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Can correlational analyses help determine the drivers of microcystin occurrence in freshwater ecosystems? A meta-analysis of microcystin and associated water quality parameters

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Abstract Microcystin (MC) is a toxic secondary metabolite produced by select cyanobacteria that threatens aquatic and terrestrial organisms over a diverse range of freshwater systems. To assess the relationship between environmental parameters and MC, researchers frequently utilize correlational analyses. This statistical methodology has proved useful when summarizing complex water quality monitoring datasets, but the correlations between select parameters and MC have been documented to vary widely across studies and systems. Such variation within the peer-reviewed literature leaves uncertainty for resource managers when developing a MC monitoring program. The objective of this research is to determine if correlational analyses between environmental parameters and MC are helpful to resource managers desiring to understand the drivers of MC. Environmental (i.e., physical, chemical, and biological) and MC correlation data were retrieved from an estimated 2,643 waterbodies (largely from the north temperate region) and synthesized using a Fisher's z meta-analysis. Common water quality parameters, such as chlorophyll, temperature, and pH, were positively correlated with MC, while transparency was negatively correlated. Interestingly, 12 of the 15 studied nitrogen parameters, including total nitrogen, were not significantly correlated with MC. In contrast, three of the four studied phosphorus parameters, including total phosphorus, were positively related to MC. Results from this synthesis quantitatively reinforces the usefulness of commonly measured environmental parameters to monitor for conditions related to MC occurrence; however, correlational analyses by themselves are often ineffective and considering what role a parameter plays in the ecology of cyanobacterial blooms in addition to MC production is vital.

Keywords Cyanotoxin · Cyanobacteria · Harmful algal bloom · Synthesis

Introduction

Cyanobacterial blooms can have harmful impacts on global freshwater ecosystems by causing drastic changes in physicochemical conditions (e.g., hypoxia, decreased light penetration, elevated pH; Paerl et al., 2001; Paerl & Otten, 2013) and by producing secondary metabolites that can be toxic to aquatic organisms, livestock, and humans (Graham et al., 2004; Malbrouck & Kestemont, 2006; Paerl et al., 2001; Rinta-Kanto et al., 2009). Of these cyanobacterial

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toxins, microcystin (MC; produced by several cyanobacterial genera, including *Microcystis*, *Nostoc*, *Oscillatoria*/*Planktothrix*, *Dolichospermum*, and *Anabaenopsis*; Martins & Vasconcelos, 2009) is commonly observed in freshwater systems with 246 structural variants documented (Meriluoto et al., 2016). The ubiquity of MC has prompted many researchers to study the environmental conditions that contribute to its occurrence (Graham et al., 2017; Rinta-Kanto et al., 2009). Field observations have begun to provide a framework of the typical conditions in which a toxigenic cyanobacterial bloom may occur; however, observations across systems indicate variability in the documented conditions most related to MC occurrence (Kotak et al., 2000; Billam et al., 2006; Wu et al., 2006; Duong et al., 2013; González-Piana et al., 2017). For instance, total nitrogen is thought to be positively correlated with cyanobacterial bloom formations (Paerl et al., 2001; Paerl & Otten, 2013) as well as MC production (Paerl & Otten, 2013), and has even been identified as the single-most driver of MC production in some systems (Giani et al., 2005). Yet, peer-reviewed field studies report wide disparities in the relationship between nitrogen and MC, with both positive and negative correlations observed (Appendix A1 Fig. l; methods to secure such data and identify/generate correlations are described in the "Materials and methods" section). These contradicting reports do not necessarily discount the importance of nitrogen to MC, but do highlight the difficulties of generalizing the specific conditions most influential to the occurrence of MC despite the wealth of literature published on this topic.

Contributing to the discrepancies in documented findings of the environmental parameters likely to influence MC occurrence is the natural variability of toxin production and the highly dynamic nature of phytoplankton blooms. This includes variation in the relative dominance of toxigenic cyanobacteria in a bloom and the abundance and diversity of cyanobacteria over space and time (Li et al., 2017; Rinta-Kanto et al., 2009; Wu et al., 2008). In addition, each freshwater system and respective bloom will be subjected to different physical, chemical, and anthropogenic influences (Rinta-Kanto et al., 2009). These factors increase the difficulty of determining the primary conditions likely to produce a cyanobacterial bloom and/or MC, especially across large geographical regions.

Although difficult, determining the relationship between environmental parameters and MC are needed as cyanobacterial blooms are a global issue, the occurrence of which persist through anthropogenic-promoted eutrophication and climate change (Paerl & Paul, 2012). The impending health effects of microcystin and other cyanobacterial toxins threaten drinking water (WHO, 2020) and recreational areas (Francy et al., 2016). Testing for MC does exist, whereby a governing authority may determine when their system has the toxin present. However, this form of monitoring only indicates when MC is present and does not assist in determining why it occurred. Solidifying the overarching trends between environmental conditions and MC occurrence may assist resource managers when choosing the environmental parameters to monitor and/or mitigate as they strive to reduce the risks associated with the toxin.

Bearing in mind the need for a greater understanding of how water quality parameters are related to MC occurrence, the objective of this research was to quantitatively assess the strength of the relationship between various environmental parameters and MC occurrence in freshwater ecosystems using meta-analysis techniques. We have observed that numerous studies in this field document correlation-based relationships between common environmental parameters and MC concentration as authors seek to distill a complex topic into practical information for the reader (Graham et al., 2004; Billam et al., 2006; Duong et al., 2013; González-Piana et al., 2017; Kotak et al., 2000; Li et al., 2017; Li et al., 2007; Liu et al., 2008; Rinta-Kanto et al., 2009; Wu et al., 2006; Xu et al., 2011; Xue et al., 2018; Yen et al., 2012; Zhang et al., 2018; Zheng et al., 2004; additional studies provided in Appendix A1). This reporting provides a relationship with MC independent of confounding factors that are also at play in the ecology of cyanobacterial blooms (e.g., species composition and biomass, cellular physiology traits, presence/absence of toxin related genotypes, nutrient preferences, and thresholds; Paerl et al., 2001; Paerl & Otten, 2013), though such factors are typically addressed within the published report as well. Although direct correlations with MC do not allow for the nuanced aspects of toxigenic cyanobacterial ecology to be delineated (and we stress that such factors need to be understood for a holistic approach to MC mitigation), nor does it provide a method to decipher if an environmental

parameter directly or indirectly contributes to MC occurrence (i.e., correlation does not equal causation), their popularity within the global literature provides a wealth of information on the potential drivers of MC in a format that is standardized. By leveraging this commonly reported measurement, we hope that the results of this study will provide (1) resource managers with a quantitative assessment of the cursory parameters to monitor to determine MC occurrence in freshwater systems, and (2) a summary to the often contradictory reports observed within literature regarding MC occurrence when using correlational analyses (e.g., A1 Fig. 1).

