

ORIGINAL ARTICLE

Network-Scale Patterns and Drivers of Microcystins in a Heavily Impacted Watershed

Jessica Lerminiaux,^{1*}  Kerri Finlay,^{1,2}  Peter R. Leavitt,^{1,2} 
Samuel G. Woodman,³  and Matthew J. Bogard³ 

¹Department of Biology, University of Regina, Regina, Saskatchewan S4S 0A2, Canada; ²Institute of Environmental Change and Society, University of Regina, Regina, Saskatchewan S4S 0A2, Canada; ³Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada

ABSTRACT

The most abundant and problematic of the cyanobacterial toxins, microcystins (MCs), degrade water quality, are lethal to livestock at high concentrations, and target the liver in humans. Most research has focused on lakes and reservoirs, while less is known about MCs in rivers, and few studies have integrated toxin threats across entire drainage networks. Here, we quantified landscape patterns of MCs and phytoplankton abundance in one of Canada's most heavily impacted watersheds. This catchment exhibits intensive agriculture and livestock production, with runoff controlled through a hydrologically modified network of regulated rivers, storage reservoirs, and a natural wetland used for slaughterhouse effluent treatment. While the headwater wetland complex exhibited greatly elevated concentrations of MCs ($> 300 \mu\text{g L}^{-1}$), there

was little evidence of MCs export to the river drainage network in years with either good or poor hydrological connectivity. Generalized linear models showed that wetland MC concentrations scaled positively with phytoplankton abundance, nitrogen concentrations, salinity, and water temperature. In contrast, MC concentrations in rivers correlated with sampling month and only weakly with salinity, with no apparent connection to river flows. Correlation of MCs with cyanobacteria-specific pigment concentrations was weaker than that for total phytoplankton, suggesting knowledge of cyanobacterial community biomass added little to the prediction of toxin patterns. A continental meta-analysis showed that MC concentrations in the effluent-receiving wetland were high but not anomalous for lentic ecosystems, whereas rivers were greatly understudied ($< 1\%$ of total observations). Our findings underscore the distinct environmental controls on MCs in lentic versus lotic ecosystems and emphasize the need for habitat-specific management strategies.

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Key words: Microcystins; Cyanobacteria; Watershed; Aquatic network; River; Wetland; Reservoir.

*Corresponding author; e-mail: jessica.lerminiaux@uregina.ca

HIGHLIGHTS

- Predictors of MCs differ in lentic vs lotic waters, with little effect of drought
- Wetland concentrations were elevated ($> 300 \mu\text{g MCs L}^{-1}$) but were similar to other surveys
- Meta-analysis shows that rivers have fewer MCs, but account for $< 1\%$ of study data

INTRODUCTION

Cyanobacterial harmful algal blooms (cyanoHABs) in inland waters are expanding worldwide in terms of frequency, magnitude, and geographical range (O'Neil and others 2012; Taranu and others 2015; Merder and others 2023). Many cyanoHABs produce harmful toxins such as microcystins (MCs), well-studied and ubiquitous hepatotoxins which are often recorded at levels exceeding the World Health Organization (WHO) guidelines (Svirčev and others 2019). Microcystins primarily target the liver (Omid and others 2018; Gu and others 2022) but can also damage the gastrointestinal tract and cause renal impairment (Wu and others 2018; Xu and others 2020). Microcystins are produced by specific cyanobacteria that include non-nitrogen-fixing taxa (e.g., *Microcystis*, *Planktothrix*) and diazotrophic genera (e.g., *Dolichospermum*, *Nostoc*) (Finlay and others 2010; Pick 2016; Wilk-Woźniak and others 2022). Even within species, individual strains can vary in their capacity to produce MCs, depending on the presence of biosynthesis-specific genes (Pick 2016). In Canada, observed MC concentrations range from < 0.1 to $> 2000 \mu\text{g MCs L}^{-1}$ (Orihel and others 2012; MacKeigan and others 2023). In the USA, large-scale surveys typically report MC concentrations ranging from < 0.1 to $225 \mu\text{g MCs L}^{-1}$ (Merder and others 2023), although higher episodic concentrations ($\sim 600 \mu\text{g L}^{-1}$) have been documented in hypereutrophic systems (Zastepa and others 2017). Microcystins can inhibit the growth and reproduction of aquatic organisms (Blanchette and Haney 2002; Harke and others 2016; Yang and Kong 2012) and are thought to protect cyanobacterial cells from environmental stresses (Ross and others 2006; Engström-Öst and others 2011; Omid and others 2018). Despite extensive research into the physiological processes that control MC production and the conditions associated with MC outbreaks, the environmental drivers of MCs are typically not well established (Banerji and others 2019; Buley and others 2022),

making it difficult to forecast the potential toxicity of surface waters.

Microcystin concentrations are often poorly predicted from simple measures of total algal biomass (Health Canada 2022), while the environmental predictors of MCs vary widely across studies. On the one hand, toxic cyanobacterial dominance and MCs have been shown to increase with water temperature (Dziallas and Grossart 2011; O'Neil and others 2012; Hayes and others 2020), particularly in deep, stratified lakes (Taranu and others 2012), and with elevated salinity (Tonk and others 2007; Wang and others 2022). In contrast, laboratory (Swarbrick and others 2019), mesocosm (Finlay and others 2010; Donald and others 2011; Bogard and others 2020) and catchment (Leavitt and others 2006) analyses show that nitrogen (N) pollution can increase total algal biomass and toxicity in hypereutrophic ecosystems, while other studies have shown a strong positive relationship between total phosphorus (P) concentrations and MCs (Kotak and others 2000; Rinta-Kanto and others 2009). At the same time, N and P enrichment frequently co-occur in eutrophic systems and may jointly influence cyanobacterial biomass and toxin production. Additionally, MC production has been shown to be greater at comparatively lower N:P values (< 23) relative to more elevated N:P conditions that favor green algal dominance in nutrient-rich waterbodies (Donald and others 2011; Otten and others 2012), while other work suggests that lower N:P is a consequence of factors that favor blooms of toxic *Microcystis* spp. (Xie and others 2003). Molecular-level analyses shed light on these controls, indicating that the production and abundance of MCs depend on the presence of complete, functional gene operons (Pick 2016). However, simply measuring gene abundance (e.g., *mcyA* and *mcyE*) is not effective for predicting concentrations of MCs (Beverdors and others 2015). Therefore, uncertainty remains regarding the factors regulating MC production in natural environments, particularly over broad hydrological landscapes (Beverdors and others 2015; Neilan and others 2013).

Little is known about how environmental drivers of MCs vary across the contrasting hydrological regimes of lakes, rivers, and wetlands, particularly within interconnected drainage networks. Most studies of in situ cyanoHABs and MCs have focused on large lentic waterbodies (MacKeigan and others 2023; Merder and others 2023), while fewer have examined rivers and entire aquatic networks (Heiskary and Markus 2001; Chételat and others 2006; Al-Tebrineh and others 2012; Svirčev and

others 2019; Graham and others 2020), despite increasing evidence that rivers can support recurrent and sometimes severe toxic blooms (Kutovaya and others 2012; McKay and others 2018; Nietch and others 2022; Patiño and others 2023; Ramos and others 2023). However, hydrological, chemical, and biological conditions vary greatly among flow regimes and habitats (e.g., Basu and Pick 1996), such that variable environmental controls may lead to heterogeneous patterns of cyanoHAB abundance, strain dominance, and thus toxin abundance throughout drainage networks (Graham and others 2020). Hydrological connectivity can regulate residence time, nutrient transport, and the downstream movement of cyanobacteria and their toxins, influencing where and when cyanoHABs may develop and persist (Klotz and others 2026). Similarly, as connectivity between waterbodies can vary seasonally and annually within individual watersheds, changes in hydrological conditions may further increase heterogeneity in the spatial distribution of MCs across lentic and lotic habitats (Kutovaya and others 2012; Graham and others 2020). Understanding how hydrological variability may influence MC distribution is, therefore, critical for predicting cyanoHAB impacts across integrated aquatic networks.

Watersheds subject to intensive agriculture may be particularly susceptible to cyanoHABs and elevated levels of MCs, such as those in the Northern Great Plains of North America (Orihel and others 2012; Dreher and others 2019; MacKeigan and others 2023). This semi-arid to sub-humid ecoregion spans three Canadian provinces and five US states, where ~80% of the land has been converted to agricultural use (Coles and others 2017; Baulch and others 2019). Soils in the Northern Great Plains naturally contain elevated P concentrations that can be exported to aquatic ecosystems, resulting in nutrient-rich waterbodies with low N:P ratios (Leavitt and others 2006; Orihel and others 2012). As a result, this landscape experiences seasonal cyanoHAB formations that respond positively to N pollution (Leavitt and others 2006; Finlay and others 2010). Wastewater effluent also contributes to freshwater eutrophication in this region (Rock and Mayer 2006), serving as a point source of N and P (Leavitt and others 2006; Nwankwegu and others 2019). Because of the naturally flat topography, these prairie catchments typically integrate wetland, river, and reservoir ecosystems into large drainage basins (> 1000 km²), making them ideal sites for investigating the spatial patterns of cyanoHAB abundance and toxicity.

