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Supporting Information for

Early and late cyanobacterial bloomers in a shallow, eutrophic lake

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Introduction

This document contains additional text, figures and tables that support the analysis and results reported in the manuscript. Texts S1 to S3 describe additional details about the analysis of cyanotoxins and nutrients. Figures show cyanobacterial biomass by species (Figure S1), the relationships between dominant cyanobacterial biomass and chl-*a* (Figure S2), nutrient concentrations (Figure S3), and detailed water temperature measurements (Figure S4). The supplemental tables include the individual analyte detection and quantification limits for cyanotoxins (Table S1) and the full results of the indicator species analysis referenced in the main manuscript text (Table S2). Figures were created in R and tables in MS Excel. Associated code and data are available from: https://github.com/biogeochem/buffalopound_blooms.

Text S1. Chemicals and standards for cyanotoxin analysis

Cyanotoxins standards were purchased from Enzo Life Sciences (Farmingdale, NY, USA: dmMC-RR, MC-RR, MC-YR, MC-LA, MC-LY, MC-LW, MC-LF, MC-WR, MC-HtyR and MC-HilR), the National Research Council of Canada (Halifax, NS, Canada: ANA-a, CYN, MC-LR and dmMC-LR), Cyano Biotech GmbH (Berlin, Germany: AP-A and AP-B), and Abraxis Eurofins (Warminster, PA, USA: HANA-a). The isotope-labelled internal standards ANA-a-13C4 and CYN-15N5 were purchased from Abraxis Eurofins, while MC-LR-15N10 was from Cambridge Isotopes Laboratories, Inc. (Tewksbury, MA, USA). Isotopically labelled D3-MMPB was from the National Research Council of Canada (Halifax, Nova Scotia, Canada) (D3-MMPB originally from Wako Pure Chemicals Industries, Ltd). All chemical reagents were obtained from Millipore Sigma (Oakville, ON, Canada) and HPLC grade solvents from Fisher Scientific (Whitby, ON, Canada).

Text S2. Instrumentation for cyanotoxin analysis

For MC total analysis, a 5-mL aliquot of the water was retrieved and filtered through GHP. A 2-mL aliquot of the filtrate was then spiked with internal standard (d3-MMPB) and subject to Lemieux-von Rudloff oxidation, which generates the MMPB moiety from permanganate oxidation (Munoz et al. 2017). Samples were submitted to on-line solid phase extraction (SPE) ultra-high performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (Thermo TSQ Quantiva). The UHPLC instrument consisted of a loading pump (Accela 600, Thermo Fisher, San Jose, CA, USA) and an analytical pump (Accela 1250, Thermo Fisher). The SPE column and the analytical column were with Hypersil Gold C18 selectivity (Thermo Fisher) with dimensions of 20x2.1mm; 12µm particle size and 50x2.1mm; 1.9µm particle size, respectively.

For specific analysis of 17 individual cyanotoxins, a 5-mL aliquot of the water was retrieved and filtered through GH polypropylene. A 2-mL aliquot of the filtrate was then spiked with internal standards and analyzed by on-line SPE-UHPLC high-resolution mass spectrometry (Thermo Orbitrap Q-Exactive). Though only one replicate was analyzed per time point, the analytical method had been previously validated for accuracy and precision through lake water matrix spikes, as detailed in Roy-Lachapelle et al. (2019). A Thermo Scientific Dionex UltiMate LPG-3400SD pump was used for on-line SPE enrichment and a Thermo Scientific Dionex UltiMate HPG-3400RS pump for the analytical separation. A Hypersil Gold aQ C18 (20x2.1mm; 12µm particle size) and a Hypersil Gold C18 (100x2.1mm; 1.9µm particle size) were used as SPE and analytical columns, respectively. Orbitrap acquisition parameters were optimized as described elsewhere (Roy-Lachapelle et al. 2019).

