Fate and persistence of microcystin congeners in lakes and lake sediments

Arthur Zastepa

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Department of Biology
Faculty of Science
Ottawa-Carleton Institute of Biology
University of Ottawa

Abstract

Cyanobacterial blooms and their toxins are a major water quality and potential health risk around the world. This thesis developed an analytical method for microcystin congeners in sediments in order to examine their fate in lakes and establish the history of toxin-producing cyanobacteria in relation to environmental change using lake sediments. A novel method for both intra- and extracellular microcystins in lake sediments was developed, consisting of accelerated solvent extraction, hydrophilic-lipophilic balance solid phase extraction and multiple reaction monitoring-based HPLC-MS/MS quantitation. The method achieved comparable recoveries of intra- and extracellular cyanotoxins based on nine microcystins and nodularin (marine analogue). The analytical method was validated using surficial and deeper sediments from seven lakes of diverse geography and trophic state. To study the fate of microcystins, a multi-year, whole lake study of *Microcystis* blooms was conducted to obtain both *in situ* and *in vitro* half-life estimates of microcystin-LA (MC-LA), an understudied, but increasingly reported microcystin. MC-LA appeared to undergo slower rates of decomposition and persist longer than the more frequently studied MC-LR. Experimentally, high light intensity increased *in vitro* decomposition of dissolved MC-LA while high temperature enhanced decomposition in the particulate phase. Sediment deposition measurements and estimates of sediment-pore water distribution coefficients, sediment accumulation rates, and diffusive fluxes indicated that microcystin congeners differ in their fate. Notably, MC-LA preferentially distributed into pore water and remobilized (by diffusion) from sediments and into overlying water while MC-RR adsorbed more strongly to

sediment particles. Finally, the sediment record of an eutrophic lake of major recreational importance was examined to identify possible drivers of toxigenic cyanobacteria and determine if the perceived increase in toxigenic cyanobacteria could be corroborated. Microcystins were detected to the bottom of the core (early 1800s), indicating that toxigenic cyanobacteria were present prior to the first permanent settlements. Microcystins were significantly correlated with changes in diatom-inferred nutrients (DI-TP and DI-TKN) within the sediment core as well as with specific algal pigments. Sediment microcystins in the upper layers also significantly correlated with a 20-year monitoring record for water column microcystins suggesting that sediment microcystins can be used as a proxy for past surface water conditions.

Résumé

Les proliférations de cyanobactéries et de leurs toxines en milieu aquatique causent des problèmes de qualité d'eau et représentent un risque pour la santé humaine. Cette thèse a développé une méthode d'analyse des congénères de microcystines dans les sédiments lacustres afin d'examiner leur sort dans les lacs et d'établir l'historique de la production de toxines par les cyanobactéries par rapport aux changements environnementaux. Une nouvelle méthode pour les microcystines intra-et extracellulaires a été élaborée pour les sédiments lacustres, composée d'extraction accélérée par solvant, l'équilibre hydrophile- extraction en phase solide et la quantification par HPLC-MS/MS. La méthode a réalisé des recouvrements comparables de cyanotoxines intra-et extracellulaires (neuf microcystines et nodularine (analogue marin)). La méthode d'analyse a été validée en utilisant des sédiments provenant de sept lacs de géographie et d'états trophiques différents. Pour étudier le sort environnemental des microcystines, une étude de blooms sur deux ans a été menée dans un petit lac clos pour obtenir des estimations de leur dégradation à la fois *in situ* et sous conditions contrôlées *in vitro*. La microcystine dominante MC- LA était un congénère peu étudié, mais de plus en plus observé et toxique envers les vertébrés. MC-LA se dégradait assez lentement et semblait persister plus longtemps que la microcystine plus fréquemment étudiée, MC- LR. Expérimentalement, une forte intensité lumineuse augmente la décomposition *in vitro* de MC -LA dissous et une température élevée mène à une augmentation de la décomposition de la phase particulaire. Des mesures de taux de déposition dans les sédiments, des estimations de coéfficients de distribution entre les sédiments et les

eaux interstitielles, les taux d'accumulation de sédiments, et les flux diffusifs ont indiqué que les divers congénères de microcystines se distinguent par leur sort environnemental. Notamment, MC -LA est distribuée préférentiellement dans l'eau interstitielle et remobilisée (par diffusion) dans les sédiments et dans la colonne d'eau. Par contre, MC- RR est adsorbée plus fortement aux particules de sédiments. Finalement, le dossier des sédiments d'un lac eutrophe d'importance récréative a été examiné pour identifier les facteurs liés aux proliférations de cyanobactéries toxiques et pour déterminer si la perception que les blooms toxiques sont plus sévères que dans le passé pouvait être confirmée. Les microcystines ont été détectées tout en bas de la carotte (début des années 1800), ce qui indique que les cyanobactéries toxiques étaient présentes avant l'arrivé des colons européens et le défrichement des terres. Les microcystines étaient significativement corrélées avec les changements dans les nutriments inférés par la composition en diatomées fossiles (DI -TP et DI- ATK) des sédiments ainsi que par la composition de pigments d'algues spécifiques. Les microcystines dans les couches supérieures des sédiments étaient également significativement corrélées avec un suivi de 20 ans des microcystines dans la colonne d'eau, ce qui suggère que les microcystines dans les sédiments peuvent être utilisées comme un indicateur des conditions d'eau de surface dans le passé.

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“I may not have gone where I intended to go, but I think I have ended up where I needed to be.” – Douglas Adam

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Chapter I: Introduction to toxic cyanobacteria and cyanotoxins in aquatic ecosystems

1.0 Cyanobacteria and cyanotoxins

Cyanobacteria are photosynthetic micro-organisms with a long history on Earth, being the first organisms to release elemental oxygen into the primitive atmosphere. They have a prokaryotic cellular organization and are comprised of unicellular and colonial (including filamentous) forms. They thrive globally, inhabiting marine, brackish and fresh waters as well as terrestrial environments (Whitton and Potts 2000).

Massive growth, or blooms of cyanobacteria, can lead to visible accumulations in summer and autumn in temperate waters and almost permanently in tropical regions (Chorus et al. 1999). These can appear in the form of surface scums along shorelines, pelagic blooms, and benthic mats in littoral zones or shallow systems. The development of cyanobacterial blooms is determined by a complex interaction of nutrients, light penetration (affected by turbidity), temperature (affecting growth rate and water column stability), pH, and at times salinity (e.g. Jacoby et al. 2000, Downing et al. 2001, Moisander et al. 2002, Tonk et al. 2007, Håkanson et al. 2007, Jöhnk et al. 2008, Taranau et al. 2012, Kosten et al. 2012, Beaulieu et al. 2013). Some have argued that the N:P ratio is of particular importance (Smith 1983, Chorus et al. 1999, Jacoby et al. 2000, Orihel et al. 2012) while others suggest the opposite (Pick and Lean 1987, Dolman et al. 2012). The ability of cyanobacteria to regulate their buoyancy and migrate vertically in search of optimal growing conditions provides a major advantage over other plankton. They can effectively shade and thus, suppress the growth of the underlying phytoplankton

community while harvesting optimal wavelengths of light themselves (Reynolds 2006; Jöhnk et al. 2008).

Anthropogenic activities have clearly led to increases in cyanobacterial dominance in lakes primarily through nutrient enrichment of surface waters (e.g. Smith et al. 2003, Anderson et al. 2002, Finni et al. 2001, Riedinger-Whitmore et al. 2005, Heisler et al. 2008). However, climate change may also be contributing to the increased reporting of cyanobacterial blooms (e.g. Hallegraeff 2010, LeBlanc et al. 2008, Paerl and Paul 2012, Winter et al. 2011). Climate change has led to a longer growing season as well as changes in the hydrological cycle. For example, increased precipitation can increase surface and ground water nutrient discharge, while drought can provide the subsequent water column stability needed for nutrient uptake and bloom development (Huisman et al. 2005; Paerl et al. 2006). This increased dominance by cyanobacteria has drawn attention to the presence of toxigenic taxa and their apparent growing risk for ecological and human health (e.g. Chorus et al. 2000; Miller et al. 2010; Pouria et al. 1998).

Some genera and species of cyanobacteria are known to produce toxins (Table I-1) however, their presence is not sufficient to confirm toxicity because toxicity is commonly determined at strain level. The presence of toxins must be confirmed by chemical analysis while the potential for toxin expression can be measured through molecular amplification of the gene cluster responsible for synthesis (Figure I-1). Unfortunately detection methods for all the known cyanotoxins and gene(s) responsible are not readily available. The most widely studied and monitored group of cyanotoxins are the microcystins (Figure I-2) due to

their relatively high toxicity and prevalence (Chorus and Bartram 1999). In vertebrates, microcystins are taken up by the liver via the bile acid transport system and can induce cytoskeletal redistribution and concomitant disruption of cell-cell interactions (reviewed in Zurawell et al. 2005). The resultant necrosis of hepatocytes causes deterioration of hepatic architecture, leading to severe intrahepatic hemorrhaging and unregulated inflow of blood. The outcome is increased liver size (swelling) and decreased systemic arterial blood pressure, ultimately leading to hypovolemic shock and death. There are extensive historical records of animal and human poisonings associated with cyanobacterial blooms and developments in analytical methods have identified the cyanotoxins responsible (Table I-2, Table I-3, Pouria et al. 1998, Miller et al. 2010). The recognized routes of human exposure to cyanobacterial cells and toxins are summarized in Table I-4. The World Health Organization (WHO) and Health Canada have implemented drinking water guidelines of 1.0 and 1.5 μg L^{-1} while guidelines for recreational exposure have been set between 10 and 20 μg L^{-1} (Codd et al. 2005).

2.0 Occurrence of toxigenic cyanobacteria and microcystins in surface waters

Microcystins have been detected in surface waters globally (Codd et al. 2005). In Canada, public attention and monitoring of cyanobacteria has increased significantly in the last decade. Alberta conducted its first provincially funded assessment of microcystins in raw and treated water in 2001. Microcystins were detected in 67% and 10% of raw and treated water samples, respectively (Zurawell 2008). A more recent assessment detected microcystins in more than 100 lakes,

reservoirs, city storm water retention ponds, and farm dugouts in Alberta (Kotak et al. 2007). Compared to western Canada, the microcystin data for central and eastern Canada is limited, however monitoring programs for toxigenic cyanobacteria are emerging. The Ontario Ministry of Environment, which has an algal monitoring group, identifies cyanobacterial species, conducts special studies in waters with recurrent blooms, and has developed a comprehensive response protocol (Winter et al. 2008). In Ontario, the number of samples received for identification is increasing every year (Winter et al. 2008). Québec has a monitoring program for cyanobacteria through the Ministère du Développement durable, de l'Environnement et des Parcs and the Ministère de la Santé et des Services sociaux. In 2004, about 20 lakes in Quebec were reported to experience cyanobacterial blooms and in 2007 that has increased to over 150 lakes affected (MDDEP 2012). Giani et al. (2005) observed microcystins in 22 lakes sampled in southern Quebec while Kotak et al. (2007) detected microcystins throughout Lake of the Woods, Ontario. Recently, microcystins have been detected in Lake Ontario including Hamilton Harbour and the Bay of Quinte as well as coastal embayments, rivers, nearshore and offshore, and upland lakes of the Lake Ontario ecosystem (Murphy et al. 2003, Boyer 2007, Makarewicz et al. 2009).

3.0 Microcystin congeners and chemistry

Microcystins are a seven amino-acid cyclic peptide containing the rare amino acid, 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyldeca-4,6-dienoic acid, abbreviated Adda. Adda is a 20-carbon non-protein amino acid that is important for

the toxicity of the compound. The general structure of microcystins is cyclo-(-D-Ala-*X*-D-MeAsp-Y-Adda-D-Glu-Mdha-), where X and Y are variable L-amino acids, D-MeAsp is D-*erythro*-β-methylaspartic acid, and Mdha is *N*-methyldehydroalanine (Figure I-2). Over 70 congeners are known (Zurawell et al. 2005), with variability arising primarily from amino acid substitutions and their modifications at the two locations noted in Figure I-2. Minor modifications throughout the structure have also been reported including addition/removal of short alkyl groups (e.g. methyl, methylene, aminoisobutyric acid) and methyl ethers.

Physicochemical data on microcystins are incomplete. The high number of congeners and amphiphilic structure of microcystins makes assessment of their environmental fate and persistence especially challenging and at present impossible to predict with accuracy. One of the first studies that attempted to determine physicochemical properties of microcystins was Rivasseau et al. (1998) using log k_w (retention factor in RP-ODS with water) to estimate K_{ow}. The log k_w of the three congeners studied (MC-YR, -LR, and -RR) was about 4, which is comparable to the most persistent organochlorine compounds withdrawn from the market (K_{ow} >4). Although this suggests microcystins are hydrophobic, the high solubility of MC-LR in water (1 g L^{-1}) and low adsorption to suspended particulate matter observed in this study suggests they will not behave like organochlorines or other hydrophobic organic compounds. In contrast, others have reported very high adsorption to sediments (or particulates), especially sediments rich in clay (~81%) (e.g. Morris et al. 2000). Microcystins contain polar groups, such as carboxylic acids and amino and amido functional groups. This combination of hydrophobicity and polar

functional groups can lead to formation of micelles and possibly ion-pairs, making predictions of their chemical behaviour challenging.

4.0 Environmental fate and persistence of microcystins

Microcystins are highly stable under laboratory conditions even at low pH and high temperatures (Harada et al. 1998). They have been observed in natural waters for prolonged periods following natural and artificial bloom lysis (Jones and Orr 1994, Miller et al. 2010). Photodecomposition is very limited, even under natural sunlight, but can be accelerated in the presence of natural pigments and other photosensitizers such as fulvic acids and dissolved organic matter (Tsuji et al. 1994, Welker and Steinberg 2000). Humic substances, when irradiated, can facilitate formation of reactive oxygen species that can in turn react with the normally photochemically inert microcystins (Welker and Steinberg 1999). Furthermore, UV has been observed to induce isomerization but this mechanism also depends strongly on photosensitizers (Tsuji et al. 1994). Microbes have been observed to rapidly degrade microcystins but the rate can strongly depend on many factors including previous exposure to microcystins (Rapala et al 1994), their initial concentration (Park et al. 2001), and ambient temperature. Several studies have shown that biodegradation can be an especially rapid process in environments previously impacted by bloom events (Christoffersen et al. 2002; Lahti et al. 1997). Several bacterial species able to degrade microcystins have been isolated including *Pseudomonas aeruginosa* (Takenaka et al. 1997) and *Sphingomonas sp*. (Bourne et al. 1996). The few bacterial isolates observed to degrade microcystins

preferentially cleaved the Adda-Arginine bond present in congeners such as microcystin-LR (MC-LR) while congeners such as MC-LF, that do not contain an arginine substituents, were not significantly degraded (Imanishi et al. 2005, Ishii et al. 2004). Consistent with these observations, Chen et al. (2008) observed different half-lives in the decomposition of different microcystin congeners. The differences were more pronounced when experiments were done with surface water compared to sediment samples. Holst et al. (2003) demonstrated that biodegradation of microcystins was possible under both aerobic and anaerobic conditions but these experiments were performed at elevated temperatures. Notably, nitrate addition to anoxic sediments promoted degradation by microbes, indicating that anaerobic microcystin degradation may be coupled to nitrate respiration (denitrification). A summary of literature investigating the decomposition of microcystins in different waters and sediments is presented in Table I-5.

Despite evidence of decomposition of microcystins in water and sediments, a few recent studies have reported the presence of toxigenic cyanobacteria and microcystins in lake sediments (Ihle et al. 2005, Chen et al. 2008, Pawlik-Skowronska et al. 2010, Efting et al. 2011). Latour et al. (2007) found active cyanobacteria in surficial sediments obtained at 45 m depth despite low temperatures, lack of oxygen, and darkness. Furthermore, Latour et al. (2007) found *Microcystis aeruginosa* cells in a 70 cm section of a sediment core taken from the same eutrophic reservoir. The cell counts were more than two-fold greater than at the sediment surface. A 25-35 cm section revealed microcystin concentrations (1 pg $cell^{-1}$) more than three-fold higher than at the sediment surface and approximately

six-fold higher than measured in summer planktonic samples (Sedmak and Kosi 1998).

Microcystins have been observed to deposit into sediments by several routes. Sedimentary deposition of intact cells can occur due to active buoyancy down-regulation, lake mixing, and end-of-season die off (Welker et al. 2007). Phenological investigations have observed *Microcystis aeruginosa* cycling through a spring to autumn planktonic phase and an overwintering benthic phase (Latour et al. 2004, Latour et al. 2004, Verspagen et al. 2005). Several studies have also reported the presence of exclusively benthic microcystin-producing species, particularly in shallow lakes. These species have been found in lakes in Switzerland (Mez et al. 1997), California (Izaguirre et al. 2007), and the Antarctic (Hitzfeld et al. 2000), some producing microcystins *in situ* that exceed the WHO drinking water guideline of 1.0 μg L^{-1}. Extracellular microcystins deposit into sediments by adsorbing to suspended particulates (clay, organic matter), fecal excrements, and dead biomass in the water column (Zakaria et al. 2007, Morris et al. 2000, Miller et al. 2001). Additionally, aquatic plants and animals have been reported to accumulate microcystins, which can then deposit into sediments post-mortem (Song et al. 2007).

5.0 Thesis objective and outline

The environmental fate and persistence of microcystins, and cyanotoxins in general, remain poorly understood and investigations have focused on MC-LR. Current drinking and recreational water quality guidelines for microcystins are

primarily based on the toxicity of MC-LR (Chorus and Bartram 1999, Falconer et al. 1994, Fawell et al. 1994, WHO 2006). Based on limited comparative modeling studies, current management and treatment practices developed in response to MC-LR are deemed suitable for other microcystin congeners (UKWIR 1997, Newcombe et al. 2003). However, increasing evidence suggests there may be significant differences in the fate and persistence of different microcystin congeners (Newcombe et al. 2003, Miller et al. 2010).

The overall objective of this thesis was to investigate the natural fate and persistence of microcystin congeners in lakes and lake sediments, providing quantitative comparisons between congeners. As a first step (Chapter II), an analytical method was developed and validated to extract, identify, and quantify a representative group of microcystin congeners in lake sediments, while addressing the limitations in existing methodologies. Chapter III follows the senescence of *Microcystis* blooms over two years in a small lake and provides estimates for *in situ* and *in vitro* half-lives of MC-LA, a congener as toxic as MC-LR but less frequently studied. Additionally, the new analytical method was applied to evaluate the extent of sedimentary deposition of different congeners (notably MC-LA and -RR) associated with cyanobacteria in lakes. In Chapter IV, microcystins were used to investigate drivers of toxigenic cyanobacteria from analyses of lake sediment records to determine whether the perceived increase in toxigenic cyanobacteria through time is valid. Finally, Chapter V estimates the distribution and transport of microcystin congeners between sediments, interstitial pore water, and water

overlying surficial sediment to assess the relative risk of exposure to different congeners from sediments.

Tables and figures

Table I-1: Toxins from some genera of fresh- and brackish water cyanobacteria (Adapted from Codd et al. 2995).

Toxin (class)	Molecular Structure	Variants	Sources	Modes of Toxicity
Nodularin (hepatotoxic)	Cyclic pentapeptide	5	*Nodularia*	phosphatase inhibitor
Microcystins (hepatotoxic)	Cyclic heptapeptide	>70	*Microcystis, Anabaena, Oscillatoria/Planktothrix, Nostoc*	tumourigenic, phosphatase inhibitor
Cylindro-spermopsin (hepatotoxic)	polycyclic uracil derivative, guanidino and sulfate groups	ca. 3	*Anabaena, Aphanizomenon, Cylindrospermopsis*	inhibits protein synthesis
Anatoxin-a (neurotoxic)	Secondary amine alkaloid	1	*Anabaena*	neuromuscular blocker
Anatoxin-a(s) (neurotoxic)	Guanidinium methyl phosphate ester	1	*Anabaena*	cholinesterase inhibitor
Saxitoxin (neurotoxic)	Alkaloid	ca. 6	*Aphanizomenon*	Na^+ channel blocker
LPS	lipopoly-saccharides	>3	*Microcystis, Lyngbya, Oscillatoria/Planktothrix*	toxic shock

Table I-2: Some historical animal poisoning incidents associated with cyanobacterial toxins (Adapted from Duy et al. 2000).

Year	Location	Animals affected	Species producing toxin
1878	South Australia, Lake Alexandrina	Sheep, horses, dogs, pigs	*Nodularia spumigena*
1882-1884	Minnesota, USA	Cattle, horse, hogs	*Gleotrichia echinulata*
1990	Minnesota, USA	Several cattle	*Aphanizomenon flos-aquae*
1917-1918	Alberta, CA	Hogs, horses, cattle, poultry, wild bird	"Blue-green algae"
1918	Minnesota, USA	1 sheep, 17 hogs, ~50 chickens	*Coelospharium kuetzzingianum. Anabaena flos-aquae*
1924	Ontario, CA	~20 cattle	*Anabaena*
1928	Lake Vesijärvi, Finland	~40 cattle	*Anabaena lemmemannnii*
1934	Lake Juska, USSR	Cats	Blue-green algal bloom
1989	Rutland Water, UK	Death of 20 sheep, 11 dogs	Microcystin-LR from *Microcystis aeruginosa*

Table I-3: Some reported human illness associated with exposure to cyanobacterial toxins (Adapted from Duy et al. 2000).

Year	Location	Affected Population	Exposure route	Sources (toxins)
1959	Saskatchewan, Canada	12 people with acute gastroenteristis	Swimming	*Anabaena*
1979	Palm Island, Queensland, Australia	139 children (2-16 yr) and 10 adults. Hepatitis-like syndrome with malaise, anorexia, vomiting, tender hepatomegaly, headache, and abdominal pain	Drinking water from open reservoir	Cylindrospermopsin from *Cylindrospermopsis raciborskii*
1996	Caruaru, Brazil	116 of 130 patients at a dialysis center. Visual disturbances, nausea, and vomiting; 63 patients died.	Dialysis water	Microcystins

Table I-4: Human exposure routes and exposure media for cyanobacterial toxins (Adapted from Codd et al. 2005).

Exposure route	Exposure medium
Oral (ingestion)	Drinking water, recreational water, food (shellfish, finfish if toxin accumulation has occurred during production; plant foods after irrigation with water containing cyanobacterial toxins) Dietary supplements (pills, capsules) if containing dried cyanobacterial cells with toxins
Pulmonary (inhalation, aspiration)	Water: aerosols, spray during recreation, work, showering
Dermal (skin, mucosal contact	Water during recreational, work, showering
Haemodialysis	Water used for haemodialysis

Table I-5: Summary of literature investigating the decomposition of microcystins in different waters and sediments. Both field and laboratory values are presented where reported.

Compartment	Degradation (post-lag)	Lag (days)	Details
Effluent from WWTP	Half-life: 0.2 to 3.6 d 40% loss in 7 d	7	Lam, Fedorak, Prepas 1995. Purified LR. Higher bacterial densities and O_2 than in lakes.
River water	>95% loss in 5 d	2 to 14	Jones et al. 1994. LR from cell lysate. Faster rates when higher initial microcystin concentration.
Whole lake	90 to 95% loss in 3 d	9	Jones and Orr 1994. Algicide treatment released MC-LR.
Lake water Surface sediment Sand sediment	LR: >90% loss in 4 d LR: >90% loss in 4 d RR: ~65% loss in 7 d LR: >87% loss in 4 d RR: ~17% loss in 7 d		Rapala et al. 1994. Cell lysates added to water and sediment.
Lake water	Half-life: 0.5 to 1.6 d		Lam, Prepas, Spink, Hrudey 1995. Following algicidal treatment, >39% LR remained inside decaying cells.
Surface water Water over sediment Surface sediment	Half-life: 1.1 to 7.7 d Half-life: 0.9 to 16.2 d Half-life: 0.6 to 2.1 d	2 to 15 2 to 20 0 to 2	Chen et al. 2008. Extracted and pure microcystins (LR, Dha^7 LR, and RR). Sediment from ponds with blooms.
Reservoir water Reservoir water Bed sediment	Half-life: 3 to 4 d >95% loss in 7 d >95% loss in 6 d		Cousins et al. 1996. Extracted MC-LR, natural diurnal light regime (17-21^{o}C).
Groundwater Water over sediment Sediment Sediment	None for 100 d >64% loss in 2 wk >71% loss in 1-2 wk >80% loss in 1 d		Holst et al. 2003. Extracted and pure MC-LR. Oxic and anoxic samples had low/undetectable bacteria. Oxic/microaerophilic incubation in dark at 10^{o}C. Oxic/microaerophilic incubation in dark at 10^{o}C. Anoxic with nitrate in dark at 21^{o}C.
Natural waters	Half-life: 90-120 d		Welker et al. 2000. Photosensitized humic acid degradation. Extracted MC-LR.

