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Microcystin and Algal Chlorophyll in Relation to Nearshore Nutrient Concentrations in Lake Winnipeg, Canada

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Abstract

Microcystin (MC) and chlorophyll *a* (chl *a*) concentrations were examined at three nearshore stations during two consecutive ice-free seasons in relation to nitrate-N (NN), orthophosphate-P (OP) and other parameters in the south basin of Lake Winnipeg, Canada. While average nutrient levels were high in both years, chl *a* levels were significantly higher in 2011 than 2010, while the reverse was true for MC. However within each year, chl *a* was correlated with MC. Chl *a* was highly correlated with OP and NN in 2010, but less strongly with OP and not with NN in 2011. The higher chl *a* levels of 2011 were more strongly related to *preceding* than to concurrent OP and NN. MC was also strongly correlated with OP and (less) with NN in 2010, but only with chl *a* in 2011. The NN/OP ratio (NPR) was unrelated to chl *a* in either year, but was inversely correlated with MC in 2010. Cross-correlation analysis for 2010 showed that while MC lagged behind changes in chl *a*, OP, NN, and inversely behind NPR, correlations for these parameters (except NPR) were stronger for concurrent rather than delayed MC values. More vigorous blooms, as reflected by the phaeophytin/chl *a* ratio, were more toxic. While in a given season shifts in nutrients and NPR may signal impending changes in bloom density and toxicity, in some years additional factors besides nutrients appear to modify phytoplankton nutrient response. Nutrients remain a primary target for lake refurbishment strategies.

Keywords: microcystin, chlorophyll, algae, nitrogen, phosphorus, eutrophication, Lake Winnipeg

1. Introduction

Eutrophication of global surface waters is an increasing environmental, economic and political issue. As human population expands, nutrient enrichment associated with agriculture, industry, wastewater, recreational activities, deforestation, wetland destruction, mining, and various inappropriate land uses (Pip, 2005) continues to escalate. Nutrient enrichment of fresh waters is accompanied by proliferation of undesirable algal blooms that lead to oxygen depletion, light limitation and reduction in ecosystem biodiversity. Cyanobacteria, prominent in such blooms, may also produce various toxins, including microcystins (small ecotoxic and hepatotoxic cyclic polypeptides), of which MC-LR is the most common variant (Dawson, 1998). These toxins may bioaccumulate in various aquatic organisms and present serious threats to human and animal health (e.g. Zurawel, Chen, Burke, & Prepas, 2005; Blaha & Marsalek, 2009).

Algal blooms have become increasingly problematic in Lake Winnipeg, the tenth largest freshwater lake in the world, and now commonly lead to beach closures and generate public concerns about water quality and economic impacts. An urgent need exists to better understand the environmental circumstances which promote algal bloom development and toxicity, specifically those factors which could be useful in prevention, management and prediction.

Considerable discussion has focused on the factors which favor growth of nuisance algae. Both phosphorus and nitrogen have been identified as primary nutrients in the promotion of cyanobacterial blooms (e.g. Rapala, Sivonen, Lyra, & Niemela, 1997; Te & Gin, 2011). While much experimental work regarding the relative importance of P and N has been undertaken (see review of Zurawel et al., 2005), field outcomes have been

various: some have identified effects of both P and N on algal proliferation (see review of Lewis & Wurtsbaugh, 2008), while others have contended that P, and more specifically the N/P ratio, govern the development of cyanobacterial blooms (Schindler et al., 2008). Furthermore, the relationships of nutrients and algal biomass to bloom toxicity under field conditions are even less clear (e.g. Sinang, Reichwaldt, & Ghadouani, 2013).

In Lake Winnipeg, a previous study (Pip & Allegro, 2010) linked soluble P and N, as well as their ratios, with microcystin patterns at nearshore sites. Nearshore areas represent zones where nutrient inputs are most immediate and intense, and where algal blooms may be initiated as a result (Izydorczyk, Skowron, Wojtal, & Jurczak, 2008). In the present study we examined two consecutive years in order to determine whether seasonal fluctuation patterns of chlorophyll *a*, phaeophytin (an indicator of algal vigor), and microcystin could be temporally related to each other, as well as to changes in water chemistry, particularly nitrate-N, orthophosphate, and soluble NN/OP ratios.