Here, we used a whole-catchment approach to quantify patterns in phytoplankton abundance and MCs across distinct lotic and lentic habitats under contrasting hydrological regimes (connected, isolated) in an impacted watershed. We tracked MC concentrations, phytoplankton community composition, physico-chemical properties, and nutrient concentrations in wetland, river, and reservoir habitats by sampling in years with (2021) and without (2022, 2023) hydrological connections between an effluent-receiving, hypereutrophic wetland and its downstream aquatic network. We further explored connections between riverine MCs and discharge rates. Given their shallow, warm, and productive conditions, we expected the wetland to support high MC concentrations and anticipated that downstream habitats would exhibit higher concentrations of MCs in 2021, when hydrological connectivity was greater (Zhou and others 2023) and headwater wetlands were potentially capable of exporting nutrients and MCs.

METHODS

Study Area

Alberta is an agriculturally intensive province with the highest cattle density in Canada (Shahbandeh 2024). The upper Little Bow River (LBR) drains 3491 km² within the Oldman River Basin in southern Alberta. Within this watershed, two ephemeral creeks (Blackie, Mazeppa) drain into a headwater wetland complex (Frank Lake), which is hydrologically sustained by wastewater inputs and which discharges into the LBR (Figure 1). The LBR and Mosquito Creek flow into the Twin Valley Reservoir (TVR) (Figure 1).

Frank Lake is a natural wetland complex situated 45 km south of Calgary, Alberta (50.57° N, 113.73° W) and spans an area of 10.1 km² (Zhu and others 2019). The wetland comprises three basins, each with regulated outflows, which release water into the LBR through a constructed canal (White and Bayley 2001; Zhu and others 2019; Bogard and others 2023). Frank Lake is one of the most important waterfowl habitats in southern Alberta (White and Bayley 2001; Lenoir 2023).

Frank Lake was completely dry during the mid-1980s and has been subject to hydrological management by Ducks Unlimited Canada to restore water levels for waterfowl and habitat conservation (White and Bayley 2001). After damming basin outlets, stable water levels were established in 1989 following the introduction of secondary-treated wastewater from Cargill Foods Ltd., a beef slaught-

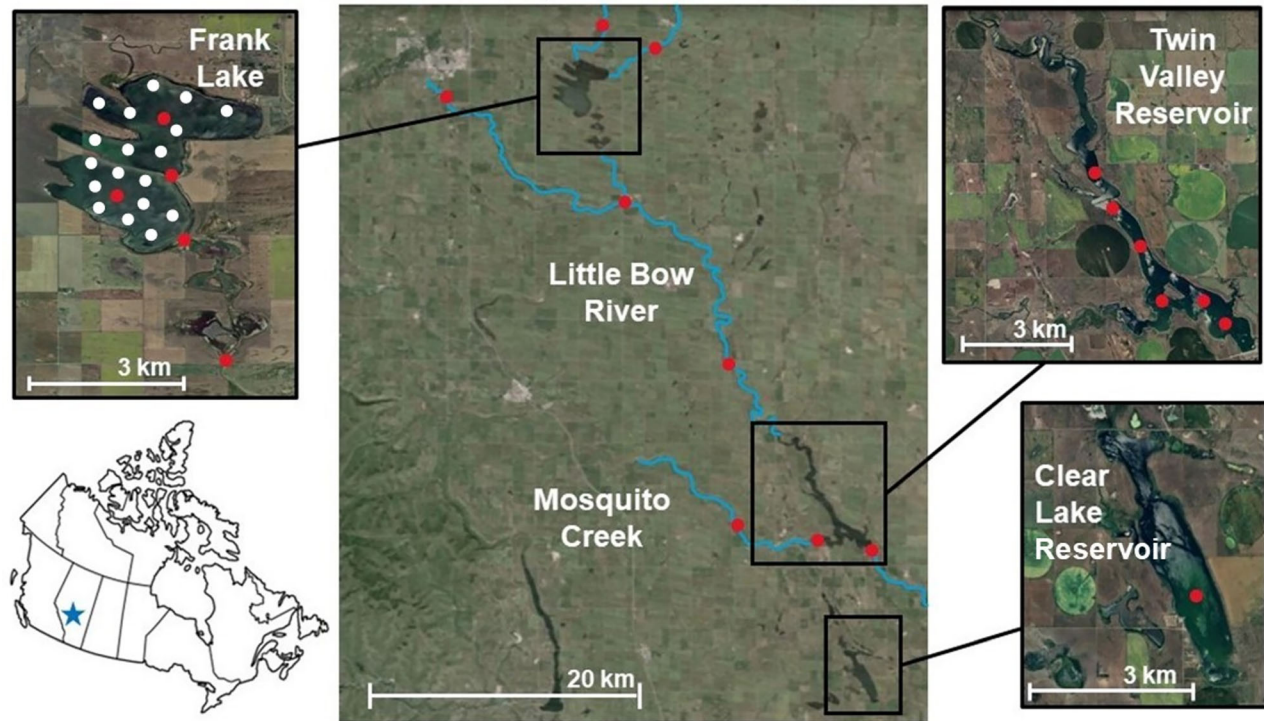


Figure 1. Map of the upper Little Bow River watershed. Red dots represent routine sampling locations, while white dots at Frank Lake denote wetland transect samples. Blue lines represent rivers. The watershed's location in reference to the rest of Canada is shown in the bottom left corner.

terhouse that processes over 4500 cattle daily (White and Bayley 2001). Since then, Frank Lake has received N- and P-rich wastewater from the meat-processing plant, secondary-treated (excluding N removal) sewage from the town of High River, and freshwater from Blackie and Mazeppa creeks (White and Bayley 2001). Such elevated nutrient influx, paired with high mean summer temperatures (17.5 ± 3.8 °C), makes Frank Lake a hypereutrophic waterbody that is subject to annual blooms of algae and cyanobacteria (White and Bayley 2001).

The water residence time (WRT) of Frank Lake varies markedly between wet and dry meteorological intervals (Zhu and others 2019), as does connectivity between Frank Lake and the Little Bow River. As reported by Zhou and others (2023), the WRT based on lateral flux observations was 16.4 years in 2021, the first year of our study, when outflow from the wetland complex into the LBR occurred. In 2022 and 2023, no outflow was observed (Denny, 2024; unpublished data), and therefore WRT could not be calculated.

Field Measurements

Limnological sampling was conducted from 2021–2023 (Figure 1). Sampling locations included the three wetland basins of Frank Lake (FLB1, FLB2, and FLB3; $n = 148$), river sites (FLDR, LB533, LBCAN, LBCAR, LBUP, MCR, MCRDIV, and TVRDR; $n = 134$), and downstream reservoir sites (CLR and TVR; $n = 32$). Site descriptions are presented in Table S1. Hydrological connectivity varied among the study years, with all three Frank Lake basins interconnected in 2021, but not 2022 or 2023, when Basin 3 was fully desiccated and the wetland complex was disconnected from downstream waterbodies. Sampling was typically conducted biweekly from May–August and monthly from September–April, depending on ice cover.

At each river site, surface-water samples (~ 0.25 m below surface) were gathered in sterilized containers from shore. For wetland and reservoir sites, surface-water samples (~ 0.25 m below surface) were collected by boat at open-water habitats near the deepest location of each basin. All samples were stored on ice in a closed cooler until returned to the laboratory for filtration. Samples for MCs were kept as whole, unfiltered surface waters. Remaining water was filtered

within 6 h of collection through pre-rinsed 0.45- μm pore FHT-45 capsule filters (Whatman) or 0.45- μm pore cellulose nitrate membrane filters (Whatman) into acid-washed bottles or pre-combusted (450 °C, 4 h) amber glass vials for various laboratory analyses. Additionally, a Yellow Springs Instrument (YSI) Multiparameter Exo-1 Sonde (YSI Inc., OH, USA) was used to record barometric pressure (mm Hg), water temperature (°C), pH, salinity (ppt), dissolved oxygen (DO; % saturation, mg L^{-1}), and turbidity (Formazin Nephelometric Unit; FNU) in surface waters. The sonde was calibrated prior to sampling trips using standard methods and is routinely serviced to ensure readings were accurate. As water depths, particularly in rivers, decreased during drought years (e.g., from 0.67 m in 2021 to 0.26 m in 2022 for TVRDR), sediment-water interactions could influence MC concentrations in the water column. However, the effects of sedimentary processes could not be quantified in this study and instead represent an important avenue for future research.

To evaluate spatial variations within a waterbody, transect surveys were conducted once in the spring (May), summer (July and August) and fall (October) on Frank Lake Basins 1 and 2, as well as the TVR. Surface water from nine transect points were typically collected at each Frank Lake basin, while 6 transect sites were sampled along the length of the TVR (Figure 1).