Materials and methods

Data collection

Three primary sources were used to acquire data, including published journal articles, the National Water Information System database of the U.S. Geological Survey (USGS, 2018), and the 2007 and 2012 National Lake Assessments conducted by the U.S. Environmental Protection Agency (EPA-NLA, 2007, 2012). Data acquisition from each source is described separately in the following paragraphs.

Published articles were retrieved using the Web of Science database. A literature search was performed in February 2018 by combining the keywords "lake," "reservoir," "environment," "parameters," "nutrients," "variables," "environmental parameters," and "environmental variables" each with "microcystin"; the search returned 3,332 articles. Studies were included in the meta-analysis if they (1) were observational field studies (i.e., not experimental in nature); (2) were from a freshwater reservoir or lake, defined as a system with little-to-no-flow (i.e., no unimpeded rivers or streams); (3) provided data for ≥ 5 samples collected at any given site; and (4) provided numerical sampling data in figures, tables, or supplementary files. Seventy-nine studies met these criteria (see Appendix A1).

The National Water Information System of the U.S. Geological Survey was accessed in February 2018 to obtain real-time field sampling data containing
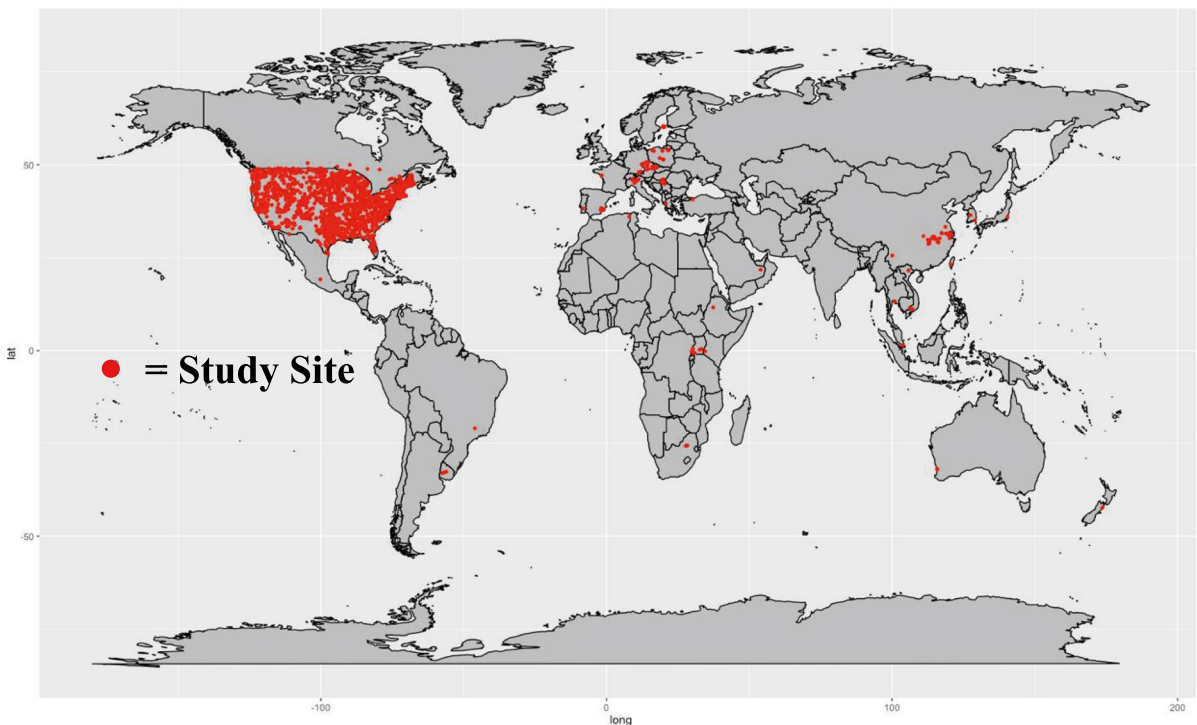

Fig. 1 Locations of waterbodies from which data were used in this study

MC values and associated environmental parameters from sites within the USA (USGS, 2018). Data were used if ≥ 5 samples were collected at any given location; fifty sites were chosen. Many sites contained a wide array of measured parameters (e.g., heavy metals, organic and inorganic chemicals, physical measurements). Parameters that were not reported in the obtained published studies were largely removed. Lastly, common symbols were reported with published values throughout the USGS dataset and remediated in various ways (A1 Table 1).

Data were also available from an estimated 1,161 and 1,137 lakes or reservoirs as part of the EPA-NLA in 2007 or 2012, respectively, which included MC data (EPA, 2007, 2012). Of these waterbodies, 460 of the 2007 dataset were estimated to be resampled in 2012, resulting in 701 newly sampled lakes or reservoirs in the 2012 dataset. If it was observed that a study used data that were also provided in the EPA-NLA or USGS datasets (e.g., Beaulieu et al., 2013; Beaver et al., 2014; Harris & Graham, 2017), the primary in-text values were not used and only the original raw data were used. The 2007 and 2012 NLAs were the largest datasets to be used in this study but generated only one correlation per environmental parameter examined per assessment.