2. Method

2.1 Sites

All three sites consisted of sandy beaches located on the east side of the south basin of Lake Winnipeg, and were sampled during the ice-free seasons of 2010 and 2011. Site 1 (96°37.03'W, 50°25.43'N) consisted of a public beach at Patricia Beach Provincial Park. Site 2 (96°34.44'W, 50°27.55'N) was located on public reserve shoreline adjacent to a cottage development. Site 3 (96°34.99'W, 50°28.46'N) was located on the north side of the public marina at Balsam Harbour. All three sites were bare of aquatic macrophytes and had high exposure to mixing effects of wind and wave action.

2.2 Sampling Procedures

The three sites were each visited twice a week (at three and four day alternating intervals) between 12 May and 28 August 2010, and every four days between 4 May and 9 November 2011. At each site, water samples were collected at the same respective time of day at each site, 10 cm below the water surface and 3-10 m from the shore, in water <1 m deep. Temperature and pH were measured *in situ* using a thermometer and portable pH meter (Radiometer, Copenhagen). Water samples for chemical analysis were filtered through Whatman No. 1 filter paper; the filters, filtered samples, as well as whole water samples for microcystin analyses were frozen at -20 °C. immediately on return to the laboratory.

2.3 Chemical Analyses

Orthophosphate-P (OP), nitrate-N (NN) dissolved organic matter index (DOMI), chlorophyll *a* (chl *a*) and phaeophytin were measured according to standard methods recommended by APHA (1995). Total dissolved solids (TDS) were measured directly using a TDSTestr (Oakton, Wards Natural Science, St. Catharines, Ontario).

Our microcystin (MC) values represented total free and lysed-cellular toxins. Whole water samples were subjected to three rapid freeze-thaw cycles to lyse the cells and were analyzed by ELISA (Lehman, 2007; Pip & Allegro, 2010) using polyclonal antibodies to bind with MC, which was quantified by dual wavelength (450 and 605 nm) spectrophotometry (Pharmacia LKB Ultrospec III, Science Park, Cambridge, England). Antibodies, reagents, standards and quality control samples were supplied by Beacon Analytical Systems Inc. (Portland, Maine). Cross-reactivity for these antibodies (vs. microcystin-LR) was: microcystin-LR 100%, microcystin-RR 73%, microcystin-YR 58%, microcystin-LW 4%, microcystin-LF 3% and microcystin-LA 2%. Our detection limit was 0.06 $\mu g\ l^{-1}$ microcystin-LR.

2.4 Statistical Analyses

Statistical tests and eligibility pretests (Sokal & Rohlf, 1981) were conducted using SPSS (Chicago, Illinois), with ln transformation as appropriate for exponential data. The critical significance level for all statistical tests was $p = 0.050$.

3. Results

3.1 Temperature, Water Chemistry and Precipitation

Temperature and water chemistry for the duration of sampling are summarized in Table 1; note the shorter sampling season in 2010. Mean NN, OP and DOMI were somewhat, but not significantly (unpaired *t*-tests) greater in 2010 than 2011; however considerably greater seasonal ranges and maxima for NN and OP were seen in 2010 (Table 1). While parameters showed unrelated fluctuation patterns at the three sites, ANOVA showed no significant differences among the sites for any environmental parameters in either 2010 or 2011; consequently for subsequent analysis sites were pooled for each year. Thus for 2010, $N = 90$; for 2011, $N = 144$.

Pearson correlation analysis indicated that NN was significantly correlated with OP ($r = 0.87$, $p<<0.001$) in 2010, but not in 2011. Both NN and OP were significantly correlated with each of DOMI and TDS in both years, but correlations of NN and OP with DOMI were much stronger in 2010.

