.,
65 2013; Carey et al., 2014). In addition, microcystin production by cyanobacteria is a complex
66 issue that might depend on their competition with other phytoplankton (e.g., Huisman and
67 Hulot, 2005; Jang et al., 2006). From these earlier studies it can be concluded that the toxin
68 concentration produced by a certain cyanobacterial biomass level might differ, depending on
69 the level of competition (i.e. cyanobacterial fraction) indicating that management should
70 consider biomass and cyanobacterial fractions concurrently.

71

72 Cyanobacterial biomass and the amount of microcystins being produced during toxic
73 cyanobacterial blooms can vary significantly on a spatial basis within and between lakes
74 (Reichwaldt et al., 2013; Sinang et al., 2013; Thi Thuy et al., 2014; Waajen et al., 2014). Past
75 studies have found large variations in the percentage of potentially toxic cyanobacteria and in

76 the microcystin concentration between spatially isolated phytoplankton communities (Sitoki
77 et al., 2012; Li et al., 2014). Furthermore, it was reported that the variability of cyanobacterial
78 biomass in lakes only explained a small fraction of the variability in microcystin
79 concentration (Sinang et al., 2013; Eva and Lindsay, 2014). These findings highlight the
80 importance to fully understand the roles of environmental factors controlling both, the
81 cyanobacteria and the microcystin variability.
82
83 It has been suggested that cyanobacterial biomass and microcystin variability largely depends
84 upon physical, chemical and biological properties of the water bodies (Engström-Öst et al.,
85 2013; Lehman et al., 2013; Paerl and Otten, 2013; Ruiz et al., 2013). A range of
86 environmental factors, including nitrogen and phosphorus concentrations (Schindler, 2012;
87 Srivastava et al., 2012; Chaffin and Bridgeman, 2014; Van de Waal et al., 2014), TN:TP ratio
88 (Smith, 1983; Wang et al., 2010b; Van de Waal et al., 2014), temperature (Davis et al., 2009;
89 Rolland et al., 2013), salinity (Tonk et al., 2007), and iron concentration (Ame and
90 Wunderlin, 2005; Nagai et al., 2007; Wang et al., 2010a) have been shown to have
91 pronounced effects on cyanobacterial biomass, cyanobacterial dominance and microcystin
92 production. Nevertheless, the results between studies differ, and there is no clear
93 understanding of the roles of these environmental factors as the triggers of cyanobacterial
94 bloom development and microcystin production. Furthermore, the occurrence of
95 cyanobacterial toxins in a system is the result of a complex interaction between abiotic and
96 biotic factors, including the competition with other phytoplankton. It therefore remains an
97 important challenge for bloom management to fully understand the mechanisms behind toxic
98 cyanobacterial bloom development and the drivers for biomass development, cyanobacterial
99 dominance (fraction) and toxin production. For instance, regardless of the fact that many
100 studies suggesting the important role of phosphorus, reduction of internal and external

101 phosphorus concentration is not always successful in preventing the occurrence of toxic
102 cyanobacterial blooms in water bodies (Lewis and Wurtsbaugh, 2008; Amano et al., 2010;
103 Koreiviene et al., 2014).
104
105 By taking into account the contrasting findings of earlier studies, including inconsistent
106 outcomes of nutrient reduction strategies, we suggest that the main environmental triggers of
107 cyanobacterial and microcystin variability may vary between water bodies due to the
108 complex, lake specific interplay of environmental conditions. Therefore, the main objective
109 of this study was to investigate the site-specificity of environmental triggers for
110 cyanobacterial biomass and microcystin variability in a local urban lake system. More
111 specifically, the objectives were to (1) determine the variability of cyanobacterial biomass
112 and microcystin concentration in a set of local urban lakes, (2) identify the site-specific
113 relationships between environmental factors and cyanobacterial or microcystin dynamics.
114

115 2 Material and methods

116 2.1 Study lakes

117 This study was carried out in Jackadder Lake (31°54′30″S, 115°47′36″E), Bibra Lake
118 (32°5′25″S, 115°49′16″E) and Yangebup Lake (32°6′56″S, 115°49′33″E) located on the
119 Swan Coastal Plain, Western Australia (Fig. 1). Sampling was carried out between January
120 and March 2010. These lakes are shallow with mean depth of 2.1 m, 1.1 m, and 2.5 m for
121 Jackadder Lake, Bibra Lake and Yangebup Lake, respectively. Jackadder Lake and
122 Yangebup Lake are permanent lakes while Bibra Lake is subjected to seasonal drying due to
123 progressive decline in groundwater levels over the Jandakot Mound. Jackadder Lake has an
124 area of 7.18 ha, is surrounded by 6.6 ha of parkland and is draining a 152 ha catchment area,
125 (Arnold, 1990; Woodward, 2008). Water levels in Jackadder Lake are maintained by the

126 input of surface runoff via 10 drain inlets (Rajah 1991, as cited in Kemp, 2009). Jackadder
127 Lake receives water from the Herdsman Lake catchment area and Osborne Park main drain
128 during dry summers (Department of Planning, 2010). Bibra Lake has a size of 135 ha with an
129 open water area of approximately 100 ha (Strategen, 2009) and is located within a 250 ha
130 catchment are. This lake is surrounded by urban areas and a golf course and serves as habitat
131 for many species of water birds (Kemp, 2009). Water enters Bibra Lake via direct rainfall
132 recharge onto the lake surface or from surface runoff from the surrounding catchment
133 (Strategen, 2009). Yangebup Lake has a total area of 90.5 ha with an open water area of
134 approximately 68 ha, and is surrounded by residential, agriculture and industrial areas.
135 Yangebup Lake is a groundwater through-flow wetland that accepts groundwater from the
136 east and discharges groundwater to the west (Dunlop, 2008). Yangebup Lake receives urban
137 runoff from three stormwater drains and additionally serves as a compensation basin for the
138 South Jandakot Drainage system with an approximate area of 200 km^2. This includes
139 receiving water from neighbouring Thomson Lake when it reaches its maximum water level.
140 Once Yangebup Lake reaches its maximum allowable water level, water is pumped into
141 nearby Cockburn Sound (Environmental Protection Authority, 1989). The hydrology of
142 Jackadder, Bibra and Yangebup lakes is mainly affected by the strong seasonal rainfall
143 pattern due to the Mediterranean climate. The region's mean annual rainfall is reported as
144 771.5 mm and monthly mean rainfall is 35.1 , 156.3 , 433.3 , and 144.2 mm during summer,
145 autumn, winter and spring, respectively (Bureau of Metereology, 2014). In response, the
146 maximum water levels in all lakes occur in September and October, and the minimum water
147 levels occur in March and April at the end of summer months (Davis et al., 1993). The
148 region's mean maximum annual temperature is 24.5 °C and monthly maximum temperature
149 are 30.9, 25.4, 18.0 and 22.6°C during summer, autumn, winter and spring, respectively
150 (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

176 well mixed with respect to physicochemical conditions (Arnold and Oldham, 1997; Song et
177 al., 2015) (Fig. 2). Water samples were stored immediately in glass bottles in the dark on ice.
178 Variables analysed from these samples were total phosphorus (TP), total dissolved
179 phosphorus (TDP), total iron (TFe), total dissolved iron (TDFe), total nitrogen (TN), total
180 dissolved nitrogen (TDN), ammonium (NH_4^+), cyanobacterial biomass, total phytoplankton
181 biomass, intracellular and extracellular microcystin fractions. Samples for dissolved nutrient
182 analyses were pre-filtered with a 0.45µm syringe filter (Acrodisc, HT Tuffryn) before
183 freezing at -20°C.
184
185 Surface water temperatures were between 19.9 and 28.7°C during the study period. However,
186 the onsite measurements of surface water temperatures were dependent on the time of
187 sampling and varied by up to 3.9°C over the course of a day. Therefore, maximum air
188 temperature on each sampling day recorded by weather stations located nearest to the studied
189 lakes was used as a substitute for surface water temperature in all analyses (Yen et al., 2007).
190

191 **2.2.1 Nutrients and phytoplankton biomass**

192 TP and TDP concentrations were analyzed using the ascorbic acid method, while TFe and
193 TDFe concentrations were analyzed with the Phenanthroline method, according to standard
194 methods (APHA, 1998). TN, TDN, and NH_4^+ were analyzed at the South Coast Nutrients
195 Analysis Laboratory, Albany, Western Australia with the standard colorimetric methods on a
196 segmented flow auto-analyser (Alpkem, Wilsonville, OR, USA). Cyanobacterial and total
197 phytoplankton chlorophyll-*a* were measured with a top-bench version of a FluoroProbe (bbe
198 Moldaenke, Germany).The FluoroProbe measures chl-*a* fluorescence and differentiates four
199 groups of phytoplankton (chlorophytes, cryptophytes, diatoms, and cyanobacteria) by their
200 specific fluorescence emission spectrum (Beutler et al., 2002). The fluorescence is used to

201 calculate total biomass of each phytoplankton group that is expressed as chl-*a* concentration
202 equivalents (µg chl-*a* L^{-1}) (Beutler et al., 2002; Ghadouani and Smith, 2005). FluoroProbe
203 chl-*a* measurements were validated against chl-*a* data of samples extracted according to
204 standard methods (APHA, 1998) (linear regression analysis: $R^2 = 0.94$, $N = 32$, $P < 0.05$). In
205 our study, chl-*a* fluorescence as measured by FluoroProbe was used as a proxy for
206 cyanobacterial biomass (Geis et al., 2000; Eisentraeger et al., 2003).
207
208 For quantification of cyanobacterial biomass and to separate the intracellular from the
209 dissolved microcystin fraction, water samples were filtered through pre-combusted and pre-
210 weighed 47 mm GF/C filter papers. Filter papers containing particulate organic matter were
211 dried for 24 hours at 60°C and re-weighed to obtain total dry weight (Harada et al., 1999).
212 These filter papers were then moistened with Milli-Q water and kept frozen (at -20°C) until
213 intracellular microcystin extraction. As we were interested in the microcystin concentration
214 per unit cyanobacterial dry mass, cyanobacterial dry mass was calculated from the total dry
215 mass (from the filters) by adjusting it to the percentage of cyanobacteria measured with the
216 FluoroProbe. Cyanobacterial dry mass was only used for microcystin quantification.
217
218 Water samples collected for cyanobacterial identification and enumeration were preserved
219 with acidic Lugol's iodine solution (5 g I_2+10 g KI, 20 ml distilled water and 50 ml of 10%
220 acetic acid) and cyanobacteria were identified to the genus level using phytoplankton
221 taxonomic guideline (Komarek and Hauer, 2011). The relative abundance of each
222 cyanobacterial genera (cells or colonies ml^{-1}) was determined from 10-50 ml of sample using
223 an inverse microscope (Utermöhl, 1958) and converted into biovolume per ml (μm^3 ml^{-1}) by
224 multiplying the mean cell or colony biovolume (μm^3) with the total cells or colonies per ml
225 (cells or colonies ml^{-1}). Mean cell or colony biovolume for each cyanobacterial genus was