From the three data sources, a total of 131 articles and datasets (hereby termed "studies") that contained physicochemical (i.e., temperature, dissolved oxygen, pH, Secchi depth (transparency), conductivity), chemical (i.e., nitrogen, nitrate, nitrite, ammonia, phosphorus, phosphate), algal abundance (total chlorophyll and chlorophyll-*a*), and cyanobacterial toxicity (microcystin) data, independent of time or season, were included. Phosphate included both phosphate and orthophosphate. If two or more environmental parameters were found to report the same estimates, they were condensed whenever possible; however, parameters that were not considered uniquely similar or had variable analytical methods (e.g., phosphate, soluble reactive phosphorus, total dissolved phosphorus) were left separated. Total microcystin measurements were used if offered, but intracellular (filtered) microcystin, microcystin + nodularin (USGS, 2018), and the microcystin variant, microcystin-LR, were all also used as an alternative if presented no other option. Broader information regarding MC variants, sampling methods, and correlation types of each study/site can be found in A1 Table 5.

Data collected during the literature review that fit the pre-determined inclusion criteria originated largely from the north temperate region of the globe. All data that fit the literature review selection criterion were utilized in the analysis reported on in the primary text of this article, but assessments of data by region (e.g., north temperate, south temperate, and tropical) are reported in Appendix 2.

Correlation coefficient development

Commonly reported correlations of the relationship between a specific environmental parameter and microcystin concentration served as the foundation for this synthesis. From the published articles, three correlation coefficients provided by the authors were primarily used, including Pearson's *r*, Spearman's *rho*, or Kendall's *tau*. Reported correlation coefficients that were not otherwise specified were treated as Pearson's *r*. It was observed that 68% of the peer-reviewed publications used in this analysis reported the relationship between environmental parameters and MC occurrence specifically using some form of correlation coefficient (A1 Table 5). In some cases, Pearson's correlation coefficients were also generated for studies that did not directly provide this value. In these instances, data were extracted from a figure using the *metaDigitise* package in R (Pick et al., 2020). The *cor.test* function in R was then used to generate Pearson's correlation coefficients from the obtained data. Lastly, Spearman's *rho* or Kendall's *tau* correlation coefficients were transformed to Pearson's *r* using the following two equations:

Kendall's *tau* to Pearson *r*, reported by Rupinski and Dunlap (1996):

$$r = \sin(t * \frac{\pi}{2})$$

where *r* = Pearson's coefficient and *t* = Kendall's *tau*.

Spearman's *rho* to Pearson's *r*, reported by Rupinski and Dunlap (1996):

$$r = 2 \times \sin(r_s \frac{\pi}{6})$$

where *r* = Pearson's coefficient and r_s = Spearman's *rho*.

Data analysis

Fisher's z-scores were used as a measure of effect size due to its improved statistical properties (Jiang & Pu, 2009; Weaver et al., 2018; Ortega et al., 2020). Prior to statistical analyses, the Pearson's correlations (r) for each environmental variable were converted to Fisher's z-scores using the following equation (Fisher, 1958) in the *metafor* package in R to normalize the distribution of the data (Viechtbauer, 2010):

$$z = 0.5 \times ln\left(\frac{1+r}{1-r}\right)$$

Effect sizes (hereby termed "correlations") were then generated from the Fisher's z-scores using the Robust Multi-Array Average function including the restricted maximum likelihood method found within the *metafor* package. The *metafor* package also produced a weighted variance and standard error for each Fisher's z-score. In addition, a test of heterogeneity (I^2) and 95% confidence intervals were generated for each environmental parameter correlated with MC, separately. Lastly, a visual test for publication bias was generated via a funnel plot depicting effect size, as Fisher's z, against standard error using the *metafor* package (A1 Fig. 2). Funnel plots depict heterogeneity of estimates, and values that fall within the 95% confidence region (triangular region) are considered to be free of publication bias (Higgins & Green, 2011). It was determined that the majority of values used in this analysis fell within this region.

To ease the interpretation of the study results, a Fisher's z-score was converted back to a Pearson's correlation coefficient using the equation:
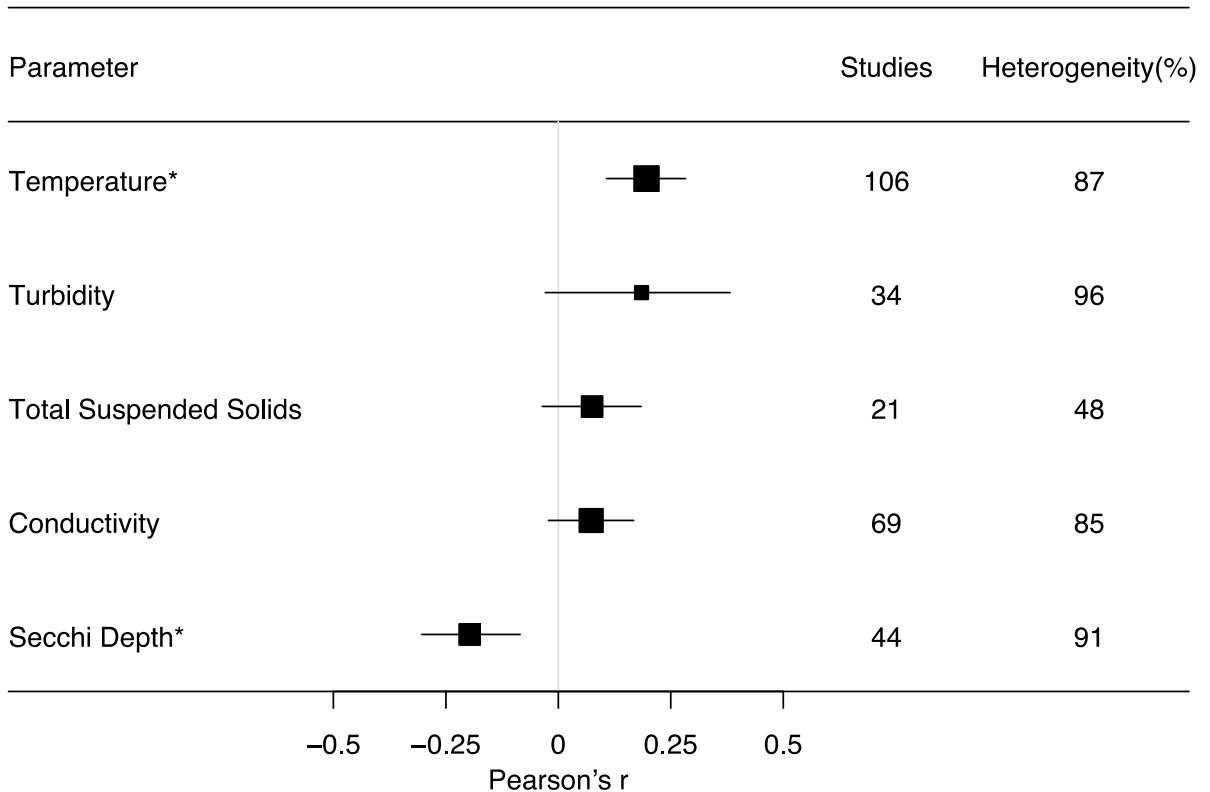
$$r = \tanh(Z_r)$$