Table 1. Summary of temperature and water chemistry at the three study sites in 2010 and 2011

	2010 (12 May - 28 August) N = 90	**2011** (4 May - 9 November) N = 144
Parameter	**Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**
Temperature °C	23.3 ± 0.3 (16 - 27.5)	15.9 ± 0.6 (2.0-26)
Total dissolved solids mg l^{-1}	330 ± 7 (140-540)	379 ± 9 (140-600)
Dissolved organic matter index (275 nm)	0.37 ± 0.01 (0.01-0.46)	0.27 ± 0.004 (0.14-0.68)
Nitrate-N mg l^{-1}	0.73 ± 0.17 (<0.01-11.5)	0.50 ± 0.05 (<0.01-2.43)
Orthophosphate-P mg l^{-1}	0.29 ± 0.05 (0.07-4.1)	0.20 ± 0.01 (<0.01-0.85)

Daily precipitation data from the Gimli Climate Station (WMO ID 71748) on Lake Winnipeg was available for 2011. Precipitation (pooled for the four days preceding each sampling day) was significantly correlated with NN ($p = 0.003$, NN ln transformed) as well as the NN/OP ratio (NPR) ($p = 0.006$, NPR ln transformed). Precipitation was also highly correlated with total suspended solids ($p < 0.0001$) (not examined in 2010), and to a lesser extent with temperature ($p = 0.002$) and TDS ($p = 0.024$).

3.2 Chl a and Phaeophytin

Chl *a* concentrations (Figures 1 & 2) were lower in 2010 compared to 2011 (mean 0.37 ± 0.34 S.E., max. 30.2 mg l^{-1} vs. mean 6.9 ± 0.01 S.E., max. 37.9 mg l^{-1} respectively). Fluctuation patterns were not correlated among the three sites in either year, and ANOVA showed no significant differences in seasonal chl *a* concentrations among the sites within each year. In 2010, chl *a* concentrations were highly significantly correlated with concurrent OP, DOMI and NN (Table 2). In 2011, chl *a* was still correlated with OP, but less strongly than in 2010, while no significant correlation was seen with either NN or DOMI (Table 2).

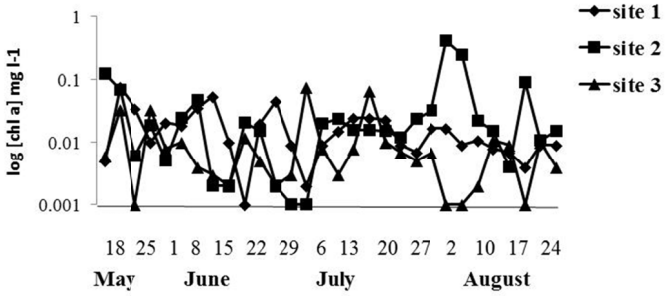

Figure 1. Log [chl *a*] at the study sites in 2010

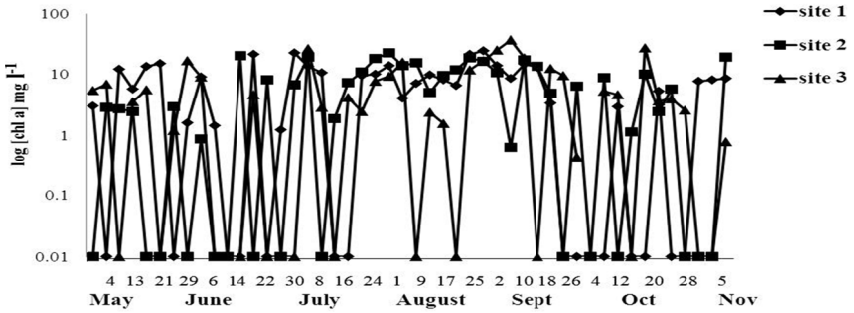

Figure 2. Log [chl *a*] at the study sites in 2011

Cross-correlation analysis (delayed chl *a*), revealed that in 2011, chl *a* concentrations were more strongly correlated with preceding nutrient concentrations, compared with those concurrent. While OP showed the strongest correlation, NN also emerged as marginally significant when paired with delayed chl *a* (Table 3). These delayed relationships were not significant in 2010.