226 calculated by finding the geometric figure that best approximated the shape of each genera,
227 and by measuring the dimension of 20 individual cells or colonies (Hillebrand et al., 1999). A
228 minimum of 200 cells or colonies of the most abundant cyanobacteria were counted for each
229 sample. Different cyanobacterial species within each genus can vary in size by several orders
230 of magnitude. However, as we measured the mean biovolume of each cyanobacterial genus,
231 differences in sizes between species are evened out as a larger mean is expected, if larger
232 species are more abundant and vice versa. The calculated mean biovolume of each
233 cyanobacterial genus was used to compute the dominant cyanobacteria genera in the studied
234 lakes.
235
236 **2.2.2 Microcystin extraction and quantification**
237 Filters were freeze-thawed twice to break the cells prior to methanol extraction (Lawton et
238 al., 1994). Filters were placed into centrifuge tubes and 5 ml of 75% methanol-water (v/v)
239 was added. Filters were sonicated on ice for 25 min, followed by gentle shaking for another
240 25 min. The extracts were then centrifuged at 3273*g* (Beckman and Coulter, Allegra X-12
241 Series) for 10 min at room temperature. Extracts were carefully transferred into conical
242 flasks, and two more extractions were done per filter. All three extracts were pooled and
243 diluted with Milli-Q to 20% methanol (v/v).
244
245 Intracellular microcystin extracts and the pre-filtered water containing dissolved
246 (extracellular) microcystin were subjected to solid-phase extraction (SPE) (Waters Oasis
247 HLB) for clean-up and concentration with a loading speed of < 10 ml min^{-1}. SPE cartridges
248 were then rinsed with 10 ml of 10, 20 and 30% methanol-water (v/v), before microcystin was
249 eluted with acidified methanol (0.1% v/v trifluoroacetic acid (TFA)) and evaporated with
250 nitrogen gas at 40°C. Finally, samples were re-dissolved in 30% acetonitrile and analysed

251 with high-performance liquid chromatography (HPLC) by using the Alliance 2695 (Waters,
252 Australia) with a PDA detector (1.2 nm resolution) and an Atlantis T3 3µm column (4.6 x
253 150mm i.d). Mobile phases used were acidified acetonitrile (0.05% v/v TFA) and acidified
254 Milli-Q water (0.05% v/v TFA). Microcystin peaks were separated using a linear gradient as
255 described in Lawton et al., (1994) but with a maximum acetonitrile concentration of 100%
256 and a run time of 37 min. Column temperature was maintained at 37.5 ± 2.5 °C. The limit of
257 detection per microcystin peak was 1.12 ng. Microcystin variants were identified based upon
258 their typical absorption spectrum detected by PDA detector at 238 nm (Meriluoto and Codd,
259 2005). Commercially available microcystin-LR standard (Sapphire Bioscience, Australia;
260 purity ≥ 95 %) was used to quantify microcystin concentrations. Throughout this manuscript
261 we refer to the total concentration of microcystin variants per sample as microcystin
262 concentration.
263
264 In this study, cellular (intracellular) microcystin concentration was expressed as µg
265 microcystin-LR mass equivalents per g cyanobacterial dry mass to illustrate cyanobacterial
266 microcystin content. Extracellular microcystin was expressed as the fraction of extracellular
267 microcystin concentration per total microcystin concentration to allow the quantification of
268 the proportion of microcystin released into the water column in comparison to the total
269 microcystin being produced.
270

271 **2.3 Data processing and statistical analyses**

272 Differences in physicochemical factors, cyanobacterial biomass and microcystin between
273 lakes were analysed with one-way ANOVA (SPSS 17.0) with post hoc test (Least
274 Significance Difference; LSD) as all assumptions for an ANOVA were met (homogeneity of
275 variances, normality). For the descriptive phase, bivariate correlation analysis (Pearson's)

276 was carried out to identify the environmental variables which significantly correlate with
277 cyanobacterial fraction, cyanobacterial biomass, cellular microcystin concentration and
278 extracellular microcystin fraction (SPSS 21.0). We used linear mixed models to identify
279 correlations between environmental variables and cyanobacterial fraction, cyanobacterial
280 biomass, cellular microcystin concentration and extracellular microcystin fraction in each
281 lake using sampling site and sampling date as random factors, and for all lakes combined
282 adding lake as random factor (SPSS 21.0). All dependent variables were ln-transformed. As
283 extracellular microcystins were only detected in five out of twelve samples in Bibra Lake,
284 this resulted in only five data points for this dependent variable in Bibra Lake, making the
285 calculation of linear mixed models for this explanatory variable impossible. Two redundancy
286 analyses (RDA) were calculated to identify the best combination of variables to explain the
287 variability of intracellular microcystin concentration, extracellular microcystin fraction and
288 either cyanobacterial fraction or cyanobacterial biomass, (R version 2.15.1) for each lake.
289 Canonical ordination (999 permutations) with forward selection was computed with
290 standardised explanatory and response variables. All data was ln transformed to meet the
291 assumption of normality. RDA analysis on Bibra Lake was conducted without the inclusion
292 of pH and temperature due to an inadequate number of data points (residual d.f < 0). In all
293 analyses, results were considered significant at $P < 0.05$, unless stated differently.

294

295 **3 Results**

296 **3.1 Physical and chemical characteristics of studied lakes**

297 On the sampling days, mean pH fluctuated between 8.2 and 9.2 (Fig. 3A) and mean air
298 temperature (Fig. 3B) ranged from 27 to 43°C in all lakes. Salinity in Jackadder and
299 Yangebup was mostly below 1.0 ppk and much lower than in Bibra Lake (Fig. 3C). The
300 sharp increase in salinity in Bibra Lake was probably due to the decreasing water level as the

301 lake dried up by end of February. Nutrient concentrations varied on a temporal basis within
302 lakes and spatially between lakes. Phosphorus concentrations were higher in Bibra Lake than
303 in Jackadder and Yangebup Lakes throughout the sampling period. Mean TP concentrations
304 (Fig. 3D) ranged from 22 to 92, from 230 to >1000, from and 28 to >150 $\mu g\ L^{-1}$ in Jackadder,
305 Bibra and Yangebup Lakes, respectively. Meanwhile, mean TDP concentrations (Fig. 3E)
306 ranged from 12 to 24, from 17 to 142, and from 14 to 37 $\mu g\ L^{-1}$ in Jackadder, Bibra and
307 Yangebup Lakes, respectively. Temporal variation of macronutrient concentrations in
308 Yangebup and Jackadder Lakes were much smaller than in Bibra Lake. The large increase of
309 TP, TDP, TN and TDN in Bibra Lake might again have been a concentration effect due to the
310 lake drying up. Mean TFe and TDFe concentrations were higher in Bibra Lake during the
311 earlier three sampling dates. Mean TFe (Fig. 3F) ranged from 77 to 247, from 147 to 220,
312 and from 51 to 110 $\mu g\ L^{-1}$ in Jackadder, Bibra and Yangebup Lakes, respectively. Mean
313 TDFe (Fig. 3G) ranged from 24 to 174, from 61 to 117, and from 21 to 89 $\mu g\ L^{-1}$ in
314 Jackadder, Bibra and Yangebup Lakes, respectively. TN (Fig. 3H) and TDN (Fig. 3I)
315 concentrations were up to one order of magnitude higher in Bibra Lakes compared to
316 concentrations in Jackadder and Yangebup Lakes. In contrast, mean TN:TP in Bibra Lake
317 were lower than the ratios in Jackadder and Yangebup Lakes (Fig. 3J). Mean TN:TP ranged
318 from 18 to 60, 16 to 38, and 29 to 115 in Jackadder, Bibra and Yangebup Lakes, respectively.
319 NH_4^+ decreased over time in Jackadder and Yangebup Lakes (Fig. 3K) and mean
320 concentrations ranged from 43 to 170, from 157 to 239, and from 40 to 143 $\mu g\ L^{-1}$ in
321 Jackadder, Bibra and Yangebup Lakes, respectively.
322
323 The three lakes were significantly different in salinity, phosphorus, nitrogen and iron, either
324 as total or dissolved forms (except TDFe) (ANOVA; Table 1), but did not show a significant
325 difference in pH, air temperature and TDFe. The posthoc tests (LSD) indicated that Jackadder

326 and Yangebup Lake did not differ in TP, TDP, and NH_4^+, however, both lakes were different
327 to Bibra Lake. Furthermore, all lakes were different in salinity, TN, TDN, and TFe. Jackadder
328 and Yangebup Lakes can be classified as eutrophic, while Bibra Lake can be classified as
329 hypereutrophic, based on the mean TP concentrations (Carlson, 1977). Nitrogen limited
330 conditions in a lake is usually defined when the TN:TP weight ratios are less than 10
331 (Graham et al., 2004). As our result indicate that TN:TP ratios below 10 were rare, the
332 studied lakes were not associated with persistent nitrogen limitation.
333