Parameter	Studies	Heterogeneity(%)
Temperature*	106	87
Turbidity	34	96
Total Suspended Solids	21	48
Conductivity	69	85
Secchi Depth*	44	91

Pearson's r

Fig. 2 Estimates of effect between microcystin occurrence and physical parameters. Estimates converted from Fisher's z to Pearson's r. Studies = number of articles or datasets incorporated into the effect size estimate. I^2 = Heterogeneity (%). Error bars represent 95% confidence intervals. * denotes parameters with statistical significance ($p \leq 0.05$). A larger effect size box denotes a smaller variance (greater weight) within the estimate

where Z_r = Fisher's z-score (Gilpin, 1993).

Pearson's correlation coefficients can range from −1 (perfect negative correlation) to +1 (perfect positive correlation). Ninety-five percent confidence intervals were used to gauge significance ($p > 0.05$). The amount of studies (n) used to generate effect size estimates were also reported.

Results

The final dataset contained correlations of 35 environmental parameters with MC totaling 1,029 unique effect sizes (i.e., correlations). These correlations included data from an estimated 2,643 unique waterbodies (note that a study could contain more than one lake in its survey; Fig. 1). Most data were collected in the USA, Europe, and China (Fig. 1). Additional studies were identified on other parts of the globe during the literature review but contained data that were not in a usable form and/or did not meet the inclusion requirements of this study.

Each of the 35 environmental parameters contained a range of studies that were used in their assessment (ranging from 3 to 106 studies per environmental parameter assessment; Figs. 2, 3, and 4; A1 Tables 2, 3, and 4), and averaged 36 studies per parameter assessment. Heterogeneity also varied widely across environmental parameters (0–98%; Figs. 2, 3, and 4). Although most correlations had high heterogeneity ($I^2 > 50\%$), I^2 for eight correlations were low ($\leq 25\%$), indicating that either the value between estimates were extremely homogenous or a small sample size precluded accurately estimating variation.
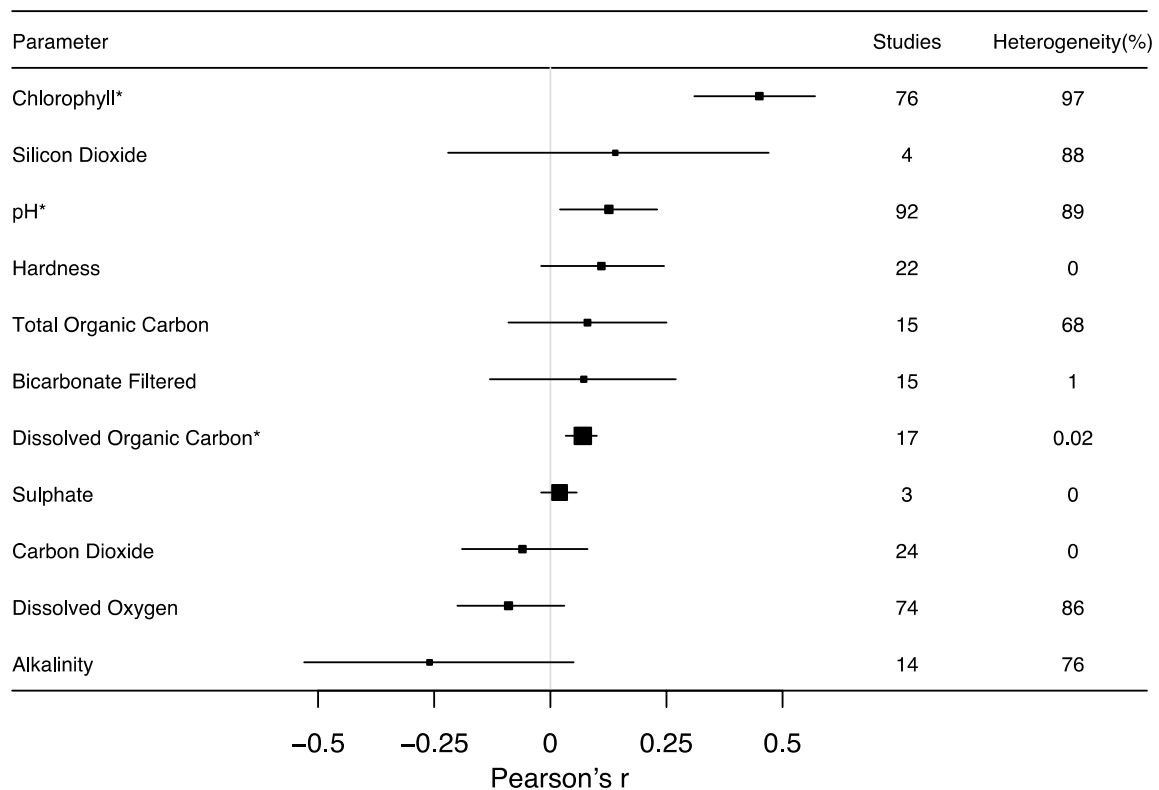

Parameter	Studies	Heterogeneity(%)
Chlorophyll*	76	97
Silicon Dioxide	4	88
pH*	92	89
Hardness	22	0
Total Organic Carbon	15	68
Bicarbonate Filtered	15	1
Dissolved Organic Carbon*	17	0.02
Sulphate	3	0
Carbon Dioxide	24	0
Dissolved Oxygen	74	86
Alkalinity	14	76