Chl *a* showed additional but lesser correlations with concurrent temperature and TDS in 2010, but these correlations were much stronger in 2011 (Table 2). In 2011 chl *a* was also strongly correlated with water pH (r = 0.31, p <0.0001) and inversely with total suspended solids (r = -0.22, p = 0.003, ln chl *a*) (not examined in 2010).

Phaeophytin, a breakdown product of chlorophyll, was strongly inversely related to chl *a* concentrations in both years (p <0.0001). However in 2010, phaeophytin was also inversely correlated with OP (p = -0.33, OP ln transformed).

Table 2. Significant correlations between chlorophyll *a* and concurrent parameter values for 2010 and 2011. NS = not significant

Chlorophyll *a*	**2010**	**2011**
vs.	N = 90	N = 144
Orthophosphate-P	r = 0.98	r = 0.26
	p<0.0001	p =0.001
Dissolved organic	r = 0.98	NS
matter index	p<0.0001	
Nitrate-N	r = 0.84	NS
	p<0.0001	
Total dissolved	r = 0.19	r = 0.25
solids	p = 0.038	p = 0.001
Temperature	r = 0.19	r = 0.29
	p = 0.038	p<0.001
	Both ln	Linear
	transformed	

Table 3. Comparison of correlations between chl *a* and each of concurrent and immediately preceding nutrient concentrations in 2011. NS = not significant. N = 144

	Nitrate-N	Orthophosphate-P
Concurrent	NS	$r = 0.26$, $p = 0.001$
Preceding	$r = 0.14$, $p = 0.05$	$r = 0.32$, $p < 0.0001$

3.3 Microcystin

MC concentrations in 2010 averaged 0.21 + 0.04 S.E. µg l^{-1}, with a maximum of 2.4 µg l^{-1} (Figure 3). However in 2011 MC were below detectable levels for much of the season (mean 0.034 ± 0.016 S.E. µg l^{-1}), although at site 1 a late (24 October) maximum of 2.14 µg l^{-1} occurred (Figure 4). Thus while seasonal chl *a* was lower in 2010 than 2011, the reverse was true for MC. Nonetheless within each season, chl *a* and MC remained positively correlated (Table 4). In 2010, MC also showed a number of significant correlations with other parameters (Table 4), but in 2011 MC was correlated only with chl *a* ($r = 0.18$, $p = 0.015$), which had also been the strongest correlation in 2010 (Table 4). Similarly, while inverse correlation of MC was apparent with phaeophytin in 2010 ($p = 0.035$), and even more so with the phaeophytin/chl *a* ratio ($p = 0.002$), these relationships were not significant in 2011.

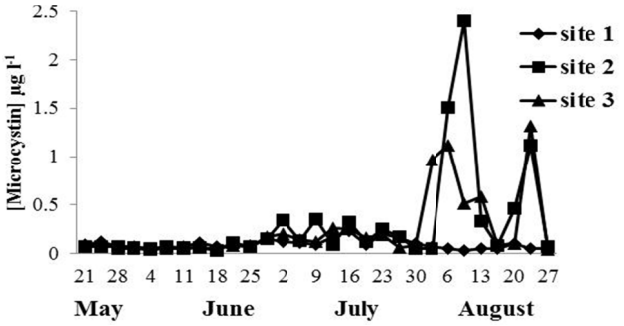

Figure 3. Microcystin concentrations at the study sites in 2010

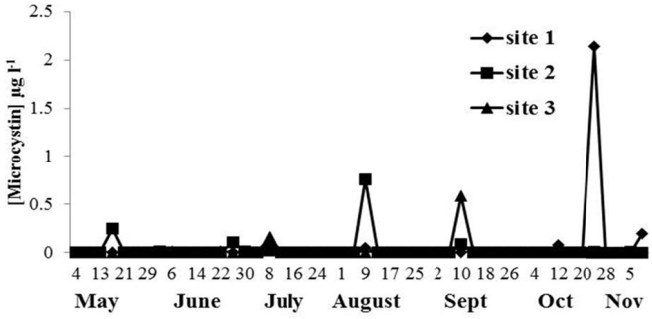

Figure 4. Microcystin concentrations at the study sites in 2011

Although MC was correlated more strongly with OP than NN in 2010, the NN/OP ratio (NPR) was inversely correlated with MC. Thus as proportion of OP increased relative to NN, MC increased, but this was demonstrable only at the higher MC levels of 2010.