Quantification of Microcystins

Surface-water samples were subjected to three freeze/thaw cycles to lyse cyanobacterial cells, then filtered through a 1.2- μm nominal-pore GF/C glass-fiber filter using a manual hand pump. This allowed both intracellular and extracellular MCs to be collected in the supernatant (Liu and others 2023). Following filtration, enzyme-linked immunosorbent assay (ELISA) kits specific for MC detection were used to quantify total MC concentrations in surface waters following Finlay and others (2010). Briefly, QuantiPlate™ ELISA Kits for Microcystins (EnviroLogix Inc., Portland, Maine) were run following kit instructions. Concentrations of MCs ($\mu\text{g L}^{-1}$) within each water sample were calculated using optical density data. The method detection limit was 0.10 $\mu\text{g MC L}^{-1}$. Therefore, in this study, MC concentrations refer to the combination of intracellular and dissolved MC concentrations. We recognize that this approach has limitations, such as the false-positive detection of MCs due to the potential presence of nodularin toxins (Aranda-Rodriguez and others 2015), and

the potential underestimation of total MC concentrations due to incomplete lysis of cyanobacterial cells; however, ELISA kits are widely used and allow direct comparison with other studies (Loftin and others 2008; Haggard and Austin 2023; Liu and others 2023). Importantly, MC concentrations in this study spanned orders of magnitude, making potential methodological uncertainties less consequential for the study of broad spatial and temporal patterns of toxin occurrence. Nonetheless, we acknowledge that cross-habitat patterns observed in this study should be interpreted as metrics of potential maximum bloom toxicity, rather than as an index of instantaneous exposure to dissolved MC alone.

Phytoplankton Analysis

Pigments from algae and cyanobacteria were analyzed as a measure of phototrophic community composition following standard protocols (Leavitt and Hodgson 2001) at the Institute of Environmental Change and Society (IECS), University of Regina, Canada. Methods are fully detailed in previous studies (Jeffrey and Humphrey 1975; Finlay and others 2010). Briefly, total phytoplankton concentration was recorded using trichromatic estimates of Chl *a* concentration using spectrophotometry (Finlay and others 2010), while high performance liquid chromatography (HPLC) was used to estimate other biomarker pigment concentrations to establish gross community composition. Phytoplankton filtered and frozen on 1.2 μm pore GF/C glass-fiber filters were extracted using a mixture of acetone: methanol: water (80:15:5, by volume; Finlay and others 2010), then solutions were passed through a 0.22 μm pore chemically resistant membrane filter and stored in the dark at -10°C until drying with 99.9% pure N_2 gas. Pigments were diluted with a standard injection solvent mixture and introduced into an Agilent 1100 Series HPLC system that separated taxonomically diagnostic carotenoid, chlorophyll, and derivative pigments (Leavitt and Hodgson 2001). All algal pigments are expressed as nmoles pigment L^{-1} , a metric that accurately captures general changes in gross community composition compared to direct microscopic enumeration (Donald and others 2013). Our approach allowed us to distinguish among total cyanobacteria (as pigment echinenone), colonial forms (as myxoxanthophyll), Nostocales (canthaxanthin), and diazotrophic species (aphanizophyll). While phycocyanin is commonly used as a proxy for cyanobacteria, quantification of this water-soluble

pigment was not compatible with our HPLC approach.

Nutrient Analysis

Total and dissolved nutrient chemistry was analyzed using multiple methods. A subset of samples was analyzed at the IECS, University of Regina. Total nitrogen (TN; $\mu\text{g N L}^{-1}$) and total phosphorus (TP; mg P L^{-1}) were measured using the Lachat QuikChem 8500 Series 2 (Hach®, CO, USA). Method detection limits and applicable ranges were as follows: TN $10 \mu\text{g L}^{-1}$, $0\text{--}6000 \mu\text{g L}^{-1}$, and TP 0.001 mg L^{-1} , $0.005\text{--}2.00 \text{ mg L}^{-1}$. The remaining samples were analyzed at a certified commercial laboratory (Element Lab) following standard methods summarized in Table S2. Unfiltered water samples were sent to the Element Lab and processed for dissolved N species, including combined nitrate/nitrite ($\text{NO}_3 + \text{NO}_2$) and total Kjeldahl N (TKN), and TN concentrations were calculated as TKN plus $\text{NO}_3 + \text{NO}_2$ concentrations. TP concentrations were also quantified. When values were below the limit of detection, we replaced them with the limit of detection.

META-ANALYSIS of Microcystin Concentrations Across Aquatic Ecosystems

To place MC concentrations measured in the upper Little Bow River watershed into a broader geographic and ecological context, we conducted a meta-analysis of both published and unpublished MC concentration data from aquatic ecosystems across North America. Data were compiled from open-access or public databases, as well as peer-reviewed literature (Table S3). Studies were included if they reported quantitative MC concentrations in surface waters and provided sufficient metadata to classify sampling sites by habitat type. When multiple measurements were reported for a given site, each observation was retained to capture temporal variability. Habitats were categorized as riverine, lake, reservoir, wetland, estuarine, or coastal based on descriptions provided in the original sources. The final dataset consisted of 16,831 MC measurements (including those recorded in this study) spanning diverse freshwater to marine environments (Table S3). Concentrations of MCs were \log_{10} -transformed prior to statistical analysis (see below) and visualization, and comparisons among ecosystem types were based on median values.

Modeling and Statistical Analyses

All analyses were conducted in R (version 4.2.3; R Core Team 2024). Kruskal–Wallis tests were conducted using the R *stats* package to assess relationships among environmental variables across waterbody types and sampling years. Dunn’s post-hoc analysis with Bonferroni correction was performed on all significant differences identified by the Kruskal–Wallis tests to determine significant inter-group differences. Bonferroni correction was chosen because it is conservative, though we acknowledge other tests (e.g., Benjamini–Hochberg) could be suitable as well. The same approach was used in our meta-analysis to compare MC concentrations with other North American ecosystems.

Spatial maps were constructed using the *rnatu-ralearth*, *rnatu-ralearthdata*, *ggspatial*, and *terra* packages in R to display MC distributions across the entire upper Little Bow River network. At each sampling location, MC concentrations were averaged across 2021, 2022, and 2023 because seasonal patterns were consistent across years. Concentrations of MCs were modeled spatially using nearest-neighbor interpolation with the *terra* and *gstat* packages to identify local hotspots of the toxin.

To assess the predictors of MC abundances, toxin concentrations were modeled as generalized linear models (GLMs) with a Gamma family distribution and a log-link function using the *stats* package in R. A set of plausible candidate models was created for both wetlands and rivers and ranked separately using AICc to correct for small sample size using the *MuMIn* package in R. Predictor variables included Chl *a*, TN, TP, TN:TP, DO, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), turbidity, salinity, pH, and temperature. River models also included discharge as a predictor (see Figure S1 for discharge rates of river sites). In most instances, we included only Chl *a* concentrations, as preliminary analysis revealed that this metric of total primary production produced better models than those derived from cyanobacteria-specific pigments (Table S4). To avoid multicollinearity, predictors with generalized variance inflation factors > 5 (GVIF, calculated using the *glmtoolbox* package) were considered collinear and were not included together in the same model. Month and Year were used as categorical predictors in the GLMs, although Month was excluded from wetland GLMs because it did not add predictive strength to models using water temperature (Tables S5–S8), and its removal aligned with our goal of using environmental predictors where possible to explain pat-

terns of MCs. Final plausible models exhibited a $\Delta\text{AICc} < 4$ (Burnham and others 2011). We present the top 3 models for each waterbody (wetland, river) in the main text, and the top five models in Tables S5–S8.

RESULTS

Limnological and Environmental Conditions

Each sampling site and habitat type exhibited unique limnological conditions. Frank Lake wetlands had the highest average temperatures, carbon content, salinity, turbidity, pH, and nutrient concentrations across habitats in the upper Little Bow River watershed (Figure 2, Table S1). Dunn's post-hoc pairwise comparisons showed that water temperatures differed significantly between rivers and wetlands ($p = 0.011$), but not between rivers and reservoirs, nor between wetlands and reservoirs (all $p > 0.05$; Figure 2A). Mean DOC and DIC concentrations (reported as mean \pm standard deviation) decreased from wetlands ($38.77 \pm 16.82 \text{ mg L}^{-1}$ and $167.83 \pm 46.19 \text{ mg L}^{-1}$, respectively) to rivers (mean DOC = $4.44 \pm 7.00 \text{ mg L}^{-1}$, mean DIC = $37.52 \pm 24.70 \text{ mg L}^{-1}$), then reservoirs (mean DOC = $4.91 \pm 2.33 \text{ mg L}^{-1}$, mean DIC = $33.28 \pm 5.39 \text{ mg L}^{-1}$) (Figure 2B and C). Wetland DIC and DOC values were significantly greater than in rivers and reservoirs ($p < 0.001$), while both were similar between rivers and reservoirs ($p > 0.05$; Figure 2B and C). Wetland average salinities (2.14 ± 0.89 ppt) exceeded river and reservoir values (0.27 ± 0.43 and 0.26 ± 0.39 ppt, respectively) ($p < 0.001$; Figure 2D). Salinity did not differ between rivers and reservoirs ($p > 0.05$; Figure 2D). Wetlands were more turbid (mean = 79.61 ± 567.87 FNU) than rivers (11.75 ± 16.07 FNU) and reservoirs (2.40 ± 3.73 FNU), although all were significantly different ($p < 0.001$; Figure 2E). Wetlands also exhibited elevated pH values ($p < 0.001$; Figure 2F), with an average pH of 8.69 ± 0.31 across all sampling years. Mean TN concentrations decreased significantly ($p < 0.01$) from wetlands ($6.16 \pm 3.15 \text{ mg L}^{-1}$), to reservoirs ($1.03 \pm 0.46 \text{ mg L}^{-1}$), then rivers ($0.45 \pm 0.73 \text{ mg L}^{-1}$) (Figure 2G). Wetland TP concentrations (mean $3.23 \pm 0.83 \text{ mg P L}^{-1}$) were also greater ($p < 0.001$) than in rivers ($0.14 \pm 0.52 \text{ mg P L}^{-1}$), and reservoirs ($0.10 \pm 0.05 \text{ mg P L}^{-1}$) (Figure 2H), and wetlands had the lowest TN:TP ratios ($p < 0.001$, Figure 2I). While TN and TP concentrations did not differ between rivers and reservoirs (both $p > 0.05$), TN:TP ratio was significantly

lower in rivers ($p = 0.031$). While values differed across habitat type, rivers had the highest DO values (all $p < 0.05$; Figure 2J), averaging $10.05 \pm 1.67 \text{ mg L}^{-1}$ (97.82% saturation).