Text S3. Methods for nutrient analyses

Nutrients including soluble reactive P (SRP), ammonium (as NH_3 + NH_4^+) and nitrate (as NO_2^- + NO_3^-) were analyzed at the Saskatchewan Water Chemistry and Ecology laboratory at the University of Saskatchewan Global Institute for Water Security in

Saskatoon, Canada. Samples were filtered through a 0.45 µm nylon syringe-tip filter and frozen until time of analysis. Ammonium samples were preserved additionally with sulfuric acid prior to storage. Samples were analyzed using a SmartChem 170 discrete chemical analyzer (Westco Scientific, USA) using standard EPA methods (SRP, EPA Method PHO-001-A; ammonium, EPA Method AMM-001-A; nitrate, EPA Method NO3-01). Method detection limits were 1.8 µg P/L, 5.0 µg N/L, and 4.4 µg N/L for SRP, ammonium, and nitrate, respectively.

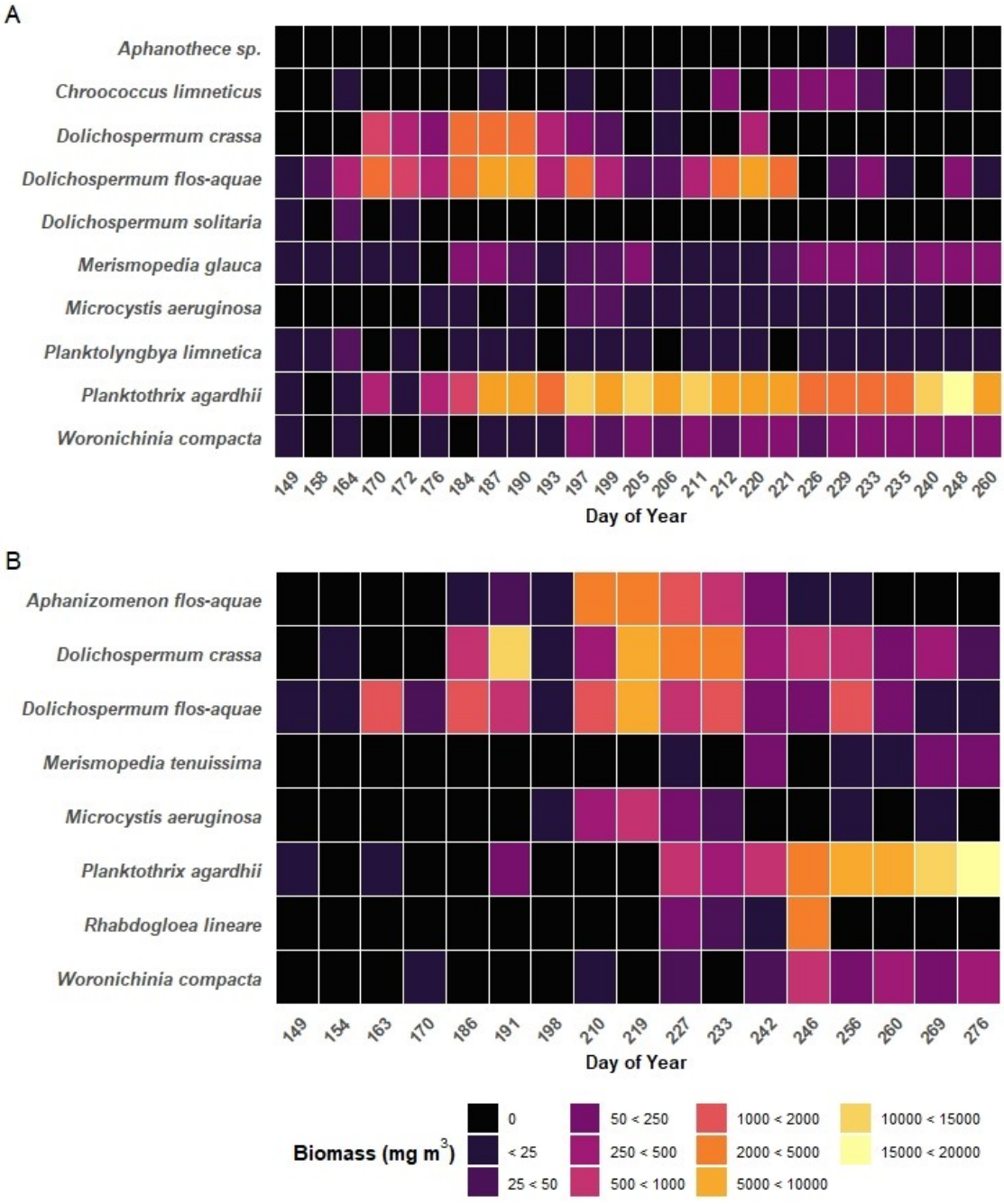

Figure S1. Biomass (mg/m^3) of cyanobacterial phytoplankton species identified (n = 10 in 2018; n = 8 in 2019) from samples collected from the 0.8 m depth of Buffalo Pound Lake, Saskatchewan in 2018 (A) and 2019 (B)