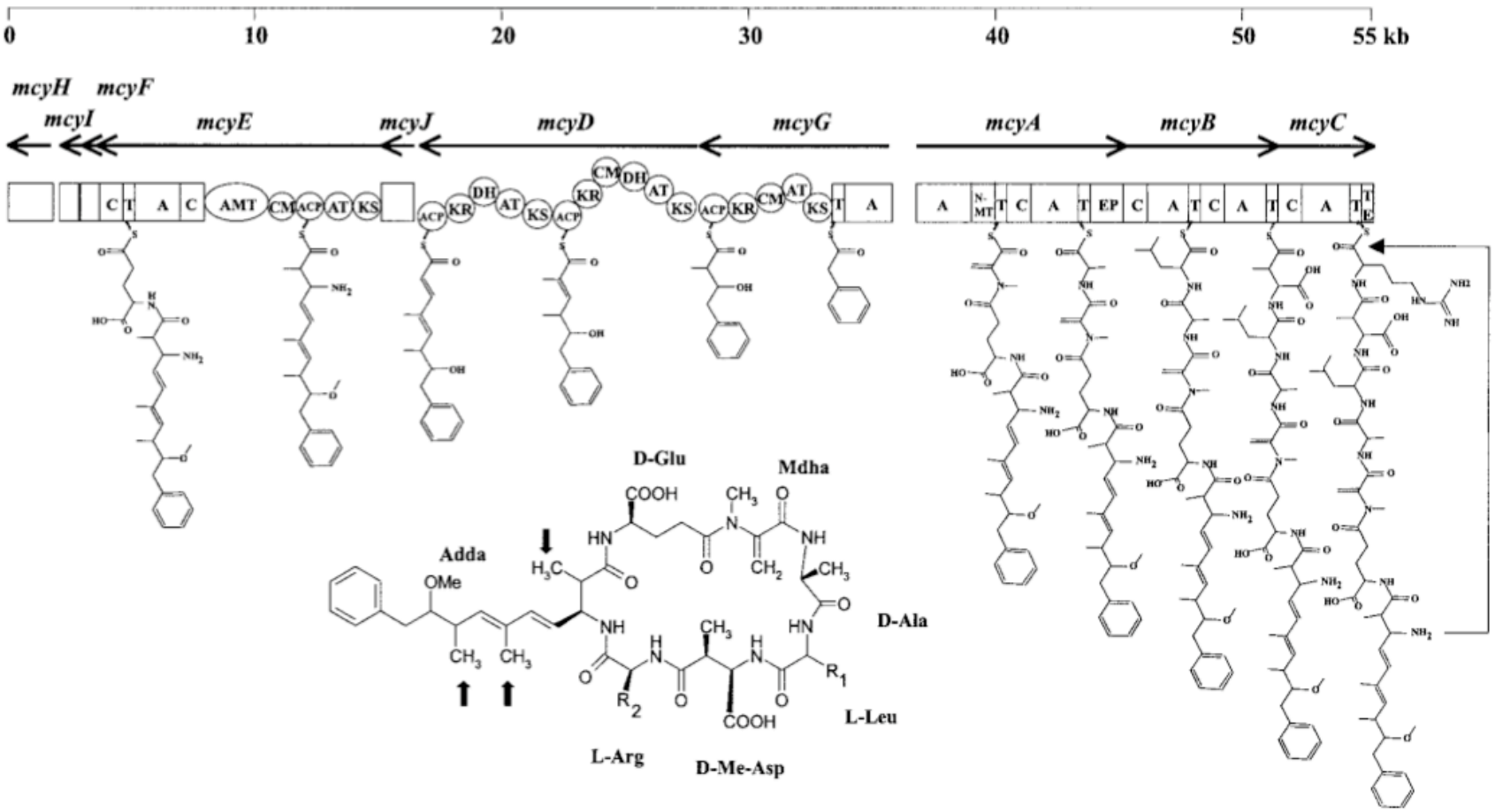

Figure I-1: Gene cluster of microcystin synthetase. Model for linear assembly of MC-LR. Domain abbreviations: A, adenylation; C, condensation; T, thiolation; NMT, N-methyltransferase; EP, epimerase; TE, thioesterase; KR, ketoacyl reductase. Three methyl groups indicated by thick arrows are putatively introduced by the CM domains. Cyclization of the microcystin precursor is indicated with an arrow on the right (Adapted from Rouhiainen et al. 2004).

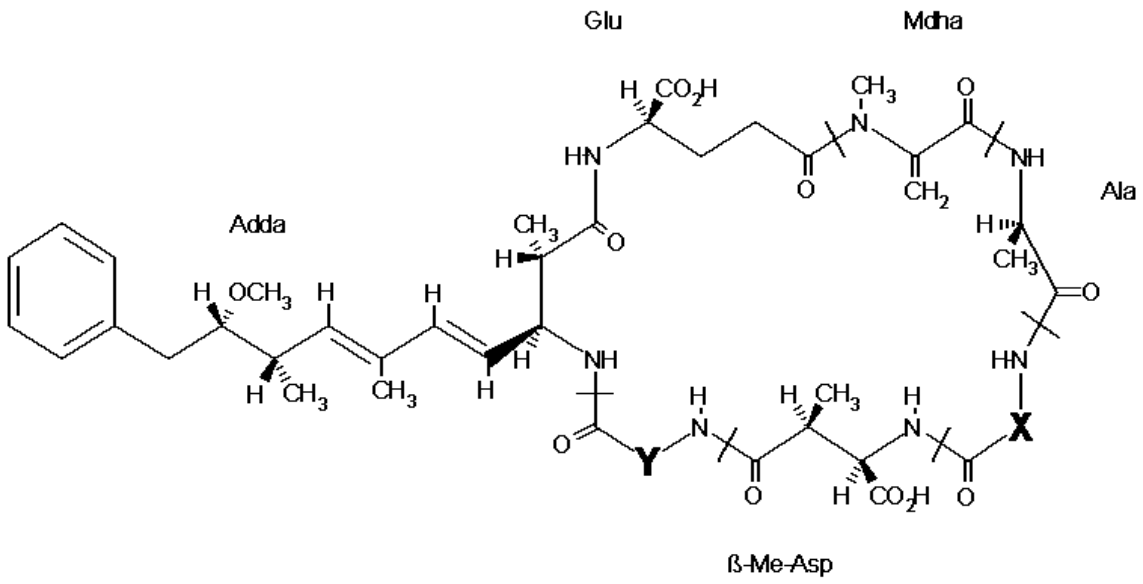

Figure I-2: General structure of the microcystin group of cyanotoxins. X and Y represent sites of amino acid substitution that give rise to different congeners. Other modifications have been observed throughout the structure.

Chapter II: Simultaneous analysis of microcystin congeners and nodularin in sediments and pore waters by optimized accelerated solvent extraction and rapid liquid chromatography-tandem mass spectrometry

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Abstract

The fate and persistence of microcystin cyanotoxins in aquatic ecosystems remains poorly understood in part because of a lack of analytical methods for microcystins in sediments. Existing analytical methods for microcystins in surface waters have had some success with sediments however, significant challenges remain. Notably, extraction methods have focused on only three to four congeners of similar chemistry and HPLC-UV analysis has proven unreliable due to selectivity issues inherent to working with sediments. Furthermore, these methods only consider extracellular microcystins yet toxic cyanobacterial cells are known to persist intact in lake sediments. We have developed the first analytical method for both intra- and extracellular microcystins in lake sediments, consisting of accelerated solvent extraction (ASE), hydrophilic-lipophilic balance solid phase extraction (HLB-SPE) and multiple reaction monitoring-based (MRM) HPLC-MS/MS quantitation. We detected and quantified nine chemically diverse microcystin congeners and nodularin (marine analogue) in sediments and pore waters at trace levels. Our ASE and HLB-SPE offered comparable recoveries for intra- and extracellular microcystins ranging between 75 and 98% with one, microcystin-RR (MC-RR), having a 50% recovery. The method detection limits achieved by MRM were 0.3 to 2.5 ng g^{-1} dry weight (dw). These detection limits are up to 50 times lower than previously reported and include important congeners not previously quantified. Chromatographic separation of ten analytes was achieved within 7.5 min, the fastest chromatographic separation of cyanotoxins in sediment extracts. The new methodology was applied to sediment cores collected from seven Canadian lakes of

diverse geography and trophic state. Microcystins were detected more than 40 cm deep in several cores and reached maximum concentrations of 830 ng g^{-1} dw in sediments and up to 130 ng mL^{-1} in sediment pore waters. These were the first measurements of cyanotoxins in sediment pore waters. MC-LR, -RR, and -LA were frequently observed while MC-YR, -LY, and -LW were less common. The analytical method enabled us to estimate distribution coefficients (Kd), which indicated that MC-RR had the greatest affinity for sediment particles while MC-LA had the lowest affinity and was preferentially distributed into pore waters. Our findings confirm that sediments serve as a reservoir for microcystins and indicate that some congeners may diffuse into overlying water thereby constituting a new route of exposure after toxic blooms have dissipated. The methodology can help determine microcystin fate and persistence in aquatic systems as well as the history of toxigenic blooms.

1.0 Introduction

Microcystins are a diverse family of over 70 cyclic heptapeptides produced by cyanobacteria in freshwaters (Zurawell et al. 2005). They are the most prevalent cyanotoxins and can reach very high concentrations in surface waters (Sivonen and Jones 1999). Microcystins can have negative health effects on animals and humans from both acute and chronic (sub-lethal) exposure leading most seriously to liver failure and possibly carcinogenesis (Kuiper-Goodman et al. 1999). While disproportionately higher levels of microcystins are found within cells and on suspended particles, dissolved microcystins are often detected during senescence of blooms (e.g. Gurbuz et al. 2009; Zastepa et al. 2013 in press). Several studies suggest that microcystins can deposit into surficial sediments by adsorbing to suspended particulates and cellular debris settling to the lake bottom (Rapala et al. 1994, Morris et al. 2000, Miller et al. 2001). These biogenic toxins also have the potential to deposit into sediments within intact cyanobacterial cells (Verspagen et al. 2005, Ihle et al. 2005, Welker et al. 2007, Wormer et al. 2011). Some groups have even reported intracellular microcystins in actively growing benthic cyanobacteria on surface sediments, particularly in shallow lakes (Mez et al. 1997, Izaguirre et al. 2007, Hitzfeld et al. 2000). Lake sediments may therefore constitute significant reservoirs of microcystins, posing long-term risks to surface waters.

Several studies have attempted to extract microcystin congeners from sediments by modifying traditional solvent-based methods (Babica et al. 2006, Chen et al. 2006). Computer models have predicted similar adsorption behaviour of a variety of congeners (UKWIR 1997, Newcombe et al. 2003) and consequently,

these methods have focused on only three to four extracellular (spiked) congeners of similar chemistry. However, increasing experimental and field evidence suggests significant differences in the chemical behaviour and environmental fate of different congeners particularly MC-LA (Newcombe et al., 2003, Miller et al. 2010, Zastepa et al. 2013 in press). Additionally, no existing extraction method has demonstrated recovery of microcystins from intact cyanobacterial cells within sediments. Extraction of microcystins even from seston and cultures using traditional solvent-based methods has been challenging, suggesting their application to recovering intracellular microcystins from within sediments could pose even greater challenges due to the inherent complexity of the sediment matrix. One study has even detected microcystins within still viable cyanobacterial colonies 35 cm deep in sediment cores, highlighting the need for an extraction method capable of rupturing cells within sediments (Latour et al. 2007). Quantitation of microcystin congeners in sediment extracts has also been very challenging. According to Schmidtkunz et al. (2009), RP-HPLC-UV analysis used by Babica et al. (2006) and Chen et al. (2006) was highly unreliable, especially at low concentrations.

This study reports the optimized extraction, enrichment, and analysis of a chemically diverse group of cyanotoxins from freshwater sediments. Our methodology separates pore waters from crude sediments to obtain semi-dry sediments before utilizing accelerated solvent extraction (ASE) to recover both intra- and extracellular microcystins. The separate analysis of pore waters affords the assessment of congener distribution while using semi-dry sediment in ASE avoids analyte recovery problems associated with sample drying. We reasoned that high

temperature and pressure conditions achievable in ASE would effectively lyse cyanobacterial cells within sediments, yielding high recoveries of both intra- and extracellular microcystins from sediments. We tested ASE with water in an effort to avoid the chemical modifications associated with acidified methanol extraction observed in previous studies (Schmidtkunz et al. 2009) and to streamline enrichment by solid phase extraction (SPE). Rapid separation by RP-HPLC and targeted multiple reaction monitoring-based (MRM) triple quadrupole mass spectrometry offered unambiguous identification and repeatable quantitation of cyanotoxins in chemically complex sediments at sub-parts-per-billion (ppb) levels. The optimized extraction and quantitation method was validated using sediment samples and their pore waters collected from seven Canadian lakes with different histories of toxic cyanobacteria. Furthermore, the analytical method enabled us to estimate distribution coefficients (K_d) and is well suited to determine the environmental fate and persistence of cyanotoxins in sediments.

2.0 Experimental

2.1 Cyanotoxin standards

Certified standards for ten cyanotoxins were obtained from Cedarlane and the National Research Council, Halifax, Canada. A standard mix of MC-LR, -^{7dm}LR, -RR, -YR, -WR, -LA, -LF, -LY, -LW, and nodularin (NOD) (Figure II-1) was prepared for the development of the LC-MS/MS separation and quantitation method as well as for spiking sediments in order to determine the efficacy and efficiency of various extraction and post-extraction procedures. Purity of each standard was reported as

> 99% (determined by flash chromatography followed by HPLC-UV). NOD is a marine analogue of microcystins and commonly used as an I.S.

2.2 Sediment sampling

Sediment cores were collected in duplicate from seven lakes in three Canadian provinces (Ontario, Alberta, and Quebec) using a modified gravity corer (Glew, 1989). Table II-1 lists the sampling lakes and their geographic coordinates, range of total phosphorus, sampling depth, and core length obtained. These lakes were chosen because historical monitoring indicated the presence of cyanobacterial blooms and/or cyanotoxins. Cores were extruded on site at 0.5 cm intervals for the length of the core, placed in pre-labeled Whirl-Pak® bags, and transported on ice in a dark cooler until placed in a freezer (–20°C in darkness) within an hour. Sediment material for recovery experiments (2 kg wet weight) was collected and pooled from a lake with a history of very low surface water concentrations of microcystins (average 0.08 μg L^{-1}) (Tillmanns et al. 2007) (Constance Lake, 0-10 cm homogenized).

2.3 HPLC-ESI-MS/MS analysis of cyanotoxins

Separations were performed on a 1200 series high performance liquid chromatograph (Agilent Technologies, Mississauga, ON, Canada) hyphenated with a 3200 QTRAP mass spectrometric system (AB Sciex, Concord, ON, Canada). Each analyte, dissolved at 10 μg mL^{-1} in LC-MS grade methanol, was infused at 10 μL min^{-1} into a Turbo V™ ion source in positive mode to determine optimal electrospray ionization conditions for each compound using Analyst® software

compound optimization wizard. Two MRM transitions were developed for each analyte by monitoring two major product ions. The transitions were merged into one method and a scouting gradient was performed (5-100% organic mobile phase) in 10 min at a flow rate of 0.3 mL min^{-1}. Source conditions were then optimized at isocratic mobile phase composition by T-infusion of each analyte at 10 µL min^{-1}. The optimized source conditions were: source temperature 350°C, voltage +4,500 V, curtain gas (N_2) pressure 20 psi and the collision-activated dissociation gas (N_2) pressure 60 psi. Notably, the declustering potential was re-optimized for each analyte at final mobile phase composition to attain optimal signal.

The tubing of the HPLC system was changed from 0.17 mm I.D. to 0.13 mm I.D. for a UPLC column (Zorbax SB-C18 HT 50 mm x 2.1 mm I.D., particle size 1.8 µm) connected with a guard column (Zorbax SB-C8, 12.5 mm x 2.1 mm I.D., particle size 5.0 µm) to improve resolution, speed, and signal to noise. The column thermostat temperature was maintained at 40°C. Injection volumes were either 1 or 10 µL. Separation was carried out at a flow rate of 0.3 mL min^{-1} with a solvent gradient from an initial composition of 90% water and 10% acetonitrile to 100% acetonitrile in 6 min after which it was held at 100% acetonitrile for 1 minute. Starting conditions were re-established over an additional 3 min. Both water and acetonitrile (LC-MS grade, J.T. Baker) were supplemented with 40 mM formic acid and 2 mM ammonium formate and sonicated before use (10 min L^{-1}). The system back-pressure was around 250 ± 5 bars after 15 min equilibration at the starting conditions. Analyst® software (version 1.4.1) was used for data acquisition.

Quantitation was based on area under the peak with an acceptable peak symmetry and peak width.

2.4 Preparation of crude sediments

Prior to freezing, crude sediment aliquots of 10 g were centrifuged (1200 x *g* at 4°C for 25 min) to separate pore water, leaving 'semi-dry' sediments for ASE (half the weight of the initial crude sediments) (Figure II-2). To determine recoveries, semi-dry sediments were spiked with the standard mix (0.5 µg of each cyanotoxin unless otherwise noted) and equilibrated at 4°C for 4 hrs prior to ASE. These incubation conditions are appropriate to simulate adsorption to sediments (Chen et. al 2006). In order to report microcystin concentrations normalized to gram dry weight (dw), water content was measured in the crude sediment aliquots (i.e. prior to separation of pore water) by lyophilization of a 0.5 g sub-sample. Water content was on average 90% by weight resulting in a spiked cyanotoxin concentration of 0.5 µg g^{-1} dw. This was lower than typically used in the assessment of microcystin recoveries from sediments (Chen et al., 2006; Babica et al., 2006).

2.5 ASE of cyanotoxins from semi-dry sediments

Extraction conditions originally developed for cyanotoxins in culture were used as a guide for ASE of cyanotoxins from sediments (Aranda-Rodriguez et al. 2005). Each stainless-steel extraction cell (33 mL capacity) was lined with a cellulose filter and packed with sediment mixed in with Hydromatrix™ (flux-calcined diatomaceous earth from Varian, treated with petroleum ether). Automated

extraction (two cycles) was performed using 75% methanol (25% water) (HPLC grade, J.T. Baker) with an ASE 200 system at 1900 psi and 80°C (Dionex, distributed by Thermo Scientific, Oakville, Ontario, Canada). Each cycle included a pre-heating (1 min), heating (5 min), and static (5 min) phase. Although 75% methanol has been shown to be one of the best solvents for efficient extraction of microcystins from seston (Fastner et al. 1998; Barco et al. 2005; Aranda-Rodriguez et al. 2005), it has not been tested on sediments. ASE using water was also evaluated.

Sediment extracts from ASE (40 mL) were collected in amber glass vials and immediately vacuum filtered (0.7 µm, 25 mm GF/F) to remove precipitating matrix components (Figure II-2). To concentrate, extracts in 75% methanol were dried by nitrogen- and heat-assisted evaporation in a TurboVap LV (Zymark Corp.) while extracts in water were lyophilized. Resultant residue was reconstituted with 2 mL of 50% methanol (50% water) and centrifuged (13000 x *g* at 4°C for 10 min) to pellet and remove precipitates. The concentration-centrifugation cycle was repeated using the supernatant to give a final volume of 0.2 mL 50% methanol. The final supernatant was syringe filtered (0.45 µm, 4 mm LH-PTFE, Millipore, Montreal, Quebec, Canda) to remove remaining particulates and stored at −20°C. In order to identify loss of cyanotoxins on adsorption to precipitating matrix components during post-ASE clean up and concentration, blank sediment extracts (40 mL) were spiked with standard mix (0.5 µg of each cyanotoxin) and loss was monitored on removal during vacuum filtration, concentration-centrifugation, and syringe filtration (Figure II-2).

2.6 Effect of ASE solvent choice and post-ASE SPE on recovery

The effect of SPE on the recovery of cyanotoxins from ASE extracts was evaluated (Figure II-2). As described previously, crude sediment samples were prepared as semi-dry sediment, spiked with standard mix (0.5 µg of each cyanotoxin), and extracted by ASE with 75% methanol or water. After ASE and subsequent vacuum filtration, sediment extracts (40 mL) were applied to SPE cartridges (500 mg / 6 mL, 55 µm Strata® C18, Phenomenex) before being subjected to the two concentration-centrifugation cycles (Figure II-2). Prior to application of extracts, the C18-SPE cartridges were conditioned with two volumes of methanol followed by two volumes of water according to manufacturer's directions. Once extracts were applied, the cartridges were washed with two volumes of 5% methanol (95% water) and eluted with 6 mL methanol.

A screen of other sorbent chemistries with standard mix revealed that a hydrophilic-lipophilic balance (HLB) cartridge (500 mg / 6 mL, 33 µm, Strata®-X, Phenomenex) was also suitable (data not shown). HLB-SPE enables the use of stronger wash (> 5% methanol) to remove matrix components co-extracted during ASE while maintaining analyte recovery. Therefore, recovery of cyanotoxins from sediment extracts using HLB-SPE with wash strengths of 5, 10, 20, 30, and 40% methanol were evaluated.

2.7 Recovery of intracellular cyanotoxins from semi-dry sediments by optimized ASE

To test the efficacy of the optimized analytical method to extract intracellular microcystins from sediments, a 1 mL sub-sample of *Microcystis aeruginosa* culture (CPCC 702) known to produce MC-LR, -^{7dm}LR, -YR, -RR, and -WR was used to spike crude sediments. To remove any extracellular microcystins, the culture sub-sample was centrifuged and the supernatant was removed. The pellet was reconstituted in 1 mL of water (HPLC grade, J.T. Baker). Prior to use, the cultures were grown for four weeks under 25°C with 12-hour light and dark cycles at 48 ± 3 µE (µmol m^{-2} s^{-1}) light of fluorescent and incandescent source (6:2). The percent recovery of intracellular microcystins from sediments was relative to the amount recovered in parallel ASE of 1 mL of culture alone. Intracellular microcystin recovery from cultures alone by ASE under optimized conditions is typically > 95% (Aranda-Rodriguez et al., 2005).

2.8 Recovery of cyanotoxins from dry sediments by optimized ASE

Since existing extraction methods used completely dry sediments, the optimized ASE-based method was tested using lyophilized sediments, 'dry sediments', and compared to centrifuged sediments, 'semi-dry sediments'.

2.9 Distribution of cyanotoxins between sediment particles and pore water

Crude sediment aliquots of 10 g were spiked with standard mix (1.0 µg of each cyanotoxin), homogenized by vortexing for 30 sec, and incubated for four hrs

at 4°C. Crude sediments were then centrifuged (2400 rpm at 4°C for 25 min) to separate pore water, leaving semi-dry sediments (half the weight of the initial crude sediments). Semi-dry sediments were subjected to the optimized ASE-based method (section 3.2) before microcystin analysis by HPLC-ESI-MS/MS. The pore water was vacuum filtered through a 0.7 μm, 25 mm GF/F filter and concentrated by lyophilization after which the resultant residue was reconstituted in 2 mL of 50% methanol (50% water) (Figure II-2). Following high-speed centrifugation (13000 x *g* at 4°C for 10 min), the supernatant was further concentrated by nitrogen- and heat-assisted evaporation and reconstituted in 0.05 mL of 50% methanol. Following a final centrifugation, the supernatant was syringe filtered (0.45 μm, 4 mm LH-PTFE, Millipore) and stored at −20°C until analysis by HPLC-ESI-MS/MS. Recoveries from pore water were > 95% for all microcystins.

2.10 Data analysis

Significant differences in recoveries were determined using Student's *t*-test analysis at $p < 0.05$.

3.0 Results and discussion

Figure II-2 summarizes the optimization of ASE-based extraction and clean up for trace level analysis of intracellular and extracellular cyanotoxins from lake sediment cores.

3.1 HPLC-ESI-MS/MS analysis of cyanotoxins

Using a RP-UPLC column and tandem mass spectroscopy, a highly selective analytical method was developed to monitor optimization of ASE-based recovery of cyanotoxins from sediments. Optimized retention times, MRM transitions, and compound-dependent source conditions are reported in Table II-2. Our SPE-cleaned sediment extracts afforded the use of a sub-two micron UPLC column and increased throughput to accommodate the large number of samples from method development and survey lakes (long and high resolution sediment cores). By the reduction of the internal diameter of HPLC tubing and the optimization of solvent gradient, flow rate, and column thermostat temperature, we developed the fastest chromatographic separation of cyanotoxins in sediment extracts. Rapid separation of 10 cyanotoxins was accomplished within 7.5 min. Due to its simplicity the method can be easily transferred to a UPLC system with potential to perform separation at least three times faster (Leandro et al. 2006).

Compound-dependent source conditions were optimized separately for two MRM transitions of each cyanotoxin by infusion into the mass spectrometer. The highest intensity MRM transition for each cyanotoxin (Table II-2) was used for quantitation while the second highest transition (not listed) was used as an identity confirmation. Figure II-3 shows MRM signals of a standard mix. Instrument detection limits were between 0.01 and 0.08 ng on column for all cyanotoxins tested and the instrument linear range was up to 20 ng on column ($R^2 > 0.997$) (Table II-3). The method detection limits (MDLs) in sediments ranged between 0.3 and 0.6 ng g^{-1} dw (parts per billion) for all cyanotoxins with the exception of MC-RR (2.5 ng g^{-1} dw)

(Table II-3). These MDLs are up to 50 times lower than reported by others and include important microcystin congeners not previously quantified (Babica et al., 2006, Chen et al., 2006, Efting et al., 2011, Rinta-Kanto et al. 2009). Our MDLs in sediment pore water ranged between 0.02 and 0.16 ng mL^{-1} and are currently the only estimates available. Relative standard deviations (RSDs) were less than 5% for standard mix injections and less than 15% for sediment sample injections (Table II-3). Some have used HPLC-UV in an attempt to determine microcystin congeners in sediment extracts (Chen et al. 2006, Babica et al. 2006) however, a detailed investigation by Schmidtkunz et al. (2009) revealed poor detection limits and identification and quantitation problems from co-eluting matrix components. Schmidtkunz et al. (2009) went on to demonstrate that even clean up by size exclusion chromatography was insufficient to obtain unambiguous identification by HPLC-UV. Therefore, to achieve unambiguous identification and repeatable quantitation of cyanotoxins in chemically complex sediments at trace levels, rapid separation by RP-HPLC and targeted MRM-based triple quadrupole mass spectrometry is more suitable. Two recent investigations have employed mass spectrometry in the analysis of microcystins in sediments but have focused on only three to four congeners (Rinta-Kanto et al. 2009, Efting et al. 2011).

3.2 Recovery of cyanotoxins from semi-dry sediments by optimized ASE

Preliminary ASE of cyanotoxins from semi-dry sediments with 75% methanol or pure water resulted in low recoveries between 10 and 50%. It was suspected that poor recoveries were due to the adsorption of cyanotoxins to precipitating matrix

components during post-ASE processing (Figure II-2, no SPE). To assess this, extracts from ASE of blank sediments (n = 4) were spiked with standard mix (0.5 ug of each cyanotoxin) and analyte loss was monitored on removal of precipitates during vacuum filtration, concentration-centrifugation, and syringe filtration (Figure II-2 and Figure II-4). We observed that 90 to 100% of cyanotoxins remained in the extract after vacuum filtration, dropping to 15 to 50% after the subsequent concentration-centrifugation cycle, and 10 to 50% remained after subsequent syringe filtration. Reduction in extract volume enhanced the formation of precipitates and their encounter with cyanotoxins, leading to the high losses observed during subsequent centrifugation and syringe filtration. Notably, MC-RR was the most difficult to recover with only 10% remaining after the three post-ASE steps.