Wetlands as Hotspots for Phytoplankton and Microcystin Concentrations

Mean total abundance of phototrophs (as Chl *a*) was greatest in wetland habitats, followed by reservoirs, and then rivers (Figure 2K) with means of $51.00 \pm 262.33 \text{ } \mu\text{g L}^{-1}$, $6.06 \pm 6.08 \text{ } \mu\text{g L}^{-1}$, and $3.11 \pm 9.79 \text{ } \mu\text{g L}^{-1}$, respectively. Frank Lake Basin 2 had the highest Chl *a* and MC concentrations (mean = $73.8 \pm 383.2 \text{ } \mu\text{g L}^{-1}$ and $10.1 \pm 39.1 \text{ } \mu\text{g L}^{-1}$, respectively; Table S1). Phytoplankton biomass was comparable between wetlands and reservoirs ($p = 0.302$), but significantly different between wetlands and rivers ($p < 0.001$) as well as reservoirs and rivers ($p = 0.036$; Figure 2K). Chl *a* concentrations differed among years in wetlands ($p < 0.003$), but not in rivers or reservoirs ($p > 0.05$). Overall, wetlands exhibited significantly higher MC concentrations (mean = $6.2 \pm 26.3 \text{ } \mu\text{g MC L}^{-1}$) than those seen in other habitats ($p < 0.001$; Figure 2L), with values over tenfold greater than those in reservoirs ($0.3 \pm 0.5 \text{ } \mu\text{g L}^{-1}$) or rivers ($0.2 \pm 0.8 \text{ } \mu\text{g MC L}^{-1}$). Lotic concentrations of MCs were greatest in the LBR directly upstream of the TVR (Table S1; max = $8.9 \text{ } \mu\text{g L}^{-1}$ in July 2023). River and reservoir MC concentrations were not significantly different ($p = 0.089$; Figure 2L). Among years, MC concentrations were greater in rivers in 2023 compared to 2021 ($p < 0.001$) and 2022 ($p = 0.002$), but neither wetlands nor reservoirs showed significant temporal variations in MC concentrations ($p > 0.05$). Spatially, Frank Lake contained the highest abundance of MCs (Figure 3A), with concentrations in the outflow of Basin 2 exceeding $300 \text{ } \mu\text{g L}^{-1}$ in July 2023. Blackie Creek, Mazeppa Creek, the LBR, Mosquito Creek, the TVR, and Clear Lake Reservoir all exhibited average MC concentrations below $1 \text{ } \mu\text{g L}^{-1}$ across all sampling years (Figure 3A). As the catchment hotspot for MCs, we explored the patterns in Frank Lake in more detail using transect surveys of Basin 1 and Basin 2 (Figure 3B). Microcystins were more abundant during summer months (July and August) than in late spring (June) or fall (October), with the northeastern region of Basin 1 exhibiting MC concentrations up to $8 \text{ } \mu\text{g L}^{-1}$ in August 2021 (Figure 3B).

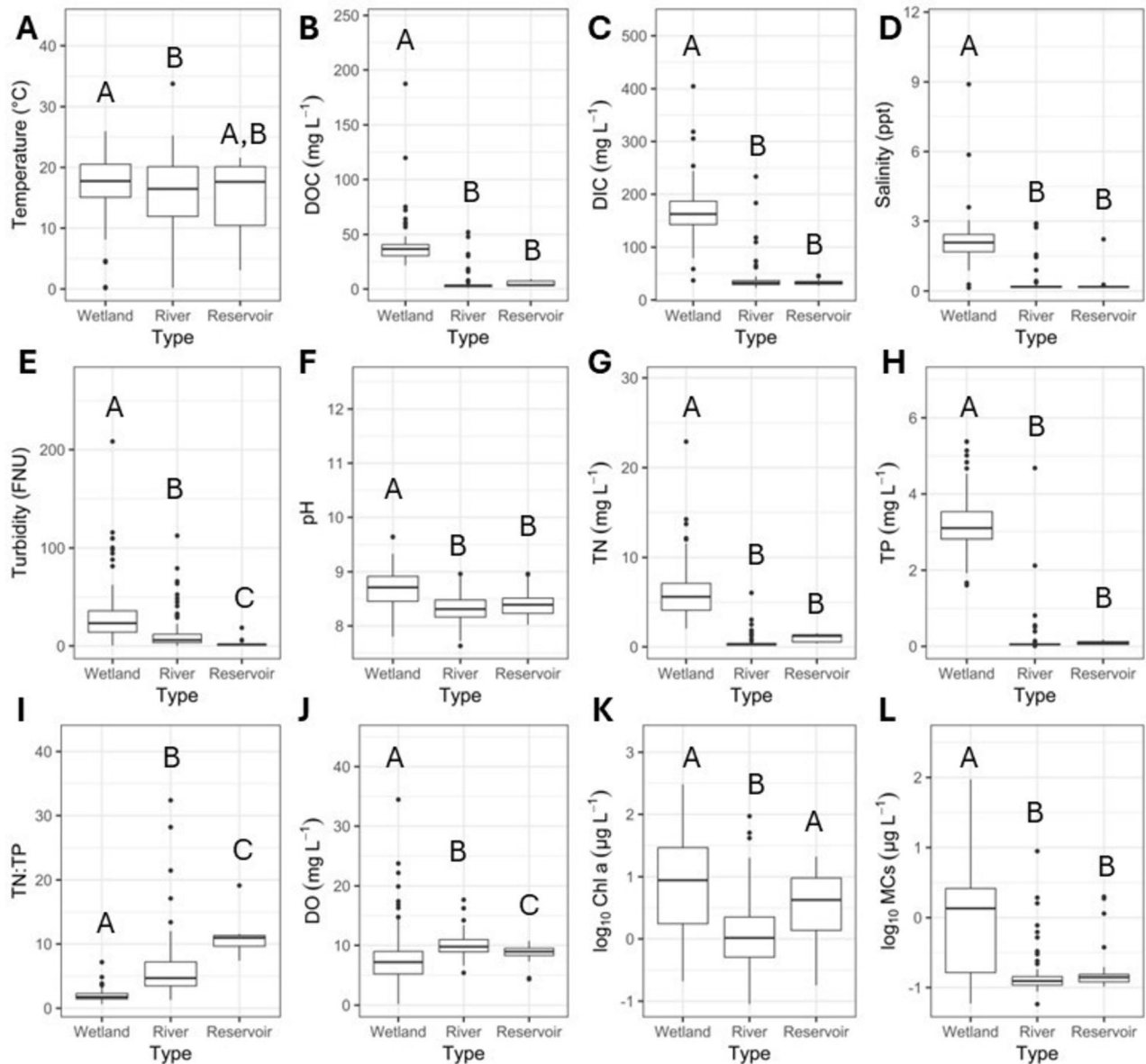


Figure 2. Boxplots of (A) water temperature; (B) DOC and (C) DIC concentrations; (D) salinity; (E) turbidity; (F) pH; (G) TN and (H) TP concentrations; (I) TN:TP ratio; (J) DO concentration; (K) Chl *a* concentration; and (L) total MCs between different habitat types (Wetland, River, Reservoir). Statistical significance was based on Dunn's post-hoc analysis following Kruskal–Wallis tests. A Bonferroni correction was applied to all comparisons to reduce Type I error. Note that extreme outliers of turbidity (> 6570 FNU), Chl *a* ($> 2975 \mu\text{g L}^{-1}$), and MC ($> 300 \mu\text{g L}^{-1}$) values are excluded to facilitate dataset visualization. Frank Lake wetland data includes open water and outflow samples. The median is denoted by the thick horizontal line, while box ends denote interquartile range (IQR), spanning from the 25th percentile to the 75th percentile. Whiskers extend to the most extreme data points within $1.5 \times$ the IQR from the box edges. Points beyond this range are shown as individual dots and represent statistical outliers.