For 2010, stepwise multiple regression analysis was undertaken, using the parameters that were correlated with untransformed MC (i.e. the first four parameters in Table 4), with MC as the dependent variable. The analysis yielded Equation (1), ($F = 46.2$, $p < 0.0001$):

$$MC\ (\mu g/L) = 0.11\ ln[chl\ a] + 0.50\ DOMI + 0.50 \quad (1)$$

where chl *a* = mg l^{-1} ($\beta = 0.46$), and DOMI = absorbance at 275 nm ($\beta = 0.39$). A model for 2011 could not be obtained due to the low MC concentrations.

Cross-correlation analysis (i.e. MC delayed by one sampling interval) for 2010 showed that MC lagged significantly behind fluctuations in OP, NN, and inversely behind NPR in descending order (Table 3). In addition, MC lagged behind chl *a*. However concurrent MC and each of OP, NN and chl *a* were more strongly correlated than the cross-correlation (delayed) MC values (Table 4).

Table 4. Significant correlations between significant concurrent and immediately preceding environmental parameters and microcystin concentrations (MC) in 2010. $N = 90$. NS = not significant

	Concurrent MC	Lagged MC (cross-correlation)
MC vs. ln chl *a*	$r = 0.67$, $p<<0.001$	$r = 0.39$, $p<0.001$
MC vs. DOMI	$r = 0.64$, $p<<0.001$	NS
MC vs. OP	$r = 0.61$, $p<<0.001$	$r = 0.38$, $p<0.001$
MC vs. NN	$r = 0.46$, $p<<0.001$	$r = 0.28$, $p=0.020$
MC vs. ln phaeo/chl *a*	$r = -0.33$, $p = 0.002$	NS
lnMC vs. ln temperature	$r = 0.27$, $p = 0.005$	NS
lnMC vs. NPR	$r = -0.25$, $p = 0.016$	$r = -0.29$, $p=0.006$
lnMC vs. lnTDS	$r = -0.22$, $p = 0.017$	NS
MC vs. phaeophytin	$r = -0.19$, $p = 0.035$	NS

4. Discussion

Nitrate and orthophosphate are highly soluble forms of N and P that are readily available for assimilation by aquatic primary producers (Pick & Lean, 1987), and thus elicit the most immediate algal response. The results of the present study indicated that both chl *a* and MC were temporally most strongly correlated with OP, as well as less significantly with NN, concurring with previous findings by others (see Zurawel, Chen, Burke, & Prepas, 2005) and for a nearby Canadian Shield lake (Pip, 1988), but the intensity of the correlations varied in different years. Temporal and spatial variability in cyanobacterial biomass as well as MC production is a recurrent problem that is responsible for much inconsistency in the published literature (Sinang, Reichwaldt, & Ghadouani, 2013), and large year-to-year differences may be encountered in a given lake (Carmichael & Gorham, 1981). In the present study, both consecutive seasons showed high soluble N and P levels, although OP and NN fluctuations as well as maxima were greater in 2010 (Table 1). However contrasting algal bloom development and toxicity were observed at the same study sites in the two years: seasonal chl *a* was significantly greater in 2011, but MC was higher in 2010, and thus toxicity was greater in the year of less intense algal blooms. Although MC levels were higher in the year that showed *lower* chl *a* concentrations, yet *within each season,* MC was positively correlated with chl *a.* These correlations supported the findings of Oh, Lee, J. H. Kim, H. S. Kim, and Yoon (2001); according to the latter authors, such association is particularly relevant to blooms that are dominated by a few toxic species or strains.