We reasoned that incorporating RP-SPE after vacuum filtration (Figure II-2) would reduce the downstream matrix precipitates that led to the significant losses observed during concentration-centrifugation and syringe filtration (Figure II-4). Indeed recovery of all cyanotoxins from semi-dry sediments significantly improved (three times better on average) when C18-SPE was included post-ASE (Figure II-5). Most dramatically, the recovery of MC-RR improved by about four times when ASE was with water and six times when ASE was with 75% methanol. Furthermore, lower RSDs were observed when SPE was included indicating improved repeatability. In general, we found little difference in using 75% methanol or water in ASE of a wide range of microcystin congeners from sediment (Figure II-5). In extraction of microcystins from bloom or culture samples, 75% methanol has been the most suitable solvent for both polar and non-polar microcystin congeners due to

its amphiphilic properties. However, our findings indicated that water can be equally effective for the extraction of a wide chemical range of microcystins from sediments (Figure II-5). ASE with water is most suitable in our methodology as it allows direct application to RP-C18-SPE, which requires sample application in aqueous mobile phase. Others have tried using acidified methanol to extract microcystins from sediments however, Schmidtkunz et al. (2009) observed the esterification of microcystins with this commonly used solvent. Our MRM transitions with predominant formation of Q3 product ion at *m/z* 135 propose that these chemical modifications could be mistaken for naturally occurring microcystin congeners. The use of pure water as the extraction solvent precludes this and other types of chemical modifications associated with acidified solvents. Therefore, water was the better choice overall with added benefits of being non-hazardous and more economical than traditional solvents.

In an effort to further improve recovery and reduce matrix effects, HLB-SPE was tested as an alternative to C18-SPE. We reasoned that the hydrophilic N-vinyl pyrrolidone and lipophilic divinyl benzene polymeric sorbent will enable the recovery of a wider range of microcystin chemistries (i.e. polar and non-polar) while using a more aggressive wash (>5% methanol). Indeed, the HLB-SPE showed between 5 and 15% improvement in recovery of the cyanotoxins with a wash strength of 20% methanol (Figure II-6). Additionally, extracts were visibly cleaner and HLB-SPE required lower elution volumes to achieve the improved recovery (based on analysis of three separate 2 mL elution fractions, data not shown).

Optimization of the ASE-based methodology (Figure II-2) demonstrated that the highest recovery of cyanotoxins from semi-dry sediments was achieved when water was used in ASE and HLB sorbent was used for SPE with a 20% methanol wash followed by elution with 4 mL of methanol. Optimal recoveries of cyanotoxins ranged from 75% to 98%, with the exception of MC-RR, which was consistently the most difficult to recover (approximately 50%) (Figure II-6). Both MC-LA and -WR had a recovery of approximately 75% while NOD and MC-YR were markedly better at 80 and 90%. MC-LW and -LY were above 90% with MC-LF, -LR, and -^{7dm}LR above 95%.

3.3 Recovery of intracellular cyanotoxins from semi-dry sediments by optimized ASE

Recovery of intracellular cyanotoxins from sediments has been overlooked by previous analytical methods (Babica et al., 2006, Chen et al., 2006). However, in addition to intracellular cyanotoxins being deposited into sediments during bloom senescence, lake mixing, and the over-wintering phase of benthic species, toxigenic colonies with good cell integrity may be settling constantly during blooms (Welker et al. 2007, Verspagen et al. 2005, Ihle et al. 2005, Wormer et al. 2011). Several studies have also found production of microcystins by permanently benthic species (Mez et al. 1997, Izaguirre et al. 2007, Hitzfeld et al. 2000). Finally, intact cells containing the cyanotoxin MC-LR have even been found in deeper sediments (35 cm) at levels exceeding what was observed in summer planktonic colonies (pg cell^{-1}) (Latour et al. 2007). Clearly, analytical method development and

validation for intracellular cyanotoxins is of major importance particularly within complex matrices such as sediments where lysing cells may be especially challenging. Our optimized ASE-based methodology was effective in extracting intracellular cyanotoxins from sediments and therefore provides a major advantage over currently available methods. Intracellular MC-LR, -^{7dm}LR, -YR, -RR, and -WR congeners were recovered with 85, 87, 80, 50, and 70 % efficiency from *Microcystis aeruginosa* cultures spiked into semi-dry sediments. These intracellular extraction efficiencies were comparable to extracellular extraction efficiencies of corresponding congeners.

3.4 Recovery of cyanotoxins from dry sediment by optimized ASE

Existing analytical methods prepare sediment samples by lyophilizing whole sediments (includes pore water) before extraction (Chen et al. 2006, Babica et al. 2006, Smith and Boyer 2009, Efting et al. 2011). We used a different approach, separating pore water from sediment by centrifugation to yield semi-dry sediments for ASE and analyzing each fraction separately. For comparison, the efficacy of our optimized ASE-base methodology was evaluated using dry (lyophilized) sediments. Although successful, we observed that ASE of cyanotoxins from dry sediments was significantly less efficient than from semi-dry sediments (Figure II-7). This suggests that there are additional losses when sediment is completely dried by lyophilization before extraction. It is thought that compounds can sorb onto sediment particles during the drying process and become more difficult to extract (Schmidtkunz and Welsch 2009). Indeed, extraction difficulties have been noted in previous attempts

to recover microcystins from dry sediments (Harada et al. 1998, Babica et al. 2006). Water was again comparable to 75% methanol in extracting cyanotoxins from dry or semi-dry sediments (Figure II-7).

3.5 Cyanotoxins in freshwater sediments

The optimized analytical method was used to quantify cyanotoxins in the sediments of seven Canadian lakes, varying in their geographic location and trophic status (Table II-1). Five of the seven lakes tested were positive for microcystins in surficial sediments and concentrations ranged from 0.4 to over 800 ng g^{-1} dw with MC-LR and -LA being the most frequently detected congeners (Table II-4). Microcystins were also detected deeper in the sediment cores of four of the lakes including MC-LA and -LR in a 40 cm layer from a Baptiste Lake core. The pore water also contained measurable levels of microcystins with MC-LA disproportionately distributing into the pore water. These were the first measurements of cyanotoxins in sediment pore waters. Extracted ion chromatograms of sediment and pore water extracts from different depths of the Lake of the Woods core are presented in Figure II-8.

3.6 Distribution of cyanotoxins between sediment particles and pore water

The majority of microcystins remained adsorbed to sediment particles in laboratory experiments with only approximately one third overall distributing into pore water (Figure II-9). This affinity for sediment particles helps explain the prevalence of microcystins in lake sediment samples (Table II-4). Unlike other

microcystins under study, MC-LA preferentially distributed into pore water indicating its potential to diffuse through sediment layers and into the water column. Lake sediments may therefore constitute significant reservoirs of microcystins, posing long-term exposure risks to surface waters. The potential diffusion of microcystins through sediment underscores the importance of our preparative approach of separating pore water when reporting microcystin concentrations in sediments. This approach can then be used to assess exposure risks to surface waters by estimating the accumulation rate and diffusive flux of microcystin congeners in lake sediments.

Using sediment-pore water distribution measurements (Figure II-9), we estimated distribution coefficients for each congener (Table II-5). Overall, they indicated moderate adsorption of microcystins to sediments (pH ~6.6) with the exception of MC-RR, which showed strong affinity for sediments. With the exception of MC-RR, this was consistent with conclusions reached from experimentally-derived octanol-water and soil-water distribution coefficients for MC-LR (De Maagd et al. 1999, Miller et al. 2001, Miller et al. 2005). Based on estimates of distribution coefficients, MC-LA had the lowest adsorption to sediments followed by -LF and -LY, -LW and NOD, -LR and -^{7dm}LR, -YR and -WR, while MC-RR had the highest adsorption. Our distribution data is in agreement with our recovery data, congeners with higher recovery predominantly distribute into pore water while those with lower recovery tend to adsorb to sediments. This strong binding could be the major reason for the lower recovery of some congeners (e.g. MC-RR). Consistent with our experimental results, Newcombe et al. (2003) observed relatively low adsorption of

MC-LA to granular activated carbon. Furthermore, Harada et al. (1998) observed that more hydrophilic microcystins such as MC-RR absorbed strongly to sediments. In contrast to our experimental estimates, predicted partition and distribution coefficients (also Table II-5) have suggested MC-RR and -YR should have had low adsorption while MC-LA should have adsorbed strongly to sediments due to its relative hydrophobicity and low molecular weight. Newcombe et al. (2003) has suggested the discrepancy between predicted and experimental estimates is likely due to the significant influence of electrostatic interactions. It is apparent that predictions of distribution coefficients for some microcystin congeners can be dramatically different than experimental estimates. Therefore, caution must be exercised when using predicted values to gain insight into environmental fate and persistence of microcystin congeners.

With over 70 known microcystin congeners (Zurawell et al. 2005), we have developed an analytical method for nine microcystins as well as NOD. These nine microcystins were chosen to represent the chemical diversity present in this group of hepatotoxic cyanotoxins. Many of the remaining microcystin congeners have minor chemical modifications such as the addition/removal of short alkyl groups (e.g. methyl, methylene, aminoisobutyric acid) and methyl ethers. These modifications are not expected to pose difficulties in the analytical method nor have dramatic differences in environmental fate and persistence compared to congeners in this study. Many others have substitutions of related amino acids such as MC-AR, -AW, and -VF, where alanine or valine was substituted for leucine in MC-LR, -LW, and -LF or MC-FR, where phenylalanine was substituted for tryptophan or tyrosine

in MC-WR and -YR. However, there remain a few congeners that may have dramatically different environmental fate and persistence than those studied. Notably, the presence of methysulfanyl groups in microcystins with methionine and methionine S-oxide substituents as well as additional carboxyl groups in microcystins with glutamic acid substituents (negatively charged at environmental pH) suggests dramatically different environmental fate and persistence. However, the lack of commercial availability of these congeners remains a significant challenge to their investigation.

4.0 Conclusions

As a first step, a rapid MRM-based LC-MS/MS method was developed to monitor the development and optimization of ASE and SPE for the recovery of nine microcystin congeners and NOD from lake sediments. The analytical method enabled us to identify and quantify ten cyanotoxins in surficial and deep sediments from seven very different Canadian lakes. Our preparative approach enabled the first measurements of cyanotoxins in pore water and highlights the need to assess the risk of cyanotoxin diffusion into water overlying sediments. Our estimates of distribution coefficients demonstrate differences in pore water distribution of congeners and highlight the advantage of our multi-toxin analytical method. Furthermore, the elevated temperature and pressure feasible under ASE addresses the pressing need for an analytical method capable of recovering both intra- and extracellular cyanotoxins from sediments. High recoveries were achieved using water in ASE, which allowed streamlined enrichment with HLB-SPE and avoided

methyl ester chemical modifications observed during extraction with the commonly used acidified methanol. The optimized and validated analytical method is well suited to provide valuable insight on many other aspects of cyanotoxin fate and persistence in sediments including in the estimation of diffusive flux, degradation kinetics, and as a paleolimnological tool to investigate past toxigenic blooms.

Tables and figures

Table II-1: Sampling lakes and their geographic coordinates, range of total phosphorus, sampling depth, and core length obtained. Range of total phosphorus (TP) measurements in the lakes over a number of years as an indication of trophic state.

	Latitude Longitude	TP (μg/L)	Coring Depth (m)	Core length (cm)
Lakeland Estates, ON	45° 14' 30.8" N 75° 34' 57.0' W	14 - 59 (3 years)	4	10
Lake Tomcod, QC	45° 32' 18.9" N 72° 2' 23.1" W	58 - 73 (3 years)	1.8	40
Lake Baptiste, AB	54° 44' 19.7" N 113° 33' 7.4" W	43 - 85 (7 years)	26.5	46
Lake of the Woods, ON (Hay Island)	49° 9' 25.6" N 94° 7' 23.2" W	38 - 67 (2 year)	13	38.5
Constance Lake, ON	45° 24' 34.8" N 75° 58' 45.8" W	16 - 38 (5 years)	3.5	40
Lake Ontario, Bay of Quinte inlet	44° 5' 15.3" N 76° 48' 49.2" W	Not analyzed	>12	10
Lac Waterloo, QC	45° 20' 5.4" N 72° 31' 8.2" W	20 - 48 (2 years)	4	35

Table II-2: Optimized retention times, multiple reaction monitoring (MRM) transitions, and source conditions for the detection and quantitation of cyanotoxins in sediments.

	LR	**7dmLR**	**RR**	**YR**	**WR**	**LA**	**LF**	**LY**	**LW**	**NOD**
Retention (min)	6.0	6.1	5.6	5.9	6.2	6.6	7.1	6.7	6.9	5.8
MRM transition[a]	995.6/ 135.1	981.4/ 135.1	520.1/ 135.1	1045.4/ 135.1	1068.4/ 135.1	910.6/ 135.1	986.6/ 135.1	1002.6/ 135.1	1025.6/ 135.1	825.5/ 135.1
DP (V)	70	70	70	70	131	70	70	70	70	70
EP (V)	10	10	10	10	11.5	10	10	10	10	10
CEP (V)	38.02	37.62	24.71	39.41	60	35.64	37.77	38.22	38.86	33.26
CE (V)	85	85	65	115	95	115	50	50	50	115
CXP (V)	3	3	3	3	4	3	3	3	3	3

a: dwell time for each transition was 20 ms

DP: declustering potential; EP: entrance potential; CEP: collision cell entrance potential; CE: collision energy; CXP: collision cell exit potential

Table II-3: Performance of the developed analytical methodology for the detection and quantitation of cyanotoxins in sediments and pore waters.

	LR	7dmLR	RR	YR	WR	LA	LF	LY	LW	NOD
ILR (ng on column)[a]	0.02-20	0.02-20	0.03-20	0.02-20	0.06-20	0.02-20	0.04-20	0.08-20	0.07-20	0.01-20
ILOQ (ng on column)[b]	0.04	0.04	0.06	0.04	0.12	0.04	0.08	0.16	0.14	0.02
Slope (area ng^{-1} mL^{-1})[c]	2.2	3.15	8.66	2.08	5.22	4.56	2.93	1.76	2.51	11.2
Linearity (R^2)[c]	0.997	0.997	0.997	0.997	0.995	0.998	0.997	0.998	0.998	0.998
Sediment MDL (ng g^{-1} dw)[d]	0.3	0.3	2.5	0.3	0.6	0.3	0.5	0.5	0.6	0.3
Pore water MDL (ng mL^{-1})[d]	0.04	0.04	0.06	0.04	0.12	0.04	0.08	0.16	0.14	0.02

a: Instrument linear range. Triplicate injections at three concentration levels gave RSDs of less than 5%.

b: Instrument limit of quantitation based on twice the instrument limit of detection.

c: Slope and linearity of LC-MRM response based on six concentrations levels within instrument linear range.

d: Method detection limit (MDL) = standard deviation $\times$ t (n = 7, α = 0.01), where t = 3.14. Standard deviation was determined by analyzing extracts of semi-dry sediment spiked to 5 ng g^{-1} dry weight (dw) and pore water spiked to 0.5 ng mL^{-1}. Triplicate injections of extracts gave RSDs of less than 15%.

Table II-4: Concentrations of microcystin congeners in different sediment layers in sediment cores collected from lakes.

	Interval Depth (cm)	Sediment (ng g^{-1} dw)	Pore water (ng mL^{-1})
Lakeland Estates, ON	0 - 2.0	LA: 20.0, RR: 10.0	Not measured
Lake Tomcod, QC	0.5 - 1.0	LR: 343.1, RR: 34.6, YR: 33.5	LR: 4.6, RR: 0.5
	2.0 - 2.5	LR: 37.8	<MDL[a]
Lake Baptiste, AB	0 - 0.5	LR: 378.1, LA: 356	LA: 131.9, LR: 72.9, LY: 1.9
	41 - 41.5	LA: 4.2, LR: 1.9	LA: 1.0
Lake of the Woods, ON (Hay Island)	0 - 0.5	LR: 254.9, LA: 19.6	LA: 1.6, LR: 1.0, LW: 0.8
	30 - 30.5	LW: 1.2, LA: 1.0, LR: 0.8, YR: 0.7, LF: 0.7	<MDL
Constance Lake, ON	0 - 0.5	<MDL	<MDL
	39.5 - 40	<MDL	<MDL
Lake Ontario, Bay of Quinte inlet	0 - 0.5	<MDL	<MDL
	7.5 - 10	<MDL	<MDL
Lac Waterloo, QC	0 - 0.5	RR: 829.1	RR: 20.0, LR: 6.4
	2 - 3	RR: 211.8	LR: 38.9, RR: 12.8, YR: 2.7

a: Below method detection limit (see Table II-3 for method detection limit)

Table II-5: Experimentally calculated distribution coefficients (Log K_d) based on sediment-pore water distribution data (**Figure II-9**) compared with predicted partition (Log P) and distribution (Log D) coefficients based on octanol-water distribution data[a].

	LR	7dmLR	RR	YR	WR	LA	LF	LY	LW	NOD
Log K_d (pH 6.3-6.8)	+0.4	+0.4	+1.3	+0.5	+0.5	-0.4	+0.1	+0.1	+0.2	+0.2
Log P[a]	-1.4	ND	+1.3, -4.0	-1.9	ND	ND	ND	+4.5	ND	+1.5
	-1.4	ND	-5.0	-1.3	ND	ND	ND	+2.8	ND	-1.2
	+2.3	ND	-0.2	+2.2	ND	ND	+5.1	+4.7	ND	+1.7
	-1.7	ND	-0.1	-0.8	-1.4	1.3	ND	ND	ND	ND
Log D[a]	-4.9	ND	-1.2, -6.5	-5.4	ND	ND	ND	-0.2	ND	-2.0
(pH 7.4)	-3.8	ND	-3.6[b]	-3.6	ND	ND	ND	-3.4	ND	-3.6

K_d: Calculated as the ratio of analyte adsorbed to sediment (pH 6.8) and that distributed into pore water (pH 6.3) fraction (section 2.9).

a: Each row represents predictions obtained by algorithms from three different sources: chemspider.com (first), chemicalize.org (second), phenomenex.com (third), and UKWIR 1997 (fourth). chemspider.com uses ACD/Log P algorithms, which uses the principle of isolating carbons. Well-characterized log P contributions have been compiled for atoms, structural fragments, and intramolecular interactions derived from >12,000 experimental log P values. A secondary algorithm is applied when unknown fragments are presented. A detailed description of the original algorithm may be found in Petrauskas and Kolovanov (2000). Details of algorithms used by chemicalize.org and phenomenex.com were not readily available.

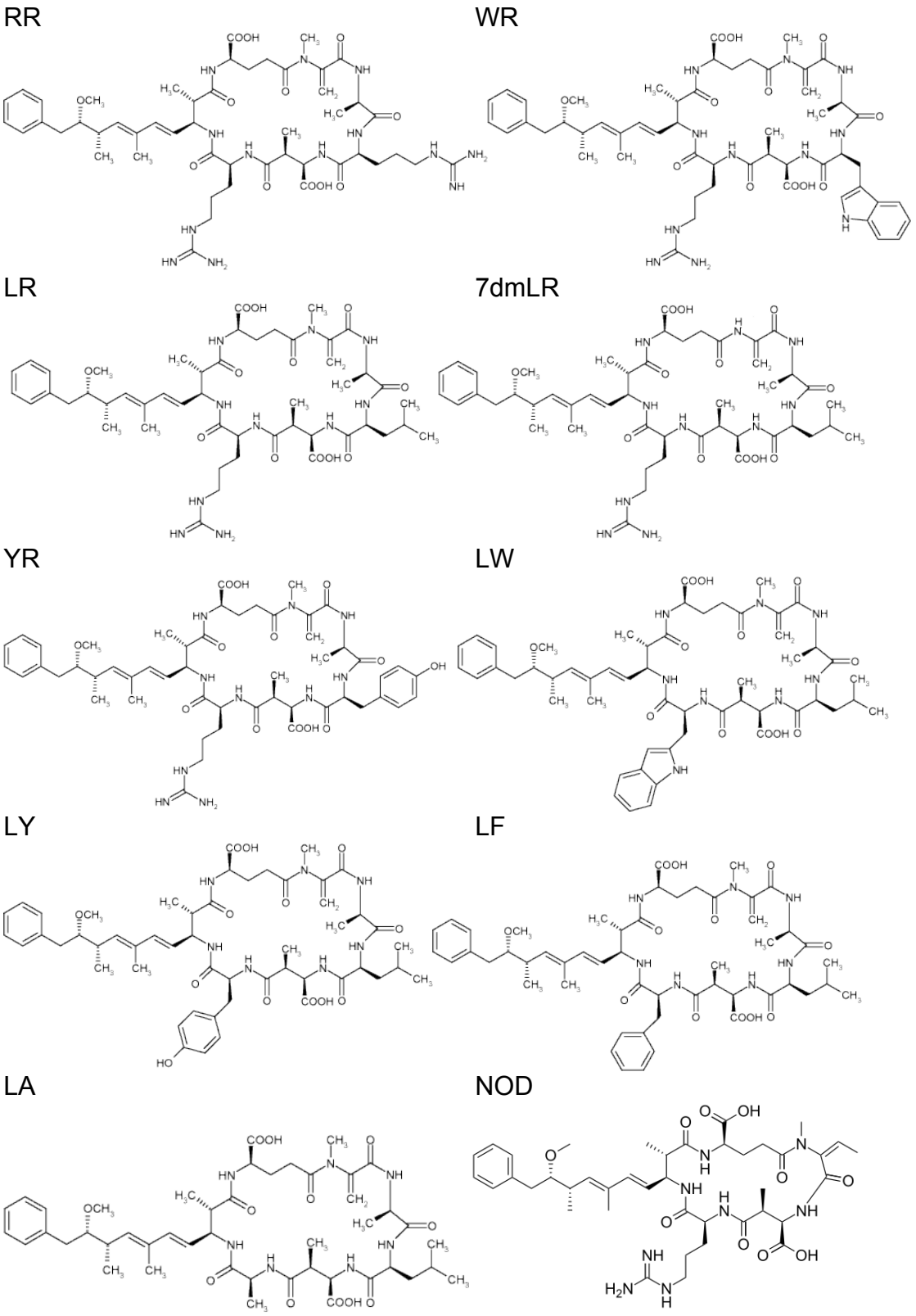


Figure II-1. Chemical structures of cyanotoxins under study.

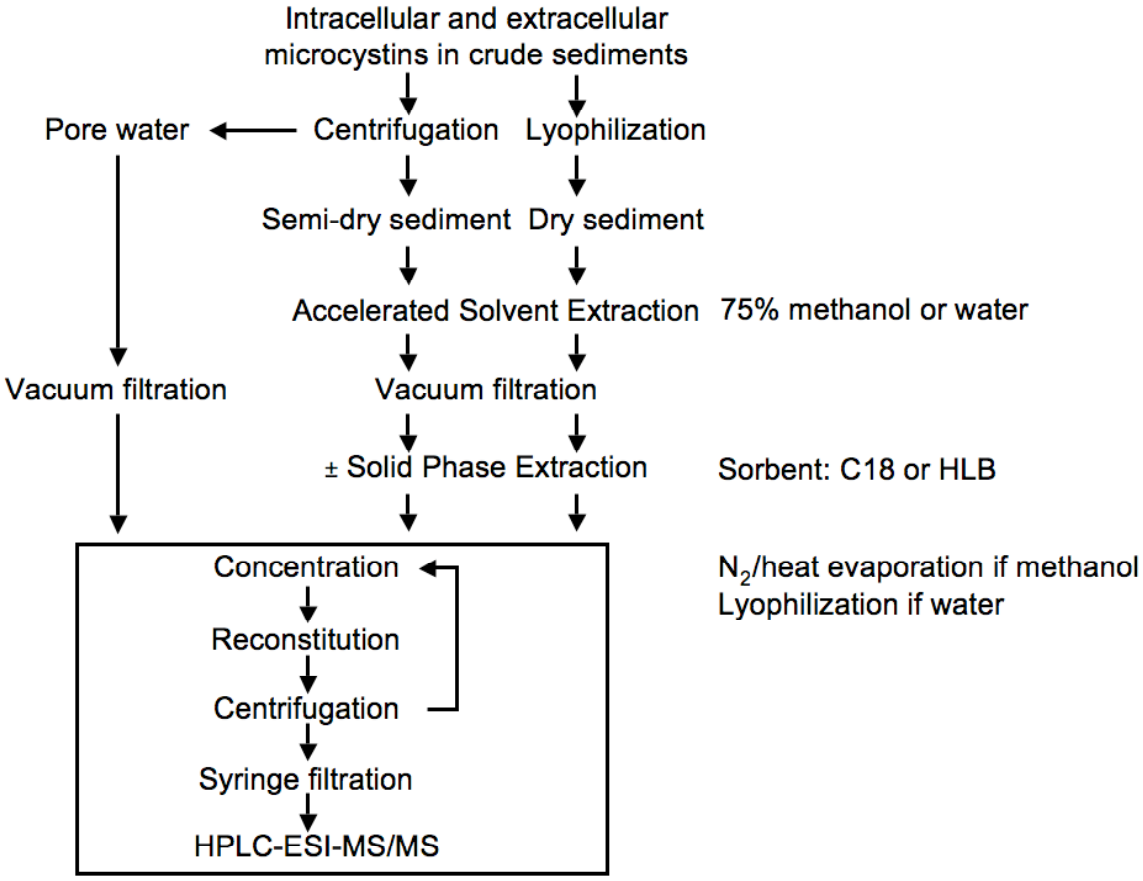


Figure II-2. A schematic for the development and optimization of an analytical method for intracellular and extracellular cyanotoxins in sediments and corresponding pore waters. HLB, hydrophilic-lipophilic balance.