Habitat-Specific Phytoplankton Community Composition and Toxicity

The relationship between MCs and suspended phytoplankton abundance differed between habitat types (Figure 4). Toxin levels were correlated positively with Chl *a* concentration in wetlands

($R^2 = 0.37$, $p < 0.001$) and reservoirs ($R^2 = 0.30$, $p = 0.06$), but not in rivers ($R^2 = 0.00$, $p = 0.76$) (Figure 4). The habitat-specific differences in MC concentrations may reflect distinct suspended phytoplankton communities and cyanobacterial types in each habitat (Figure 5A). As proportions of group-specific pigment biomass, cyanobacteria

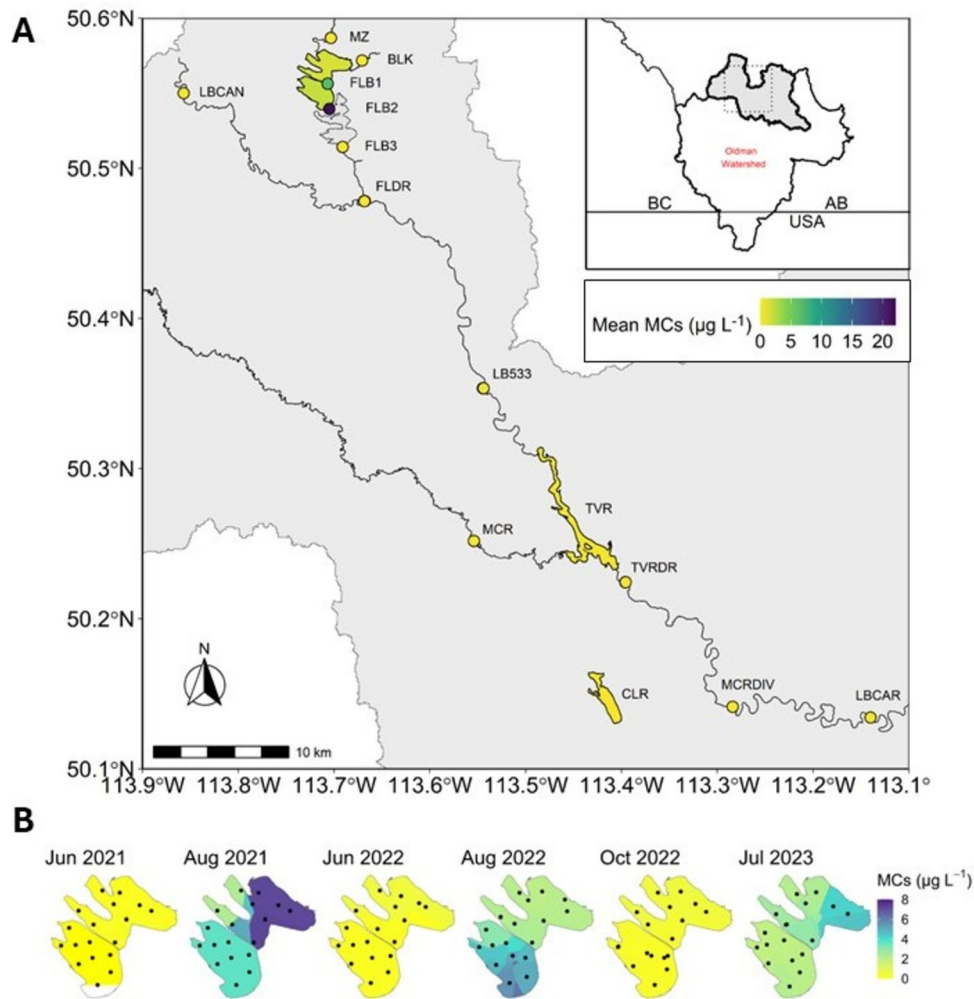


Figure 3. (A) MC distribution along the upper Little Bow River watershed from 2021 to 2023. All sampling points represent MC averaged across all three sampling years. Frank Lake Basin 1 and Basin 2 include an additional outflow sampling point. The sub-watershed is denoted within the broader Oldman River Watershed with a black dashed line (see inset map). (B) Within the Frank Lake wetland complex, distribution of MCs is shown for Basins 1 and 2 based on seasonal transect surveys of open-water habitats. Spatial maps of MC concentrations reflect Nearest Neighbor interpolation.

were often more abundant in lentic than lotic ecosystems, although there was marked variation in community composition among years, with elevated abundance of N_2 -fixing cyanobacteria in 2022 (Figure 5A). River sites exhibited the lowest proportion of cyanobacteria of all habitats. We assessed the relationship between MCs and Chl *a*, echinenone (total cyanobacteria), canthaxanthin (Nostocales) and myxoxanthophyll (colonial cyanobacteria) in both wetlands and rivers (Table S4) to compare various biomarker pigments for total algal biomass and cyanobacterial biomass. Chl *a* was the best predictor for both habitat types, followed by echinenone. To evaluate patterns of cyanobacterial biomass across habitat types, we also examined the relationship between Chl *a* and

echinenone (total cyanobacteria) concentrations (Figure 5B). As Chl *a* concentrations increased, cyanobacterial biomass also increased across all habitat types (Figure 5B). This relationship was strongest in wetlands ($R^2 = 0.89$, $p < 0.001$), followed by rivers ($R^2 = 0.57$, $p < 0.001$), then reservoirs ($R^2 = 0.42$, $p = 0.061$), and indicates that Chl *a* is a reliable indicator of trends in cyanobacterial biomass. Cyanobacterial biomass was significantly higher in lentic ecosystems than in lotic habitats ($p < 0.001$; Figure 5C).

Drivers of Microcystin Concentrations

We modeled MC concentrations using generalized linear models (GLMs), ranked by AICc, for wetland and river habitats (Table 1). We excluded reservoir

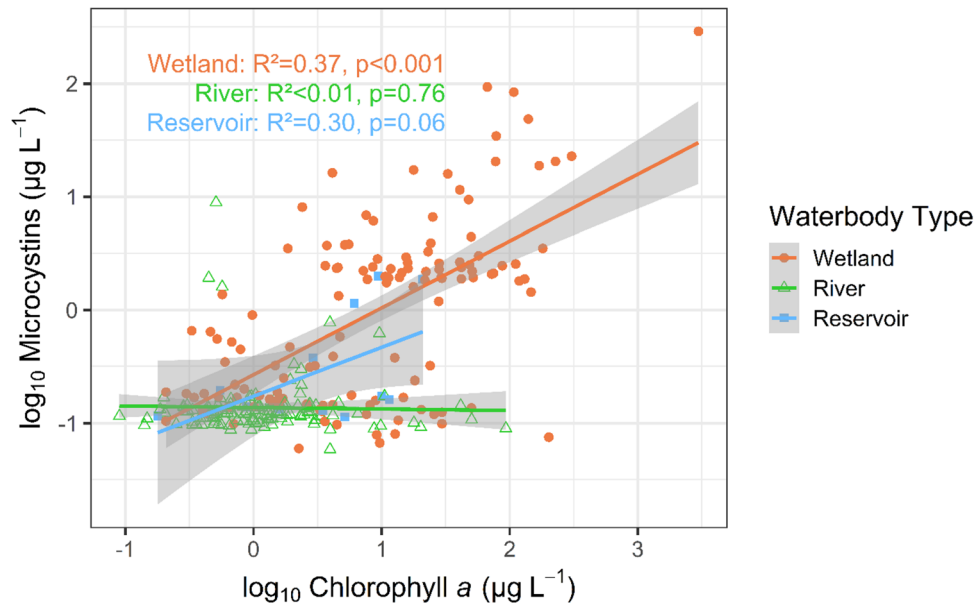


Figure 4. Linear regressions of log-transformed Chl *a* and MC concentrations within distinct habitats (Wetland, River, Reservoir). R^2 and p -values for each relationship are shown in the top left. Detection limit is -1.0 ($\log_{10}(0.10 \mu\text{g L}^{-1})$) for MC concentrations.

data as our sample sizes were too small for these analyses. The best-fit model for predicting MC abundance in wetland habitats included Chl *a*, temperature, and salinity as predictors (Figure 6A; $R^2 = 0.81$). When we substituted echinenone (total cyanobacteria) for Chl *a* (total phototrophs), model performance declined slightly ($R^2 = 0.79$) in all habitats, while models with more taxonomically diagnostic pigments (canthaxanthin, myxoxanthophyl) performed substantially worse (Table S4). This pattern demonstrates that quantifying cyanobacterial biomass as a predictor of toxin concentrations showed no statistical advantage, likely because cyanobacteria represented a substantial proportion of total phytoplankton biomass (up to 44.2% in 2022; Figure 5A). When Chl *a*, temperature, or salinity was removed, the ΔAICc value increased substantially to include only implausible models ($\Delta\text{AICc} > 19$). Nutrients, turbidity, and Year (a categorical variable) were included in weaker models, but had limited predictive power (Table S5).

Performance of GLMs was much lower for rivers than wetlands (Table 1; $R^2 = 0.53$) irrespective of the precise phytoplankton predictor used. Specifically, models based on total cyanobacteria (echinenone) again performed worse than those based on Chl *a* (Table S4). In all cases, the best models included Month (a categorical variable) as a predictor (Table 1), while four of the five top models also included salinity (Table S7). However, unlike

for wetlands, MC concentrations and salinity were inversely related in rivers (Figure 6B). When we removed Month, water temperature was included in the next best candidate models (Table S8), suggesting that Month captured an unsampled aspect of seasonal environmental change. However, because inclusion of temperature weakened the model performance substantially (R^2 declined by 0.21; Tables S7–S8), we infer seasonal changes included more than just the thermal regime. All top models included either Month, or salinity, or both, and had ΔAICc values ≤ 4 (Table S7), indicating these models effectively predicted river MC concentrations over other candidate models. The addition of other environmental predictors (turbidity, Chl *a*, or discharge) added little predictive power to our river models.