Fig. 3 Estimates of effect between microcystin occurrence and biological, biochemical, or chemical parameters. Estimates converted from Fisher's z to Pearson's r. Studies = number of articles or datasets incorporated into the effect size estimate. I^2 = Heterogeneity (%). Error bars represent 95% confidence intervals. * denotes parameters with statistical significance ($p \leq 0.05$). A larger effect size box denotes a smaller variance (greater weight) within the estimate
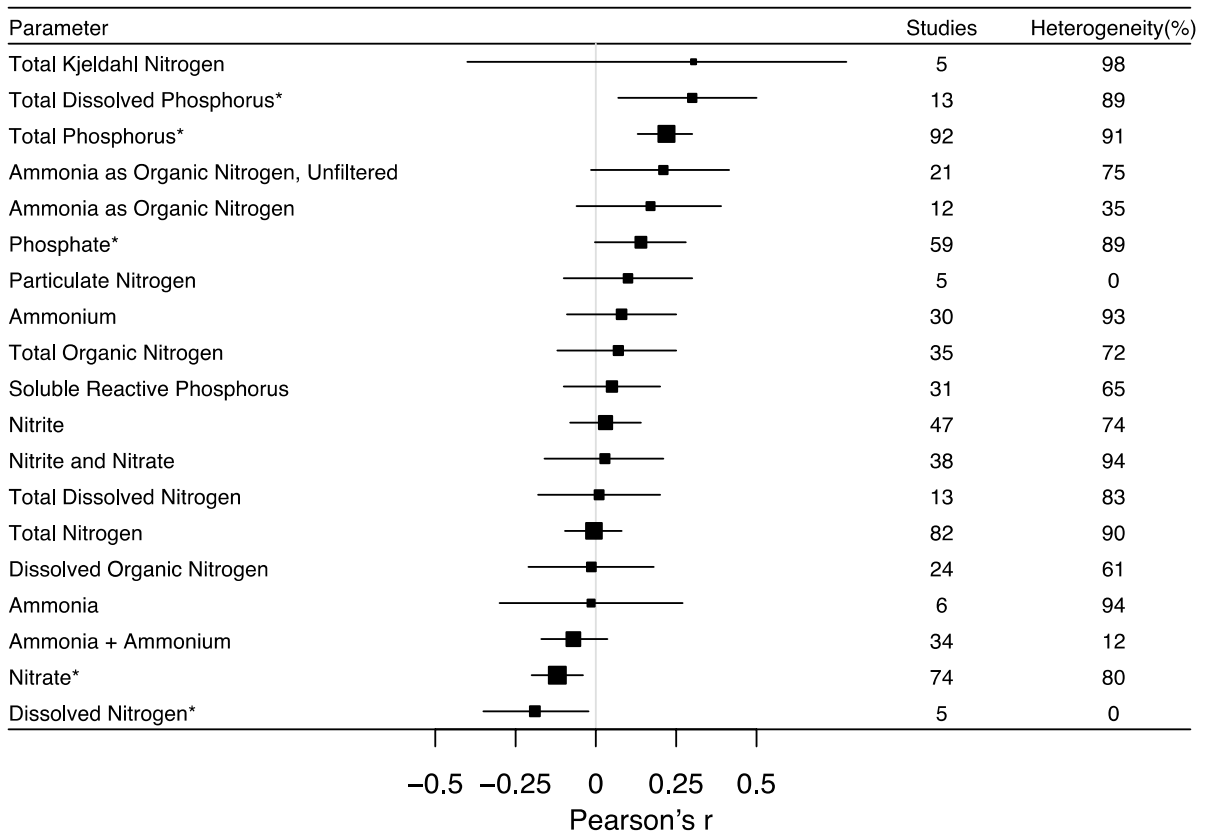
Parameter		Studies	Heterogeneity(%)
Total Kjeldahl Nitrogen		5	98
Total Dissolved Phosphorus*		13	89
Total Phosphorus*		92	91
Ammonia as Organic Nitrogen, Unfiltered		21	75
Ammonia as Organic Nitrogen		12	35
Phosphate*		59	89
Particulate Nitrogen		5	0
Ammonium		30	93
Total Organic Nitrogen		35	72
Soluble Reactive Phosphorus		31	65
Nitrite		47	74
Nitrite and Nitrate		38	94
Total Dissolved Nitrogen		13	83
Total Nitrogen		82	90
Dissolved Organic Nitrogen		24	61
Ammonia		6	94
Ammonia + Ammonium		34	12
Nitrate*		74	80
Dissolved Nitrogen*		5	0

−0.5 −0.25 0 0.25 0.5

Pearson's r

Fig. 4 Estimates of effect between microcystin occurrence and nitrogen or phosphorus parameters. Estimates converted from Fisher's z to Pearson's r. Studies = number of articles or datasets incorporated into the effect size estimate. I^2 = Heterogeneity (%). Error bars represent 95% confidence intervals. * denotes parameters with statistical significance ($p \leq 0.05$). A larger effect size box denotes a smaller variance (greater weight) within the estimate

Physical parameters

A statistically significant ($p \leq 0.05$) positive correlation was found between MC and temperature ($r = 0.20$, $n = 106$; Fig. 2; A1 Table 2). Secchi depth (measurement of transparency) was the only physical parameter that had a significant negative correlation with MC ($r = -0.20$, $n = 44$). The three other physical parameters, turbidity ($r = 0.18$, $n = 34$), total suspended solids ($r = 0.08$, $n = 21$), and conductivity ($r = 0.07$, $n = 69$), assessed in this meta-analysis were not significantly correlated with MC.