The composition of Lake Winnipeg phytoplankton has been examined by Kling (1998), who found the communities varied between blooms prevailed by diatoms (*Aulacoseira*) and cryptomonads in some years*,* and in other years by massive cyanobacterial blooms dominated by *Anabaena* and *Aphanizomenon*, although *Microcystis* and others also occur. The two years of the present study therefore reflected this variation. However bloom composition is not a dependable or consistent indicator of its toxicity. A given cyanobacterial species may have a variety of toxigenic and nontoxic genetic strains, which cannot be distinguished morphologically (Davis, Berry, Boyer, & Gobler, 2009). Furthermore known toxigenic strains may not produce toxins under certain conditions (Vazquez-Coriano, 2011). Thus cyanobacterial abundance alone, even of known MC producers such as *Microcystis* and *Anabaena*, often cannot be related to toxin concentrations due to inherent diversity in toxigenic activity (Chen, Burke, Mosindy, Fedorak, & Prepas, 2009).

MC has been reported to be correlated with high concentrations of both P and N (e.g. Vezie, Rapala, Vaitomaa, Seitsonen, & Sivonen, 2002; Te & Gin, 2011), and toxigenic cyanobacterial strains may have greater N and P requirements than nontoxic strains (Vezie et al., 2002). In the present study, as soluble N decreased relative to P, MC increased (but this effect was identifiable only at the higher MC levels of 2010). Furthermore the results of a

geographic survey of Canadian fresh waters found that the probability of higher MC was elevated when (total) N/P ratios were low (Orihel et al., 2012). This contrasted with reports of cell culture studies (Oh, Lee, Jang, & Yoon, 2000; see Zurawel et al., 2005) which found that MC-LR production may increase with increasing P limitation.

However in 2010, MC changes were also correlated with *preceding* chl *a* concentrations (Table 3). Since preceding OP and NN concentrations were more strongly related to chl *a* than were concurrent values, and since OP was the more important of the two factors for chl *a* in both 2010 and 2011, monitoring of OP patterns could signal impending changes in algal density.

The present study showed that under the higher MC concentrations of 2010, MC could also be related to preceding changes in OP and NN, and inversely following shifts in NPR. Under lower nutrient levels at these study sites in 2007 (Pip & Allegro, 2010), MC was similarly correlated with preceding OP levels, as well as inversely with preceding NPR, but for NN in 2007, MC was correlated with concurrent values only (sampling intervals in 2007 were longer, i.e. weekly). But in 2011, no significant relationships, concurrent or delayed, could be detected between MC and nutrient concentrations or ratios, likely due to the generally low, often undetectable, MC levels in that year. Thus while OP and NN patterns, as well as declining NPR, may have applications in predicting toxic blooms and thus anticipating possible beach closures in some years, these parameters seem less useful at low MC concentrations.

In the present study, the relationships between fluctuations in OP, NN and NPR, and MC in 2010 (and 2007 (Pip & Allegro, 2010)) supported the assertion that in some years lower relative availability of dissolved inorganic nitrogen may favor those cyanobacteria that through nitrogen fixation are able to circumvent dissolved nitrogen limitation (Smith, 1983), and thus greater abundance of toxigenic cyanobacteria might be implied, even though these relationships were much weaker in the high chl *a* conditions of 2011. Pawlik-Skowronska, Kalinowska, and Skowronski (2013) similarly reported that increases in dissolved inorganic NPR were accompanied by lower cyanobacterial biomass. However the critical N/P ratios vary for different nitrogen-fixing species (Lehman, 2007), and probably for their individual strains.

A dichotomy of thought regarding the relative importance of P and N in driving development and toxicity of algal blooms has led to two types of lake refurbishment strategies: reduction of both N and P (e.g. Jeppesen, Sondergaard, Meerhoff, Lauridsen, & Jensen, 2007), or reduction of P only (e.g. Schindler et al., 2008). From a review of the literature, Downing, Meyer, Gehringer, and van de Venter (2005) concluded that P and N concentrations are more consistently related to algal biomass than to N/P ratios, based on observations that algal production may be limited by either nutrient (e.g. Stanley, Clarke, McNeal, & MacLeod, 2003; Lewis & Wurtsbaugh, 2008). In Lake Winnipeg, while P appears to be the primary limiting factor during the first part of the season, evidence of increasing late summer N limitation has been reported (Kling, 1998; Pip & Allegro, 2010).