DISCUSSION

Catchment-scale assessments of cyanoHAB abundance and MC distribution are rare (e.g., He and others 2021), but they provide an integrated picture of whole-watershed water quality. Here, we used our multi-year, cross-habitat assessment of cyanoHAB patterns in the upper Little Bow River watershed to fill this knowledge gap by monitoring the total extractable MC pool (intracellular and extracellular MCs). While most previous work examined community dynamics or physiological, cellular-scale controls of MC production (Neilan

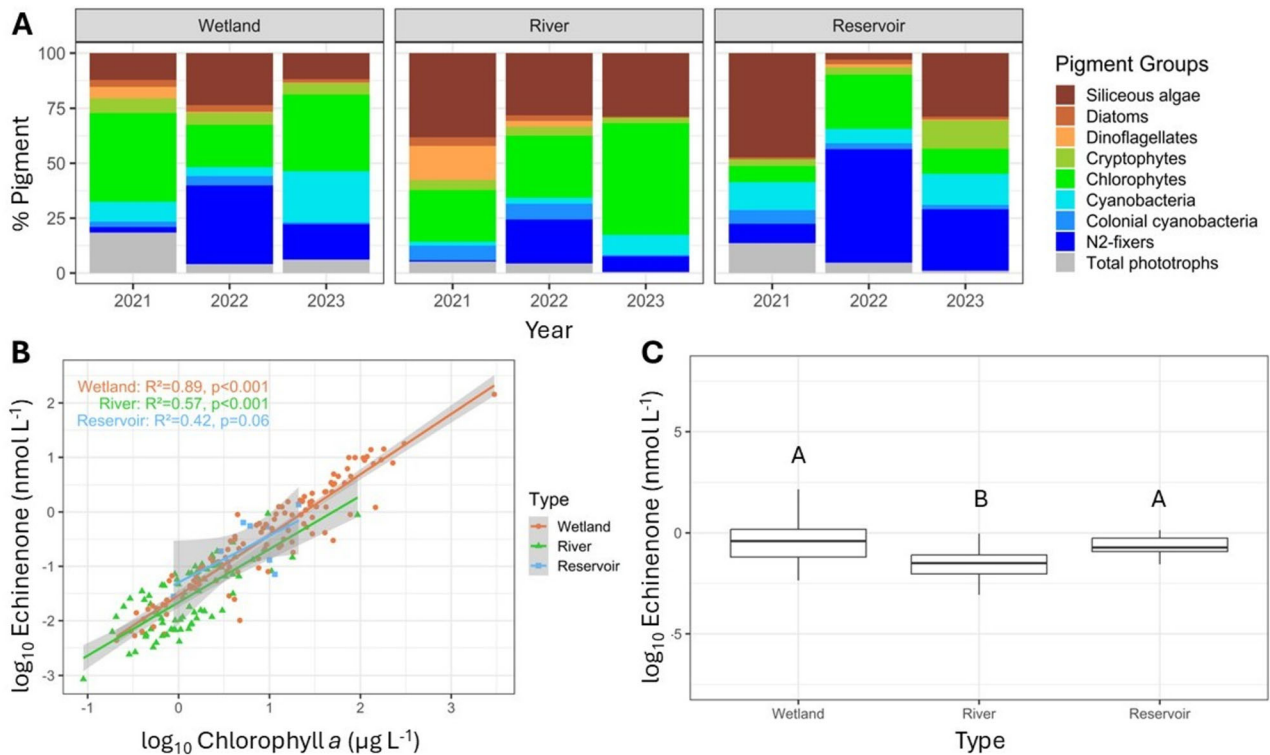


Figure 5. Patterns in cyanobacterial biomass and other phytoplankton. **(A)** Proportion of cyanobacteria relative to other phytoplankton community groups for wetlands, rivers, and reservoirs, from 2021 to 2023. Carotenoid and chlorophyll pigment groups included cyanobacteria (measured as echinenone and canthaxanthin), colonial cyanobacteria (measured as myxoxanthophyll), N₂-fixers (measured as Aphanizomenon), siliceous algae (measured as fucoxanthin), diatoms (measured as diatoxanthin), dinoflagellates (measured as diadinoxanthin), cryptophytes (measured as alloxanthin), chlorophytes (measured as chlorophyll *b* and lutein-zeaxanthin), and total phototrophs (measured as beta-carotene). **(B)** The relationship between Chl *a* concentrations and total cyanobacterial biomass (echinenone and canthaxanthin) within distinct habitats (Wetland, River, Reservoir). Lines represent the linear regression models and shaded areas are the 95% confidence intervals for each model. R² and p-values for each relationship are shown in the top left. **(C)** Concentrations of total cyanobacterial biomass grouped by habitat type. Statistical reporting as for Figure 2.

Table 1. Top Wetland and River Generalized Linear Models

System	Null df	Null deviance	Models	R ²	Residual df	Residual deviance	ΔAICc
Wetland	84	333.5	~ Chl + Temp + Salinity	0.81	81	92.80	0.00
			~ Chl + Temp + Salinity + TP	0.81	80	91.99	1.45
			~ Chl + Temp + Salinity + Turbidity	0.81	80	92.25	1.73
River	69	95.62	~ Month + Salinity	0.53	62	49.66	0.00
			~ Month + Salinity + Turbidity	0.54	61	48.69	1.20
			~ Month + Salinity + Chl	0.54	61	49.05	1.72

Note: Generalized linear models (GLMs) for MC concentrations in wetland and riverine habitats. The top three wetland and river GLMs are ranked by ΔAICc. Chl = chlorophyll *a*, TP = total phosphorus, Temp = temperature, df = degrees of freedom.

and others 2013; Beversdorf and others 2015; Graham and others 2020), we focused on identifying and comparing ecosystem-scale predictors of MCs across the entire catchment. The top predictors of MC concentrations differed among flowing

and standing waters, suggesting unique controls of MCs in each habitat. We found that MC concentrations were greatest in the effluent-receiving wetland complex (Figure 3A), sometimes exceeding thresholds for high risk of health effects from

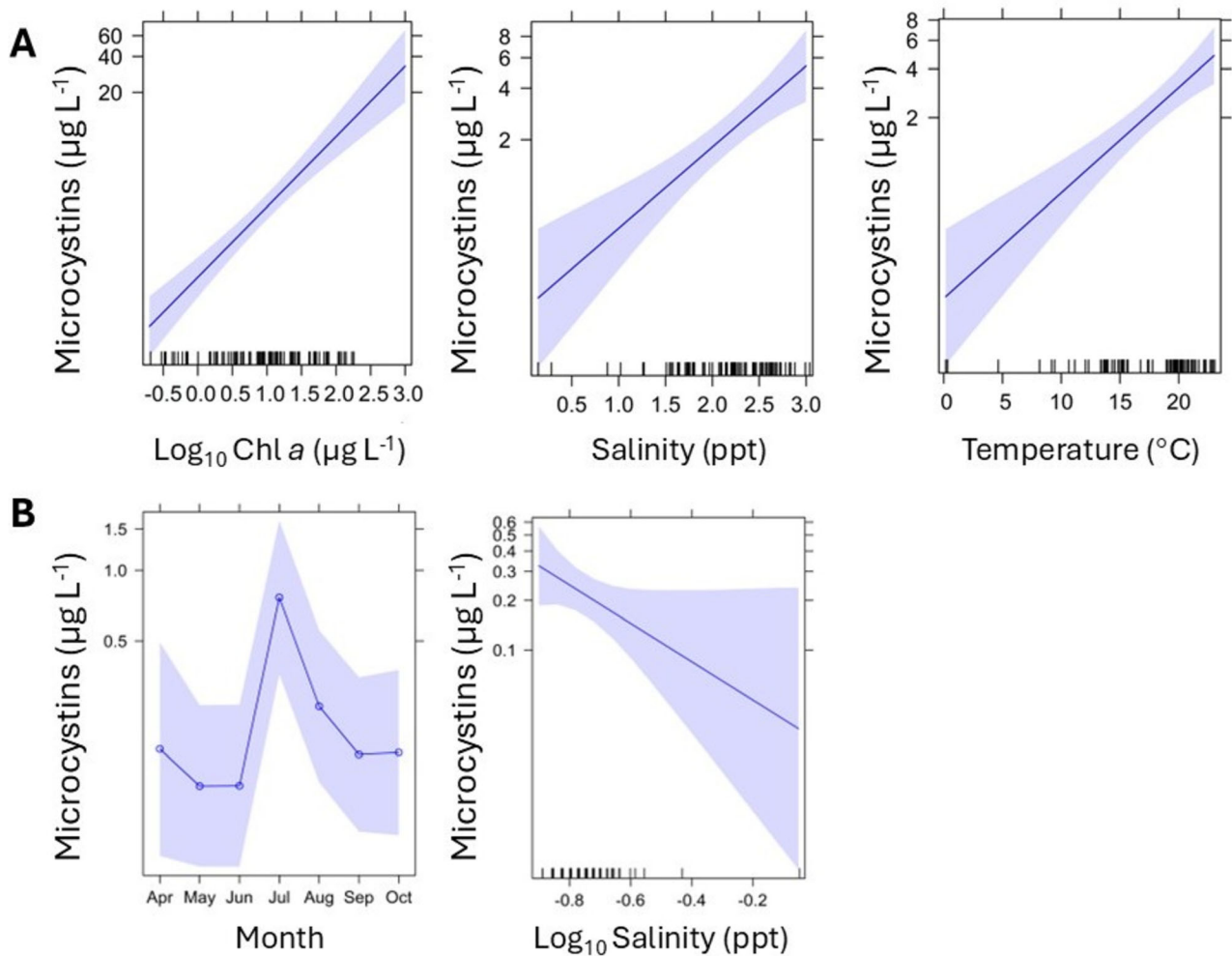


Figure 6. (A) Top wetland GLM. Predictors include log-transformed total algal biomass (measured as chlorophyll *a*), water temperature, and salinity. The R^2 for this model is 0.81. (B) Top river GLM. Predictors include month and log-transformed salinity. The R^2 for this model is 0.53. Blue shaded areas represent 95% confidence intervals. Hash marks along the x-axes (rug plots) indicate the distribution of observed data points for each predictor variable. Y-axis tick marks are shown at intervals reflecting the range and distribution of predicted values.