334 **3.2 Variability of cyanobacterial biomass and microcystin concentration**

335 Cyanobacterial communities in all lakes contained potentially toxin-producing cyanobacteria
336 including *Microcystis* spp., *Planktothrix* spp., *Anabaenopsis* spp., *Anabaena* spp and
337 *Nodularia* spp. (Fig. 4) with *Microcystis* spp. being the most abundant cyanobacterial genera
338 in all lakes. Mean total cyanobacterial biomass was 5.41 µg L^{-1}, 29.60 µg L^{-1}, 15.14 µg L^{-1} in
339 Jackadder, Bibra and Yangebup Lake, respectively (Fig. 5A). Cyanobacterial biomass varied
340 within an order of magnitude on a temporal basis in Bibra and Jackadder Lake (Jackadder: 1 -
341 12 µg L^{-1}, Bibra: 5 - 83 µg L^{-1}, Yangebup: 8 - 32 µg L^{-}1). Although cyanobacterial biomass
342 was significantly higher in Bibra Lake compared to the other two lakes ($F_{(2,45)}$= 7.62, $P <$
343 0.05), the cyanobacterial fraction (the ratio of cyanobacterial chlorophyll-*a* to total
344 phytoplankton chlorophyll-*a*) in this lake was significantly lower than in Jackadder and
345 Yangebup Lake ($F_{(2,45)}$= 3.59, $P < 0.05$) (Fig. 5B). Cyanobacterial fraction ranged between
346 0.05 to 0.71 in Jackadder Lake, 0.16 to 0.68 in Yangebup Lake, and 0.11 to 0.51 in Bibra
347 Lake. The post hoc tests indicated that Jackadder and Yangebup Lakes did not differ in
348 cyanobacterial biomass and cyanobacterial fraction, but both lakes were different to Bibra
349 Lake.
350

351 Cellular microcystin concentration (mg g^{-1} cyanobacterial dry mass) varied over three orders
352 of magnitude in Jackadder Lake, and two orders of magnitude in both Bibra Lake and
353 Yangebup Lake (Fig. 5C) throughout the sampling events. Mean cellular microcystin
354 concentrations were 0.407 mg g^{-1} in Jackadder Lake, 0.233 mg g^{-1} in Bibra Lake, and 0.150
355 mg g^{-1} in Yangebup Lake. Cellular microcystin concentration was not significantly different
356 between lakes ($F_{(2,45)}$= 2.07, P >0.05). Mean extracellular microcystin fraction was 0.18 in
357 Jackadder Lake, 0.04 in Bibra Lake, and 0.26 in Yangebup Lake (Fig. 5D). The post hoc tests
358 indicated that Bibra Lake was the only lake that had a significantly different extracellular
359 microcystin fraction when compared to other lakes ($F_{(2,45)}$= 6.49, P <0.05).
360

361 **3.3 Relationship between environmental factors and cyanobacterial fraction,**
362 **cyanobacterial biomass, or microcystin concentration**

363 Most analysed nutrients were weakly, but significantly correlated with cyanobacterial
364 fraction, biomass and microcystin concentrations when data from all lakes were combined
365 (Table 2, 3). The correlations presented in Tables 2 and 3 suggested that, in general,
366 cyanobacterial dominance in the phytoplankton community was favored at relatively lower
367 nutrient concentrations as it was negatively correlated to TP, TDP, TFe, and TDFe. In
368 contrast, cyanobacterial fraction was positively correlated with TN:TP ratio, potentially due
369 to relatively lower TP concentrations in comparison to TN concentrations. Cyanobacterial
370 biomass on the other hand was positively correlated to salinity, TN, TDN and NH_4^+, but
371 negatively correlated with TDFe. Cellular microcystin concentration was positively
372 correlated with phosphorus and iron, but not with nitrogen. TDFe showed the strongest
373 positive correlation with cellular microcystin concentration, followed by TP, TFe, and TDP.
374 Cellular microcystin was also negatively correlated with TN:TP ratio (Table 3). In contrast to
375 cellular microcystin, extracellular microcystin fraction was negatively correlated with

376 salinity, TP, TDP, TN, TDN, and positively correlated with TN:TP ratio (Table 3).
377 Correlating environmental variables with cyanobacteria or microcystin for each lake
378 separately, the correlations that were significant (Pearson's) were different between lakes
379 (Table 2, 3).
380
381 Using data from all lakes combined in linear mixed models, cyanobacterial fraction was
382 negatively correlated to TP, TDP, TFe, TDFe (Fig. 6A-D), and positively to TN:TP (Fig. 6
383 E). However, within each lake, the correlations with cyanobacterial fraction were significant
384 only for TP, TDP and TDFe in Jackadder Lake and TP in Yangebup Lake. Cellular
385 microcystin concentration was on the other hand positively correlated to TP, TDP, TFe and
386 TDFe (Fig. 7A-D) and negatively to TN:TP (Fig. 7E). Within each lake, these correlations
387 were only significant for TP, TFe, TDFe in Jackadder Lake (Fig. 7A, C, D), for TDP in Bibra
388 Lake (Fig. 7B) and for TP in Yangebup Lake (Fig. 7A). When combining all lakes,
389 extracellular microcystin fraction was negatively correlated to salinity (linear mixed model;
390 $p<0.1$), TP and TDP, but positively to TN:TP (Fig. 8A-D). Jackadder Lake was the only lake
391 showing significant correlations between extracellular microcystin fraction and salinity
392 (positive, Fig. 8A) and TP (negative, Fig. 8B). Using linear mixed models, cyanobacterial
393 biomass was only significantly correlated to TDP and TDFe when combining all lakes (Fig.
394 8E, F), with Bibra Lake showing a significant negative correlation to TDFe (Fig. 8F). The
395 95% confidence intervals of the slopes of the correlations between TP and cyanobacterial
396 fraction or extracellular microcystin fraction in Jackadder Lake and in all lakes combined
397 (Fig. 6A, 8B) or between salinity and extracellular microcystin fraction in Jackadder Lake
398 and in all lakes combined (Fig. 8A) did not overlap, providing a conservative estimate that
399 the slopes were significantly different (Payton et al., 2003).
400

401 **3.4 Multivariate analysis of site-specific environmental factors and the variability of**
402 **cyanobacteria and microcystin concentration**
403 The first RDA analysis showed significant relationships ($P < 0.05$) between the measured
404 environmental factors and the combined variability of cyanobacterial fraction, cellular
405 microcystin concentration and extracellular microcystin fraction for each lake. The canonical
406 ordination indicated that 75% (Jackadder Lake; $R^2_{adj.} = 0.75$; F=5.726), 80% (Bibra Lake; R^2
407 $_{adj.} = 0.80$; F=5.888) and 75% (Yangebup Lake; $R^2_{adj.} = 0.75$; F=5.804) of the combined
408 variability of cyanobacterial fraction, cellular microcystin concentration and extracellular
409 microcystin fraction can be explained by the measured environmental factors (Fig. 9A - C).
410 The second RDA analysis, which sought to find relationships between environmental factors
411 and absolute cyanobacterial biomass, cellular microcystin concentration and extracellular
412 microcystin fraction for each lake found that 71% (Jackadder Lake; $R^2_{adj.} = 0.71$; F=4.725),
413 80% (Bibra Lake; $R^2_{adj.} = 0.80$; F=5.806) and 66% (Yangebup Lake; $R^2_{adj.} = 0.66$; F=3.953)
414 of the combined variability of absolute cyanobacterial biomass, cellular microcystin
415 concentration and extracellular microcystin fraction can be explained by the measured
416 environmental factors (Fig. 10A - C).
417
418 In both sets of analyses, many of the environmental factors that were closely correlated to
419 cyanobacteria and microcystins were slightly different between lakes. TDP was only
420 correlated to either cyanobacteria fraction or cellular microcystin concentration in Bibra and
421 Jackadder Lakes (Fig. 9A, B) but not in Yangebup Lake (Fig. 9C). Additionally, TFe was
422 positively correlated to cyanobacteria only in Bibra Lake (Fig. 9B, 10B) but not in the other
423 two lakes (Fig. 9A, 9C, 10A, 10C). In comparison to the other factors, TDFe was always
424 negatively correlated to cyanobacterial fraction and biomass and positively correlated to
425 cellular microcystin concentration variability (Fig. 9, 10).

426

427 ## 4 Discussion

428 The relationships between the environmental factors and cyanobacterial and microcystin
429 variability were different between lakes. This is an indication that the relevance of factors
430 that drive cyanobacteria and their toxin production depends on their site-specific
431 combinations. Our results suggest that the site-specificity of environmental triggers may be
432 related to spatial heterogeneity of the respective environmental factor, as each factor can be
433 present at different concentration regimes in each lake. Graham et al. (2004) and Dolman et
434 al. (2012) have suggested that the correlations between the environmental factors and
435 cyanobacterial biomass and microcystin concentration could change when the concentrations
436 of the respective environmental factors increase from low to high in systems. Our results
437 support these previous findings as the relationships between cyanobacterial fraction,
438 cyanobacterial biomass and cellular microcystin concentration with TFe and TDFe were
439 closely related to the concentration levels of TFe and TDFe in each lake. Mean TFe
440 concentration in Bibra Lake was one order of magnitude higher than in Jackadder and
441 Yangebup Lakes, while mean TDFe concentrations in all lakes ranged within the same order
442 of magnitude (Table 1). This could explain why the relationship between cyanobacterial
443 fraction or cellular microcystin and TFe was different for between lakes, while TDFe was
444 not. Further, the correlation between cyanobacterial fraction and TP was only significant in
445 Yangebup and Jackadder Lake, which both had lower TP concentrations than Bibra Lake, in
446 which no significant correlation was found. Meanwhile, the correlation between cellular
447 microcystin concentration and TFe was negative only in Bibra Lake, where TFe was present
448 at significantly higher concentrations compared to the other two lakes. This indicates that the
449 effect of environmental factors on cyanobacterial and microcystin variability may depend on
450 site-specific factors such as concentration regimes, even in non-nutrient limited lakes.