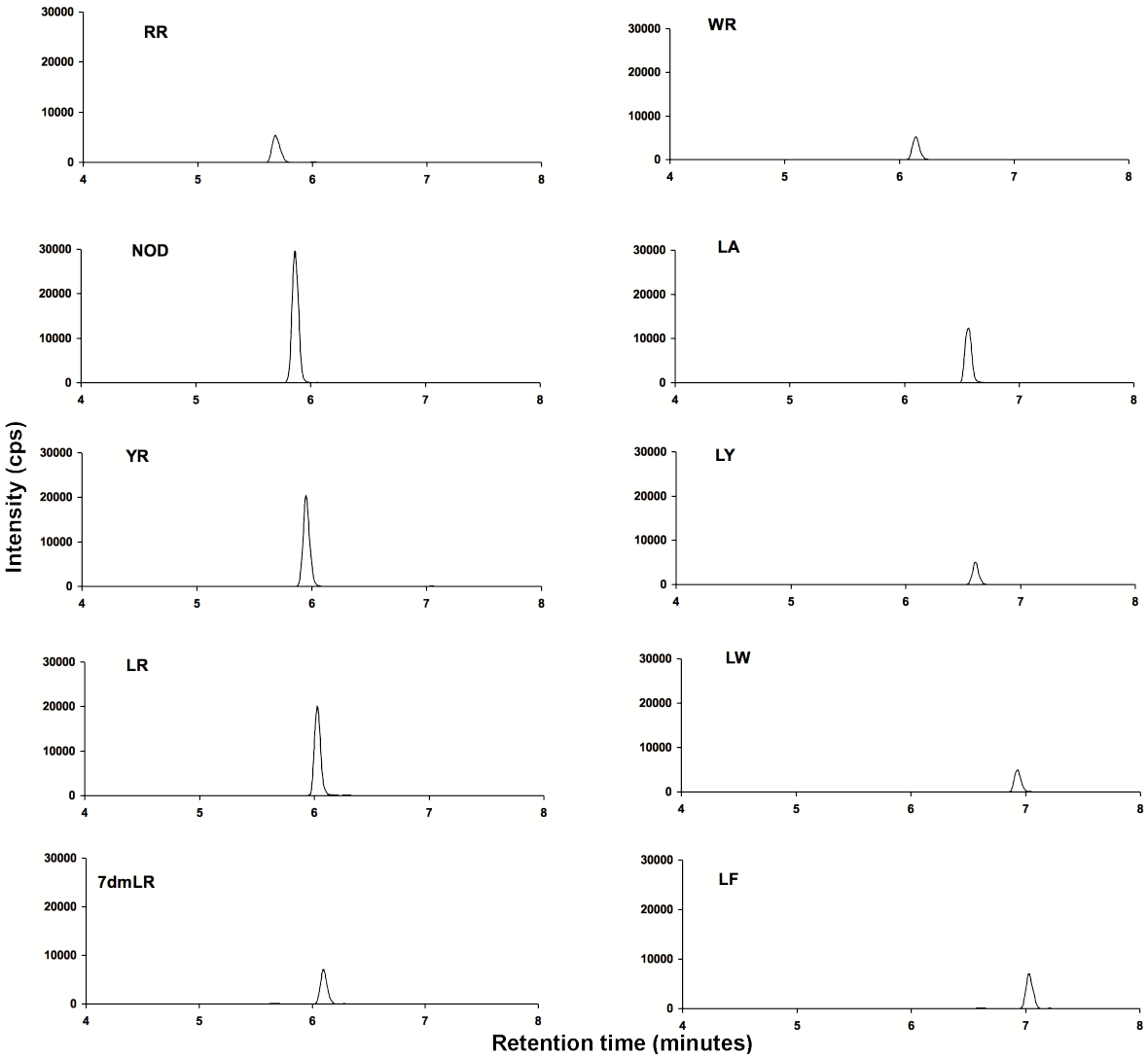


Figure II-3. LC-MRM chromatograms of standard mix containing microcystin-RR, -YR, -LR, -^{7dm}LR, -WR, -LA, -LY, -LW, -LF, and nodularin (NOD).

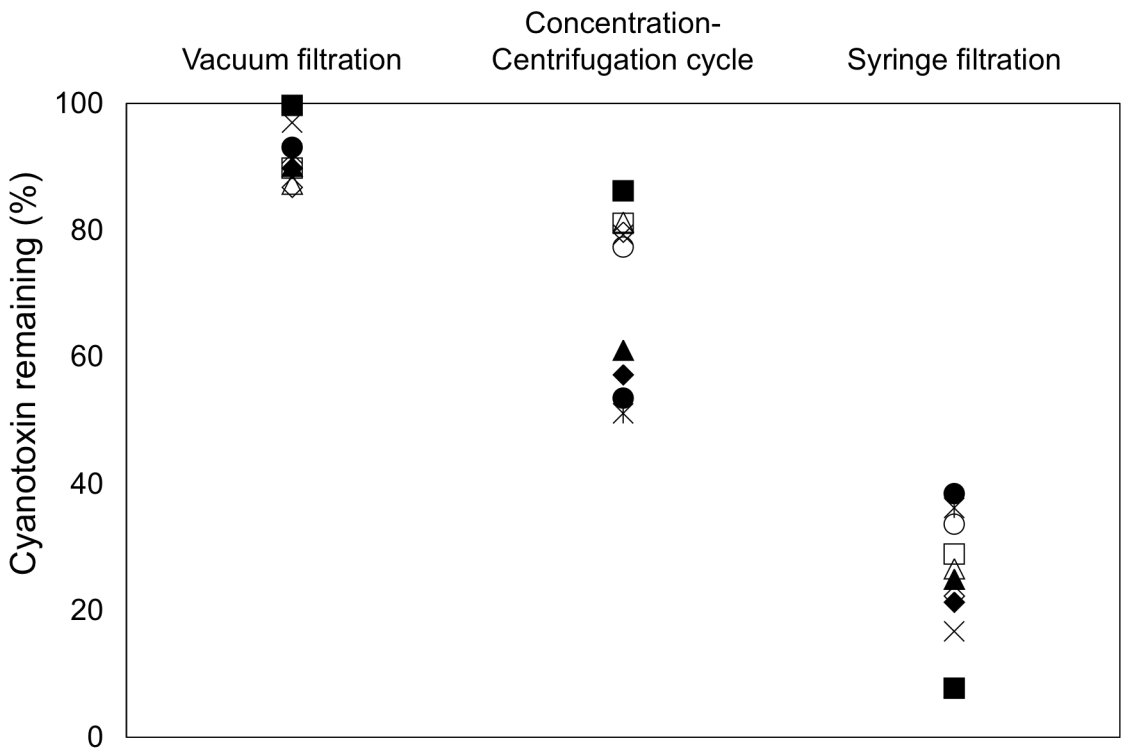


Figure II-4. Cyanotoxins remaining (%) on sequential precipitate removal from extracts of semi-dry sediments (n = 4, RSD < 15%) according to methodology in Figure II-2. 75 % methanol was used as the extraction solvent with no solid phase extraction clean up. ● LR, ✳ ^{7dm}LR, ■ RR, ▲YR, ◆WR, ○ LA, □ LF, △LY, ◇LW, × NOD.

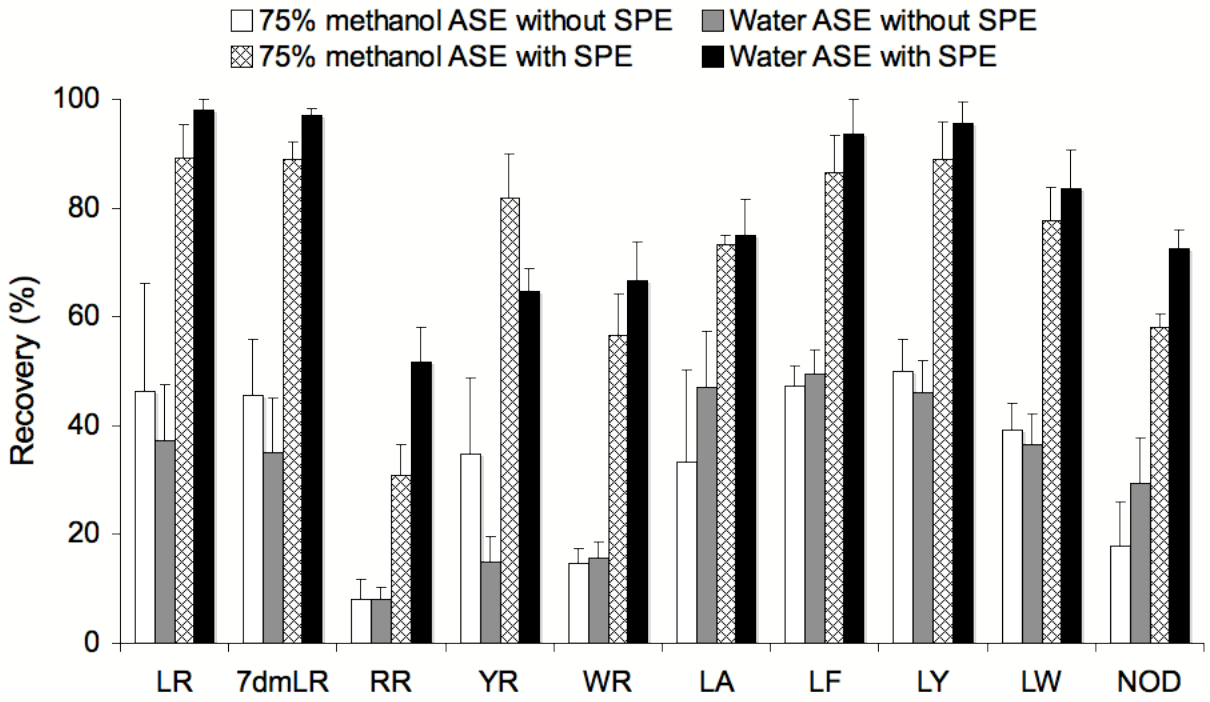


Figure II-5. Recovery (%) of cyanotoxins from semi-dry sediments using 75% methanol or water in ASE with and without C18-SPE clean up of extracts (n = 4).

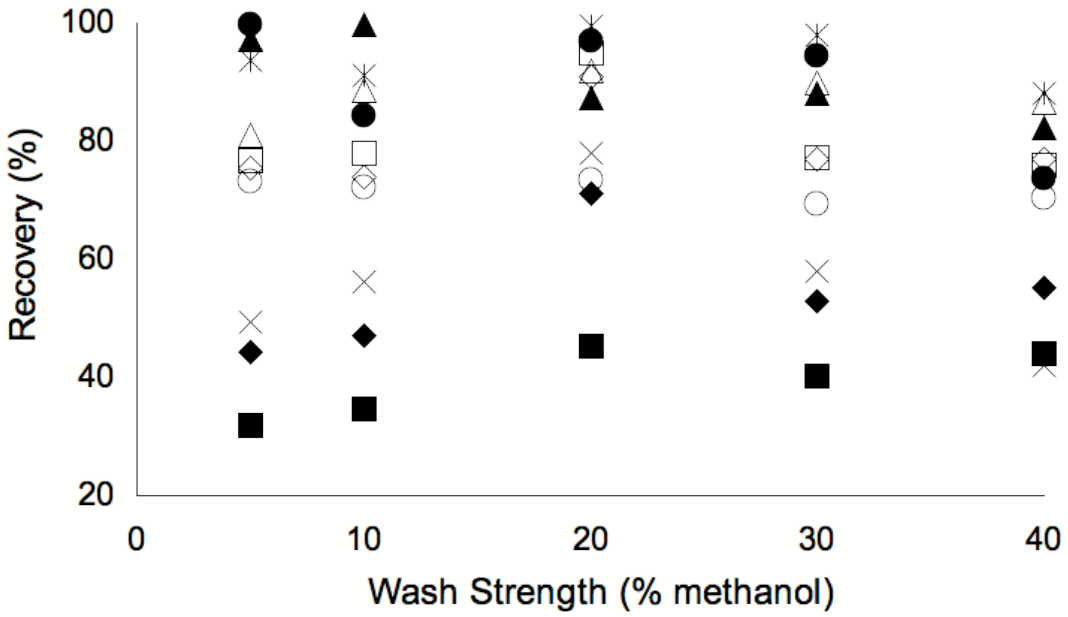


Figure II-6. Recovery (%) of cyanotoxins from semi-dry sediments using ASE with water followed by HLB-SPE clean up using increasing wash strengths (n = 4, RSD < 10%). ● LR, ✳ ^{7dm}LR, ■ RR, ▲YR, ◆WR, ○ LA, □ LF, △LY, ◇LW, ×NOD.

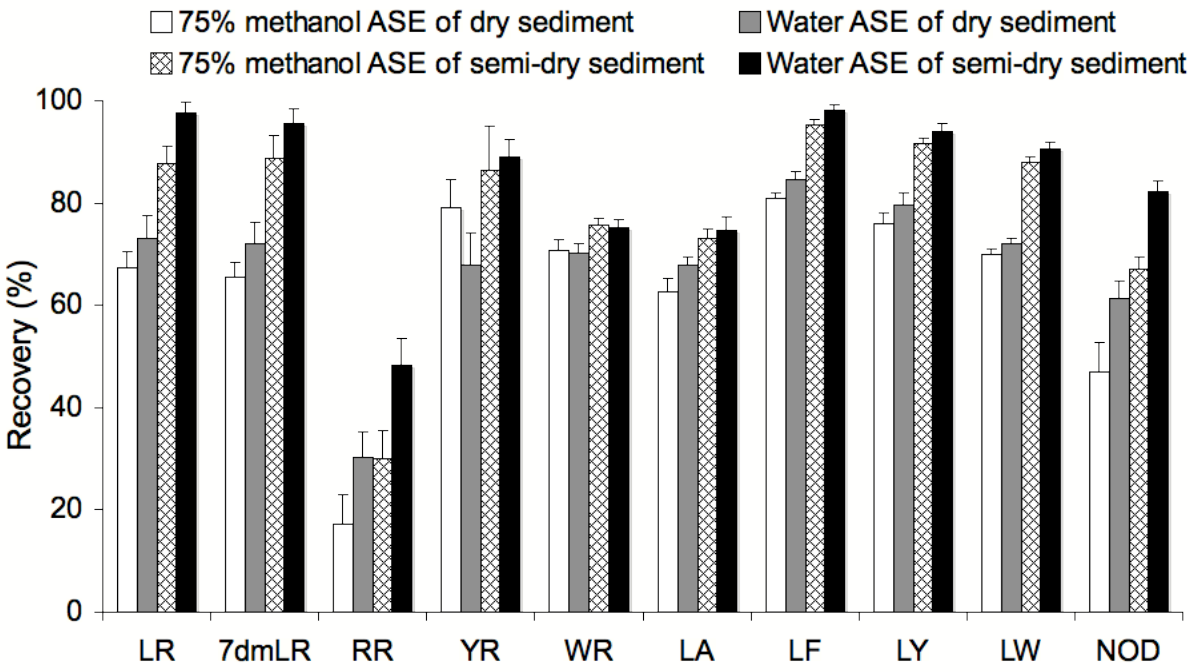


Figure II-7. Recovery (%) of cyanotoxins from dry or semi-dry sediments using 75% methanol or water as ASE solvents (n = 4) followed by post-ASE clean up by HLB-SPE.

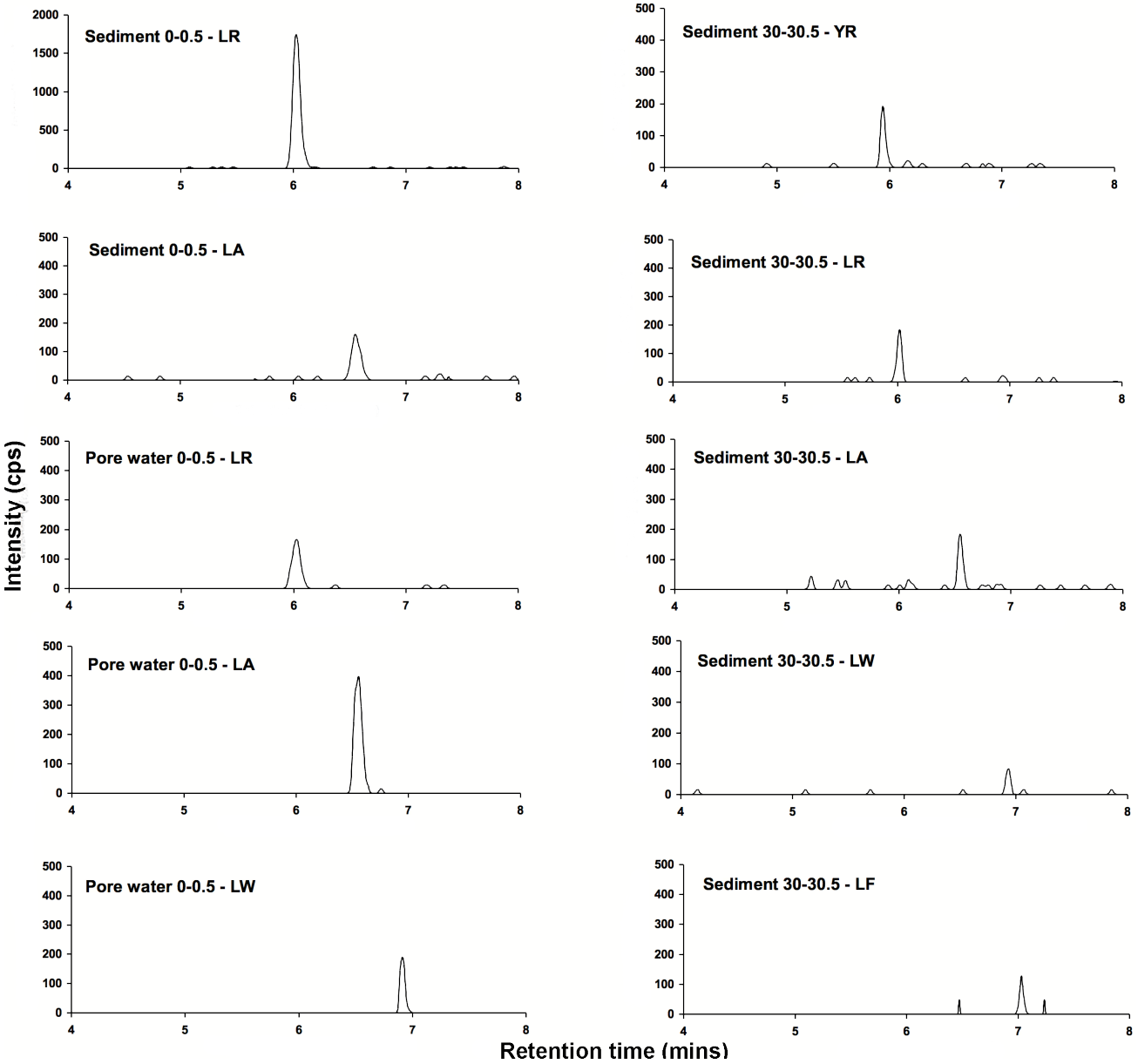


Figure II-8. LC-MRM chromatograms of sediment and pore water extracts from 0-0.5 and 30-30.5 cm depth sections of the core obtained from Lake of the Woods, ON (Hay Island site). Chromatograms showing signals at an expected retention time and noise at other retention times.

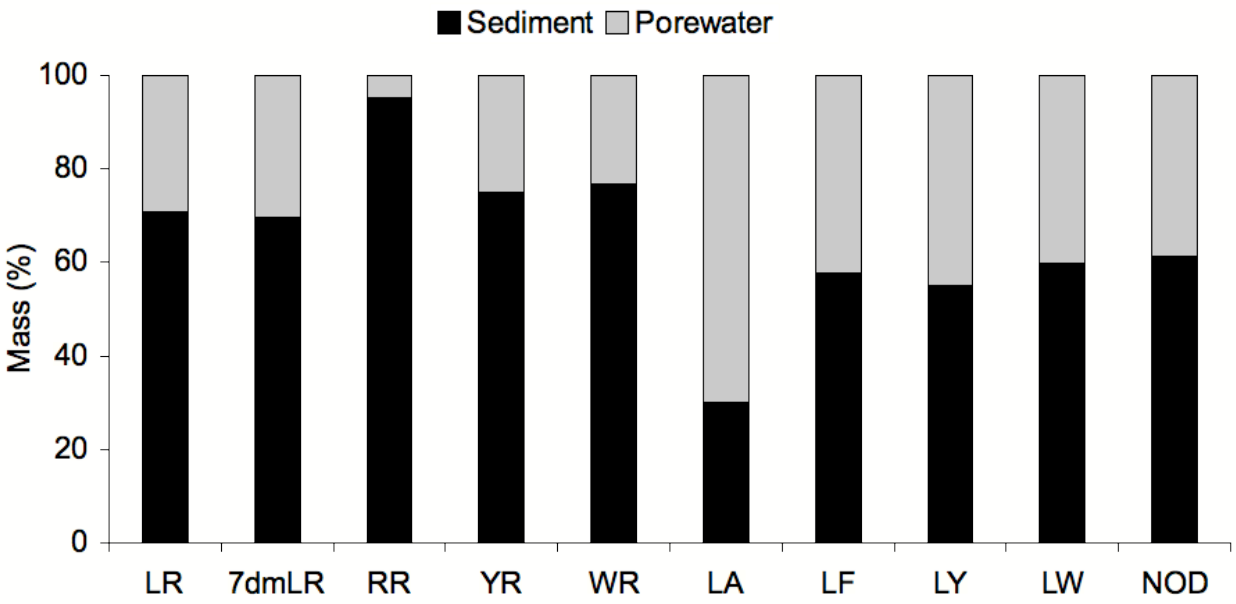


Figure II-9. Distribution of cyanotoxins between sediment and pore water phase (n = 7, RSD < 10%).

Chapter III: Fate and persistence of particulate and dissolved microcystin-LA from *Microcystis* blooms

Modified from A. Zastepa, F. R. Pick, J. M. Blais. 2013. Human and Ecological Risk Assessment: An International Journal. In press.

Abstract

Hepatotoxic microcystins frequently occur in surface waters prone to cyanobacterial blooms, posing ecological and human health risks. Few studies have estimated the fate and persistence *in situ*, making risk assessment challenging. We determined the fate and persistence of microcystin congeners during two years of *Microcystis* blooms in a small, shallow, closed-basin lake in Eastern Ontario, Canada. *In situ* microcystin half-lives were compared to estimates obtained *in vitro* under controlled temperature and light. The blooms produced elevated microcystin-LA (MC-LA) (maximum ~4.2 mg L^{-1}) with minor MC-LR, -RR, and -YR. Dissolved MC-LA declined more slowly and persisted longer than particulate MC-LA with *in situ* half-lives (total 1.5–8.5 days) shorter than *in vitro* (total 6.8–60.0 days). Half-lives in 2010 were two to eight times shorter compared to 2009 likely due to differences in bloom phenology and species/strain composition between years. *In vitro*, higher temperature (4 ^{o}C → 25^{o}C in dark) and irradiance (dark → 45 µE m^{-2} s^{-1} → 260 µE m^{-2} s^{-1} at 25^{o}C) accelerated particulate and dissolved MC-LA decline, respectively. MC-RR accumulated in surface sediments while MC-LA was near the detection limit despite elevated surface water concentrations. MC-LA appears to persist longer in surface waters than the equally toxic MC-LR, requiring in one year almost the entire recreational season (9.5 weeks) to reach guideline concentrations (20 µg L^{-1}). In order to assess human and ecological health risks, the environmental fate of different microcystin congeners needs to be specifically considered.

1.0 Introduction

As a consequence of eutrophication (e.g. Finni et al. 2001; Riedinger-Whitmore et al. 2005; Smith 2003) and possibly climate change (e.g. Hallegraeff 2010; LeBlanc et al. 2008; Paerl and Paul 2012), toxigenic cyanobacteria are a growing risk for ecological and human health (e.g. Chorus et al. 2000; Miller et al. 2010; Pouria et al. 1998). The most common toxins produced by cyanobacteria are the hepatotoxic microcystins, a large family of cyclic heptapeptides. Amino acid substitutions result in differences in chemistry and toxicity among microcystin congeners (Table III-1) (Chorus and Bartram 1999). Most studies have focused on a few congeners thought to be the most common in surface waters, namely MC-LR, -RR, and -YR. However, over 70 congeners have been characterized (e.g. Zurawell et al. 2005) with only a handful typically measured due to limited commercial availability of analytical standards. Current guidelines for microcystins in drinking water (e.g. WHO, 1.0 μg L^{-1}, Health Canada 1.5 μg L^{-1}) and recreational water (WHO 20 μg L^{-1}) (Chorus et al. 2000; WHO 2006; Bartram et al. 2003) were developed based primarily on the toxicity and persistence of MC-LR (Chorus and Bartram 1999; Falconer et al. 1994; Fawell et al. 1994; WHO 2006).

Several studies have investigated microcystin fate and persistence but have done so under laboratory conditions or in microcosms (e.g. Christoffersen et al. 2002; Welker and Steinberg 2000). They have collectively documented an initial lag period of up to 20 days before microcystins decline resulting in half-lives of less than one day to as long as 22 days (e.g. Chen et al. 2008; Edwards et al. 2008; Harada and Tsuji 1998; Ho et al. 2012). Most of these studies used dissolved microcystins

prepared as pure standards or solvent extracts, which were then added to surface waters. However, in nature, microcystins along with other cellular components undergo partitioning between the particulate and dissolved phase during cell lysis and decomposition. Only three whole-lake studies have been conducted to investigate the natural fate and persistence of microcystins. Jones and Orr (1994) measured dissolved MC-LR following a *Microcystis* bloom (lysed artificially with copper sulphate) in a small recreational lake in Australia. A more recent study investigated the fate of total microcystins released from a reservoir upstream of the Kansas River, USA, observing long distance transport through the system (Graham et al. 2012). A third study, simultaneously conducted mesocosm experiments and surrounding lake water analyses to determine the persistence of MC-LR in particulate and dissolved phase during the decline of a cyanobacterial bloom in Finland (Lahti et al. 1997). In addition to the fate of microcystins in the water column, whole-lake studies should consider the fate of microcystins in surficial sediments as evidenced by recent reports that have analyzed this compartment (Caldwell-Eldridge et al. 2013). Furthermore, as in laboratory and microcosm studies, half-lives in whole-lake studies have only been estimated for MC-LR, -RR, and –YR but other congeners are suspected to behave differently (e.g. Cook and Newcombe 2008, Miller et al. 2010; Newcombe et al. 2003; Watanabe et al. 1992). In particular, MC-LA is of growing concern in some parts of the world as a toxic cyanobacterial bloom producing this congener recently led to the deaths of sea otters off California (Miller et al. 2010).