While in the present study OP was clearly more important than NN with respect to bloom toxicity (MC), both OP and NN were highly significantly correlated in 2010 with *total* algal biomass as estimated by chl *a* (Table 2). Nitrogen availability may boost the non-nitrogen-fixing cyanobacterial (Sivonen, 1990) and eukaryotic algal components. Indeed in the present study, NPR, concurrent or preceding, did not significantly affect total chl *a*. For example, *Microcystis* is an ubiquitous cyanobacterial bloom producer, but does not fix nitrogen; under N-rich conditions, its growth and toxin production have been shown to increase (Gobler, Davis, Coyne, & Boyer, 2007). Conversely, nutrient reduction programs may not significantly address chrysophytes, and may indeed aggravate them in eutrophic systems (Watson et al., 2008). Thus management of algal blooms by restricting P loadings only may not solve the problem of algal proliferation, and the ensuing problems of toxicity, light limitation and oxygen depletion.

Traditionally bloom toxicity has been evaluated from a standpoint of public safety on the basis of MC concentrations (1 μg/L Guideline in Canada), but algae, particularly cyanobacteria, produce a very large variety of toxins (Blaha & Marsalek, 2009). During low dissolved N/P ratios, for example, anatoxin-a production may be favored compared to MCs, and different toxins may show unrelated patterns of production under bloom conditions (Pawlik-Skowronska, Kalinowska, & Skowronski, 2013). Thus low MC may not necessarily signify low bloom toxicity.

Higher MC was also correlated with lower phaeophytin/chl *a* ratios, suggesting that senescent blooms were less toxic than those that were more actively growing. This finding supported experimental work which found that MC peaks during the late exponential phase of the growth cycle (van der Westhuizen & Eloff, 1985). Linked to

this was the observed inverse relationship between phaeophytin and OP; thus blooms were more vigorous at higher OP levels.

While relationships between each of chl *a* and MC with OP, DOMI and NN were much more pronounced in 2010 than 2011, temperature, and to a lesser extent TDS, gained greater importance in 2011 (Table 2). Water pH and total suspended solids were also significant factors in 2011 (not examined in 2010); Wicks and Thiel (1990) and Sinang, Reichwaldt, and Ghadouani (2013) reported MC relationships with pH and salinity respectively as well. Thus nutrients alone appeared to play a comparatively reduced role in 2011 with respect to chl *a* and MC, indicating that bloom composition differed in the two years, including dissimilar proportions of actively toxigenic algae. According to Rapala, Sivonen, Lyra, and Niemela (1997), the type of toxin produced may differ with temperature: these workers found that MC-LR and MC-RR produced by *Anabaena* were favored at lower and higher temperatures respectively. Davis, Berry, Boyer, and Gobler (2009) found that higher temperatures were associated with more toxic *Microcystis* blooms, but a large MC maximum at the end of October 2011 in the present study (Figure 4) indicated that warm temperatures are not requisite for development of a toxic bloom. Temperature effects may be obfuscated by competing processes: for example temperature may affect associated bacterial populations that degrade cyanobacterial toxins (Dziallas & Grossart, 2011).