451 Therefore, a generalization by only using concentrations of nutrients might not be sufficient
452 for future management of lakes.
453
454 The site-specificity of the environmental triggers of cyanobacterial and microcystin
455 variability may also be a consequence of the variation of cyanobacterial communities
456 between the systems. TFe was negatively correlated to cyanobacterial fraction in Jackadder
457 and Yangebup Lake, and positively in Bibra Lake. The cyanobacterial community in
458 Jackadder Lake was composed of only one nitrogen-fixing cyanobacterial genera (Fig. 4). In
459 contrast, multiple nitrogen-fixing cyanobacterial genera were present in Bibra Lake.
460 Nitrogen-fixing cyanobacteria are known to utilize more iron in comparison to non nitrogen-
461 fixers (Wilhelm, 1995). Therefore, the site-specific correlation between TFe and
462 cyanobacterial fraction may be explained through a greater iron requirement of the
463 cyanobacterial community in Bibra Lake, in comparison to the cyanobacterial community in
464 Jackadder Lake.
465
466 Currently, in the absence of lake-specific information, cyanobacterial management strategies
467 are based on knowledge derived from general trends of the relationship between
468 environmental factors and cyanobacteria or their toxins. Our study clearly indicates that the
469 environmental variables explaining the variability in cyanobacteria and their toxins might be
470 lake-specific and, more importantly, that these lake-specific correlations might also be
471 different to the correlation derived from combining all data (e.g., 6A, 8A, B). This strongly
472 supports the conclusion that site-specific conditions have to be taken into account for
473 managing lakes with cyanobacterial blooms. Due to the site-specific environmental triggers
474 of cyanobacterial and microcystin variability, the results presented in this study are important
475 for the management of these lakes or lakes with similar physical, chemical and biological

476 characteristics. In this study, the cyanobacterial fraction was negatively related with TP, TDP,
477 TFe, TDFe, and positively correlated with TN:TP ratio. These relationships illustrate that in
478 our study, cyanobacteria may dominate under lower phosphorus availability (Amano et al.,
479 2010). Although the lakes in our study were not limited in phosphorus *per se*, the differences
480 in phosphorus levels could have been responsible for the differences in the phytoplankton
481 communities between lakes. At high concentration, phosphorus had been shown to
482 potentially limit the ability of cyanobacteria to become dominant in the phytoplankton
483 community, even though cyanobacteria as a group can dominate under a wide range of
484 conditions (Chorus and Bartram, 1999; Reynolds et al., 2006). One reason for that is the
485 higher growth rate of other phytoplankton groups compared to cyanobacteria, and, as such,
486 their ability to utilize nutrients faster under high nutrient conditions. This can explain the
487 negative correlation between cyanobacterial fraction and phosphorus concentration found in
488 our study, and, maybe as a consequence of this, a positive correlation with TN:TP. In terms
489 of iron, low availability was correlated to high cyanobacterial fraction in these lakes. This
490 result indicated that cyanobacteria pose a competitive advantage to dominate the
491 phytoplankton community under low iron availability. Cyanobacteria are capable to alter
492 their cellular iron requirements, and increase the ability to utilize iron at a low concentration,
493 through the present of siderophores (Boyer et al., 1987; Lee et al., 2011). As reported in the
494 Nagai et al., (2007), cyanobacteria including *Microcystis* spp. and *Planktothrix* spp. can
495 produce siderophores and become a superior competitor under iron limited conditions. These
496 results indicate that phosphorus and iron reduction in water bodies might not be a sufficient
497 remedial strategy against the occurrence of toxic cyanobacterial bloom.
498
499 In contrast to cyanobacterial fraction, cellular microcystin concentration was positively
500 related to TP, TDP, TFe, TDFe and negatively correlated to TN:TP in all lakes. High

501 availability of phosphorus relative to other nutrients is required for energy and material
502 supply in microcystin biosynthesis as microcystin production in cyanobacterial cells is an
503 energy intensive process (Vezie et al., 2002). This is further supported through the observed
504 negative relationship between cellular microcystin and TN:TP ratio, as low microcystin
505 production is expected under conditions where phosphorus is present at lower concentrations
506 in relation to other nutrients. In addition, the positive correlation between iron and cellular
507 microcystin concentration is in agreement with earlier studies which suggested that iron plays
508 an essential role in many metabolic pathways including microcystin biosynthesis in
509 cyanobacteria (Jiang et al., 2008; Wang et al., 2010a). Our results illustrate that reducing
510 phosphorus and iron concentrations in water bodies could potentially reduce the overall
511 toxicity of cyanobacterial bloom, even though it might not completely prevent the occurrence
512 of cyanobacterial bloom.
513
514 Environmental conditions influencing the release of microcystin into the environment,
515 besides cells lyses, are-not well understood (Rohrlack and Hyenstrand, 2007; Barrington et
516 al., 2013). Our results showed that correlations exist between extracellular microcystin
517 fraction and nutrients, however, the correlations could be direct or indirect ones. If they are
518 direct, our results suggest that regardless of the potentially low microcystin production,
519 cyanobacteria may release microcystins at lower nitrogen and phosphorus concentrations.
520 This would support by the hypothesis that microcystin is involved in nutrient competition in
521 the phytoplankton community (Huisman and Hulot, 2005).
522
523 Based on the RDA results, the measured environmental factors were able to better predict the
524 variability of cyanobacterial fraction than the variability of absolute cyanobacterial biomass
525 in two out of three lakes (Yangebup and Jackadder Lakes). Both descriptors are important

526 indicators for management. The competition with other phytoplankton, described by the
527 cyanobacterial fraction in this study can affect the toxin production within a cell through
528 allelopathy (Huisman and Hulot, 2005). Therefore, understanding the importance of site-
529 specific drivers of both, biomass and the cyanobacterial fraction is of highest importance to
530 develop successful and sustainable management strategies.
531
532 **5 Conclusions**
533 The current approach to water body restoration and the prevention of toxic cyanobacterial
534 blooms relies on reducing nutrient loading into water bodies and limiting the availability of
535 nutrients in the water column. This approach might not always be successful in preventing
536 the occurrence of cyanobacterial blooms, due to the roles of physicochemical factors on
537 cyanobacteria and microcystin variability being dependent on the site-specific combination of
538 environmental factors. Our study clearly highlights the importance of taking between-lake
539 heterogeneity in the management of toxic cyanobacterial blooms into account. Site-specific
540 studies may be required to determine the factors causing cyanobacterial dominance and
541 microcystin production in different systems with different characteristics such as the
542 hydrology, land use and water chemistry.
543
544 In our study, the dominance of cyanobacteria in the phytoplankton community is correlated to
545 lower phosphorus and iron concentrations in the systems. In contrast, cyanobacteria required
546 higher phosphorus and iron concentrations in the water column to produce a high amount of
547 microcystin. Therefore, reducing phosphorus and iron concentration in the water column
548 might not be a sufficient remedial strategy against the occurrence of toxic cyanobacterial
549 bloom, if these nutrients are still available in sufficient amount to support the growth of

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

552

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560

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769 Table 1. Physical and chemical properties of the three lakes throughout the sample period (Jan – March 2010), including analysis of differences
770 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	$F_{(2,45)} = 19.51$ *

771 N = number of samples
772 SD = standard deviation
773 * = $P < 0.05$
774

775 Table 2: Pearson's correlation coefficients (R) between the environmental factors and cyanobacterial fraction (%) or cyanobacterial biomass
776 (µg chl-*a* L^{-1}) analysed for each lake and for all lakes combined using bivariate correlation analysis. The dependent variables are ln
777 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

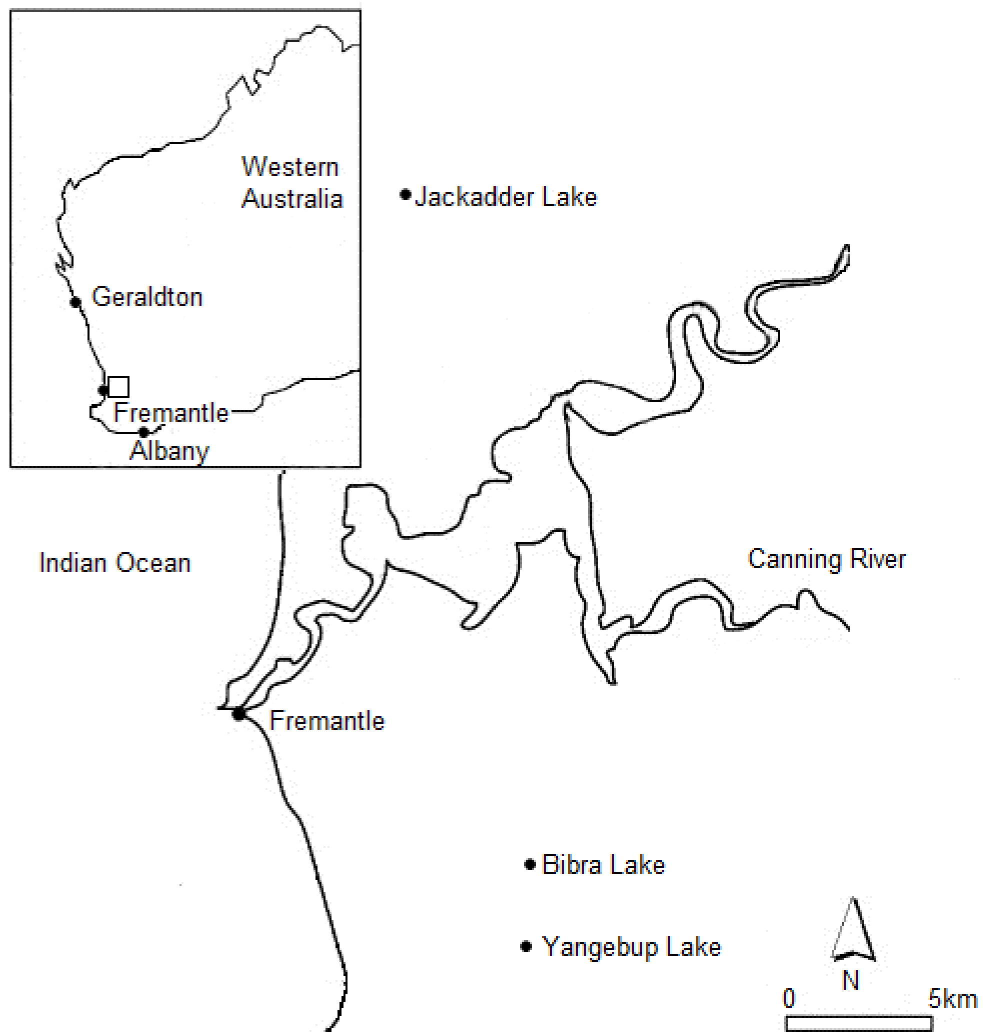
778 Significant (P<0.05) correlations are highlighted in bold.