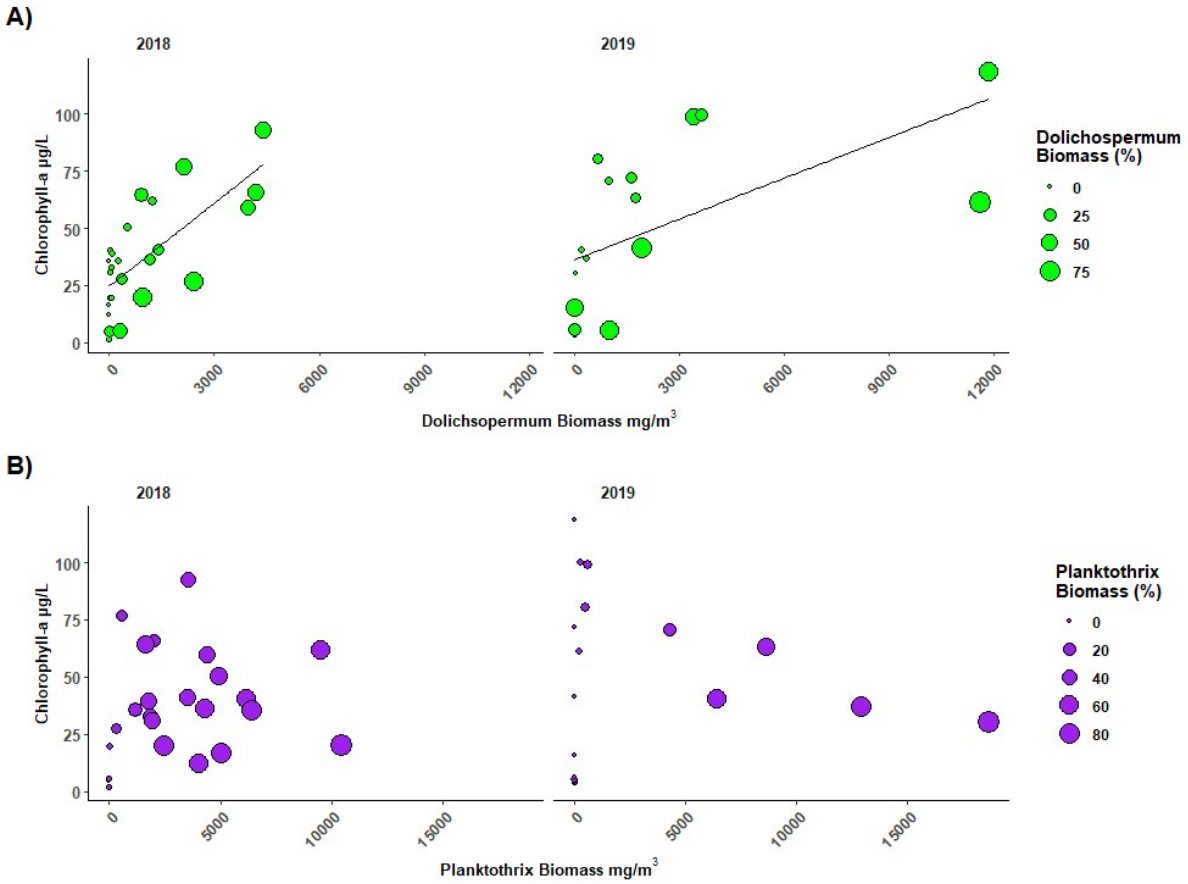

Figure S2. Relationships between biomass (mg/m^3) of dominant cyanobacterial genera A) *Dolichospermum* and B) *Planktothrix* and chlorophyll-*a* (chl-*a*) in samples collected from the 0.8 m depth of Buffalo Pound Lake, Saskatchewan in 2018 and 2019. Point size represents % contribution of each genus to total phytoplankton biomass. Significant correlations between *Dolichospermum* and chl-*a* are indicated by a line of best fit in 2018 ($p<0.001$, Spearman's rho=0.66) and 2019 ($p<0.001$, Spearman's rho =0.80).