recreation ($> 20 \mu\text{g MC L}^{-1}$) (Figure 2L) (WHO 2022). The complex appears to be a localized hot-spot for MCs, as there was little evidence of toxin transport downstream from the wetland basins to the LBR or the TVR (Figure 3A). In part, this pattern reflects effects of basin desiccation and disconnection during drought years (Zhou and others 2023). Given that our sampling took place during an extended arid period, the limited hydrological connectivity in 2021 was unlikely to have facilitated the substantial export of MCs to downstream habitats, and we found little influence of hydrological variability on MC patterns in the network. In fact, the highest riverine MC concentrations were observed during years when rivers were disconnected from wetlands suggesting that local production or in situ processes, rather than up-

stream transport, governed MC occurrence at lotic sites. Together, these findings indicate that hydrological connectivity was not a primary driver of MC distribution within this watershed and that the internal ecological processes within individual habitats likely exerted stronger control over toxin dynamics. The absence of robust predictive models for MCs in rivers also suggests that the mechanisms regulating toxin production are complex or cryptic and warrant further research.

Wetlands as Toxin Hotspots

The Frank Lake wetland complex is a hotspot for MC production (Figure 3), with elevated cyanobacterial biomass (Figure 5C) throughout the wetland network. Most studies have focused on

regional or broader-scale comparisons of lentic systems that use a space-for-time substitution approach to predicting MCs (e.g., MacKeigan and others 2023; Merder and others 2023). Consequently, the features we identified as top predictors of MCs in Frank Lake differed somewhat from past work (water temperature, salinity, Chl *a*) but together strongly predicted MC concentrations through space and time in the wetland (Table 1).

Given the high relative abundance of cyanobacteria (Figure 5C), it was not surprising that estimates of total production derived from Chl *a* were correlated strongly with MC concentrations, particularly in hypereutrophic lentic ecosystems (Figure 5B). Toxigenic strains of cyanobacteria may constitute an important fraction of this population (Pick 2016), and using genetic analysis to further quantify these strains could improve our model performance beyond that based on total phototroph or cyanobacteria-specific pigment measurements (Table S4). While this genetic approach was not practical given the landscape focus of our study, it should be considered in future investigations of the environmental controls of MC occurrence. Ultimately, the positive link between the concentrations of Chl *a* and MCs is consistent with earlier demonstrations for lentic ecosystems that MCs tend to be most abundant when cyanobacteria are abundant and actively growing (Finlay and others 2010; Banerji and others 2019; Buley and others 2022). Although toxin quotas can vary across growth phases and environmental conditions (Long and others 2001; Horst and others 2014), cyanobacterial biomass remains an important predictor of total MC concentrations in highly productive systems (Paerl and Otten 2013). Similarly, significant effects of water temperature on MC abundance are not surprising, as previous work has shown pronounced seasonality of cyanobacterial growth and concentrations of MCs in lentic habitats in this region (Donald and others 2013; Hayes and others 2020; Bogard and others 2020). In general, growth of MC-producing cyanobacteria is greatest in mid-to-late summer, when water temperatures routinely exceed 20–25 °C (Yang and others 2020), a range commonly experienced in Frank Lake (Table S1). Finally, the inclusion of salinity in predictive models of MC concentrations may reflect long gradients in both salts and nutrients across the diverse habitats (Table S1). Further, increased salinity in wetlands may favor salt-tolerant cyanobacterial taxa that produce MCs (e.g., *Microcystis aeruginosa*; Tonk and others 2007) and the potential upregulation of *mcyB* genes (Wang and others 2022). High salt levels can also initiate

the release of MCs through cell lysis (Jia and others 2018). Such a pattern is consistent with the assumptions that MCs serve as a defense mechanism when cells are exposed to stressful environments (Omid and others 2018 and references therein).

Continental lake surveys often identify N and P concentrations as the main drivers of MCs in comparisons among discrete ecosystems (Orihel and others 2012; MacKeigan and others 2023). Decades of research have shown that pollution with both N and P interactively controls total phytoplankton production as well as the dominance of toxigenic strains of cyanobacteria (Pick 2016 and references therein). However, unlike prior research, we found that nutrient concentrations were not significant predictors of spatial or temporal variation in MCs in the Frank Lake drainage basin, possibly because all sites are hypereutrophic (mean TN \sim 5–8 mg L⁻¹; mean TP \sim 3.1–3.5 mg L⁻¹) (Table S1). While the second-best wetland GLM included TP concentrations, adding nutrients did not significantly increase the R^2 value, suggesting only marginal value for improving MC predictions (Table 1). Similarly, while TN was included in an early version of the top wetland GLM (not shown), the model was ultimately discarded in favor of one with Chl *a*, likely because TN reflects particulate-bound N in phytoplankton biomass. In support of this assessment, Chl *a* and TN were highly correlated (GVIF = 8.15) and could not be included in the same model. In the future, assessments of algal growth limitation (e.g., enzymatic or nutrient bioassays) could help isolate the effects of nutrient supply on regulating patterns of MC abundance at the landscape scale.

Limited Predictive Power of River Microcystin Models

River MC concentrations did not vary significantly as a function of measured planktonic biomass or physico-chemical parameters. Our proxy for phytoplankton abundance (Chl *a*) was also a poor predictor for concentrations of MCs in rivers (Figure 4; Table S7), likely reflecting the fact that these lotic systems had a low proportion of cyanobacteria in the phytoplankton (\sim 15% (Figure 5A)), based on HPLC analysis of biomarker pigments (Figure 5C). As suggested above for lentic ecosystems, it seems likely that total- or cyanobacterial-specific biomass measurements may not reflect the presence of key toxigenic cyanobacteria strains or the factors that favor their growth (Otten and others 2015; Graham and others 2020). Fortunately, our

GLMs explained $\sim 50\%$ of the temporal and spatial variation in MC concentrations using only the categorical variable Month (Table 1). While the physiological basis underlying this relationship is unclear, given the lack of physico-chemical predictors, the presence of this relationship may be a useful management tool for estimating when toxin concentrations are greatest in flowing waters, particularly if it can be validated over a wider range of catchments.

The inclusion of Month as a top predictor of river MC content suggests that other unmeasured but seasonally variable factors may have influenced lotic toxin production. The absence of temperature and discharge in the top model suggests that river toxin regulation is not driven by physico-chemical features as commonly proposed in other studies. Seasonal control of riverine MCs has been observed in agriculturally impacted tributaries, where early season blooms and toxin production occurred prior to peak summer conditions (McKay and others 2018). In our study, salinity was also not linked to cyanobacterial dominance or increased MC concentrations, as observed in wetlands (Figure 6A). Although increasing salinity can promote the release of extracellular MCs through cell lysis (Jia and others 2018), this effect was unlikely given that most river sites exhibited very low salinities (Table S1). Alternatively, given the productive nature of these rivers (Janvier 2024) and the importance of phyto-benthos in some shallow prairie creeks (Bergbusch and others 2021), it is possible that MC production was controlled by growth of as-yet-unmeasured benthic cyanobacterial populations. Similarly weak or context-dependent relationships between environmental predictors and MC concentrations have been reported in other river systems, highlighting the challenges of forecasting toxin risk in lotic environments (Nietch and others 2022; Patiño and others 2023). While further research is required to resolve the precise mechanisms regulating MCs in these streams, it is evident that MC abundance in lotic and lentic ecosystems is subject to distinct controls, suggesting that managers may need to employ site-specific strategies to remediate elevated toxin levels.