779

780 Table 3: Pearson's correlation coefficients (R) between the environmental variables and cellular microcystin concentration (µg g^{-1}) or
781 extracellular microcystin fraction (%) analysed for each lake and for all lakes combined using bivariate correlation analysis. The dependent
782 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

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



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

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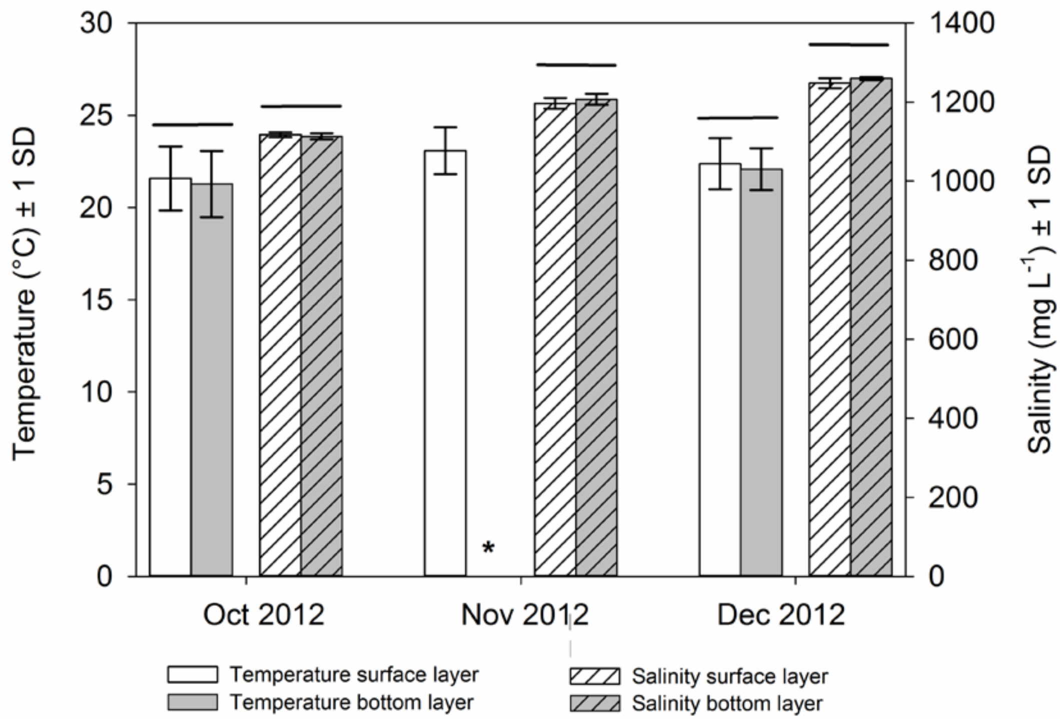

792

793 **Fig. 2.** Temperature (°C) and salinity (ppm) in the surface and bottom layers measured at 7

794 sites over three months in Lake Yangebup during a previous study in 2012. * = missing data;

795 horizontal line indicates that no significant difference between layers were detected (t-test)

796 (from Song et al., 2015).

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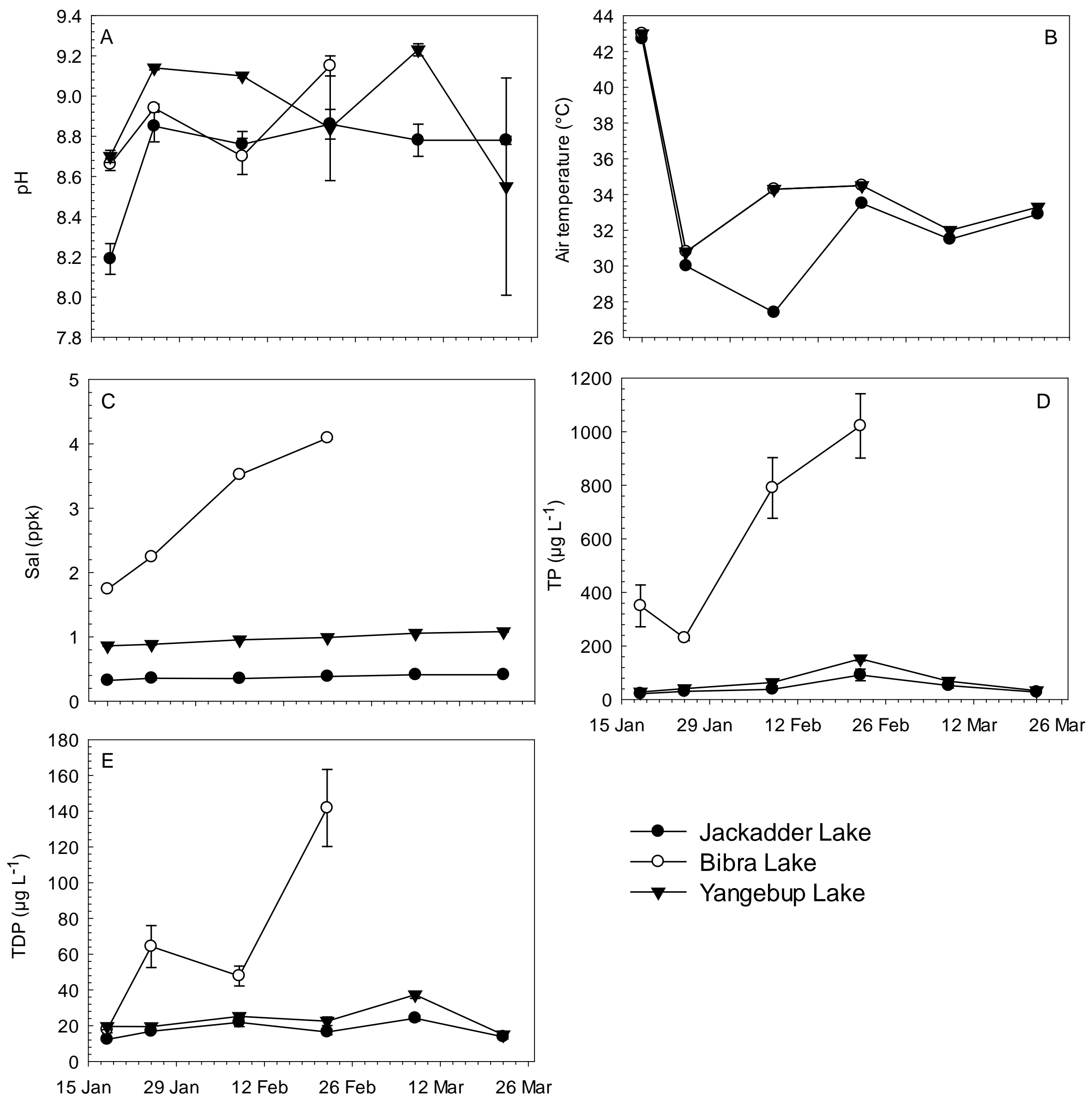

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800 **Fig. 3.** Mean values (± one standard error) of physicochemical variables over time

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

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

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

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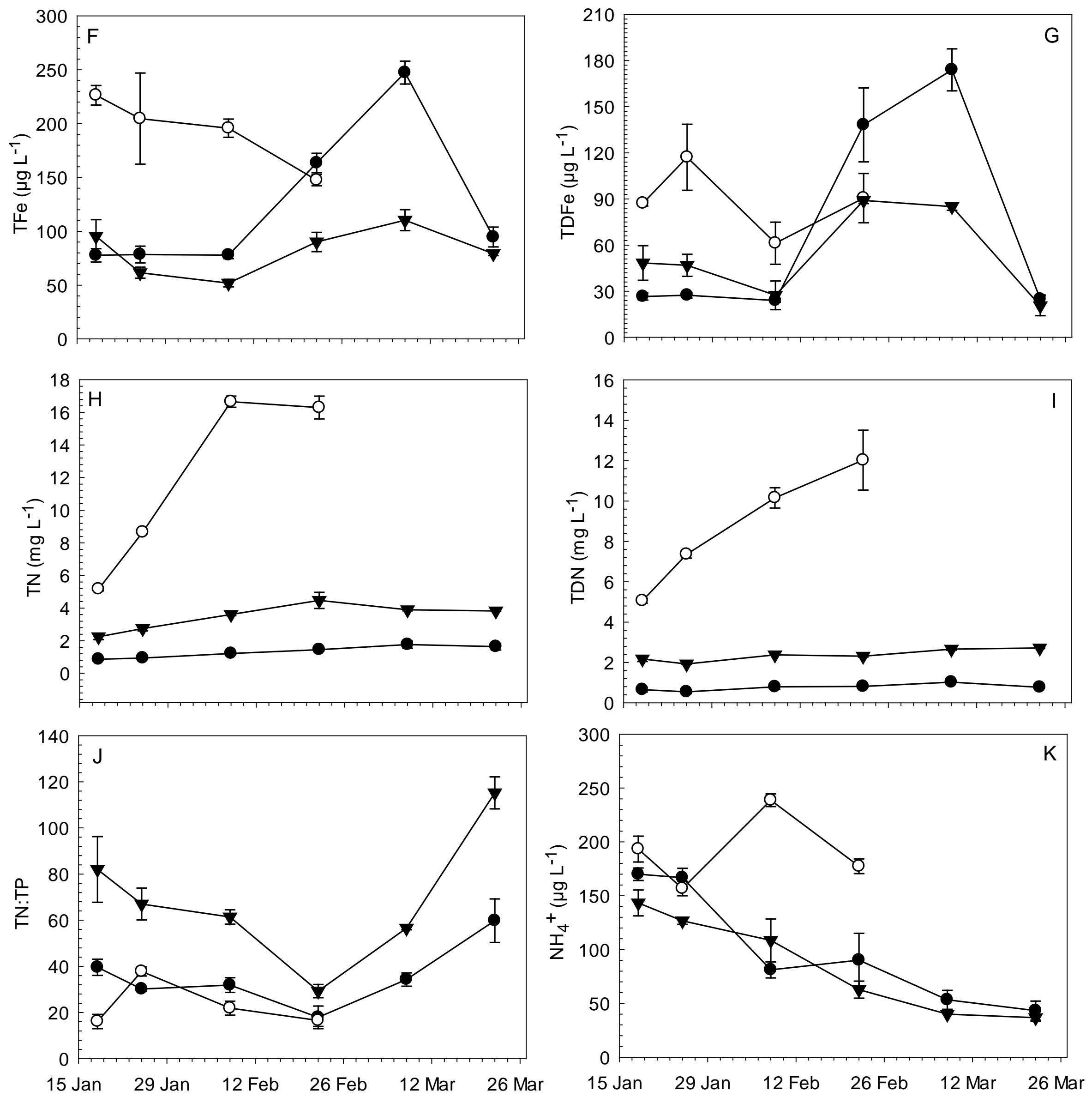