In the following study, two MC-LA dominant cyanobacterial bloom events were studied in consecutive years in a small lake in Eastern Ontario, Canada in order to determine the fate and persistence of microcystins in both the particulate and dissolved phase. We compared decomposition both *in situ* (whole lake) and under controlled temperature and light conditions *in vitro*. Temperature (Imanishi et al. 2005; Ishii et al. 2004; Jones et al. 1994) and light (Tsuji et al. 1994; Welker and Steinberg 2000) are considered the most important environmental factors in microcystin degradation. The results will aid in the assessment of exposure risk to microcystins in recreational waters and the development of better lake management practices (e.g. when to re-open affected beaches) (Frank, 2002; Bowling 2010; Health Canada 2012; Wolf and Frank 2002).

2.0 Methods

2.1 Study Site

The study site is a small (~ 13.2 ha) and shallow (depth: mean 2.4 m, max 4.5 m) privately owned lake, south of the City of Ottawa, Canada (45°14'30.25" N, 75°34'56.65" W). The lake is polymictic and has 4-5 months of ice cover. The site was originally a stone quarry, has no inflow or outflow channel, and the principal water input and output are precipitation and evapotranspiration. The water residence time was estimated to be approximately 1.8 years using precipitation and evapotranspiration rates obtained from the National Climate Data and Information Archive and The Hydrological Atlas of Canada. The sediments are composed of gravel and sand with little organic matter accumulation. The lake, along with an

adjacent pond, serves as the focal point of a surrounding residential community and is used for recreational activities (swimming and non-motorized boating). The lake was created in the mid-1970s over 7-8 years and the first homes were built in the mid-1980s. Early on, the lake became dominated by Eurasian milfoil (*Myriophyllum spicatum*), which was first controlled by harvesting and then by periodic chemical treatment starting in 1995.

During the time frame of this study, the lake was not chemically treated or harvested for aquatic plants in 2009, but in 2010, the lake was chemically treated in the spring and again in mid-summer (ionized mineral matrix Cu^{2+} and/or diquat dibromide). During both years of study, the lake was mesotrophic to eutrophic, with total phosphorus ranging from 14 to 59 μg L^{-1} during the ice-free season. In spring 2009, a toxic cyanobacteria bloom led local public health authorities to issue an advisory for recreational purposes. We followed the environmental fate of microcystins associated with this bloom and a subsequent one in 2010. Prior to the present study, the only record of toxic cyanobacterial blooms in this lake was an Ontario Ministry of Environment report from 2008.

2.2 Water and Sediment Sampling

To obtain an estimate of the *in situ* decline of microcystins, sub-surface (0.5 m) water was collected at up to five sites across the lake through time following the start of each *Microcystis* bloom (mid-May in 2009 and late August in 2010). Water was stored in 2 L Nalgene® polyethylene bottles and kept at 4°C in the dark until transported to the laboratory (within the hour) for microscopic examination of

phytoplankton and microcystin analyses. Lake water (0.1 to 1.0 L depending on bloom density) was filtered through pre-ashed (500 °C for 2 hours) and pre-weighed Whatman GF/C filters (approximate pore size 1.2 µm). Both the filter (particulate/cell-bound) and filtrate (dissolved) fractions were collected and stored at -20°C in the dark until subsequent extraction and analyses for microcystins.

To examine the fate of microcystins, surficial sediment samples were also analyzed. Sediment cores were collected in duplicate at each site using a modified gravity corer (Glew 1989). The top 2 cm of the cores were extruded on site at 0.5 cm intervals, placed in pre-labelled Whirl-Pak® bags, and kept at 4°C in the dark until stored (within the hour) at -20°C in the dark for subsequent extraction and analyses of microcystins.

2.3 *In vitro* Experiments

To obtain estimates of microcystin decline under controlled laboratory conditions, a 2 L water sample from the cyanobacterial bloom (one in 2009 and one in 2010) was divided into 160 mL aliquots in 250 mL Erlenmeyer flasks and subjected to duplicate experimental conditions of 4°C darkness, 25°C darkness, 25°C low light (45±3 µE m^{-2} s^{-1}), and 25°C high light (260±15 µE m^{-2} s^{-1}). The light was of fluorescent and incandescent source (6:2) with the light cycle extending for 12 hours per day for up to 40 weeks. Sub-samples of 5 mL were taken through time and filtered through pre-ashed and pre-weighed Whatman GF/C filters as above. Both the filter (particulate/cell-bound) and filtrate (dissolved) fractions were collected

and stored at -20°C in the dark until subsequent extraction and analyses for microcystins.

2.4 Microcystin Extraction

The particulate fractions (filters) were extracted using Dionex ASE 200 Accelerated Solvent Extraction (ASE) guided by the method of Aranda-Rodriguez et al. (2005). Filters were placed in 11 mL stainless steel compartments at 80°C and high-pressure (1900 psi) for 22 minutes in 75% aqueous methanol in order to lyse the cyanobacterial cells and extract the toxin. The 20 mL extract was subsequently concentrated by N_2 stream- and temperature-assisted (59°C) evaporation on a Zymark Turbovap II concentration station. Samples were evaporated to dryness and re-constituted in 10 mL of 100% methanol. A subsequent evaporation to dryness was done and re-constituted in a final volume of 0.2 mL of 50% aqueous methanol. The concentrated extract was syringe filtered using a 0.45 µm, 4 mm PVDF membrane (Millex®) and stored in amber vials at -20°C in the dark until analysis. Preliminary analyses found that MC-LA was the dominant congener. Hence, sub-samples of 5 mL of non-toxic cyanobacterial culture (*Microcystis aeruginosa* CPCC 632 isolated from Lake Mendota, Madison, WI, USA) were filtered as above and spiked with MC-LA certified standard (Cedarlane Corporation) to determine extraction efficiency alongside each batch of environmental samples (mean efficiency of 73.5 ± 7.6%). The dissolved fraction (filtrate) was analyzed directly following syringe filtration as described above for filter extracts.

The sediment samples (10 g wet weight) collected had their interstitial pore water removed by centrifugation and were subsequently extracted using ASE. The sediment method used water as the extraction solvent, which was better suited than 75% aqueous methanol for extracting microcystins from sediment. Suspended particulates in sediment extracts were precipitated overnight at 4^{o}C and removed by vacuum filtration using a GF/C filter. microcystins were then isolated from matrix components and simultaneously concentrated by hydrophilic-lipophilic balanced solid-phase extraction. Elution with methanol was followed by cycles of N_2 stream- and temperature-assisted (59°C) evaporation, reconstitution in 50% aqueous methanol, and removal of precipitating particles by high-speed centrifugation. The final extract was syringe filtered before analysis by LC-ESI-MS/MS.

2.5 Microcystin Analysis

Reverse-phase high-pressure liquid chromatography (RP-HPLC) with detection by photodiode array (PDA) was used to quantify microcystins extracted from particulate and dissolved water fractions. Certified standards obtained from Cedarlane Corporation and the National Research Council, Halifax, Canada were used for instrument calibration. These included MC-RR, -YR, -LR, -7dmLR, -WR, -LA, -LY, -LW, and -LF, as well as nodularin. The HPLC system was an HP series 1100 HPLC-PDA fitted with a Zorbax Eclipse XDB-C18 column (i.d. 3.0 mm, length 150 mm, particle size 5 µm), operating at 40°C. The flow rate was set to 0.5 mL min^{-1} with a solvent gradient from an initial composition of 90% water and 10% acetonitrile to 100% acetonitrile in 43 minutes. Both water and acetonitrile (HPLC

grade) were supplemented with 0.05% trifluoroacetic acid. microcystins were identified based on retention time and their distinct UV spectrum peaking at 239 nm. The HPLC-PDA system had an instrument limit of detection (ILOD) ranging between 0.6 and 1.4 ng on column for the various cyanotoxins while the method detection limit (MDL) in water ranged from 60 to 160 ng L^{-1}.

Sample extracts in need of more detailed analysis or below reliable detection by RP-HPLC-PDA (such as sediment extracts) were analyzed using LC-MS/MS. Briefly, an Agilent 1200 series LC system was coupled to a triple-quadrupole mass spectrometer (MS) (AB Sciex 3200) equipped with a turbo electrospray ionization (ESI) source operated in positive mode at atmospheric pressure, 350°C, and a voltage of 4,500 V. The MS was operated under multiple reaction monitoring (MRM) mode. Precursor to product transitions were monitored between 100 and 1100 *m/z* with a dwell time of 20 ms for each transition. The LC-MS/MS system had an ILOD ranging between 0.01 and 0.08 ng on column for the various cyanotoxins while the MDL in sediment ranged from 0.3 to 2.5 ng g^{-1} d.w.

2.6 Data Analysis

Regression analyses were conducted to evaluate the strength and significance of the relationship between microcystin concentration and time. Simple linear regression was used with natural log-transformed MC-LA data to calculate decline rates, which were then converted to half-lives. Total MC-LA concentrations were obtained by summing the particulate and dissolved concentrations at respective time points and subsequently transforming the data by the natural

logarithm. Significant differences between half-life estimates were determined using *t*-test analysis. Concentration and half-life values reported are means ± standard deviation unless otherwise noted.

3.0 Results

3.1 Cyanobacterial Bloom 2009

In mid-May 2009 a cyanobacterial bloom developed in the lake, which on microscopic examination was dominated by *Microcystis aeruginosa* but also included other species of *Microcystis*. MC-LA was the dominant microcystin congener. The other congeners tested (MC-RR, -YR, -LR, -7dmLR, -WR, -LA, -LY, -LW, and -LF) were not detected.

In the lake, initial particulate and dissolved MC-LA concentrations were 2472 ± 1336 μg L^{-1} (1036 - 4211) and 203 ± 14 μg L^{-1} (190 - 221) respectively (Figure III-1) such that dissolved MC-LA was on average about 8% of the total MC-LA. Particulate and dissolved MC-LA declined immediately (no lag phase) and steadily through time over 12 weeks (Figure III-1). By the end of the sampling period, concentrations had declined to 0.84 ± 0.03 μg L^{-1} (0.81 - 0.86) for particulate and 7.4 ± 1.5 μg L^{-1} (5.2 - 8.6) for dissolved MC-LA such that the dissolved fraction was about 90% of the total MC-LA. There was a highly significant negative relationship between concentration and time following a log-linear relationship (as in a first order reaction) for both the particulate (R^2 = 0.98, $p < 0.001$) and dissolved phase (R^2 = 0.97, $p < 0.001$) (Figure III-1) resulting in *in situ* half-life estimates of 6.5 ± 0.4 and

15.8 ± 1.0 days respectively (Table III-2). For total MC-LA, a half-life of 8.5 ± 0.5 days was determined (R^2 = 0.97, $p < 0.001$).

In vitro, cell lysis and particulate MC-LA loss ensued immediately (no lag) and progressed gradually under all conditions (Figure III-2). At 15 weeks, particulate MC-LA underwent rapid loss at 25^{o}C in the dark in concordance with the peak in dissolved MC-LA before its decline ensued. Dissolved MC-LA increased at 25^{o}C under both low and high light conditions before decline ensued after about five weeks. At 4^{o}C in the dark, dissolved MC-LA levels increased very slowly and only slightly until a slow decline began at five weeks.

Generally, half-life estimates were based on concentration measurements using all time-points since decline ensued immediately (no lag). However, in certain cases such as the dissolved phase *in vitro* where an initial increase occurred, half-life estimates were based only on measurements during the decline phase. For example, in 2009 at 25°C and high light, the estimates were based on measurements starting at six and a half weeks. Initial *in vitro* concentrations were 4415 µg L^{-1} ± 44 and 82.3 ± 1.6 µg L^{-1} in the particulate and dissolved phase respectively (Figure III-2). The longest half-life for both particulate and dissolved MC-LA was at 4^{o}C in the dark (Table III-2). For particulate MC-LA, an increase in temperature to 25^{o}C in the dark significantly enhanced the degradation (*t*-test, $p < 0.005$) reducing the half-life from almost eight weeks to three weeks. At this higher temperature, light had a significant inhibitory effect on the degradation of particulate MC-LA ($p < 0.01$) increasing the half-life by about three weeks. In contrast, temperature had much less of an effect on the degradation of dissolved MC-LA.

However, high light significantly enhanced the decline ($p < 0.005$), reducing the half-life to the lowest among the treatments. Dissolved MC-LA had longer half-lives than particulate MC-LA under all experimental conditions ($p < 0.05$). After more than 40 weeks, MC-LA continued to be measurable under all incubation conditions, ranging from 5-140 μg L^{-1} and 50-93 μg L^{-1} in the particulate and dissolved phases.

Although no other congeners were detected for which standards were available, RP-HPLC-PDA analysis revealed a potential microcystin (UV spectrum peak = 239 nm) eluting within half a minute of the MC-LA standard. The suspected peak had a molecular to product ion transition characteristic of MC-LA (910.6 → 135.1 *m/z* observed by LC-MS/MS) with a marginally different fragmentation profile. The peak was classified as an MC-LA isomer (MC-LA-iso) based on independent confirmation by M. Quilliam and K. Berki (National Research Council, Halifax). Particulate and dissolved MC-LA-iso was measured at much lower concentrations *in situ* than MC-LA, initially at 989 ± 90 μg L^{-1} (886 - 1105) and 82 ± 11 μg L^{-1} (74 - 98). MC-LA-iso decreased more rapidly *in situ* than MC-LA resulting in half-life estimates of 4.0 ± 0.02 and 25.9 ± 1.0 days for particulate and dissolved MC-LA-iso respectively (data not presented). MC-LA-iso was not as persistent as MC-LA as it was no longer detectable after six weeks. *In vitro* MC-LA-iso dynamics (cell lysis and toxin release) were similar and half-lives were within five days of those of MC-LA in their respective incubation conditions.

3.2 Cyanobacterial Bloom 2010

In 2010, a cyanobacterial bloom did not appear in the lake until the end of August. As in 2009, the 2010 bloom was dominated by *Microcystis aeruginosa* but also included other species of *Microcystis*. MC-LA was again the dominant congener with initial lake concentrations of 49.4 ± 13.1 μg L^{-1} (33.4 - 73.2) particulate and 1.3 ± 0.3 μg L^{-1} (0.8 - 1.7) dissolved. The MC-LA-iso observed in 2009 was not detected in 2010. However, other congeners were present in the particulate phase at low concentrations (maximum MC-LR: 0.87 μg L^{-1}, MC-YR: 0.15 μg L^{-1}, and MC-RR: 0.18 μg L^{-1}). As in 2009, both particulate and dissolved MC-LA declined through time in a log-linear fashion (R^2 = 0.99, $p < 0.01$) with no lag phase. However, in 2010 *in situ* half-lives were shorter ($p < 0.001$) at 1.5 ± 0.03 days for particulate and 2.8 ± 0.3 days for dissolved MC-LA (Table III-3). For total MC-LA, a half-life of 1.5 ± 0.06 days was determined (R^2 = 0.99, $p < 0.01$).

In vitro, the loss of particulate MC-LA began rapidly at 25°C in the dark while a more gradual decline was observed under the remaining conditions (Figure III-3). As in 2009, all conditions showed an initial increase in dissolved MC-LA, indicating some transfer from cells and particulate material into the dissolved phase, before concentrations declined. This was especially evident at 25°C under high light where a relatively rapid increase in MC-LA occurred in the dissolved phase before rapid decline.

As described for 2009, half-life estimates were based on concentration measurements during the period of decline. *In vitro* experiments had initial concentrations of 128.1 ± 2.6 μg L^{-1} particulate and 1.1 ± 0.02 μg L^{-1} dissolved MC-

LA (Figure III-3**)**. Half-life estimates were significantly shorter ($p < 0.001$) than estimated in 2009 (up to eight times shorter). Temperature and light effects were consistent with 2009 results with one major difference; the half-life of dissolved MC-LA in 2010 at 4^{o}C in the dark was comparable to that estimated at 25^{o}C in the dark (Table III-2 and 3). *In vitro* MC-LA was still measurable in the particulate phase after 22 weeks at 4^{o}C under dark (3.2 µg L^{-1}). In the dissolved phase, MC-LA was measurable at around 1 µg L^{-1} at both 4^{o}C under dark and 25^{o}C under high light after 15 weeks incubation.

3.3 Sediment Deposition

In 2009, the bloom was no longer visible in the lake after about six weeks (July 22) when particulate and dissolved MC-LA still remained high (8.8 and 3.4 µg L^{-1} respectively, Figure III-1). At the same time MC-LA was measured in the surficial sediment (top 0-2 cm) at 0.02 µg g^{-1} d.w. at two of five sites around the lake, whereas it was not detected in three others. These were the only measurable concentrations of MC-LA in the sediments throughout all of the 2009 sampling. No MC-LA-iso was detected in the surficial sediments in 2009. Throughout 2010, neither MC-LA nor MC-LA-iso was detected in the surficial sediments (top 0-2 cm). However, MC-RR was detected at 0.01 µg g^{-1} d.w. at one of five sites, nine days after the appearance of the bloom.

4.0 Discussion

The dominant microcystin found in surface water in both years was MC-LA, a congener with the same mammalian toxicity as MC-LR (LD_{50} = 50 μg kg^{-1}, Table III-1) (Chorus and Bartram 1999). Although MC-LA is not often included in analyses, there are recent reports of its occurrence. For example, a *Microcystis* bloom in a lake in California was reported by Miller et al. (2010) to contain high MC-LA concentrations reaching 2,100 mg L^{-1} in scum samples. Although concentrations dropped significantly following transport into a downstream bay, bioaccumulation of MC-LA in marine invertebrates was linked through trophic transfer to the deaths of sea otters. In the present study, the highest initial MC-LA concentration was over 4 mg L^{-1} at one lake station (Figure III-1). About a week prior to our sampling, the Ontario Ministry of Environment measured an MC-LA concentration of 11 mg L^{-1} (OMOE unpublished data).

4.1 Decline of MC-LA

No lag phase was evident in the decline of MC-LA *in situ* in either year (Figure III-1). Although a lag phase has been seen in some studies (e.g. Chen et al. 2008; Lahti et al. 1997; Watanabe et al. 1992), it is not always observed. In fact, others have suggested that the decline of microcystins begins immediately (no lag phase) in water bodies that have had previous exposure to the cyanotoxins, presumably because of the presence of microcystin-degrading bacteria within the microbial community (Jones et al. 1994; Jones and Orr 1994; Rapala et al. 1994).

The study lake was also previously exposed, having a microcystin-producing cyanobacterial bloom in 2008.

In both years of the study, the half-life of particulate and dissolved MC-LA *in situ* was significantly lower than any *in vitro* condition including under high light and temperature (Table III-2 and 3). This is likely due to the additional loss processes *in situ* including dispersion, UV radiation, and sorption, which are difficult to mimic in the laboratory, highlighting the need for more studies on the decomposition of microcystins in natural environments.

All *in situ* and *in vitro* MC-LA half-life estimates were higher in 2009 than 2010 (Table III-2 and 3). These differences could be due to differences in environmental conditions, initial microcystin concentrations, physiological condition of the bloom (age/progression) as well as species or strain composition between years (Lahti et al. 1997). The 2009 bloom arose in cooler May temperatures that, in part, could explain the difference in *in situ* MC-LA rates of decline compared to 2010 (August). Furthermore, the 2009 bloom dominated the entire lake, had much higher initial microcystin concentrations and was clumping at the time of sub-sampling, suggesting a later stage of bloom progression, whereas the 2010 bloom was a smaller, near-shore accumulation with lower initial microcystin concentrations. Lastly, the difference in microcystin congener composition between years suggests a difference in cyanobacterial species or strain composition between years as congener composition is a strain specific trait. We were able to obtain an additional independent estimate of *in situ* half-life for MC-LA using Ontario Ministry of Environment particulate MC-LA concentrations recorded in 2008 (five dates

sampled from late August to mid-October). The estimated half-life of 8.9 ± 1.7 days was in agreement with our 2009 estimates (Table III-2).

In order to compare with literature *in situ* data on other congeners, we calculated the *in situ* half-life for MC-LR using concentration declines over time reported by the three published whole lake studies (Table III-4). Based on data from Jones and Orr (1994), we estimated an initial half-life of one day (initial concentration ~ 1.8 mg L^{-1}) followed by slower loss (from ~ 0.1 mg L^{-1}) corresponding to a half-life of approximately five days for dissolved MC-LR. Based on data from Graham et al. (2012), we estimated a half-life of about two days for total MC-LR equivalents from an initial concentration of 150 mg L^{-1}. From Lahti et al. (1997), the regression models provided were used to obtain a half-life of 4.7 days and 10.0 days for particulate and dissolved MC-LR (initial concentrations < 10 µg L^{-1}). A comparison to our *in situ* half-lives (Tables 2 and 3) suggests that MC-LA decline is slower than that of MC-LR (Table III-4).

4.2 Persistence of MC-LA

The cooler spring water temperatures and limited loss by advection (no channelized inflows and outflows) was conducive to the greater MC-LA persistence observed in 2009 relative to 2010. Due to the high initial MC-LA concentration and long half-life, it took five weeks for mean MC-LA concentrations in the particulate phase to fall below 20 µg L^{-1} (Figure III-1). The 20 µg L^{-1} corresponds to a moderate probability of adverse health effects during recreational exposure (Chorus and Bartram 1999; Codd et al. 2005; WHO 2006). However, some parts of the lake still

exceeded this concentration by more than three-fold. Dissolved MC-LA took even longer (nine and a half weeks) to fall below the 20 μg L^{-1} recreational guideline (Figure III-1). The *Microcystis* bloom was no longer visible after about five weeks while high levels of particulate and dissolved MC-LA continued to persist and spread throughout the almost kilometre-long lake. Based on the recreational guideline, this means that the lake should have been closed all summer. Although microcystins are known to be quite resistant to decomposition (Harada and Tsuji 1998), such extended persistence has not been demonstrated especially *in situ* where microcystins have the potential to be lost by additional processes such as UV decomposition and sediment adsorption. For example, Lahti et al. (1997) observed over a 90% reduction of particulate and dissolved MC-LR *in situ* after two and four weeks, respectively. Jones and Orr (1994) measured dissolved MC-LR for three weeks from a bloom that was lysed with algaecide in a deliberately isolated site within the lake. The limited *in situ* data on microcystin fate and persistence and a lack of comparisons with other congeners leaves much uncertainty in developing policies and practices for managing surface waters, particularly for recreation (Bowling 2010; Frank 2002; Health Canada 2012; Wolf and Frank 2002). The common practice is to post warnings or close the recreational shoreline (beach, boating) immediately adjacent to where the potentially toxigenic bloom has occurred. If cyanotoxins are detected, the site remains closed usually for two weeks but sometimes for as little as 24 hours (NCC 2009). The site is usually re-opened without follow-up cyanotoxin testing, especially if the bloom has disappeared. The results of our *in situ* (whole lake) study demonstrate that microcystins can persist

well beyond the disappearance (senescence) of the bloom, especially in the dissolved phase, and that some congeners, such as MC-LA, can persist longer than what is currently understood from the more commonly studied MC-LR. Therefore, recreational closures extending two weeks may be insufficient to mitigate risk of exposure to microcystins and follow-up analysis is recommended, particularly if authorities detect congeners for which little environmental fate information exists. Furthermore, sites outside the immediate vicinity of the bloom may also be at risk as suggested by reports of long-distance transport of microcystins within lakes and from lakes to river and costal systems (Graham et al. 2012; Miller et al. 2010).

4.3 Effects of Light and Temperature

Increasing temperature *in vitro* from 4^{o}C to 25^{o}C in the dark enhanced MC-LA decline especially in the particulate phase (Tables 2 and 3). Increasing irradiance (dark → low → high) at the higher temperature (25^{o}C) enhanced the rate of decline of dissolved MC-LA in both years. This is in contrast to the findings of Tsuji et al. (1994) where no significant decomposition of dissolved MC-LR was observed under laboratory irradiation even with pigment extracts. Our results suggest cellular material and dissolved organic matter in unaltered environmental samples may be involved in catalyzing photodecomposition of dissolved MC-LA even in the absence of UV as under our light conditions.

Literature *in vitro* estimates of microcystin half-lives (MC-LR, -RR, -YR) range from about one to 22 days (e.g. Edwards et al. 2008; Chen et al. 2008; Ho et al. 2012; Jones et al. 1994; Rapala et al. 1994; Welker and Steinberg 2000). This is

much shorter than the *in vitro* half-life estimates for MC-LA in this study. These *in vitro* results suggest that the decline of MC-LA is much slower than that of MC-LR, -RR, and -YR. Consistent with our findings, there is evidence to suggest that MC-LA may not be as susceptible as other microcystins to biodegradation. The few bacterial isolates observed to degrade microcystins preferentially cleaved at the Adda-Arginine bond present in variants such as MC-LR while variants such as MC-LF, that do not contain arginine substituents, were not significantly degraded (e.g. Imanishi et al. 2005). One study has isolated a strain of *Sphingomonas* capable of degrading a wider range of microcystin variants (MC-LR, -RR, -LY, -LW, -LF), yet it is still not known to what extent, if any, MC-LA is susceptible to biodegradation (Ishii et al. 2004). It is important to stress that future *in situ* and corresponding *in vitro* studies compare half-lives of different congeners (e.g. MC-LA and -LR) within the same bloom (or bloom incubation) in order to control for different experimental and environmental conditions. For example, Watanabe et al. (1992), in one of the few comparisons of microcystin congeners, observed different rates of decline for two arginine containing microcystins, MC-LR (half-life = 15 days) and MC-YR (half-life = 11 days) in the presence of heterotrophic bacteria. Although a culture (single-celled *Microcystis aeruginosa*) rather than a bloom sample (colonial morphology) was used, this design allowed the authors to simulate the natural lysis of cyanobacterial cells and microcystin partitioning and decline within the particulate and dissolved phase.

4.4 Sediment Deposition

Although MC-LA was detected in 2009 in surficial sediments, it was near the method detection limit despite very high concentrations in the water column. This could be due to the high sand/gravel and low organic matter composition of the lake sediment, which is thought to lead to low adsorption of microcystins (Miller et al. 2005; Wu et al. 2011). The following year, the bloom was again dominated by MC-LA but also contained minor amounts of MC-RR. Despite its low concentrations in the surface waters, MC-RR was detected in the sediments. In contrast, MC-LA was not detected in sediments despite elevated surface water concentrations. This suggests some congeners may deposit into sediments while others such as MC-LA remain preferentially in the water phase. This lack of sediment deposition may help to further explain the extended persistence of MC-LA in the surface waters.