Biological and chemical parameters

Chlorophyll ($r = 0.45$, $n = 76$), total dissolved phosphorus ($r = 0.30$, $n = 13$), total phosphorus ($r = 0.22$, $n = 92$), phosphate ($r = 0.14$, $n = 59$; $p = 0.055$),

dissolved organic carbon (DOC; $r = 0.07$, $n = 17$), nitrate ($r = -0.12$, $n = 74$), dissolved nitrogen ($r = -0.19$, $n = 5$), and pH ($r = 0.12$, $n = 92$) were found to be significantly ($p \leq 0.05$) correlated with MC (Figs. 3 and 4; A1 Tables 3 and 4). Chlorophyll had the largest positive correlation with MC of any parameter assessed in this synthesis ($n = 76$). Of the fifteen nitrogen water quality parameters, correlations for only two parameters were statistically significant and both were negatively correlated with MC (dissolved nitrogen, nitrate; Fig. 4). The number of studies included for specific nitrogen parameters varied widely (n range = 5–82; A1 Table 4) as did heterogeneity ($I^2 = 0$–98%). Total nitrogen displayed a nearly-zero, non-significant correlation despite having data from a large number of studies included in its estimate ($n = 82$). Ammonia, and its derivatives, showed mostly positive,

albeit statistically insignificant, correlations with MC (Fig. 4).

Interestingly, and in contrast to the nitrogenous parameters, all four reported phosphorus parameters displayed positive correlations across a range of studies ($n = 13$–92; A1 Table 4). Total phosphorus had one of the largest number of studies incorporated into its estimate and displayed a statistically significant positive correlation ($r = 0.22$; $n = 92$; $p \leq 0.05$; Fig. 4). Total dissolved phosphorus ($r = 0.3$; $n = 13$; $p \leq 0.05$) and phosphate ($r = 0.14$; $n = 59$; $p = 0.55$) were also positively correlated with MC (Fig. 4). Soluble reactive phosphorus was the only phosphorus type not statistically related to MC.

Discussion

This meta-analysis synthesized water quality data from both single- and multi-lake surveys by utilizing study correlational matrix tables to identify environmental parameters significantly correlated with MC. A number of large, multi-lake surveys have occurred in recent decades, including those conducted in Canada (Kotak et al., 2000; Giani et al., 2005), the USA (Graham et al., 2004; EPA-NLA, 2007, 2012), China (Wu et al., 2006), Europe (Mantzouki et al., 2018), and the Czech Republic (Jančula et al., 2014). However, no surveys have assessed the strength of such relationships with MC across as many environmental parameters as this study. Although data from an estimated 2,643 waterbodies were used for this meta-analysis, data largely originated from sources in the northern hemisphere, despite attempts made to secure data elsewhere within the literature review (analysis on break down of data from the northern and southern temperate, and tropical regions found in Appendix 2). The unbalanced sources of global limnological data are documented issues (Lewis, 2000; Ramírez et al., 2020), and the continued availability of open-sourced data and further assessments of lakes in the southern temperate and tropical regions will assist in addressing global questions.

Despite the wealth of monitoring data collected (Kotak et al., 2000; EPA-NLA, 2007, 2012; Wu et al., 2006), relatively few environmental parameters (29%; 10 of 35; including temperature, pH, Secchi depth, chlorophyll, DOC, total phosphorus, total dissolved phosphorus, phosphate, nitrate, and dissolved nitrogen) were significantly correlated with MC in this study. The limited significance within these 35 variables is not surprising given that the function of most cyanobacterial secondary metabolites, including MC, as well as the factors responsible for their production are poorly understood (Gągała et al., 2014; Henao et al., 2020; Holland & Kinnear, 2013; Kaebernick & Neilan., 2001; Paerl & Otten, 2013; Sivonen, 2009). We do know that toxigenic cyanobacterial taxa must be present for toxins to be produced, but the presence of these taxa alone is no guarantee for toxin production (Horst et al., 2014; Lyck, 2004; Wiedner et al., 2003). Additionally, toxin production can vary strongly across genotypes (Lyck, 2004; Watanabe et al., 1989; Wilson et al., 2006; Yinxia et al., 2017).

This meta-analysis determined the environmental factors most significantly correlated with MC that water resource managers may consider using when monitoring for the toxin. The across-study variation we observed in the correlations drawn directly between environmental parameters and MC could suggest that these correlational analyses alone do not accurately address the complex factors taking place in the ecology of a cyanobacterial bloom that lead to the eventual production of MC. As such, we suggest that future research consider the usefulness of reporting direct correlations with MC.

Despite the possible limitations correlational analyses have when drawing conclusions between environmental parameters and MC directly, we maintain that the wealth of information found within such correlation assessments should not be disregarded as they may be used to help provide input to what group of parameters are the best to be incorporated in a monitoring program (Neilan et al., 2013). Significant parameters will be described in the following paragraphs, in which examples will be given as to how parameters relate to toxigenic cyanobacteria as well as to MC itself. Such examples are meant to highlight the possible pathways in which a parameter may generate a significant correlation with MC. Further, the relationships drawn in this study were of areas affected by MC, and, as such, findings should not be extrapolated as critical parameters to areas experiencing blooms of non-toxic phytoplankton taxa.

Physical parameters

Temperature

In general, warmer temperatures lead to an increase in toxic cyanobacterial growth and toxin production (Paerl & Huisman, 2008). Numerous optimal temperature ranges for toxigenic cyanobacteria have been reported, stating that temperatures greater than 20 °C are generally beneficial to the formation of toxic blooms (15–20 °C, Billam et al., 2006; ~23 °C, Li et al., 2007; 18–35 °C, Gągała et al., 2014; >23 °C, Rigosi et al., 2015; >25 °C, Boutte et al., 2008). Interestingly, studies have also shown that increased temperatures cause an upregulation of the *mcyB* gene (which, in part, helps regulate MC production) in *Microcystis aeruginosa*, with peak upregulation occurring at 25 °C (Kim et al., 2005; Scherer et al., 2016). This finding may support the hypothesis that microcystin and cyanobacterial toxins serve as radical scavengers in cells seeking to limit oxidative stress during higher temperatures and solar irradiances (Dziallas & Grossart, 2011). Due to the significance of temperature in relation to MC, it is recommended that this easily measured parameter be used by resource managers.