The inverse relationship between total suspended solids and chl *a* (examined in 2011) was expected as turbidity and light availability have been shown to affect chl *a* concentrations (Pip, 1988). Total suspended solids were in turn related to precipitation (see *3.1*). Utkilen and Gjolme (1992) proposed that maximal MC content of *Microcystis aeruginosa* would occur near the water surface, while Bottcher, Chorus, Ewald, Hintze, & Walz, (2001) suggested that in natural environments, light intensity may have a relatively minor effect on MC. The deleterious effects of ultraviolet (UV) light on cyanobacterial metabolism and vigor through direct photolytic damage as well as formation of reactive oxygen species have been reviewed by Leao, Engene, Antunes, Gerwick, and Vasconcelos (2012), although cyanobacteria have evolved a number of UV defence mechanisms (e.g. Ding, Song, & Sedmak, 2013). MC may undergo direct photodegradation (Wormer, Huerta-Fontela, Cires, Carrasco, & Quesada, 2010), the rate of which may be prolonged by MC adsorption onto fine sediment and suspended particles (Morris et al., 2000). In the present study the algae were vertically circulated in a shallow turbulent nearshore environment, while seasonally, UV exposure effects would have been greatest in spring and early summer (Ding, Song, & Sedmak, 2013), when according to the latter authors UV may favor some MC producing strains over non-producing variants. However in the present study most of the MC production occurred in late summer and fall. Nevertheless light intensity and temperature may affect the type of MC produced (Rapala & Sivonen, 1998).

The use of copper as an algaecide in Lake Winnipeg has been reported to be inversely correlated with MC (Pip & Allegro, 2010), and this may have been a confounding factor. Control of algal blooms with copper sulphate requires repeated treatments and is not recommended from a management perspective. It induces algal cell lysis (Jones & Orr, 1994), but is toxic to a large array of aquatic organisms, including macrophytes, zooplankton, molluscs and fish, and accumulates in the bottom sediments (van Hellebusch et al., 2003; de Oliveira-Filho, Lopes, & Paumgartten, 2004), with possible adverse long-term effects on the ecosystem (Schuler, Hoang, & Rand, 2008). Treatments with copper sulphate may also promote increases in bacterial biomass following mass plankton death (Havens, 1994), and repetitive application can favor growth of copper-resistant mutants in *Microcystis* populations (Garcia-Villada et al., 2004).

In conclusion, this study indicated that great year-to-year variability exists in algal behavior and response to nutrients in Lake Winnipeg, and that development of blooms cannot be consistently simplified as dependent only on one or a few factors. High nutrient concentrations were accompanied by differing levels of chl *a* and MC in different years, but within each season, chl *a* and MC were correlated. While OP was more important than NN in promoting chl *a*, and MC increased as N relative to P decreased, NPR did not significantly affect total chl *a* concentrations. Thus OP and to a lesser extent NN were the primary parameters for chl *a*, and total MC was related in descending order to OP, NN, and inversely and less so with NPR. More vigorous blooms were more toxic, and toxic blooms could develop in warm as well as cool conditions.

While nutrient reduction strategies remain the most effective long-term initiatives to control algal blooms, other factors also need to be considered. Further study is needed to ascertain why summer algal blooms differ in different years, even though nutrients are plentiful. Other factors, such as heavy metals (see Zurawel, Chen, Burke, & Prepas, 2005; Pip & Allegro, 2010) and various other chemical (e.g. essential trace elements, algaecides, pollutants), physical (e.g. light), and biological (e.g. microbiological, allelopathic, herbivore) components may exert modifying effects on algal populations. Furthermore, physiological properties of individual algal taxa and their genetic strains may contribute to variable responses: not only do different species

respond differently to given conditions (Lehman, 2007), but responses may differ at different nutrient concentrations (Downing, Meyer, & Gehringer, 2005).

In the Great Lakes, altered patterns of P cycling (Bootsma, 2007 in Englebert, McDermott, & Kleinheinz, 2008) as well as increased water clarity (Auer et al., 2010), resulting from zebra mussel (*Dreissena polymorpha*) invasion, have contributed to problem resurgence of the green alga *Cladophora,* despite P management policies. Zebra mussels have already been sighted in Lake Winnipeg in the last two years (Pip, unpublished data). Given this new threat, and the urgent challenges of addressing existing nutrient sources and land use practices in an enormous watershed that spans, and requires, the cooperation of, many political and economic jurisdictions, the future of Lake Winnipeg remains uncertain, yet measures to save it must be diligently pursued.

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