Although early evidence from modelling and laboratory experiments suggested microcystin congeners should be similarly or increasingly reactive and adsorptive (UKWIR 1997), some have since observed a significant difference in the adsorption of microcystin congeners. Cook and Newcombe (2008) used activated carbon to demonstrate that MC-RR was the most adsorbed variant followed by MC-YR, MC-LR, and MC-LA. This trend was unexpected considering that the hydrophobicity (based on octanol-water partition coefficient) and molecular weight relationship would predict the opposite (Rivasseau et al. 1998). However, all microcystin congeners contain two carboxyl groups that become negatively charged at ambient pH (7-8). Cook and Newcombe (2008) suggest that the low adsorption of MC-LA to sediments may be due to its net -2 charge as it lacks ionizable

substituents to balance the two carboxylate ions. In contrast, two positively charged arginine substituents ensure a net neutral MC-RR that can be readily adsorbed to sediments. Considering these differences, the authors advised water authorities to consider the microcystin congeners present in water sources when deciding on management options.

4.5 Implications for management

Currently, management decisions are often based on microcystin measurements obtained by congener-independent enzyme-linked immunosorbent assay (ELISA). This is a simpler analytical approach designed to capture the concentration of total microcystins. However, most commercial ELISA kits exhibit lower cross-reactivity to congeners other than MC-LR. This is the case for MC-LA in particular, which means that ELISA measurements may underestimate the actual concentrations of microcystins and hence the risks to human health. Considering our *in situ* observations of differences in the environmental fate of MC-LA relative to MC-LR, a congener-specific approach is needed in management decisions for recreational waters and beaches susceptible to toxigenic cyanobacteria.

5.0 Conclusion

- There exist few *in situ* (whole lake) studies on the fate and persistence of microcystin congeners (in particular MC-LA). This has led to uncertainty in developing policies and practices for managing surface waters, particularly for recreation.

- This study followed the decline of microcystins both *in situ* (whole lake) and *in vitro* during two separate bloom events.
- *In situ* estimates (especially in 2009) suggest that the dominant congener MC-LA has a longer half-life and greater persistence than MC-LR, -YR, -RR. This is consistent with *in vitro* estimates when compared to similar lab-based experiments.
- Temperature enhanced decline of MC-LA in the particulate phase while irradiance enhanced the decline in dissolved phase following natural cell lysis even in the absence of UV.
- In contrast to MC-RR, MC-LA was not observed to readily deposit onto lake sediments, demonstrating congener-specific deposition.
- Recreational policies and management practices should be re-visited in light of these findings given that MC-LA is as toxic as MC-LR.

Tables and figures

Table III-1. Comparison of the properties of microcystin congeners. Adapted from Newcombe et al., 2003.

	MC-LA	MC-LR	MC-YR	MC-RR
Toxicity (LD_{50} µg/kg)	50	50	70	600
Net charge (pH 7)	-2	-1	-1	0
Molecular weight	909	994	1044	1037
Amino acid substituents	Leu, Ala	Leu, Arg	Tyr, Arg	Arg, Arg
Hydrophobicity		Decreasing → → → →		

Table III-2. Estimates of MC-LA half-life based on the May 2009 *Microcystis* bloom (±SD).

Conditions	Half life of microcystin-LA (days)		
	Particulate	**Dissolved**	**Total**
In situ (n=5)	6.5 ± 0.4	15.8 ± 1.0	8.5 ± 0.5
In vitro (n=2)			
25 ^{o}C (260 µE m^{-2} s^{-1})	44.9 ± 0.7	63.5 ± 5.3	47.4 ± 1.0
25 ^{o}C (45 µE m^{-2} s^{-1})	42.8 ± 0.7	120.4 ± 1.0	55.6 ± 0.7
25 ^{o}C (Dark)	23.8 ± 2.4	131.5 ± 7.5	41.6 ± 0.2
4 ^{o}C (Dark)	54.6± 0.5	251.0 ± 35.9	60.0 ± 0.1

Table III-3. Estimates of MC-LA half-life based on the August 2010 *Microcystis* bloom (±SD).

Conditions	Half life of microcystin-LA (days)		
	Particulate	**Dissolved**	**Total**
In situ (n=5)	1.5 ± 0.03	2.8 ± 0.3	1.5 ± 0.06
In vitro (n=2)			
25 ^{o}C (260 µE m^{-2} s^{-1})	9.2 ± 0.7	10.9 ± 0.3	11.6 ± 0.3
25 ^{o}C (45 µE m^{-2} s^{-1})	10.5 ± 0.9	26.5 ± 0.9	9.4 ± 1.0
25 ^{o}C (Dark)	5.0 ± 0.1	33.8 ± 2.2	6.8 ± 0.04
4 ^{o}C (Dark)	24.2 ± 1.3	31.3 ± 1.8	25.7 ± 4.2

Table III-4. Estimates of *in situ* (whole lake) MC-LR half-life based on data provided in cited studies.

	Half life of microcystin-LR (days)		
	Particulate	**Dissolved**	**Total**
Jones and Orr, 1994	No data	1 to 5	No data
Lahti et al., 1997	4.7	10	No data
Graham et al., 2012	No data	No data	2

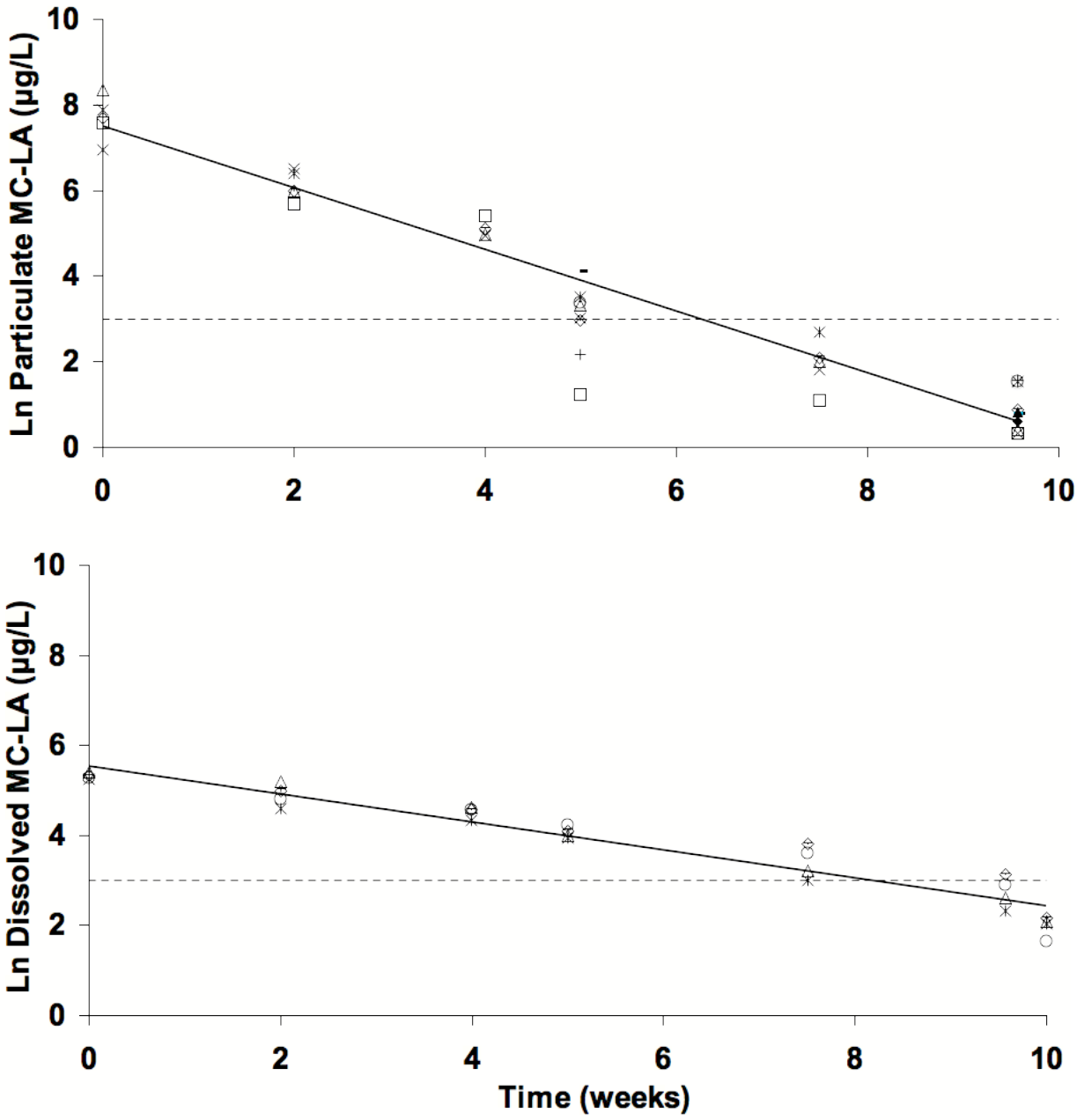


Figure III-1. *In situ* (whole lake) decline of particulate (cell-bound) (R^2 = 0.98, $p < 0.001$) and dissolved (extracellular) (R^2 = 0.97, $p < 0.001$) MC-LA (µg L^{-1}). Each symbol represents a different station of the lake sampled through time beginning in May 2009. Dashed horizontal line denotes recreational guideline of 20 µg L^{-1}, which corresponds to a moderate probability of adverse health effects during recreational exposure (WHO, 2006; Chorus and Bartram, 1999; Codd et al., 2005).

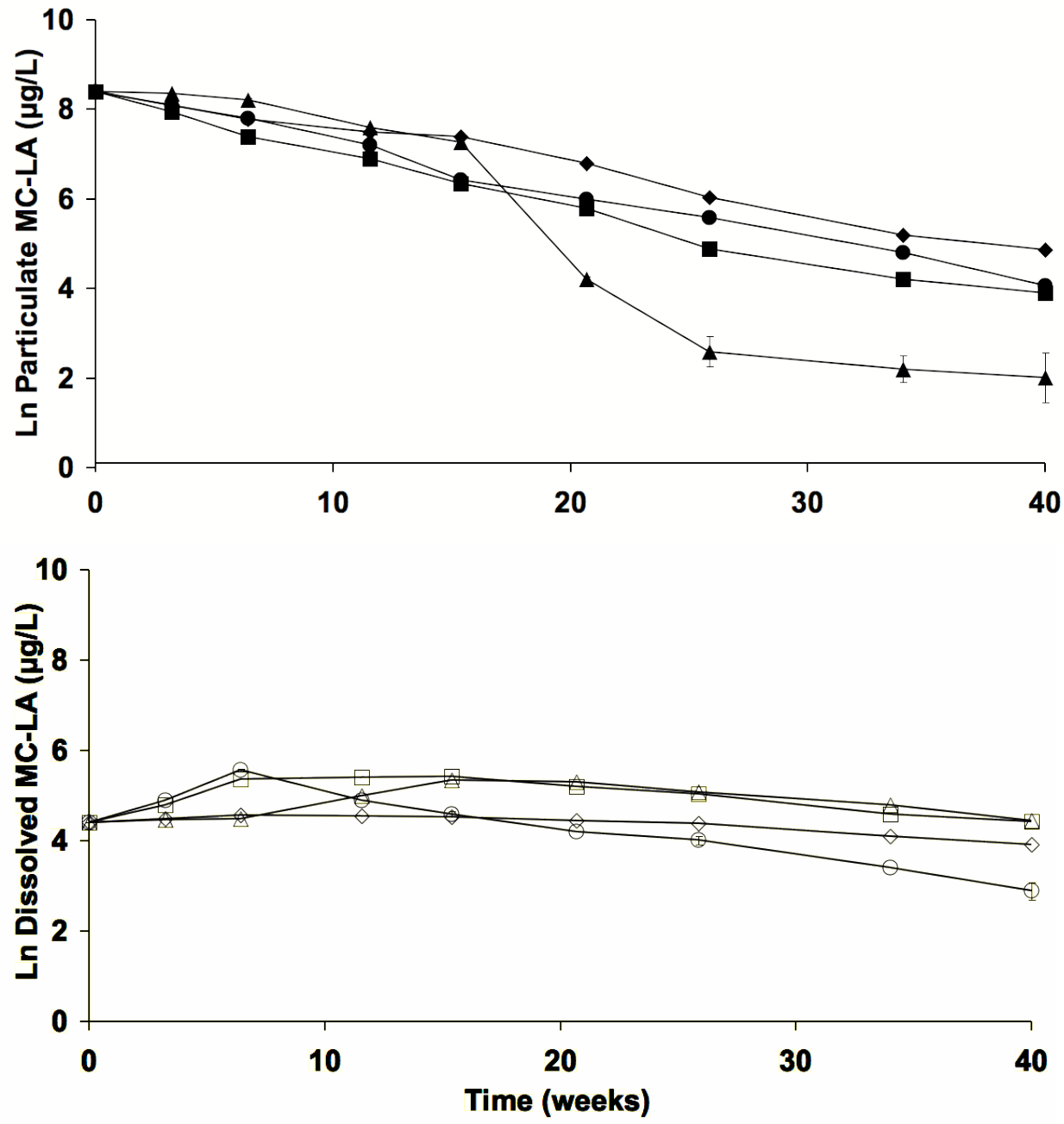


Figure III-2. The decline of particulate (cell-bound) (filled symbols) and dissolved (extracellular) (open symbols) MC-LA (µg L^{-1}) under *in vitro* conditions of 4°C dark (diamonds), 25°C dark (triangles), 25°C low light (squares), and 25°C high light (circles). Duplicate incubations were carried out using bloom material collected in 2009.

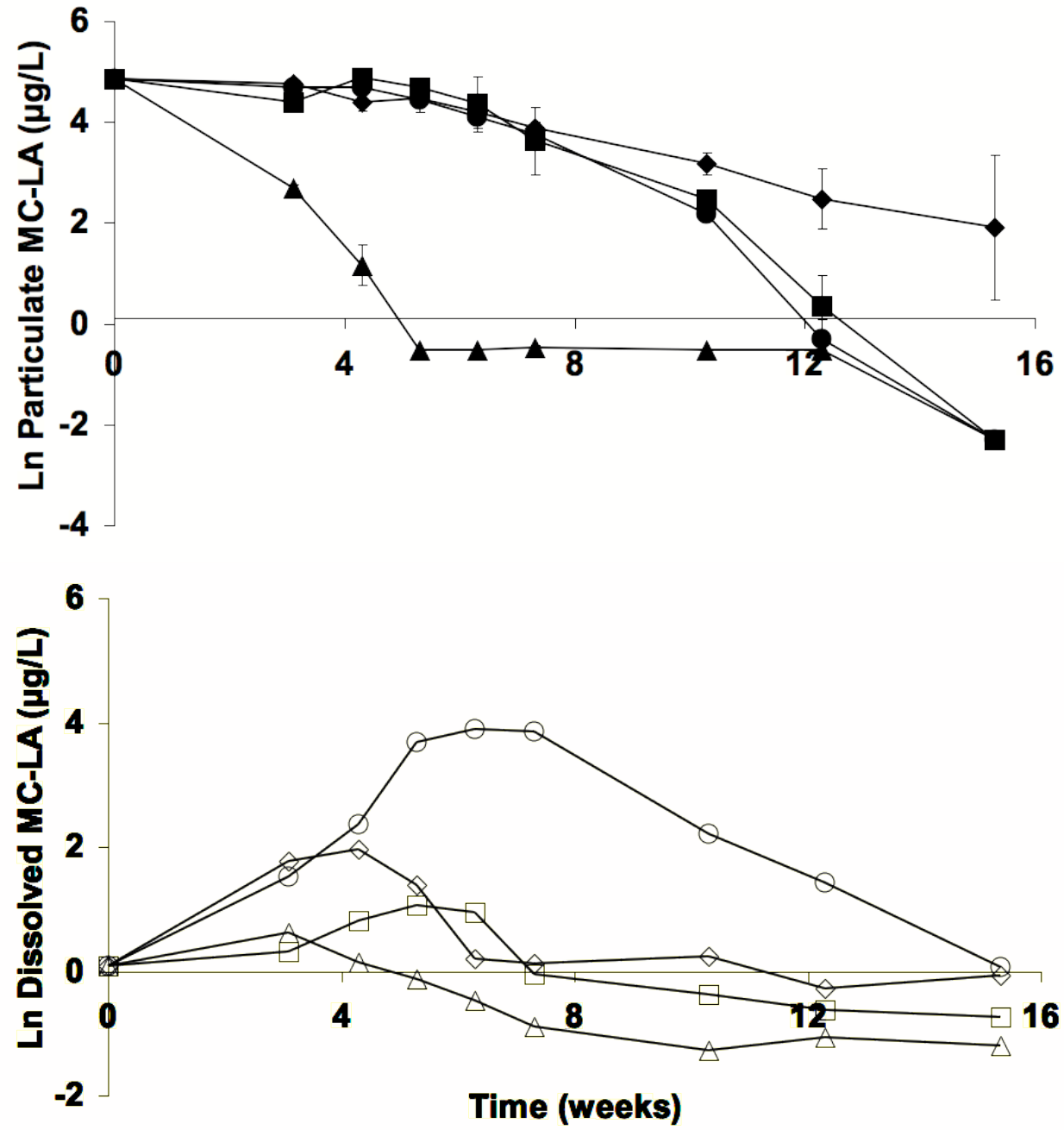


Figure III-3. Decline of particulate (cell-bound) (filled symbols) and dissolved (extracellular) (open symbols) MC-LA (µg L^{-1}) under *in vitro* conditions of 4°C dark (diamonds), 25°C dark (triangles), 25°C low light (squares), and 25°C high light (circles). Duplicate incubations were carried out using bloom material collected in 2010.

Chaper IV: Investigating drivers of toxigenic cyanobacteria from analysis of lake sediment records of microcystins

Prepared for submission to Limnology and Oceanography with Z.E. Taranu, R.W. Zurawell, L.E. Kimpe, I. Gregory-Eaves, J.M. Blais, F.R. Pick.

Abstract

Toxin-producing blooms of cyanobacteria appear to be increasing globally due to eutrophication and climate change. However, most contemporary datasets cannot determine whether this is a true increase or the result of better reporting and increased sampling. Here, we examined the sediment record of Baptiste Lake in boreal Canada to determine whether cyanobacteria and/or cyanotoxins have been increasing through time and to identify the possible drivers of toxigenic cyanobacteria. Microcystins and algal pigments were used to track changes in toxigenic cyanobacteria and phytoplankton communities respectively while diatoms were used to infer past nutrient concentrations. Microcystins were found throughout the entire 46 cm sediment record dating back to the early 1800s, much earlier than any significant alteration of the watershed, which began in the 1880s. Starting in the 1990s (~ 9.0 cm), the concentration of microcystins in the sediment record increased by approximately three orders of magnitude. Over this 20-year period, sediment concentrations of microcystins were correlated with surface water concentrations of microcystins, suggesting that sediment microcystins can be used to track the history of microcystins in the surface waters of the lake ($r = 0.88$, $p = 0.04$, $n = 5$). Sediment microcystins were also strongly correlated with diatom-inferred total phosphorus (DI-TP) ($r = 0.81$, $p < 0.001$, $n = 23$) and diatom-inferred total Kjeldhal nitrogen (DI-TKN) ($r = 0.80$, $p < 0.001$, $n = 22$); both nutrients have been increasing over the past two decades coincident with the intensification of agriculture. The rise in sediment microcystins in recent sediments was much greater than the rise in sediment cyanobacteria and nutrients (as indicated by sediment

echinone and DI-TP). Possible causes of the discrepancy are discussed. In contrast, we found no relationship between sediment microcystins and climate-related variables (open-water season mean air temperature, rainfall, and water column stability) over the 20-year period. Toxigenic cyanobacteria appear to be part of the history of this lake and this should be considered in efforts to restore the ecosystem to a state prior to significant anthropogenic disturbance.

1.0 Introduction

Several paleolimnological studies have found that the timing and rate of nutrient and cyanobacterial increases in aquatic ecosystems coincides with recent human expansion, yet others have identified eutrophic systems sustaining cyanobacterial populations that are not directly the result of anthropogenic activities (Hall et al. 1997; Bianchi et al. 2000; Riedinger-Witmore et al. 2005). The rapid proliferation of cyanobacteria is of particular concern to public and ecological health because of the potential of some bloom-forming genera to produce toxins. Monitoring studies and anecdotal evidence suggest that these toxigenic blooms are increasing in frequency and intensity due to human-induced eutrophication (Anderson et al. 2002; Heisler et al. 2008). However, cyanotoxins have been periodically detected in oligo- and mesotrophic waters (Zurawell 2010). Climate-related stressors have also been associated with increased production of certain cyanotoxins underscoring the complex physiology of toxigenic species and the interdependence of environmental factors in their proliferation (Paerl and Huisman 2009; Cerasino and Salmaso 2012).

The apparent increase in toxigenic blooms may simply be due to better reporting given advances in analytical capabilities and increased public attention (Carmichael et al. 2001; Perez and Aga 2005). There are historical records of toxigenic blooms dating back two millennia and some speculation that cyanotoxins were preserved in sediments of a Pleistocene lake basin (Braun and Pfieffer 2002; Huisman et al. 2005) suggesting that toxigenic blooms are not a new phenomenon.

Most historical data sets of cyanotoxins are too limited in time to determine whether increases in toxigenic blooms have occurred or are due to differences in sampling frequency over time and a disproportionate focus on water bodies with a known history of toxic blooms. Paleolimnological studies can help determine when cyanobacterial proliferation first occurred, whether cyanobacteria are increasing, and what might be the underlying causes of such changes (Smol et al. 2001, Riedinger-Whitmore et al. 2005). However, whether blooms are becoming more toxic has not been determined.

Here we analyzed the sediment record for microcystin cyanotoxins and algal pigments from radiometrically-dated lake sediment cores to reconstruct the history of toxic cyanobacterial blooms. Microcystins were chosen because they are the most widely and frequently produced family of cyanotoxins as well as the most persistent (Harada and Tsuji 1998, Huisman et al. 2005). A few studies have observed the deposition of microcystins in sediments and their persistence in fairly deep sediment layers (Latour et al. 2007, Wormer et al. 2011). The sediment cores used in this study were obtained from Baptiste Lake (Alberta, Canada), a deep, eutrophic lake with an anoxic depositional environment that is conducive to the formation of a reliable fossil record (Prepas and Mitchell 1990). Baptiste Lake is one of the few lakes, particularly in Canada, to have monitoring data for microcystins extending back to the early 1990s and hence, allows a comparison of water column concentrations to the fossilized record. The specific objectives of the study were to: (1) determine if sediment microcystins can serve as a proxy for past microcystin concentrations in the surface waters of the lake by assessing their concordance with

20 years of surface water measurements; (2) test the relationship between sediment microcystins and other paleolimnological (e.g. phosphorus, nitrogen, algal pigments) and climatic (e.g. air temperature) indicators to identify drivers of toxigenic cyanobacterial proliferation; (3) determine if the toxicity of cyanobacterial blooms has changed through time; and (4) characterize reference conditions in order to provide guidance in the development of lake management policies and practices.

2.0 Methods

2.1 Study site

Baptiste Lake, located in central Alberta, Canada about 165 km northwest of Edmonton (+54° 45' 23.9754", ─113° 33' 38.0016"), has two distinct basins of similar size connected by a shallow channel (max depth 5.5 m). The deeper and more strongly stratified south basin has an area and mean depth of 4.7 km^2 and 11.9 m (max depth 27.5 m). The bottom waters are anoxic during the summer and winter (Prepas and Mitchell 1990). Baptiste Lake was eutrophic even prior to the first permanent settlements (1880s) in the watershed and has experienced increasing productivity, possibly linked to land-use changes and/or a climate-induced change in its mixing regime (meromictic to complete mixing becoming more frequent) (Hickman et al. 1990). Livestock poisoning cases, suspected to be associated with toxic algae in Baptiste Lake, were reported in the 1950s (O'Donoghue and Wilton 1951).

2.2 Sediment core sampling

Two sediment cores were obtained (+54° 44' 19.7", ─113° 33' 7.4") from the south basin in June 2010 using a modified gravity corer (Glew, 1989) at a depth of 26.5 m. Cores were extruded at 0.5 cm intervals to 46 cm, placed in pre-labeled Whirl-Pak® bags, and immediately transported on ice in a dark cooler to be frozen in the laboratory (-20^{o}C in darkness). Approximately 15 lyophilized sub-samples spanning the length of each core were prepared for dating. The two cores were dated by measuring ^{210}Pb and ^{226}Ra activities with a high purity germanium detector in a gamma spectrometer (Core A: DSPec Spectrometer linked to Maestro II Software by Ortec, Tennessee, USA; Core B: Canberra Industries Inc.© Well Detector) according to methods of Appleby (2001). Unsupported ^{210}Pb was estimated by subtracting supported ^{210}Pb (measured as ^{226}Ra activity) from total ^{210}Pb. As a result of nuclear arms testing circa 1954, ^{137}Cs was used as an independent chronostratigraphic marker, peaking in 1963 prior to the Nuclear Test Ban Treaty. The constant rate of supply model, constant initial concentration model, and constant flux constant sedimentation model was evaluated to establish chronology in each core (Binford 1990, Appleby and Oldfield 1978, Blais et al. 1995).

2.3 Limnological measurements

At the time of sampling the cores, the Secchi depth was 4.5 m. Basic limnological parameters were measured *in situ* with depth down to 11 m:

Temperature dropped from 15.2 to 7.2 ^{o}C, pH from 8.5 to 8.1, specific conductivity from 240 to 234 µS cm^{-1}, and dissolved oxygen from 10.0 to 8.4 mg L^{-1}.

2.4 Extraction and analysis of microcystin cyanotoxins, algal pigments, and diatoms

To achieve as detailed a chronological resolution as possible, 0.5 cm intervals were extruded as described above and as a result, there was insufficient sediment material in intervals from a single core to conduct all required analyses. Therefore, one core (core A) was used for extracting and quantifying microcystins while the second core (core B) was used for algal pigment and diatom analysis. Intervals from the two cores were aligned based on ^{210}Pb dating profiles described above.