Secchi depth

Transparency (measured as Secchi depth) displayed a significant negative correlation with MC occurrence. As Secchi depth can be measured with relative ease, it is recommended that it be incorporated into water resource monitoring programs. However, this measurement alone may not accurately be related to toxic cyanobacterial blooms, and therefore training to delineate the meaning of Secchi depth measurements is needed. For instance, high levels of turbidity, algal cells (both non-toxic cyanobacterial strains and other phytoplankton species), dissolved organic matter (which can be comprised of various compounds; to be discussed), or suspended solids may each reduce Secchi depth measurements, but can equate to very different ecological stressors or processes in freshwater systems (Swift et al., 2006). Despite this, it has been suggested that certain toxigenic cyanobacterial species are capable of excelling in turbid environments, as select species can, for example, remain at the water surface using gas vesicles to maintain buoyancy,

circumventing these conditions (e.g., *Microcystis*; Paerl & Huisman, 2008). Bonilla et al. (2012) also found in an assessment of 940 lake samples from the Northern and Southern Hemisphere that *Planktothrix agardhii* biovolume was positively correlated with the turbidity of a system, and *Cylindrospermopsis raciborskii* had a high phenotypic plasticity, which allowed the species to thrive in similar turbid and low-light conditions.

Biological and chemical parameters

Chlorophyll

Chlorophyll was observed to have the greatest correlation slope ($r = 0.45$) to MC in this entire synthesis and is as such recommended to be measured in systems experiencing the threat of MC occurrence. Other algal pigments, such as phycocyanin, could also be measured to a similar effect and may provide a more specific relationship with cyanobacteria (Kasinak et al., 2015; McQuaid et al., 2011); however, these measurements are collected at a lesser extent by water resource managers globally. Chlorophyll and subsequent algal pigments are a beneficial measurement of phytoplankton abundance; however, these values alone do not infer that MC will be produced as not all blooms will possess cyanobacterial species capable of producing cyanobacterial toxins, not all cyanobacterial species will possess the genes required for toxin production even if toxigenic cyanobacterial species were to dominate, and not all blooms consist of cyanobacteria (Rinta-Kanto et al., 2009). For instance, Wilhelm et al. (2011), who studied Lake Taihu, China, found the presence of the *mcyA* genes needed for MC production in only three of the ten sites sampled despite all sites having DNA from *Microcystis* present. Despite these factors, measurements of cyanobacterial pigments, such as chlorophyll and/or phycocyanin, are some of the most effective methods to quickly monitor the direct density of an algal or cyanobacterial bloom.

Dissolved organic carbon

Dissolved organic carbon displayed the weakest slope, yet a statistically significant correlation with MC. As observed with other environmental parameters, the mechanism in which DOC is important to

MC production and cyanobacterial cell growth is questioned within the literature. Some suggest that the correlation between cell density and DOC occurrence (as dissolved organic matter) is produced as a by-product of cyanobacterial cells (Tessarolli et al., 2018) or are released during cyanobacterial cell lysis (Paerl et al., 2001). Therefore, the relationship between DOC and MC occurrence may exist because DOC is a product of the cyanobacterial bloom itself. Others suggest that DOC is important to cyanobacteria as DOC is assimilated directly either by cyanobacteria or by the bacterial matrix surrounding or residing within the mucilage of cyanobacterial colonies (Paerl et al., 2001; Znachor & Nedoma, 2010; Cook et al., 2020). Further delineation on whether the correlation observed between dissolved organic carbon and MC occurrence is attributed to DOC fueling toxigenic cyanobacterial abundances or if DOC is a by-product of established blooms is needed.

Total phosphorus, total dissolved phosphorus, phosphate, nitrate, and dissolved nitrogen

Total phosphorus and the other phosphorus parameters in this meta-analysis had positive correlations with MC and were typically greater than those of nitrogenous parameters, which reported largely low-to-negative correlations. The findings of this meta-analysis reflect the conclusions of previous studies that reported phosphorus had a larger importance to eutrophication and algal/cyanobacterial biomass rather than nitrogen or nutrient ratios (i.e., C:N:P; Schindler et al., 1974; Schindler et al., 2008), and that cyanobacteria often experience phosphorus limitation in freshwater systems (Dignum et al., 1970). Also, it has been suggested that non-toxic *Microcystis* strains outcompete toxic strains in low nutrient conditions, but toxic strains dominate at higher nitrogen and phosphorus concentrations (Vezie et al., 2002). Moreover, *M. aeruginosa* grown in phosphorus-limited conditions produced MC with the addition of phosphorus (Oh et al., 2000). It is postulated that MC may serve as a deterrent against grazing zooplankton and will increase in toxicity when more nutrients (particularly phosphorus) become available (Oh et al., 2000).

Of the phosphorus parameters reported, total dissolved phosphorus (TDP) had the greatest positive correlation to microcystin. It is possible that TDP components are released during the breakdown phase of a bloom, and this release coincides to the time where microcystins are also present and at greater concentrations. Cyanobacteria can uptake and store phosphorus within their cells for later use, a process known as luxury phosphorous uptake (Crimp et al., 2018). This phosphorus is stored as polyphosphate (Sanz-Luque et al., 2020). The breakdown of the bloom during its decay phase would release phosphate and this would register as TDP in a water quality analysis. It has been observed that up to 67% of the phosphorus released during the decay phase of a bloom is orthophosphate (Zheng et al. 2018). Also occurring during the late stages of a bloom's exponentiation phase and stationary phase are higher levels of microcystin (Watanabe, 1989), which may also be released during the decay phase. This increase of microcystin and subsequent release of phosphate may equate to a greater, positive correlation value between the two parameters.

To minimize the importance of nitrogen to MC occurrence is not the objective of this study. Numerous surveys have shown that nitrogen is correlated to MC occurrence, and nitrogen is needed for the peptide formation of the MC structure (Giani et al., 2005; Graham et al., 2004; Wilhelm et al., 2011). It is possible that the low-to-negative effect sizes of nitrogen parameters may be attributed to the ability of nitrogen to vary between dissolved, gaseous, and particulate forms, and leave or enter a system though processes, such as denitrification and nitrogen fixation; processes that may make nitrogen concentrations less static than phosphorus (Scott et al., 2019). In addition, the low-to-negative effect sizes of nitrogen parameters observed may be attributed to the ability of select cyanobacterial species to utilize N_2-fixation (e.g., *Anabaena/Dolichospermum*, *Cylindrospermopsis/Raphidiopsis*; Chorus & Bartram, 1999). Interestingly, it was found that the correlation coefficients between total nitrogen and MC occurrence used in this study ranged from $r = 0.92$ (*sample size = 48*), reported by Oberholster and Botha (2010), to $r = -0.69$ (*sample size = 12*) reported by Xue et al. (2016). If the cyanobacterial species composition is compared between these reports, *M. aeruginosa*, a species incapable of N_2-fixation, dominated the system reported by Oberholster and Botha (2010), whereas the cyanobacterial bloom reported by Xue et al. (2016) shifted from *Microcystis* to a diazotroph, *Dolichospermum*. This is an example between two

extremes, but it does reflect the importance of understanding the cyanobacterial species composition of a freshwater body in question as the nutrient requirements of species will vary over space and time.