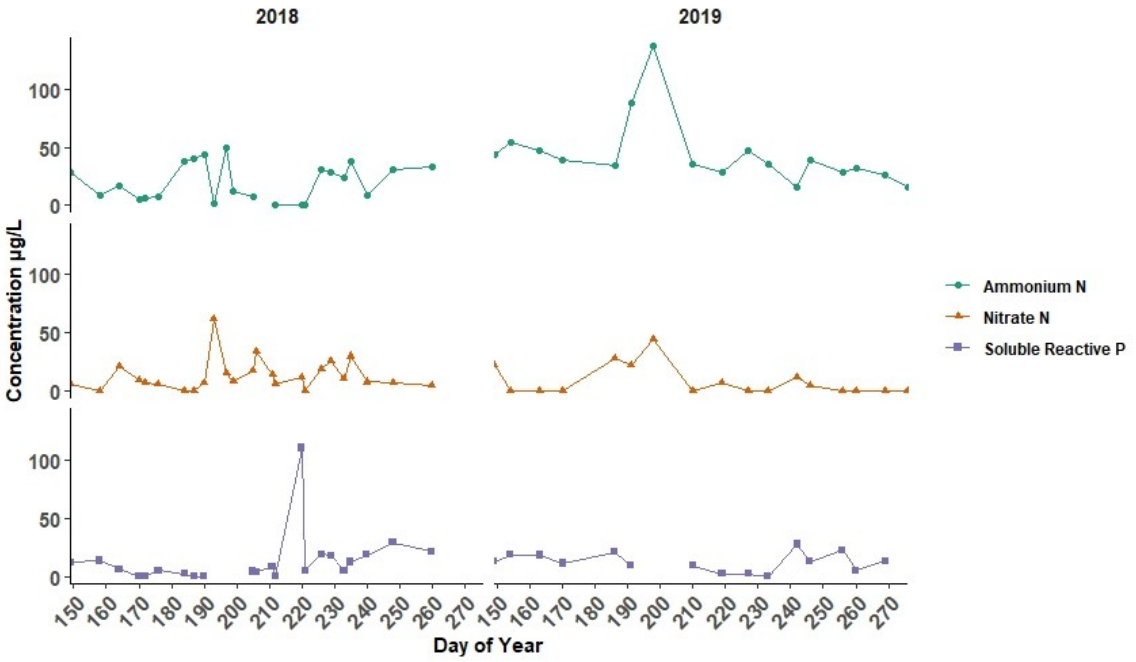

Figure S3. Concentrations of ammonium (as NH_3 + NH_4^+; µg N/L), nitrate (as NO_2^- + NO_3^-; µg N/L) and soluble reactive phosphorus (µg P/L) in water collected from the 0.8 m depth of Buffalo Pound Lake, Saskatchewan in 2018 and 2019.