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806 **Fig. 3. continued**

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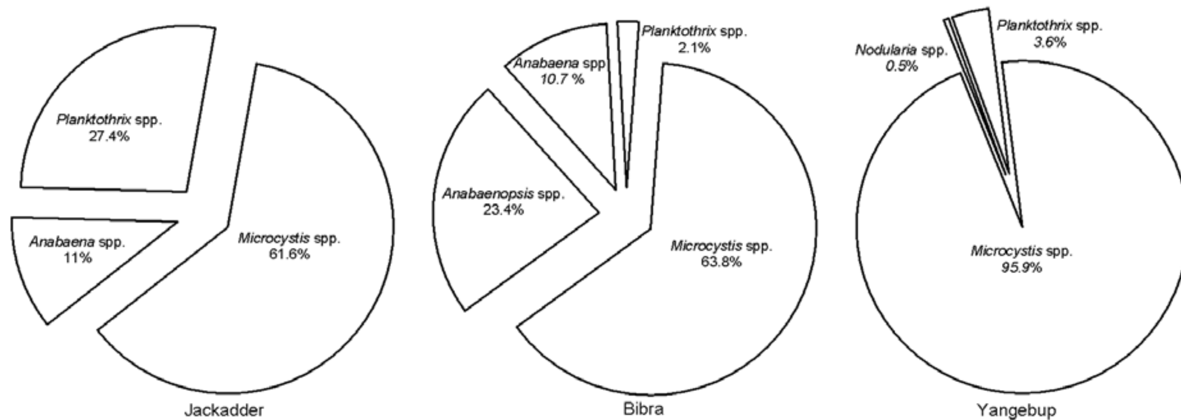

810 **Fig. 4.** Mean biomass (μm^3 mL^{-1}) proportions of potentially toxic cyanobacterial genera in

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

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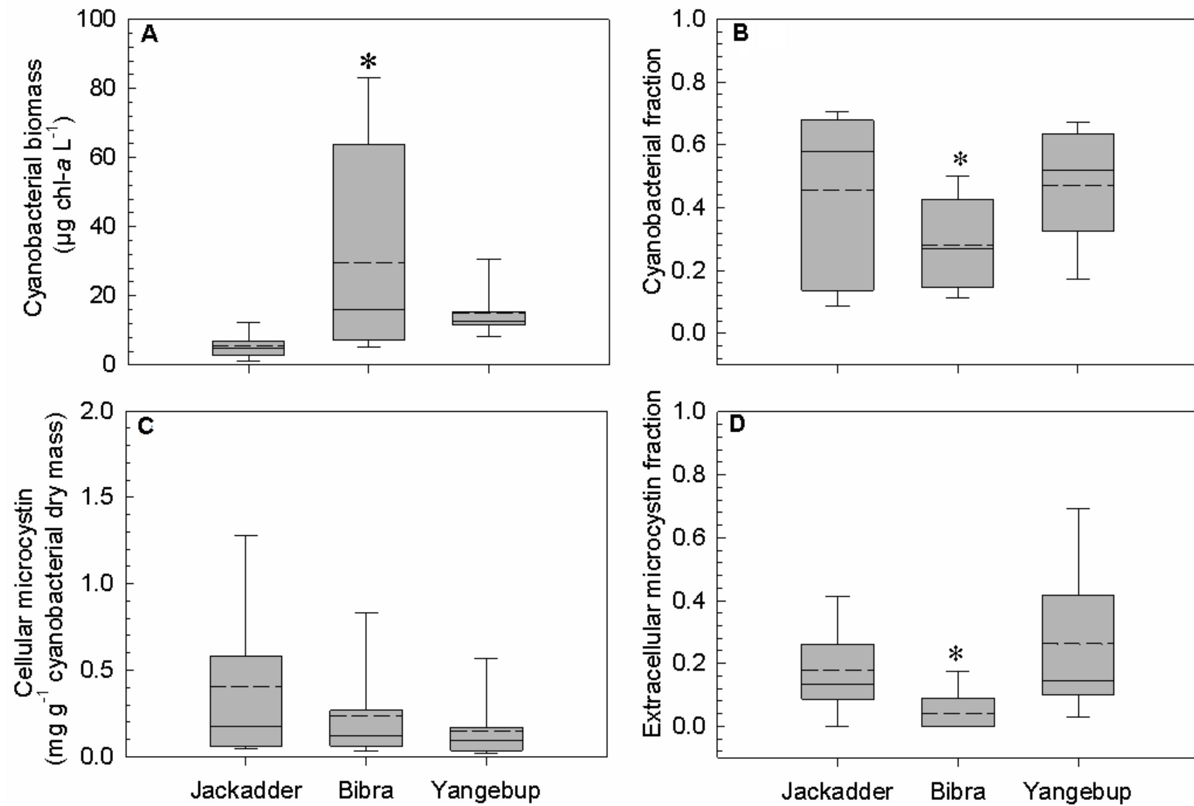

814 **Fig. 5.** The variability of (A) cyanobacterial biomass (µg chl-*a* L^{-1}), (B) cyanobacterial

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

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

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

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

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

820 lakes.

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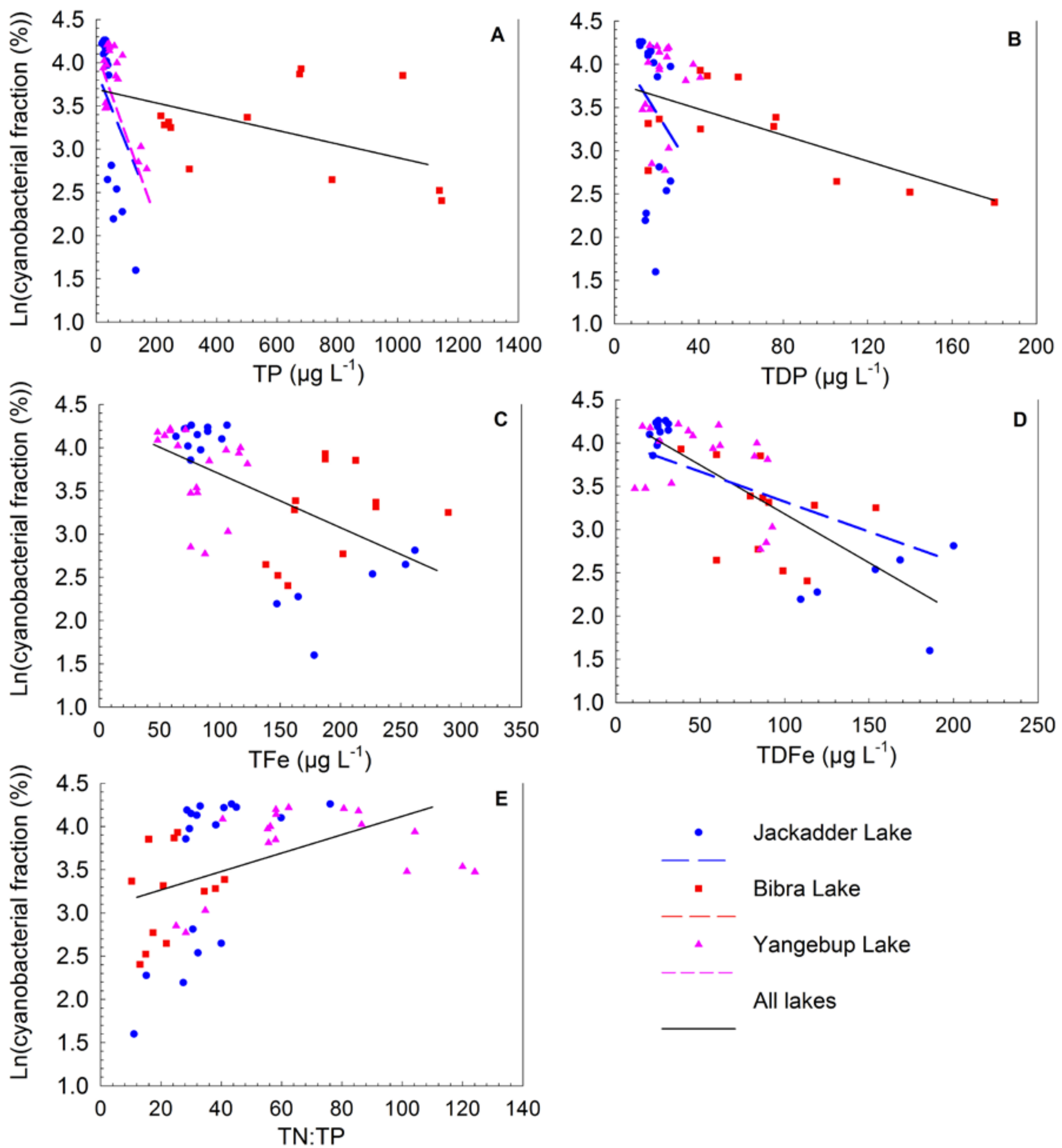

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823 **Fig. 6.** The correlations between cyanobacterial fraction and (A) TP, (B) TDP, (C) TFe, (D)
824 TDFe, (E) TN:TP in Jackadder, Bibra and Yangebup lakes during the study period.
825 Regression curves for each individual lake were calculated by linear mixed models with site
826 and date as random factors on data from each lake (broken lines) while all data points were
827 combined for the overall regression using a linear mixed model adding lake as random factor
828 (solid line). Only significant ($p<0.05$) regressions are shown.