Dissolved nitrogen was observed to have a significant negative relationship with MC. This could be due to the use of nitrogen in the production of MC. In addition to nitrogen being a key nutrient for cyanobacterial growth and function, it is also a building block in the MC compound (Hotto et al., 2008; Wagner et al., 2019). During a bloom, the cyanobacterial population requires large amounts of nitrogen for the production of additional cells as well as for MC (if the bloom should be MC-producing). Dissolved forms of nitrogen have been reported to be removed from systems quickly as they are the most energetically favorable form of nitrogen to use (Flores & Herrero, 2005). Gladfelter et al. (2022) observed that after the addition of various forms of nitrogen into an outdoor limnocorral experiment, extracellular nitrogen concentrations quickly decreased from the system and coincided with the increase of phycocyanin (a pigment) in the cells of the cyanobacteria. If nitrogen is being used for cyanobacterial function or toxin production, this could equate to low amounts of nitrogen in the water and higher MC concentrations, leading to a negative correlation between the parameters.

It is possible that the relationship between MC concentration and nitrogenous parameters is nonnormal. Such trends between MC occurrence and nitrogen have been observed prior (Graham et al., 2004), and are not reflected in the correlation type analyses that are routinely reported in this field. We recommend that researchers assess their data to look for non-linear trends and thresholds between environmental parameters and MC or consider making their raw data available at time of publication.

pH

Little consensus exists regarding the role of pH in MC production and optimal cyanobacterial growth. For instance, laboratory studies have observed that toxic strains of *Microcystis* will have a greater cell density at both higher temperatures and pH (Song et al., 1998; Watanabe & Oishi, 1985). Yet, Cuichao et al. (2013) observed the effects of pH on the peak growth rates of *M. aeruginosa* to be more nuanced, finding that *M. aeruginosa* growth in the exponential phase benefited by a higher pH of 9.5, while a pH of 7.5 supported the greatest growth when the culture was in its stationary phase.

The field studies assessed in this meta-analysis reveal a positive correlation between MC and pH. It is possible that higher pH conditions may allow for bicarbonate to become more bioavailable, which has shown to be favorable to cyanobacteria (Boyd, 2015; Mokashi et al., 2016). Alkaline environments may also contribute to the increased occurrence of MC, as has been suggested in past laboratory studies (Song et al., 1998; Watanabe & Oishi, 1985).

Although pH may be a measurement related to MC, resource managers should note that cyanobacterial bloom density and the time of day during which a sample is taken will affect the reported value of pH. As phytoplankton uptake carbon dioxide during daylight hours (increasing pH) and respire at night (decreasing pH; Boyd, 2015), the presence of a substantial algal bloom will greatly influence ambient pH. The majority of studies utilized in this analysis were taken during daylight hours. In general, a further delineation of the role of pH in MC occurrence is needed.

Microcystin variants used in this study

This meta-analysis considered total MC, intracellular MC, MC-LR, intracellular MC-LR, and MC + nodularin, as these were the most reported MC variants documented in the studies that met our inclusion criteria. Extracellular MC as well as over 246 variations in the chemical structure of MC have also been documented in past studies, but are reported to a much lesser extent in monitoring publications (Park et al., 1998; Meriluoto et al., 2016). Repeating this meta-analysis to include other MC fractions or variants may alter our findings; however, the limited presence of these MC variants and our large sample size likely preclude significant interpretational shifts. Extracellular toxins typically occur at a lesser amount than that of intracellular fractions due to rapid microbial degradation, and often an excess of extracellular toxins is observed only during the decay phase of a bloom (Park et al., 1998; Zheng et al., 2004; Li et al., 2017). For instance, Park et al. (1998) monitored the intracellular and extracellular MC fractions in Lake Suwa, Japan, over four growing seasons, and observed

that intracellular MC was on average ~25× higher to that of extracellular MC.

Conclusions

Findings from this quantitative synthesis reveal environmental parameters that are significantly correlated with the hepatotoxin, MC, in freshwater lakes and reservoirs. For example, if findings are taken together, eutrophic systems that are warmer, and alkaline, and contain elevated phosphorus concentrations tend to have greater MC occurrence. Such findings from a dataset with samples that ranged widely over both space and time may reflect the usefulness of the parameters found to be significant to be incorporated into MC monitoring programs, and managing such conditions may assist in mitigation attempts to reduce MC.

Studies incorporated a wide range of sampling and reporting methodologies (A1 Table 5), and were subsequently utilized in this analysis with limited bias. Additionally, this assessment was performed independent of season and rather focused on the quantitative assessment of all pertinent data. Seasonality may have an effect on correlations, as, for example, major stages of a cyanobacterial bloom formation coincide with different times of the year and may place a varying reliance on select parameters (e.g., nitrogen may have a greater importance during bloom formations (see Gobler et al., 2016; Otten et al., 2012)). The authors of this article strive to assess the question of seasonality in future studies.

Direct correlations between environmental parameters and MC occurrence provide an efficient method to refine a complex topic into a functional unit of information; however, such statistical analyses may fail to address the specific mechanism(s) in which an environmental parameter relates to the ecology of cyanobacterial blooms and MC production. Considering that the environmental parameters associated with significant correlations can be both directly or indirectly related to MC production (e.g., increased temperature may lead to greater toxigenic cyanobacterial biomass, pH may influence MC production or be influenced by bloom density), further laboratory and field studies that research the mechanisms that mediate interactions related to the promotion of cyanobacteria will help water resource managers better understand the conditions that contribute to the production of MC.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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