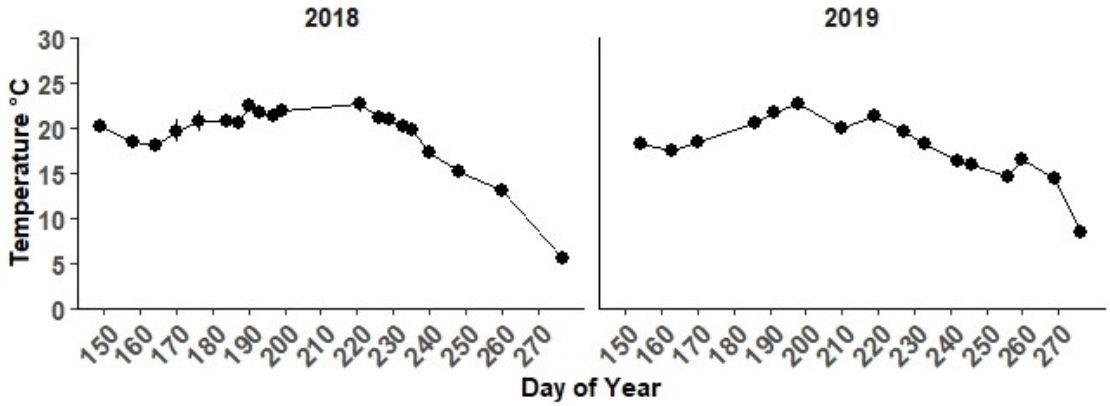

Figure S4. Mean water temperature in Buffalo Pound Lake, Saskatchewan in 2018 and 2019; vertical lines indicate standard deviation around the mean (n = 19; measured from depths in 0.2 m increments).

Table S1. Analytical levels of detection and quantification (ng/L) of cyanometabolites analyzed from samples collected from 0.8 m depth in Buffalo Pound Lake, Saskatchewan in 2018 and 2019

Analyzed Metabolite	Text Abbreviation	Level of Detection (ng/L)		Level of Quantification (ng/L)	
		Intracellular	Extracellular	Intracellular	Extracellular
Total Microcystins	MC Total	1	5	2	15
Cylindrospermopsin	CYN	2	41	5	135
Anatoxin-a	ANA-a	1	18	2	59
dmMicrocystin-RR	dmMC-RR	0	5	1	17
Microcystin-RR	MC-RR	0	11	1	37
Microcystin-YR	MC-YR	0	10	1	34
Microcystin-HtyR	MC-HtyR	1	27	4	88
Microcystin-LR	MC-LR	1	15	2	49
dmMicrocystin-LR	dmMC-LR	0	9	1	30
Microcystin-HilR	MC-HilR	1	14	2	45
Microcystin-WR	MC-WR	1	37	5	123
Microcystin-LA	MC-LA	0	10	1	32
Microcystin-LY	MC-LY	1	29	4	97
Microcystin-LW	MC-LW	0	8	1	27
Microcystin-LF	MC-LF	0	9	1	31
Homoanatoxin-a	HANA-a	0	12	2	39
Anabaenopeptin A	AP-A	1	20	3	67
Anabaenopeptin B	AP-B	0	6	1	19

Table S2. Results of indicator species analysis on groups including combinations for the 2018 and 2019 phytoplankton community collected from 0.8 m depth in Buffalo Pound Lake, Saskatchewan

	Group	Species	IndVal	p-value
2018	A	*Dinobryon sertularia*	0.944	0.011
		Monoraphidium minutum	0.857	0.038
		Large chrysophyte spp.	0.828	0.034
	B	*Dolichospermum crassa*	0.931	0.001
	C	*Woronichinia compacta*	0.963	0.001
	A + B	*Actinastrum sp.*	0.816	0.044
	A + C	Small chrysophyte spp.	0.917	0.043
	B + C	*Planktothrix agardhii*	1.000	0.009
		Peridinium goslaviense	0.975	0.017
		Scenedesmus quadricauda	0.975	0.010
		Selenastrum copricornutum	0.972	0.014
		Dictyosphaerium pulchellum	0.960	0.021
		Fragilaria capucina	0.956	0.024
		Cryptomonas erosa	0.951	0.035
		Cyclotella michiganiana	0.944	0.014
2019	A	*Dinobryon sertularia*	1.000	0.001
		Asterionella formosa	1.000	0.002
		Cryptomonas reflexa	0.866	0.006
	B	*Aphanizomenon flos-aquae*	0.997	0.001
		Ceratium hirundinella	0.890	0.030
	C	*Planktothrix agardhii*	0.990	0.001
		Woronchinia compacta	0.984	0.001
		Dictyosphaerium pulchellum	0.972	0.003
		Euglena acus	0.951	0.001
		Selenastrum copricornutum	0.872	0.010
		Stephanodiscus niagarae	0.869	0.009
		Scenedesmus quadricauda	0.858	0.038
		Closterium kuetzingii	0.845	0.006
		Merismopedia tenuissima	0.834	0.017
		Closterium sp.	0.743	0.024
	A + B	*Salpingoeca frequentissima*	0.866	0.022
	B + C	*Dolichospermum crassa*	1.000	0.001
		Aulacoseira italica	0.96	0.044
		Pediastrum duplex	0.953	0.034