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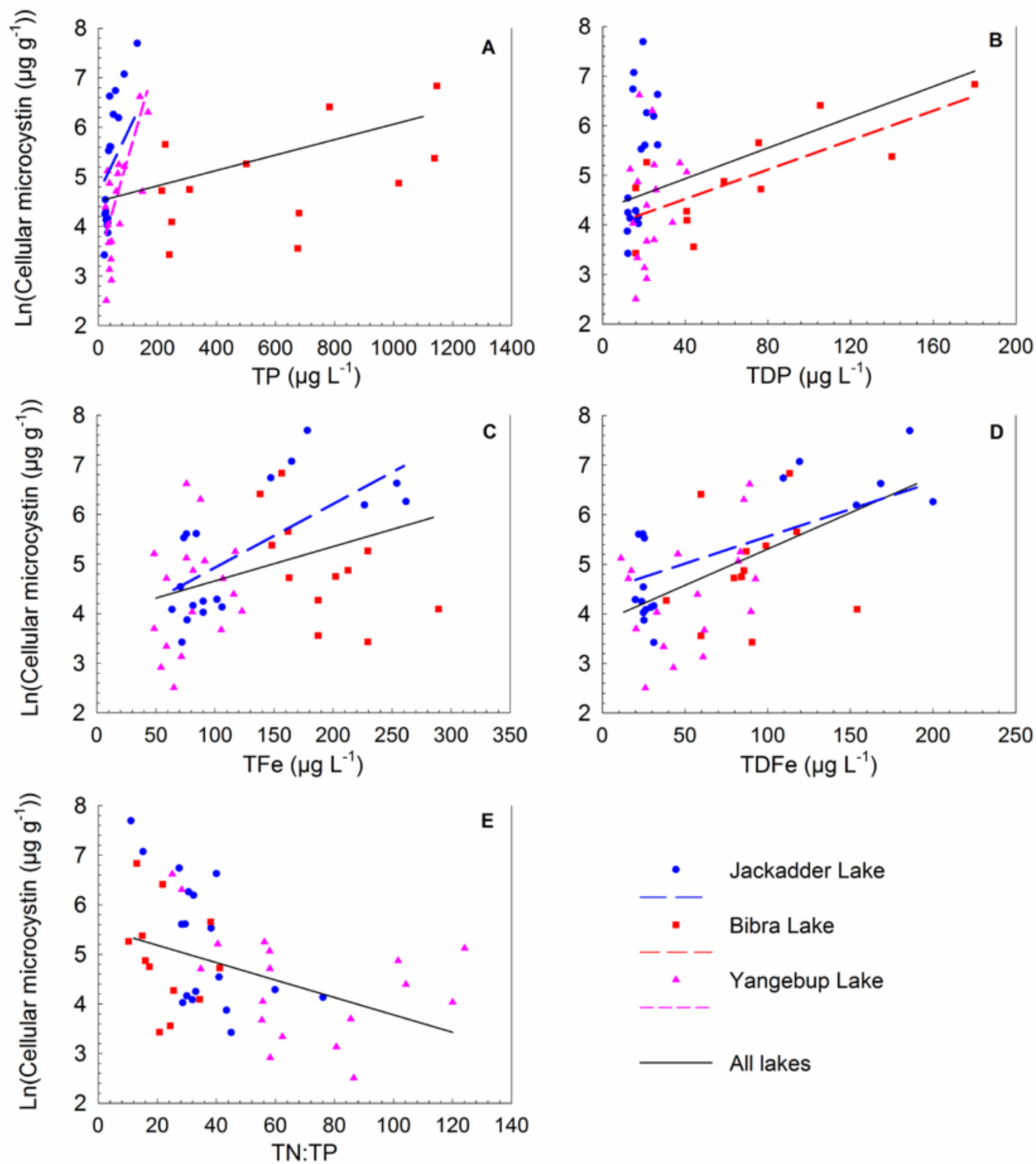


830
831 **Fig. 7.** The correlations between cellular microcystin concentration and (A) TP, (B) TDP, (C)
832 TFe, (D) TDFe, (E) TN:TP in Jackadder, Bibra and Yangebup lakes during the study period.
833 Regression curves for each individual lake were calculated by linear mixed models with site
834 and date as random factors on data from each lake (broken lines) while all data points were
835 combined for the overall regression using a linear mixed model adding lake as random factor
836 (solid line). All regression shown are $p<0.05$, except for the regression calculated for all lakes
837 combined in panel A, which is $p<0.1$.

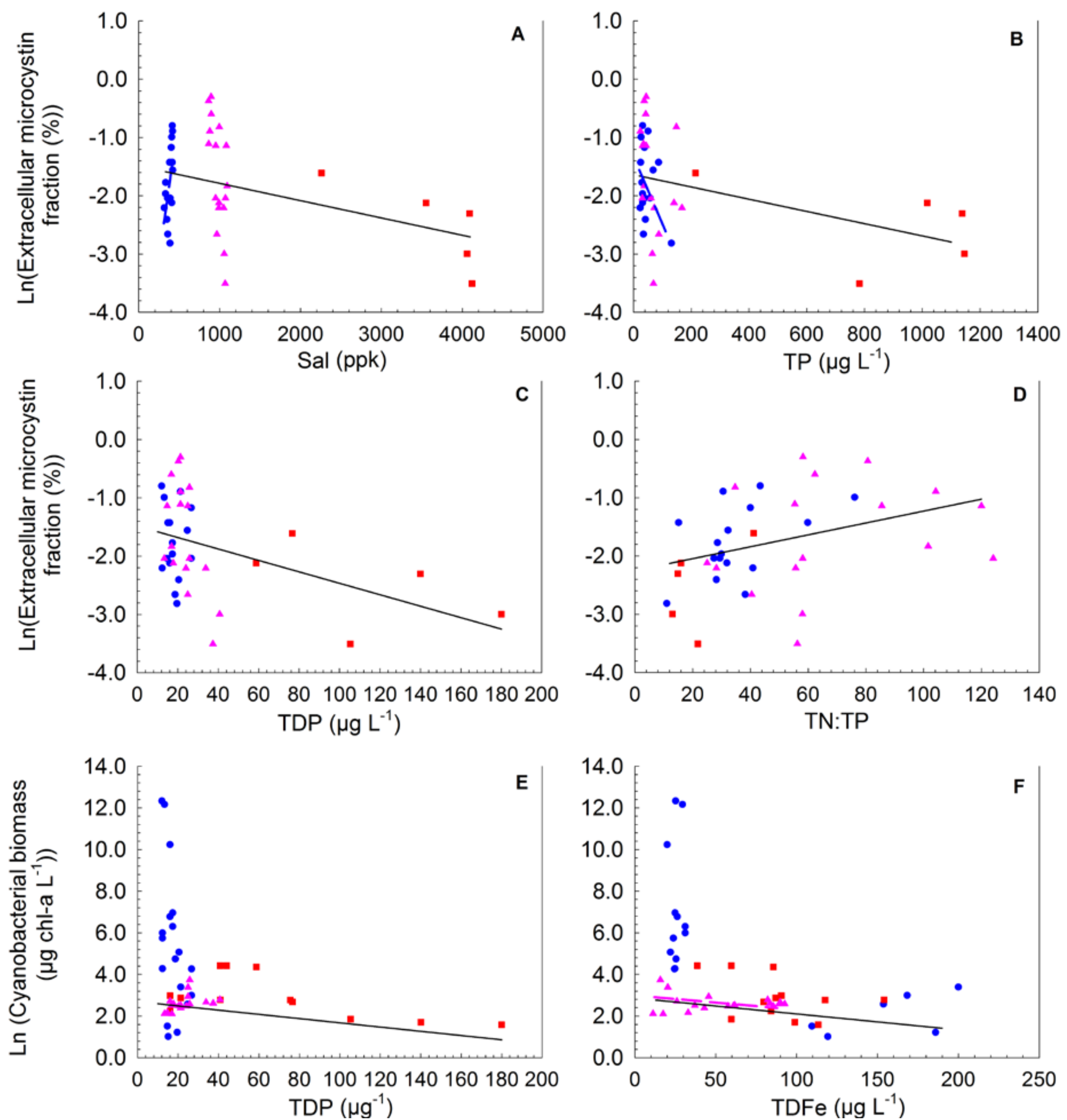
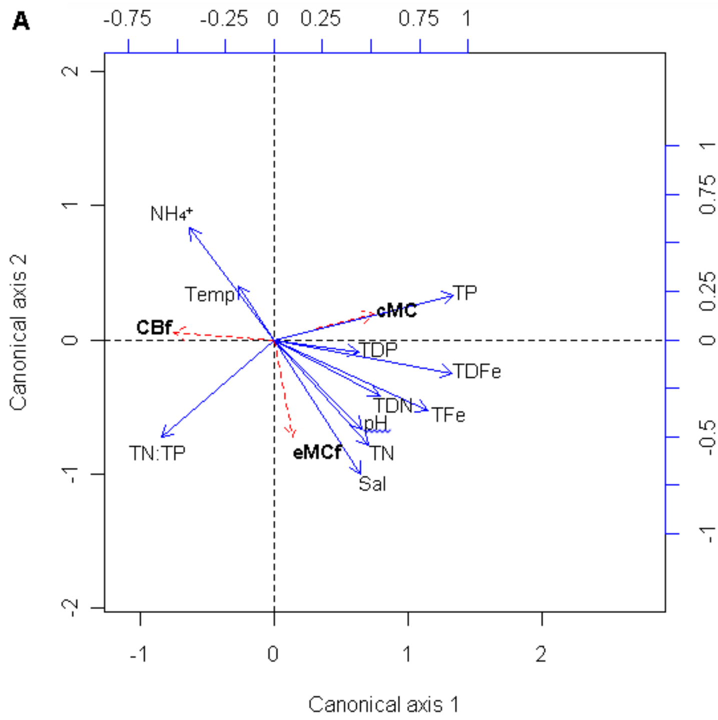