For microcystin analysis, core sections were extracted and analyzed according to Chapter II. Total microcystin values reported were based on analysis of the dominant congeners microcystin-LR, -^{7dm}LR, -RR, -YR, -WR, -LA, -LF, -LY, -LW with method detection limits (MDLs) ranging between 0.3 and 2.5 ng g^{-1} dw. Triplicate injections of extracts of sediments gave relative standard deviations (RSDs) of less than 15%.

Diatom analysis and reconstruction of past changes in total phosphorus (TP) and total Kjeldhal nitrogen (TKN) from the diatom assemblages were conducted by K. Adams of McGill University following methods described in Adams et al. (2013). These are referred to as diatom-inferred TP and TKN (DI-TP and DI-TKN). Sedimentary pigments were extracted, analyzed, and expressed by Z.E. Taranu of McGill University following standard techniques described in Zapata et al. (2000)

and Heiri et al. (2001). The focus of pigment analyses was on echinone and zeaxanthin as these xanthophylls are mainly found in cyanobacteria and have been used as a proxy for this group of algae in sediments (Leavitt 1993, Leavitt and Findlay 1994).

2.5 Historical limnological measurements

Water quality (nutrients, phytoplankton biomass) and total microcystin measurements for Baptiste Lake were collected as part of Alberta Environment and Sustainable Resource Development's (ESRD) long-term water quality programme. Integrated euphotic-zone water samples were collected from ten sites and combined to obtain a composite water sample for the lake at a given time. Selected sites were representative of the entire basin and included near- and off-shore areas and influences of embayment. The lake was sampled up to ten times during the open-water season (May and October) and results from each outing were used to calculate annual averages over the 20-year record (1985-2005). Total microcystin concentrations were determined in the water samples using colorimetric protein phosphatase inhibition assay according to An and Carmichael (1994). Phytoplankton enumeration was conducted during this time frame according to methods described by Zurawell (2010). Additional details on the sampling and analytical methodology are reported in the Alberta Environment Cyanotoxin Program Status Report (Zurawell 2010).

As part of the correlation analyses described below, water column stability was calculated using the Schmidt Stability Index equation (SSI in g cm^{-1}, Soranno

1997), where temperature profiles were used to obtain freshwater density (Kalff 2002). Temperature profiles were obtained four to six times during the open-water season (May to October) and were used to calculate annual averages as far back as 1985.

2.6 Data analysis

We conducted correlation analyses to assess the concordance of sediment microcystins with other measurements in sediments including DI-TP, DI-TKN, and algal pigments as well as water column measurements during open-water season including total microcystins, TP, TKN, and water column stability, and meteorological data such as air temperature and rainfall. Meteorological data were obtained from the Athabasca weather station courtesy of Environment Canada's historical climate data.

3.0 Results

3.1 Sediment dates and accumulation rates

The cores represent a depositional record spanning approximately 150 years based on measurements of ^{210}Pb activity (Figure IV-1). All dating models evaluated were close to the dates calculated by the constant rate of supply (CRS) model, suggesting relatively constant sedimentation rates during this 150-year period, and CRS dates were calculated throughout. The sedimentation rate varied within a narrow range of 0.02 and 0.07 g cm^{-2} year^{-1} with error approaching 50% RSD in deeper sections of the cores (Figure IV-1). Comparison of ^{210}Pb dating profiles

constructed from the replicate cores showed good agreement back to the early 1900s (Figure IV-1).

3.2 Microcystins, nutrients, and pigments in sediments

Organic carbon content was relatively constant throughout the cores with only slight decreases at 4, 38, 42, and 45 cm (Figure IV-2). Expressing sediment microcystins per unit organic matter (data not shown) to reflect the degree of microcystin preservation relative to that of the organic matrix produced a similar pattern as microcystins expressed per unit dry weight (Figure IV-2). Therefore, the concentration profiles of sediment microcystins and algal pigments were presented per dry weight along with the organic carbon profile (Figure IV-2).

Microcystins were detected throughout the entire 46 cm sedimentary record with the deepest interval (45.5-46 cm, ~1824) containing 1.5 ng g^{-1} dw of MC-LA (> 3x MDL) (Figure IV-2). The highest total microcystin concentration was over 3000 ng g^{-1} dw in the 1.0-1.5 cm interval (~2007). Below ~9 cm (pre-1990s), there was no clear directional trend in sediment microcystins with fluctuations resembling a 'saw tooth' pattern (Figure IV-2). From the 1990s until 2007, there was an increase in sediment microcystins of approximately three orders of magnitude (Figure IV-2). There was a decrease in sediment microcystins from this 2007 peak followed by a slight increase in the uppermost intervals. DI-TP also fluctuated throughout most of the sedimentary record until about the end of the mid-1990s (~ 8.5 cm) when an almost two-fold increase began, peaking in 2007 and subsequently declining (Figure IV-2). A similar trend was observed with DI-TKN. The DI-TKN to DI-TP ratio did not

show a directional trend and was on average 24.0 ± 0.9. Surface water TP concentrations from 1985 to 2005 did not show a strong directional trend and were highly variable (data not shown).

Sediment microcystins were significantly correlated with DI-TP ($r = 0.81$, $p < 0.001$, $n = 23$) and DI-TKN ($r = 0.80$, $p < 0.001$, $n = 22$) across the sediment record. We also observed a positive trend between sediment microcystins and surface water TP (1985-2005) but the sample size was low and the relationship was not significant ($r = 0.78$, $p = 0.12$, $n = 5$). Furthermore, we found no significant correlation between sediment microcystins and possible climatic drivers such as open-water season mean air temperature ($r = 0.05$, $p = 0.77$, $n = 35$), rainfall ($r = 0.09$, $p = 0.62$, $n = 35$), and water column stability expressed as the Schmidt Stability Index ($r = 0.11$, $p = 0.86$, $n = 5$).

Sediment microcystins were significantly correlated with sediment pigments representative of cyanobacterial occurrence: echinone ($r = 0.66$, $p < 0.001$, $n = 59$) and zeaxanthin ($r = 0.38$, $p = 0.003$, $n = 59$) (Figure IV-2). Sediment microcystins were also positively correlated with chlorophyll *a* ($r = 0.40$, $p < 0.005$, $n = 58$) and fucoxanthin ($r = 0.65$, $p < 0.001$, $n = 59$) in the sediments, the latter a pigment commonly found in diatoms. Finally, sediment microcystins were significantly correlated with the water column concentrations in Baptiste Lake over the past 20 years, despite the low sample size ($r = 0.88$, $p = 0.04$, $n = 5$) (Figure IV-3). Using the historical records of phytoplankton in the water column, we observed a positive trend between sediment microcystins and cyanobacterial biomass ($r = 0.66$, $p =$

0.22, n = 5) as well as the arcsin-transformed percent cyanobacteria (r = 0.83, p = 0.08, n = 5), but the relationships were not significant (similarly low sample size).

4.0 Discussion

4.1 Sedimentary trends of microcystins, nutrients, and pigments

The presence of microcystins in the earliest layers of the core (45.5-46 cm corresponding to ~1824) suggests that toxic cyanobacteria likely occurred in the lake much earlier than any significant alteration of the watershed, which began after 1880 (corresponding to ~ 36 cm) in this part of central Canada (Hickman et al. 1990). The lake was probably naturally eutrophic as suggested by previous paleolimnological studies (e.g. Hickman et al. 1990). The 'saw tooth' pattern of sediment microcystins (ranging from approximately 5 to 20 ng g^{-1} dw) between approximately 1836 and 1978 (~ 140 years) may be indicative of natural year-to-year variation in toxic cyanobacterial populations. A similar pattern is evident with echinone, an indicator pigment of cyanobacteria (ranging from approximately 600 to 2500 ng g^{-1} dw). Microcystins appear to have increased three orders of magnitude while echinone has increased almost five-fold since approximately 1990.

The correspondence of troughs and peaks between sediment microcystins, DI-TP, and DI-TKN, suggests nutrients may play an important role in the sediment microcystin pattern observed (Figure IV-2). All three variables showed an increasing trend and sediment microcystins were significantly correlated with both DI-TP and DI-TKN. Our findings are consistent with observations that eutrophic lakes have elevated microcystins (Graham et al. 2004, Dolman et al. 2012). Although high

microcystin concentrations have been reported to occur predominantly at low nitrogen-to-phosphorus ratios (< 23) in nutrient-rich Canadian lakes (Orihel et al. 2012), the ratio of DI-TKN to DI-TP in Baptiste Lake has remained fairly constant and relatively high (24.0 ± 0.9) over approximately 150 years while sediment microcystins appear to be increasing.

Although sediment microcystins, echinone, zeaxanthin, DI-TP, and DI-TKN were correlated, the magnitude of the recent sediment microcystin increase (peaking in 2007) greatly exceeded the increase in the other measurements (three orders of magnitude vs. approximately two- to five-fold) (Figure IV-2). This greater rise in microcystins relative to nutrients as well as cyanobacterial pigments indicates that cyanobacterial populations may have become more toxic in the past 20 years. Within cyanobacteria, the cellular cyanotoxin content can vary by more than three orders of magnitude (per dry weight) between strains of the same species (Zurawell et al. 2005). It could also be that the degradation rate of microcystins and the time to reach sediment equilibrium concentrations is not as rapid as that of algal pigments. Also, the decline in DI-TP and DI-TKN in the last two to three years from the peaks in 2007 was not paralleled by a decline in sediment microcystins. The disproportionate increases and recent divergence suggest there are other factors aside from nutrients influencing sediment microcystin concentrations. The disproportionate rise in microcystins relative to nutrients and cyanobacterial pigments may be partly explained by *in situ* production of microcystins in surficial sediments. In Chapter V it was found that diffusion of microcystins from surficial sediments into overlying water greatly exceeds sediment accumulation rates. This

high remobilization suggests net production *in situ*. Furthermore, Misson et al. (2012) found the presence of *mcyB* RNA in every benthic *Microcystis* population they investigated, even in those buried for six years in sediments. The presence of *mcyB* RNA in sediments indicates the ability to initiate microcystin production and is consistent with the high remobilization observed in Chapter V.

4.2 Interpretation of the sedimentary record

The reliability of paleolimnological investigations depends on whether the potential indicators preserved in sediments are proportional to their production in the water column and whether their preservation is consistent through time. Diatom species composition and phytoplankton pigments in sediment cores used in this study have been used for decades to indicate historical changes in nutrients and algal abundance (Smol et al. 2001). An improved understanding of pigment production, deposition, and decomposition has increased the confidence in interpreting their sediment record (Leavitt 1993, Smol et al. 2001). For example, it has been observed that substantial decomposition of pigments occurs in the water column during sinking due primarily to photo-oxidation. Once deposited, decomposition occurs mainly in the most recent three to five years of sediment deposits (Leavitt and Findlay 1994). In order to assess the reliability of pigments as indicators of phytoplankton abundance, several studies have compared sediment pigment records to records of phytoplankton abundance in the water column (Leavitt and Findlay 1994). Comparisons of sediment pigment profiles with historical data

and/or geographic gradients of productivity are among the best ways to assess their reliability (Gorham et al. 1974, Leavitt 1993).

Microcystins have been observed to deposit into sediments preferentially within intact cells, which significantly limits decomposition (Wormer et al. 2011). Extracellular microcystins are relatively persistent molecules but can undergo decomposition in the presence of photosensitizers or by microbes previously exposed to the toxins (Welker and Steinberg 2000, Rapala et al. 1994). In a productive system, such as Baptiste Lake, a high standing crop of phytoplankton reduces irradiance through self-shading, which likely means reduced photodecomposition of microcystins as they sink. Additionally, it is well known that preservation is relatively good in deepwater sediments (Swain 1985, Leavitt 1993). The limited light and oxygen reaching the deepwaters of Baptiste Lake means limited photo-oxidation and biologically-mediated decomposition in sediments. The productive nature of Baptiste Lake further reduces light and oxygen in deepwaters by shading the sediments and consuming oxygen during crop decay. Although a couple studies have observed that anoxic decompostion of microcystins can occur, the experiments conducted were at elevated temperatures (>10^{o}C) and with supplementation (Holst et al. 2003, Chen et al. 2010). Thus, the significance of natural processes remains unclear. Currently, there are no estimates available on microcystin stability in lake sediments.

Previous paleolimnological studies have suggested Baptiste Lake has been eutrophic throughout its history (Hickman et al. 1990), which means preservation conditions have likely been consistent through time. The relatively constant organic

carbon content throughout the cores is supportive of constant preservation conditions (Figure IV-2).

Baptiste Lake is one of very few lakes in Canada with a monitoring program that includes surface water measurements of microcystins extending back more than 20 years. The concordance of sediment microcystins with historical microcystin measurements in surface waters suggests that the sediment microcystin record may accurately reflect surface water concentrations (Figure IV-3). This provides confidence in the microcystin sedimentary record as indicative of the relative microcystin concentrations in the lake beyond the 20 years of historical data. Still, one must exercise caution in interpreting sediment records of microcystins as currently there are no estimates available on microcystin stability in lake sediments and, as discussed, there is evidence that there may be *in situ* production of microcystins in surface sediments (Misson et al. 2012, Zastepa Chapter V).

4.3 Factors contributing to increased toxic cyanobacterial blooms

The increase in sediment microcystin concentrations, DI-TP, and DI-TKN (Figure IV-2) coincides with the intensification of agriculture in the Baptiste Lake watershed at the end of the 20th century (Prepas and Mitchell 1990, Carlson 2008). Trew et al. (1987) and Cooke and Prepas (1998) showed that runoff from agriculture contributes to elevated nutrients in Baptiste Lake. In spite of a recent decline in DI-TP, sediment microcystins continue to increase (Figure IV-2). The legacy of land-use changes may be continuing to drive ecosystem conditions and sustaining increased microcystin levels (MacDonald et al. 2012). Changes in phosphorus

sources or internal loading from agriculture intensification may be changing community structure to favour toxigenic cyanobacteria and explain the continued increase in sediment microcystins while DI-TP decreases.

Given the eutrophic history of Baptiste Lake, it is likely that the recent increase in microcystins is from more than just phosphorus. The recent divergence of the relationship between sediment microcystins and DI-TP as well as the lack of significance in the correlation with the last 20 years of surface water TP measurements further suggests that other factors are involved. Concerns about frequent algal blooms began in the 1970s (Carlson 2008), well before the dramatic increase in DI-TP. O'Donoghue and Wilton (1951) described a suspected algal poisoning of livestock in 1950 along the shore of Baptiste Lake although analytical confirmation was not available at that time. Climate warming is suspected to have a significant influence on toxigenic cyanobacteria (Jacoby et al. 2000, Butterwick et al. 2005, Paerl and Huisman 2009, Beaulieu et al. 2013). However, we were not able to identify any potential climatic drivers of microcystin production in this study (i.e. no correlation with sediment microcystins). In contrast, Cerasino and Salmaso (2012) found that water temperature was correlated with anatoxin-a concentrations in a survey of nine lakes in Italy. The role of climate in cyanotoxin production needs further investigation.

According to a report by Carlson (2008) on the state of the Baptiste Lake watershed, there is some evidence that trophic interactions in Baptiste Lake have been altered and food web changes may be having a significant effect on phytoplankton communities. The primary piscivorous fish (walleye and pike) have

declined in size and abundance since the mid-twentieth century (Carlson 2008). This decline has led to changes in the zooplankton community consistent with a trophic cascade (Carpenter and Kitchell 1988) (e.g. average length of *Daphnia* decreased by one-third between 1976 and 1995) and an increase in phytoplankton biomass (based on chlorophyll *a*) greater than predicted from phosphorus concentration alone. The lake's low biodiversity and abundance of *Daphnia* make it more susceptible to trophic cascades (Polis 1994, Schindler 2006) and may be a contributing factor in the proliferation of cyanobacteria (Adams et al. 2013).

4.4 Implications for lake management

We used the relationship between DI-TP and sediment microcystins to perform a linear regression analysis to extrapolate the DI-TP value at which sediment microcystin concentration would be zero (Figure IV-4). The DI-TP value obtained (33.5 μg L^{-1}) was close to the threshold for the onset of cyanobacterial dominance in lakes obtained by Downing et al. (2001) in an analysis of 99 temperate lakes. The authors determined that the risk of cyanobacterial dominance rises abruptly from 30 to 70 μg L^{-1} TP, where above 30 μg L^{-1} usually means that nuisance algae blooms occur with increasing frequency. This demonstrates that even at phosphorus levels where the average risk of cyanobacteria dominance is low (30 μg L^{-1}), microcystin occurrence in the lake can be recorded in the sediment record.

In the development of lake management policies and practices, the sedimentary record provides reference conditions for restoration of an aquatic

system to a state prior to significant anthropogenic disturbance. In light of the eutrophic nature of Baptiste Lake and the presence of microcystins throughout the 150-year-old sediment record (Figure IV-2), restoration efforts should consider that high productivity and toxigenic cyanobacteria are part of the history of this ecosystem. Even a reduction in phosphorus does not necessarily mean an immediate decline in phytoplankton and the return to the prior species composition (Dokulil and Teubner, 2005). Therefore, a most prudent goal would be a reduction in the intensity of toxigenic cyanobacterial blooms rather than complete elimination. The use of sediment microcystins as a proxy for toxigenic cyanobacteria can provide historical information and context critical in these lake management decisions. This is especially important for lakes and lake monitoring programs with limited or non-existent records of microcystin concentrations in surface waters, as is often the case.

Figures

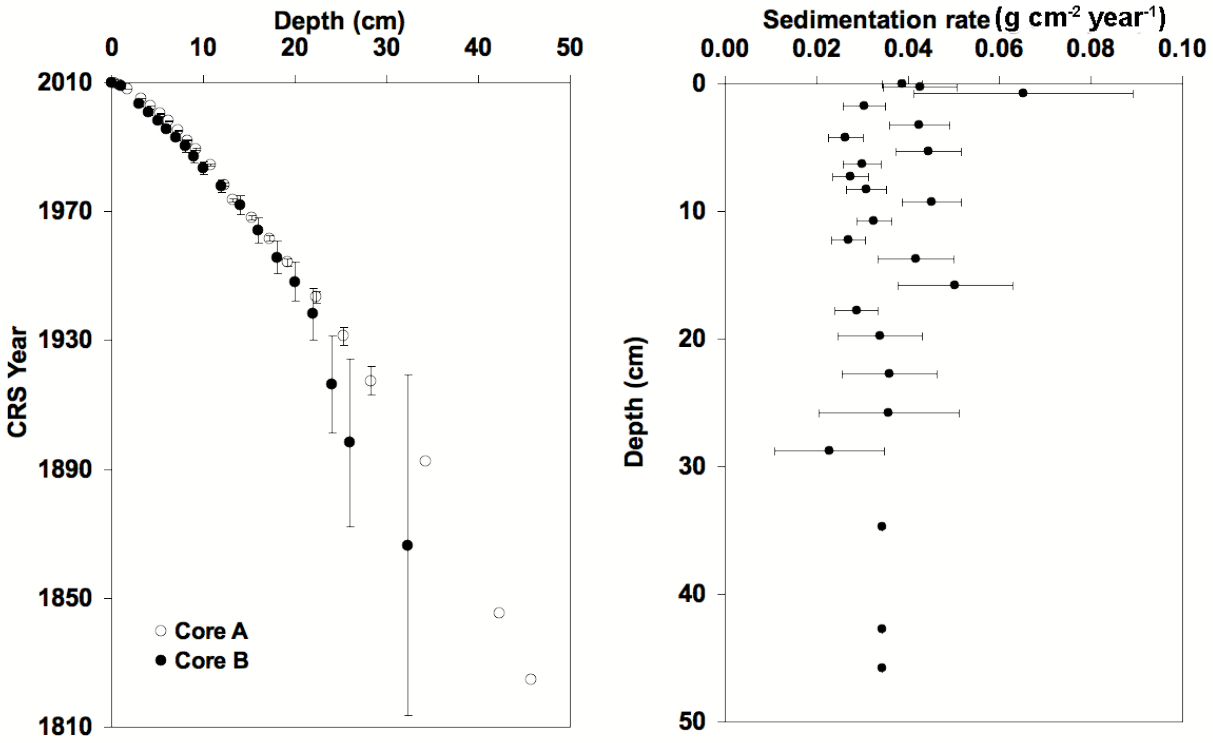


Figure IV-1: Age-depth relationship and sediment accumulation rate from Baptiste Lake (south basin), Alberta sediment cores using the constant rate of supply model (CRS). One core (core A) was used for microcystin analysis while a duplicate core (core B) was used for diatom-inferred total phosphorus, diatom-inferred total Kjeldhal nitrogen, and pigment analysis.

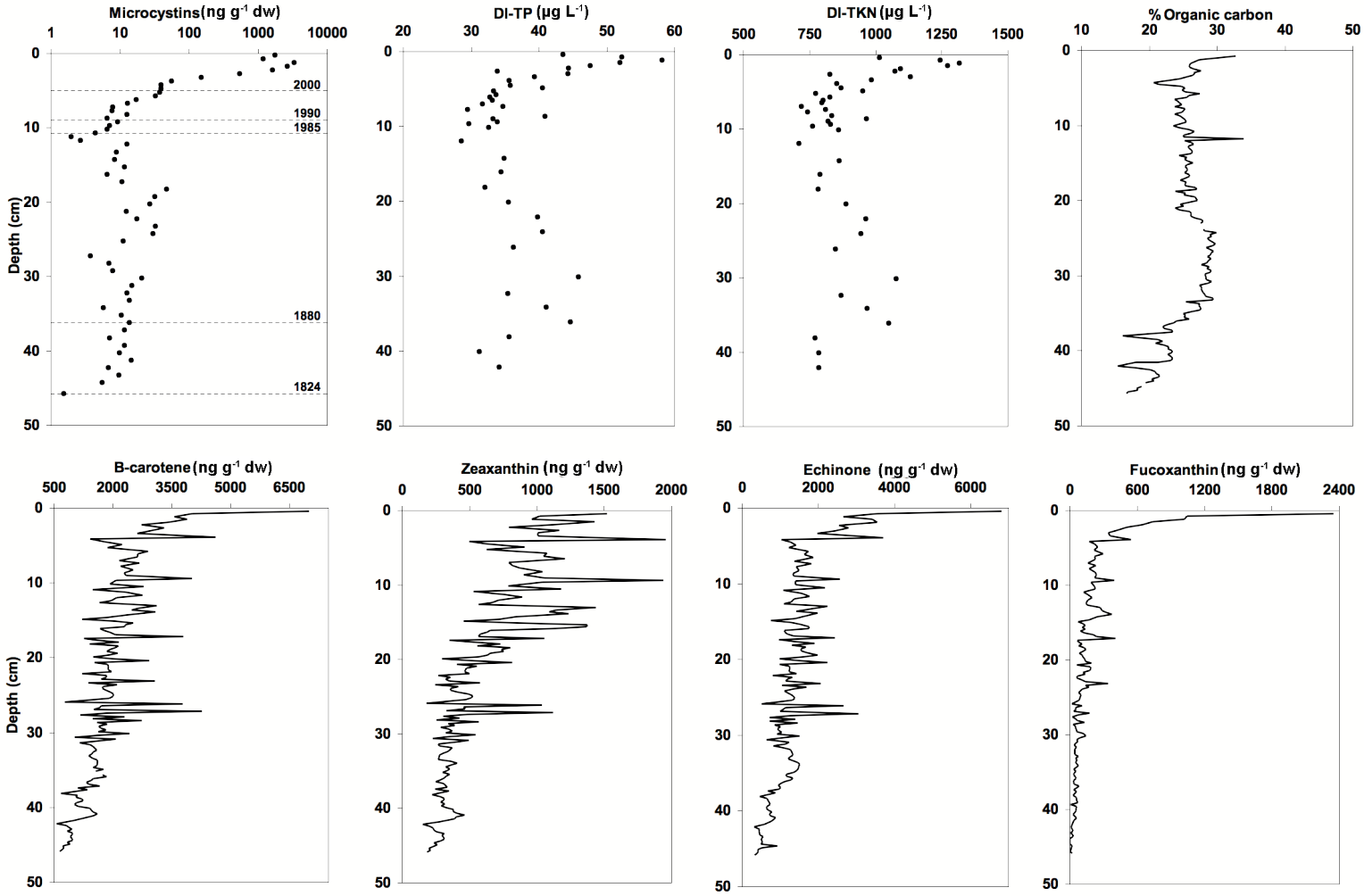


Figure IV-2: Profile of total microcystin concentration, diatom-inferred total phosphorus (DI-TP), diatom-inferred total Kjeldhal nitrogen (DI-TKN), % organic carbon, and pigment concentrations in sediment cores taken from Baptiste Lake (south basin), Alberta.

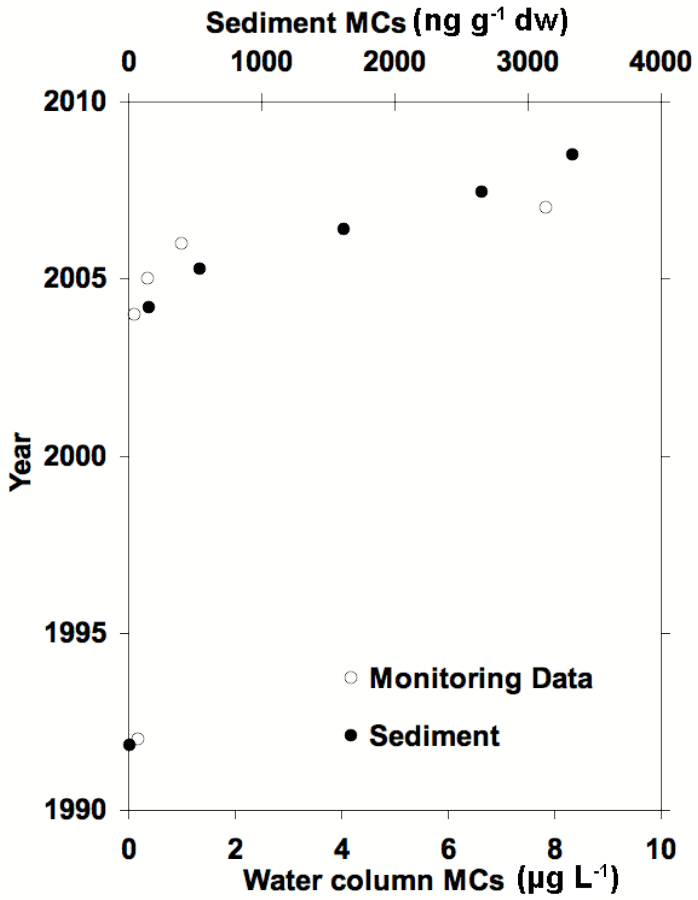


Figure IV-3: Changes in microcystin concentrations in sediment and measured in the water column over a 20-year record in Baptiste Lake (south basin), Alberta.