Limited Effect of Hydrologic Variability on Microcystin Concentrations

We found little evidence that downstream MC export or hydrologic variability influenced MC cycling across the river network. As our study was conducted during a particularly arid low-flow per-

iod from 2021 to 2023, we observed riverine connectivity only in the spring of 2021, when outflow from Frank Lake (Zhou and others 2023) could have reached the mid-stem site in the LBR (LB533). However, when Frank Lake was hydrologically connected to the LBR, its WRT was 16.4 years (Zhou and others 2023), reflecting the wetland's large storage capacity relative to outflow and suggesting minimal water export to the downstream river. Consistent with this inference, a first-order estimate of MC export from Frank Lake relative to MC flux within the LBR upstream of the wetland-river confluence showed minimal effect of wetland toxin export on river toxin concentrations. Specifically, the product of springtime MC concentrations in Frank Lake Basin 3 in June 2021 (mean = 0.09 mg m^{-3}) and total water outflow ($0.61 \times 10^6 \text{ m}^3$; from Zhou and others 2023) was 54.9 g of MCs exported, a value only 5.2% of the springtime flux of MCs in the upstream river outflow from May and April (mean MCs = 0.13 mg m^3 , discharge = $2.37 \text{ m}^3 \text{ s}^{-1}$, lotic MCs exported = 1064.8 g). Thus, even during intervals when wetlands exported water, it is unlikely that hydrologic connectivity substantially influenced downstream MC distributions, at least in these small low-discharge rivers. In larger rivers, bloom development and cyanotoxin occurrence may be more closely linked to hydrologic variability, as well as local in situ conditions (e.g., Klotz and others 2026; Ramos and others 2023). Instead, the lack of an observable impact of variable discharge and connectivity here may be due to other, poorly understood drivers of MCs concentrations within the river network. Finally, the lack of a measurable hydrological effect may reflect the relative consistency of river discharge during our study period, in part due to both drought conditions (Mi and others in review) and industrial stabilization of river flow for agriculture (Hillman and others 2016). Further research will be required to expand these observations over a wider range of meteorological and runoff conditions, allowing us to also explore potential lagged responses of phytoplankton to past nutrient inputs and mobilization.

Patterns of MCs Along the Freshwater-Marine Continuum

The Frank Lake wetland complex had high median MC concentrations compared to other aquatic ecosystems in North America (Figure 7), although maximum values were up to 200-times lower than those recorded in some other freshwater habitats ($n = 16,831$, $p < 0.001$). The highest median val-

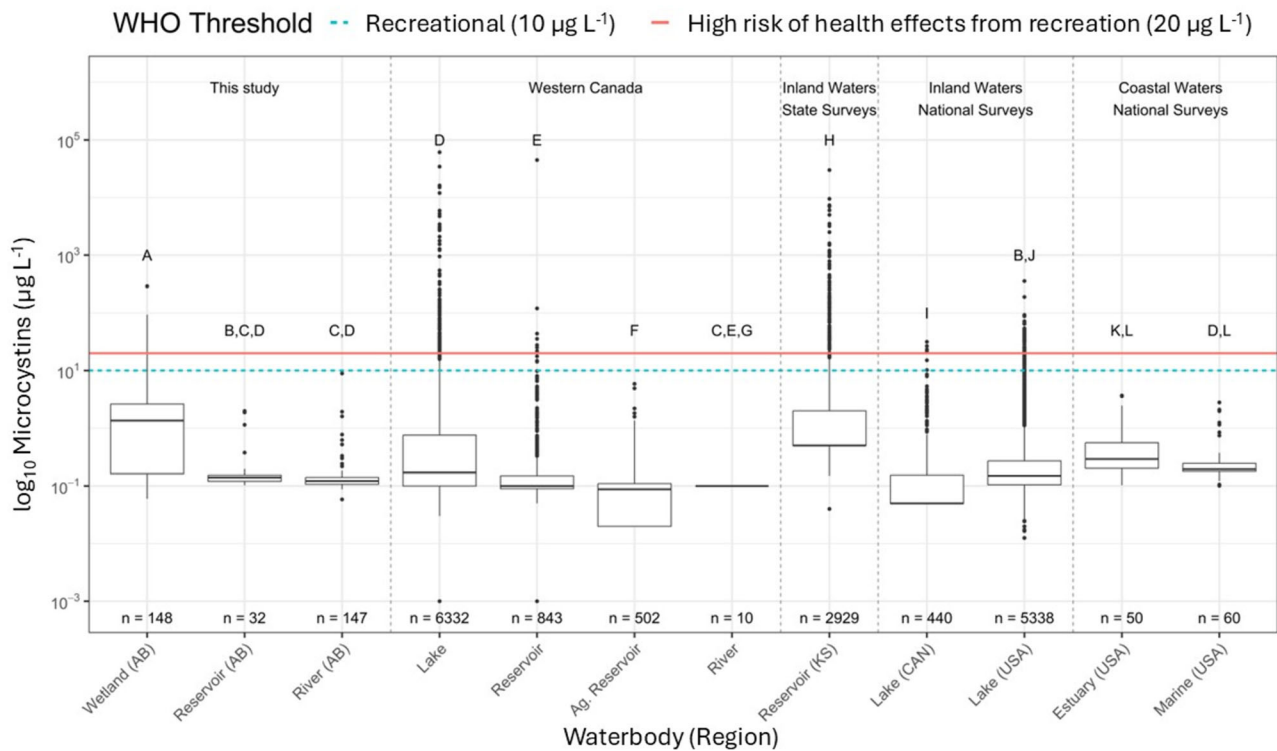


Figure 7. Comparison of MC concentrations across habitat types in the upper Little Bow River watershed versus other diverse waterbodies in North America. Horizontal dashed (blue) and solid (red) lines indicate the World Health Organization (WHO) thresholds for recreational water ($10 \mu\text{g L}^{-1}$) and high risk of health effects from recreation ($20 \mu\text{g L}^{-1}$), respectively. Significance labels are denoted above each group. Boxplots show the distribution of MC concentrations (\log_{10} -transformed), with sample sizes indicated below each category. Boxplot features are defined in Figure 2. Data are grouped based on measurements in this study, western Canada, lake and reservoir surveys at the state (Kansas) and national scales in North America, and national surveys of coastal US waters. All data sources are listed in Table S3.

ues of MCs were recorded for Frank Lake, while the next highest were in large reservoirs in Kansas (Trevino-Garrison and others 2015), another heavily agricultural region of central North America. Small agricultural reservoirs in Canada had relatively low concentrations of MCs, despite being comparatively N- and P-rich and supporting high levels of Chl *a* (Table S9), suggesting that there may be unique features to these constructed ecosystems that limit cyanoHAB development. Our meta-analysis across the entire aquatic continuum (riverine, lake, wetland, estuarine, and coastal ecosystems) showed that median MC concentrations were relatively high in estuarine habitats but lacked the order-of-magnitude extremes found in hypereutrophic lentic waters (Figure 7). Elevated estuary MC concentrations are notable because these sites are also of intermediate-to-high salinity, an important predictor of MC concentrations in Frank Lake (Figure 6A). Further mechanistic investigations are needed to define the precise role that elevated salinity may play in regulating MCs in our region, and how these processes may align with

mechanisms operating in other sub-saline and saline waters. We suspect that the high median values in Frank Lake may reflect that we sampled this system routinely and captured peak MC values throughout the growing season.

Our meta-analysis confirmed that river ecosystems are relatively understudied in surveys and predictive assessments of MC concentrations. Although not exhaustively surveyed, rivers appeared to account for only 0.9% of the over 16,000 MCs observations in our synthesis (Figure 7). This disparity persists despite growing evidence that large river systems (e.g., Ohio River, tributaries of the Laurentian Great Lakes) experience recurring cyanobacterial blooms and episodic toxin production that can pose risks to downstream users (Nietch and others 2022; Patiño and others 2023). Among river sites, MC concentrations were typically low but often exceeded values recorded for many small agricultural reservoirs in western Canada, as well as median values from nationwide Canadian lake surveys (MacKeigan and others 2023). Of note, riverine values recorded in the

Frank Lake catchment ranged from < 0.1 to $8.9 \mu\text{g L}^{-1}$, occasionally approached the threshold for recreational activities (Figure 7), and greatly exceeded the upper end values reported for MCs measured in US rivers (0.1 to $0.38 \mu\text{g L}^{-1}$; Graham and others 2020). These patterns confirm that flowing waters are subject to similar challenges as are lentic ecosystems (Otten and others 2015; He and others 2021) and suggest that urban centers reliant on rivers for drinking water or waste disposal should include monitoring of MCs in their management processes.

CONCLUSION

Our whole-catchment assessment of MC distribution showed that lentic and lotic habitats had distinct concentrations, patterns, and drivers of MCs. This observation underscores the need for managers to consider unique strategies for cyanobacteria control in flowing and standing water ecosystems. The Frank Lake wetland complex, with its consistently high temperatures, cyanobacterial biomass, and salinity, emerged as the network hotspot for MCs. Extreme N and P loading likely supports elevated production of MCs here, but nutrients were not included in our models possibly because values were uniformly greater than thresholds for nutrient limitation across most of the study habitats. The main permanent basins of Frank Lake had some of the highest sustained MC concentrations in North American ecosystems, though maximum values observed here were much lower than those in some lentic systems, indicating that toxic conditions in our watershed could worsen if poorly managed. In contrast, rivers exhibited lower MC concentrations, more complex and uncertain relationships with environmental factors, and were notably under-sampled in North American surveys. Given these ecosystem-level differences, we infer that a single management strategy is unlikely to be effective at controlling MC contamination across diverse lentic and lotic habitats. Instead, watershed managers should adopt habitat-specific strategies that address the distinct drivers of MCs in each system.

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DATA AVAILABILITY

Openly accessible data associated with this study are available at the Federated Research Data Repository (FRDR; <https://doi.org/10.20383/103.01637>). A subset of chemical data used in the study is available upon request to Cargill Ltd. or from coauthor MJB upon request. All additional hydrologic, meteorological, chemical, and limnological data used here are publicly available from a previous publication (Zhu and others 2019).

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have influenced this research.

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