Fig. 8. The correlations between extracellular microcystin fraction and (A) Sal, (B) TP, (C) TDP, (D) TN:TP, and between cyanobacterial biomass and (E) TDP, (F) TDFe in Jackadder, Bibra and Yangebup lakes during the study period. Regression curves for each individual lake were calculated by linear mixed models with site and date as random factors on data from each lake (broken lines) while all data points were combined for the overall regression using a linear mixed model adding lake as random factor (solid line). All regression shown are $p<0.05$, except for the regression calculated for all lakes combined in panel A, which is

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



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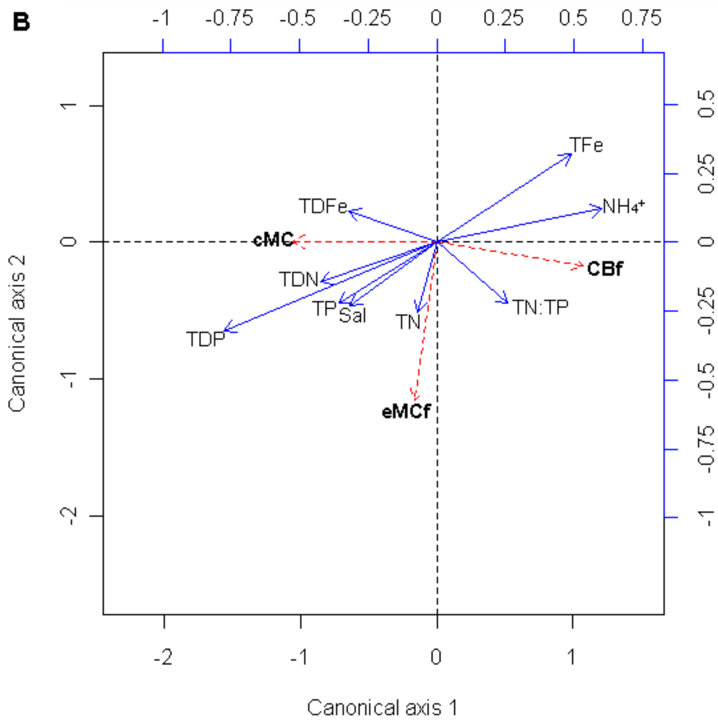

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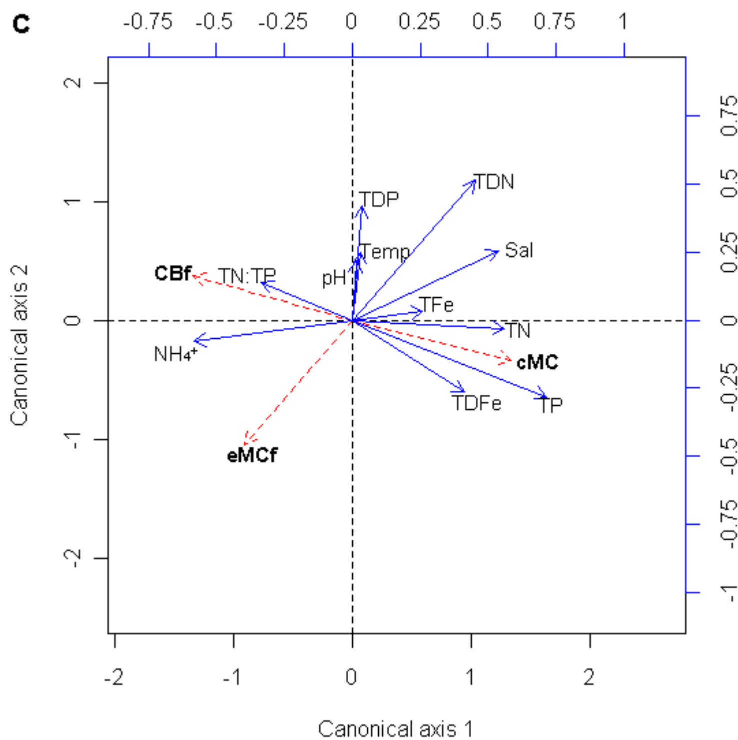

850

851 **Fig. 9.** RDA biplots for the environmental variables and the cyanobacterial fraction (CBf),
852 cellular microcystin (cMC) and extracellular microcystin fraction (eMCf) in (A) Jackadder
853 Lake, (B) Bibra Lake, (C) Yangebup Lake; solid arrows = environmental variables; short
854 dashed arrows = response variables. Canonical axis 1 and 2 represents a linear combination
855 of the environmental variables, and axes are scaled by the square root of their eigenvalues.
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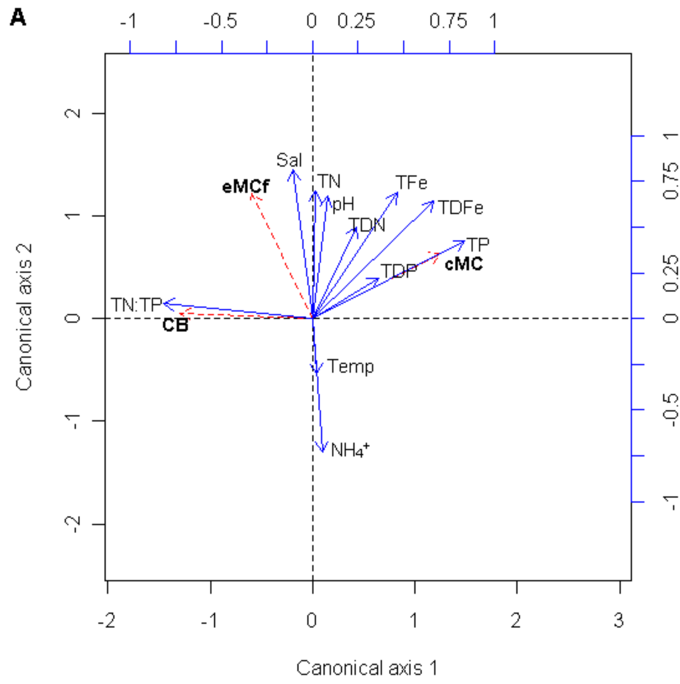

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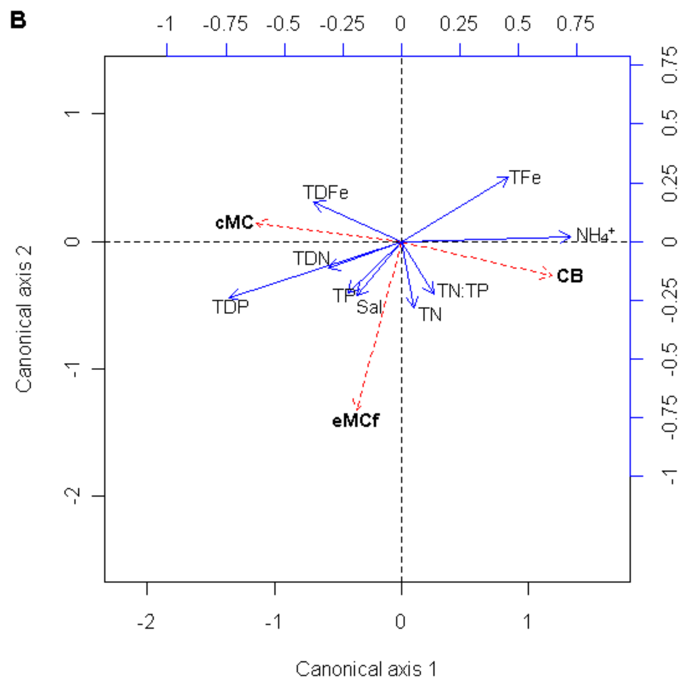

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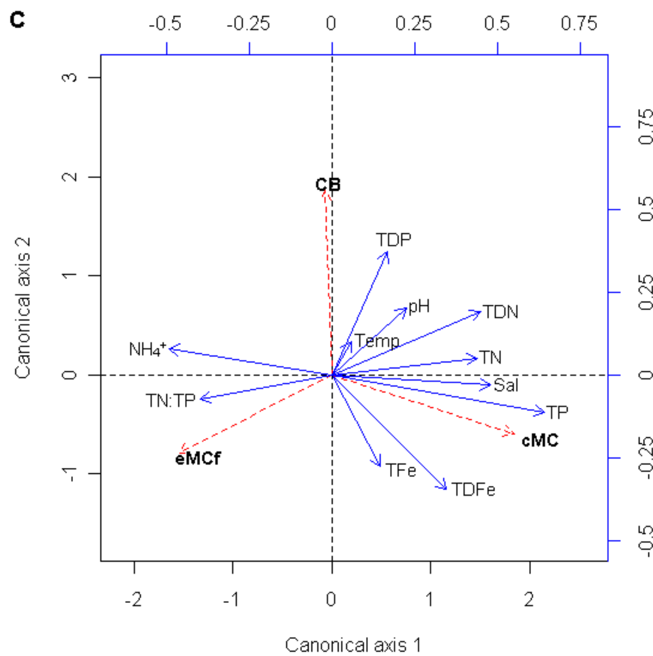

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866 **Fig. 10.** RDA biplots for the of environmental variables and the absolute cyanobacteria

867 biomass (CB), cellular microcystin (cMC) and extracellular microcystin fraction (eMCf) in

868 (A) Jackadder Lake, (B) Bibra Lake, (C) Yangebup Lake; solid arrows = environmental

869 variables; short dashed arrows = response variables. Canonical axis 1 and 2 represents a

870 linear combination of the environmental variables, and axes are scaled by the square root of

871 their eigenvalues.

872