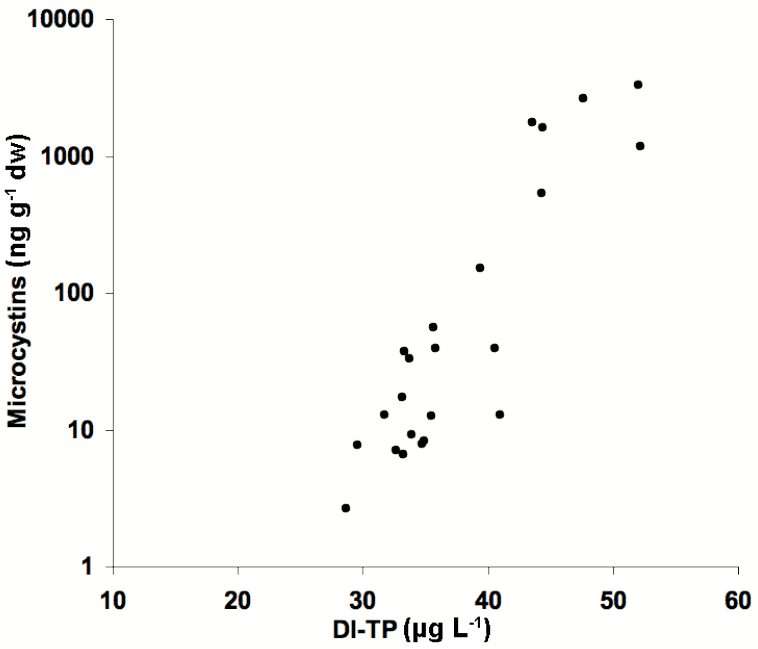


Figure IV-4: Correlation between sediment microcystins and diatom-inferred total phosphorus (DI-TP) ($r = 0.81$, $p < 0.001$, $n = 23$) throughout an approximately 150 year sedimentary record from Baptiste Lake, Alberta.

Chapter V: Distribution and flux of microcystin congeners in sediments

Prepared for submission to Science of the Total Environment with A. Paterson, F. R. Pick, J. M. Blais.

Abstract

Sediment concentrations of microcystin congeners and their exchange across the sediment-water interface were determined in two temperate lakes prone to toxigenic cyanobacterial blooms (Baptiste Lake, Alberta and Lake of the Woods, Ontario). Sediment cores were used to examine the historical occurrence of microcystin congeners. The most abundant microcystins throughout the cores were MC-LA and -LR with a greater diversity of congeners observed in the upper core sections (MC-LR, -LA, -RR, -YR, -7dmLR, -WR, -LF, -LY, -LW). MC-LA and -LR were also distributed in the pore waters whereas MC-RR and -YR were more strongly adsorbed to sediment particles. MC-LA and –LR accumulation rates were determined from the product of the concentration on sediment particles (ng g^{-1} dw) and the burial rate (based on ^{210}Pb radiochronology (g cm^{-2} yr^{-1})). Overall, burial rates were low across sites (0.05 to 7.05 ng cm^{-2} yr^{-1}) when compared to diffusion of microcystins from sediments to overlying water (11.1 to 54.6 ng cm^{-2} yr^{-1}). Diffusion from sediments was estimated from the concentration gradient between the pore water of surficial sediments and overlying water using Fick's first law. The fact that microcystins appear to remobilize at a much higher rate than their rate of burial suggests there is a reservoir of microcystins in the sediments either from previous episodes of toxic blooms or from the *in situ* production of microcystins within sediments. Sediments therefore represent a potential ongoing risk for human exposure to microcystins in the absence of visible surface blooms. The higher diffusion fluxes and lower accumulation rates of MC-LA compared to MC-LR point to differences in their environmental fate. In order to assess human and ecological

health risks, the environmental fate of different microcystin congeners needs to be specifically considered.

1.0 Introduction

Toxins produced by cyanobacteria represent a major water quality problem around the world and the hepatotoxic microcystins have garnered the most attention due to their high toxicity (TDI 0.04 μg kg^{-1} bw day^{-1} MC-LR) and prevalence in freshwater ecosystems (Chorus and Bartram 1999). With over 70 different congeners described (Zurawell et al. 2005), chemical analyses and risk assessment of these compounds represent a significant challenge.

An understanding of the environmental fate of microcystins in aquatic ecosystems is currently limited due to a lack of a standardized analytical method for various congeners and environmental compartments. Recent analytical advances, including those developed in Chapter II, indicate that microcystins can be found in surficial and deeper sediments in lakes prone to cyanobacterial blooms (Latour et al. 2007). In Chapter II, microcystins were also detected in sediment pore waters from the survey of seven Canadian lakes. This sediment reservoir of microcystins raises concerns about the potential for microcystins to remobilze into the overlying water, thus posing human and ecological health risks. Furthermore, an assessment of the potential of microcystin diffusion through sediments is of critical importance in the accurate interpretation of their historical record in sediments.

Despite a high number of microcystin congeners with a wide range of chemical properties, measurements in sediments have been limited to a few congeners of similar chemistry (MC-LR, -YR, and -RR) (Chen et al. 2006, Babica et al. 2006). Growing experimental and field evidence indicates significant differences in the environmental fate of microcystin congeners, in particular MC-LA (Newcombe

et al. 2003, Miller et al. 2010, Zastepa et al. 2013 in press, Zastepa Chapter II). Congener-specific estimates of post-depositional mobility will achieve increased accuracy in assessing risk to human and ecological health and in the interpretation of historical records of microcystin cyanotoxins in sediments. The specific objectives of this study were: (1) to determine the distribution of microcystin congeners between sediment particles and pore waters in lake sediment cores; (2) to estimate sediment deposition rates of microcystin congeners; and (3) to determine whether sediments are a significant source of microcystins to the overlying water via diffusion and whether there are congener-specific differences in these sediment-water fluxes. To achieve these objectives, sediment cores from two temperate lakes prone to toxigenic cyanobacterial blooms were used: Lake of the Woods, Ontario and Baptiste Lake, Alberta (Chen et al. 2007, Zurawell 2010).

2.0 Methods

2.1 Study sites

Two lakes were sampled to obtain sediments and associated pore waters for the analysis of microcystins. Lake of the Woods and Baptiste Lake both have a history of toxic cyanobacterial blooms, but differ in their underlying geology and surrounding land use (Chen et al. 2007, Zurawell 2010). Lake of the Woods is a large (~ 3850 km^2), morphologically and hydrologically complex water body with variable depth (mean ~ 8 m, max. ~ 66 m). It is made up of several interconnected basins occupying the Canadian provinces of Ontario and Manitoba as well as the US state of Minnesota. Lake of the Woods has a large drainage basin of

approximately 70,030 km^2 (excluding lake area) (Schupp and Macins 1977). Water flows northward from the main inlet, the Rainy River, to the main outlet, the Winnipeg River. A strong gradient of total phosphorus generally tracks the flow of the Rainy River from the south, where the highest phosphorus loading occurs. Ice covers the lake from approximately November to April. The northern region of the lake is surrounded mostly by boreal forest and underlain by Precambrian Shield granitic bedrock, whereas the southern region is surrounded mostly by agricultural land and consists of old lake sediments deposited by glacial Lake Agassiz (Johnston 1915), resulting in shallower, more turbid, and more productive waters (Rusak and Mosindy 1997). Lake of the Woods has a history of human influence including land clearing by European settlers, logging and sawmill operations, and several dams constructed for water level management and hydroelectric power (Yang and Teller 2005, Serieyssol et al. 2009, Serieyssol-Bleser 2010).

Baptiste Lake, located in central Alberta, Canada about 165 km northwest of Edmonton (54° 45' 24.0" N, 113° 33' 38.0" W), has two distinct basins of similar size connected by a shallow channel (max depth 5.5 m). The deeper and more strongly stratified south basin has an area and mean depth of 4.7 km^2 and 11.9 m (max depth 27.5 m). The bottom waters are anoxic during the summer and winter (Prepas and Mitchell 1990). Baptiste Lake was likely eutrophic prior to the first permanent settlement (1880s) and has experienced continued eutrophication, possibly linked to a climate-induced change in mixing regime (meromictic to complete mixing becoming more frequent) (Hickman et al. 1990). Baptiste Lake has a history of toxic

algae with the earliest probable case of animal poisonings dating from 1950 (O'Donoghue and Wilton 1951).

2.2 Sediment core collection and dating

Lake of the Woods was sampled in July 2010 at two northern sites and one southern site. A 46 cm core was retrieved from polymictic Rat Portage Bay (49° 43' 20.7" N, 94° 33' 12.7" W) at a depth of 23 m. A 40 cm core was retrieved from dimictic Bigstone Bay (49° 40' 10.7" N, 94° 19' 43.8" W) at a depth of 23 m. Bigstone Bay thermally stratifies during the summer. A third core (40 cm) was retrieved from southern Hay Island (49° 9' 25.6" N, 94° 7' 23.2" W) at a depth of 13 m.

The south basin of Baptiste Lake (54° 44' 19.7" N, 113° 33' 7.4" W) was sampled in June 2010 at the deepest site (26.5 m) to obtain a 46 cm core.

Each sediment core was obtained using a modified gravity corer (Glew, 1989). The overlying water from the top of the sediment core was also collected from the Lake of the Woods for diffusion calculations (500 mL). Cores were extruded on-site at 0.5 cm intervals, placed in pre-labeled Whirl-Pak® bags, and immediately transported to the laboratory on ice in a dark cooler for pore water separation before being frozen in the dark at -20 ^{o}C. Up to 22 lyophilized sub-samples spanning the length of each core were prepared to determine age and sedimentation rate. This was achieved by measuring ^{210}Pb and ^{226}Ra activities with a high purity germanium detector in a gamma spectrometer (DSPec Spectrometer linked to Maestro II Software by Ortec, Tennessee, USA) according to methods of

Appleby (2001). Unsupported ^{210}Pb was estimated by subtracting supported ^{210}Pb (measured as ^{226}Ra activity) from total ^{210}Pb. As a result of nuclear arms testing circa 1954, ^{137}Cs was used as an independent chronostratigraphic marker, peaking in 1963 prior to the Nuclear Test Ban Treaty. The constant rate of supply model, the constant initial concentration model, and the constant flux constant sedimentation model were evaluated to establish chronology in each core (Binford 1990, Appleby and Oldfield 1978, Blais et al. 1995).

2.3 Limnological parameters

Total phosphorus (TP) and pH at the time of sampling and across the three sites in Lake of the Woods were as follows: Rat Portage TP = 20 μg L^{-1}, pH = 7.66; Bigstone Bay TP = 18 μg L^{-1}, pH = 7.9; and Hay Island TP = 38 μg L^{-1}, pH = 7.64. For Baptiste Lake, nutrient and other limnological measurements have been collected as part of Alberta Environment and Sustainable Resource Development's (ESRD) long-term water quality programme. Samples taken up to ten times between May and October provide annual averages of TP concentrations between 43 and 85 μg L^{-1} for the south basin of Baptiste Lake between 1985 and 2005.

2.4 Extraction and analysis of microcystin congeners

Sediment core intervals were separated into a pore water and sediment particles fraction by centrifugation before microcystin extraction and analysis according to Chapter II. The analytical method was developed, optimized, and validated for the extraction and quantitation of microcystin-LR, -7dmLR, -RR, -YR, -

WR, -LA, -LF, -LY, -LW with method detection limits ranging from 0.3 to 2.5 ng g^{-1} dw. Method detection limits for pore water ranged from 20 to 160 ng L^{-1}.

2.5 Microcystin accumulation rate calculations

Accumulation rates of microcystins in surficial sediments were calculated as the product of the concentration of microcystins on sediment particles (ng g^{-1} dw) and the particle sedimentation rate, which was obtained from ^{210}Pb measurements (g cm^{-2} yr^{-1}).

2.6 Microcystin flux calculations

Fick's first law was used for estimating diffusion across the sediment-water interface, assuming steady state (Schwarzenbach et al. 2003). Diffusion of microcystin congeners from sediments was estimated based on the concentration gradient between the pore water of surficial sediment (top 0.5 cm) and water overlying sediment. Diffusive flux was calculated at the three sites in Lake of the Woods, but not at Baptiste Lake where no overlying water was collected.

$$\text{diffusive flux } (F) = -(\phi D_w / \theta^2)(\delta C / \delta x) \quad (1)$$

where F is the flux of (ng cm^{-2} yr^{-1}) of a solute with concentration C at depth x. ϕ is the porosity calculated using the following equation:

$$\phi = 1 - (\text{bulk density} / \text{particle density}) \quad (2)$$

where a particle density of 2.65 g cm^{-3} was used (Brady and Weil 2002). θ is the tortuosity (dimensionless) and is related to porosity by the empirical relationship of Bourdeau (1996):

$$\theta = 1 - \ln(\phi^2) \quad (3)$$

Finally, D_w is the diffusion coefficient of the solute in water in the absence of sediment matrix. A diffusion coefficient of 1.4 x 10^{-6} cm^2 s^{-1} was calculated for both MC-LR and MC-LA using molecular volume according to Schwarzenbach et al. (2003).

3.0 Results

3.1 Congener distribution in lake sediments

Lake of the Woods

Microcystins were detected in the Hay Island sediment core down to 6.5 cm, which corresponds to year 2001 ± 0.2 (Figure V-1). This is slightly earlier than the first report of microcystins in the Canadian portion of the Lake of the Woods in 2004 (Chen et al. 2007). Below 6.5 cm, the concentrations were below the congener detection limits of the method. The sediment core profile had two major peaks of total microcystins (sediment particles plus pore water) at a depth of 0.25 and 4.25 cm, which correspond to year 2010 and 2004, respectively. MC-LR and -LA were the dominant congeners in most intervals with the highest total concentration

(sediment particles plus pore water) being 271 ng g^{-1} dw MC-LR. MC-RR, -^{7dm}LR, -YR, -LW, and –WR were generally present in relatively minor amounts with the exception of MC-RR at the 4.25 cm interval, where it was the dominant congener at a total concentration of 113 ng g^{-1} dw. MC-LA was detected in the pore waters of more than half the intervals where it was detected and typically more than half of MC-LA in each interval was distributed into the pore water (Figure V-2, Table V-1). In contrast, MC-LR and -RR, despite relatively higher concentrations, were only associated with sediment particles in all of the intervals where they were detected (Figure V-1, Table V-1).

Baptiste Lake

Microcystins were detected throughout the entire 46 cm sediment core (dated as going back to 1824) and MC-LR and -LA were again the dominant congeners (Figure V-3). The first report of microcystins in the surface waters of Baptiste Lake dates from 1991 (Kotak unpublished), but a case of animal poisonings likely from toxic algae was reported as early as 1950 (O'Donoghue and Wilton 1951). The highest total concentrations (sediment particles plus pore water) measured for MC-LR and -LA were almost 1800 and over 3000 ng g^{-1} dw and were much higher than those in the sediments of Lake of the Woods (Figure V-1). MC-^{7dm}LR and -YR were frequently observed throughout the top half of the core with maximum total concentrations of 60 and 9 ng g^{-1} dw. MC-LY was observed down to 3.25 cm in the core (dated as 2004) with a maximum total concentration of almost 300 ng g^{-1} dw.

Other congeners included MC-LW, -LF, and -WR, which were detected down to 2.75 cm in the core (dated as 2005).

In general, the proportion of microcystins in each interval that distributed into the pore water decreased with core depth in Baptiste Lake (Figure V-4). Table V-1 summarizes the number of intervals where microcystin congeners were detected and the percentage distributed into pore waters. MC-^{7dm}LR was only detected down to a depth of 3.25 cm (dated as 2004) in pore waters though it was present on sediment particles down to 18.5 cm (dated as 1960) (Figure V-3 and 4). MC-YR was detected on sediment particles in 21 intervals down to 12.5 cm (dated as 1978) but it was not detected in the pore water phase.

3.2 Microcystin congener deposition and diffusion

Microcystin concentrations and composition were measured in the water above the sediments at three sites in Lake of the Woods (Table V-2). Averaged over the three sites, 32 ± 18% of total microcystins in the water overlying sediment was MC-LA while 62 ± 18% was MC-LR. In the surficial sediment, about 15 ± 8% of total microcystins was MC-LA while 85 ± 8% was MC-LR.

Concentrations in surficial sediments at the three sites in Lake of the Woods ranged from ~ 2 to 20 ng g^{-1} dw for MC-LA and ~ 7 to 250 ng g^{-1} dw for MC-LR (Table V-2). Particle sedimentation rates ranged from 0.023 to 0.060 g cm^{-2} yr^{-1}. Using the surficial sediment concentrations and the particle sedimentation rates determined from ^{210}Pb measurements, we estimated the accumulation rates of two microcystin congeners (Table V-2). Microcystin accumulation rates at the three sites

were relatively low (0.05 to 7.05 ng cm^{-2} yr^{-1}) with MC-LR accumulation rates higher than MC-LA.

Microcystin pore water concentrations at the sediment surface (top 0.5 cm) of the three sites sampled in Lake of the Woods were approximately two to seven times higher than the concentrations in the overlying water for MC-LA and up to almost three times higher for MC-LR (Table V-2). Diffusive flux (from sediment surface) at the three sites ranged from 23.6 to 54.6 ng cm^{-2} yr^{-1} for MC-LA and from 11.1 to 29.1 ng cm^{-2} yr^{-1} for MC-LR (Table V-2). Diffusive fluxes were lowest at the Hay Island site. A schematic summary of the accumulation rates and diffusive fluxes of microcystin congeners (MC-LR and -LA) in surficial sediments (0-0.5 cm) and at the sediment-water interface at three sites at the Lake of the Woods is presented in Figure V-5.

4.0 Discussion

4.1 Microcystin congener distribution in lake sediments

Of the nine congeners studied, the most frequently found and in the highest concentrations throughout the sediment cores of both Lake of the Woods (Hay Island) (Figure V-1) and Baptiste Lake (Figure V-3) were MC-LR and -LA. These two congeners have the highest mammalian toxicity reported for the congeners tested to date (Chorus and Bartram 1999, Miller et al. 2010).

MC-LA had a tendency to distribute into the pore water phase and this was consistent in both the Lake of the Woods (Hay Island) and Baptiste Lake sediment cores (Figure V-2 and 4, Table V-1). This is consistent with laboratory (Chapter II)

and field (Chapter III) observations of a low affinity of MC-LA for sediment particles (also see Zastepa et al. 2013 in press). The low affinity of MC-LA for sediment particles may be caused by electrostatic repulsion and/or high solvation resulting from the ionization (2-) of MC-LA in sediment pore waters. Newcombe et al. (2003) concluded that electrostatic interactions likely do play a role based on experiments measuring the effect of increasing ionic strength on adsorption of MC-LA.

MC-LR also had a tendency to distribute into the pore water phase but less so than MC-LA (Figure V-2 and 4, Table V-1). The distribution of MC-LR into pore waters was only observed in Baptiste Lake sediments. Furthermore, this was not consistent throughout the length of the core suggesting that adsorption of MC-LR to sediments may be a function of different sediment properties and/or differential decomposition in pore waters throughout the core. In contrast, MC-RR and -YR had a clear tendency to adsorb to sediment particles; neither congener was detected in pore waters despite their relatively high concentrations on sediment particles (MC-RR in Figure V-1 and MC-YR in Figure V-3, Table V-1). This is consistent with laboratory results indicating strong affinity of both MC-RR and -YR for sediment particles (Harada et al. 1998, Zastepa Chapter II). Results for the remaining congeners were mixed and no clear tendencies of preferential distribution were evident. Generally, a greater diversity of congeners was observed in the upper sections of the Baptiste Lake and Lake of the Woods (Hay Island) sediment cores. Only MC-LR and -LA were observed in deeper intervals of these cores. This changing congener composition may be reflective of changing toxigenic cyanobacterial communities possibly due to recent changes in environmental

conditions (Rühland et al. 2010, Adams et al. 2013) or alternatively may be due to differential preservation throughout the sediment core. To more accurately interpret sedimentary archives of microcystins, quantitative studies on the natural, post-depositional decomposition of different microcystin congeners in sediments are necessary.

4.2 Microcystin congener deposition and diffusion in Lake of the Woods

The surficial sediment concentrations of microcystins (ng g^{-1} dw) in the Lake of the Woods (Table V-2) were within the range of concentrations recently reported for other lake sediments (Babica et al. 2006, Chen et al. 2006, Zastepa Chapter II). In general, the microcystin sediment accumulation rates estimated in this study (based on ^{210}Pb measurements) (0.05 to 7.05 ng cm^{-2} yr^{-1}) were low. They were close to MC-LR sedimentation rates reported by Kankaanpaa et al. (2009), which were based on sediment trap measurements made in the northern Baltic Sea (maximum of 0.56 µg m^{-2} d^{-1} or 20.4 ng cm^{-2} yr^{-1}). They were much lower than those estimated by Wormer et al. (2011), also using sediment traps (10 - 2500 µg m^{-2} d^{-1} or 365 - 91,250 ng cm^{-2} yr^{-1}). Wormer et al. (2011) cautioned that the use of sediment traps may overestimate sedimentation rates, as being so close to the lake bottom they likely also trapped resuspended microcystins. This was supported by observations in another reservoir where microcystins were found in sediment traps even though they were not detected in the water column (Wormer et al. 2011). The higher sedimentation rates reported by Wormer et al. (2011) may be further explained by the wide-spread presence of a toxic bloom that produced high surface

water concentrations of microcystins (up to 65 μg L^{-1}) during the course of their sediment trap deployment.

Concentration gradient measurements between the pore water of surficial sediments (top 0.5 cm) and the overlying water enabled the calculation of the diffusive fluxes of microcystins (Table V-2). Microcystin concentrations in the pore water of Lake of the Woods sediments were within the range of concentrations reported in Chapter II in the survey of seven Canadian lakes (between 500 and 130,000 ng L^{-1}). The diffusive flux estimates reported in Table V-2 suggest that a return of microcystins into the water column may be significant in lakes, as the rates observed exceed diffusive flux estimates for some anthropogenic xenobiotics at contaminated sites (Delongchamp et al. 2010). Furthermore, diffusion of microcystins from sediments exceeded microcystin sediment accumulation rates. The ratio of diffusive flux to sediment accumulation rate indicates the proportion of deposited microcystins that is remobilized from the sediments. The observation that microcystins are remobilized at a higher rate than they are buried in sediments suggests there is a reservoir of microcystins in the sediments. This may derive from previous episodes of toxic blooms or the production of microcystins within sediments. Several studies have found microcystins in samples of permanently benthic species, however active production *in situ* was not confirmed (Mez et al. 1997, Izaguirre et al. 2007, Hitzfeld et al. 2000). The most compelling evidence of *in situ* production thus far was reported by Misson et al. (2012). The authors found the presence of *mcyB* RNA in every benthic *Microcystis* population they investigated, even in those buried for six years in sediments. The presence of *mcyB* RNA

indicates the ability to initiate microcystin production and is consistent with the high remobilization observed in this thesis. It is important to note that these flux estimates are diffusive fluxes and do not consider additional fluxes caused by bioturbation, bioirrigation, and resuspension of sediments. As such, these flux estimates based solely on molecular diffusion may underestimate the exposure risk.

MC-LR had a higher sediment accumulation rate than MC-LA and this was reflected in the higher proportion of MC-LR (of total microcystins) in the surficial sediments compared to the water above the sediments. In contrast, a higher proportion of MC-LA (of total microcystins) was found in the water above the sediments compared to the surficial sediments. Evidence from laboratory experiments similarly suggests that different congeners may have different sedimentary fate especially due to differential adsorption to sedimentary particles (Harada et al. 1998, Newcombe et al. 2003, Zastepa Chapter II). In contrast, field observations thus far have found no differences in congener composition between the water column and sediments (Welker et al. 2007). However, this could be because microcystins preferentially deposit into sediments within intact cells (Wormer et al. 2011) and therefore, the adsorption of dissolved microcystins to settling particles may be relatively minor. When high levels of dissolved microcystins have been present during toxic bloom senescence, differential sedimentary fate has been observed. For example, Chapter III showed (see also Zastepa et al. 2013 in press) that MC-RR preferentially deposited into sediments while MC-LA remained in the water column.

Diffusion of microcystins from sediments represents a potential ongoing risk for human exposure in the absence of visible surface blooms. For example, diffusion of microcystins from sediments means a potential risk to drinking water supplies that withdraw from deep waters. In shallow systems used for recreation, sediments may provide a new route of exposure to dissolved microcystins in the absence of blooms. Risk management strategies therefore, cannot simply rely on macroscopic (bloom) or microscopic identification of potentially toxigenic species of cyanobacteria; testing for microcystins may be necessary even in the absence of bloom episodes particularly in lakes with a history of toxigenic blooms.

Furthermore, if diffusion of microcystins is not limited to surficial sediments but extends throughout the pore waters of a sediment core, the use of microcystins as a paleolimnological tool in the reconstruction of the historical occurrence of toxigenic cyanobacteria may be compromised (Pawlik-Skowronska et al. 2010, Efting et al. 2011, Zastepa Chapter IV). However, the gap in the presence of microcystins in the pore waters of sediment layers between 8.75 cm and 13.25 cm despite their presence on sediment particles as well as in the pore waters above and below suggests a lack of diffusion in deeper sediment layers (Figure V-3 and V-4). It is important to assess the reliability of the sediment record with historical surface water concentrations where available (Chapter IV).

Finally, the higher diffusive flux and lower sediment accumulation rate of MC-LA compared to MC-LR meant higher remobilization of MC-LA into the waters overlying sediments. These findings and the recent implication of MC-LA in the deaths of marine mammals by trophic transfer through invertebrates (Miller et al.

2010) underscore the importance of congeners-specific assessment of environmental fate and exposure risk of microcystins.

Tables and figures

Table V-1: The number of intervals where microcystin congeners were detected and the percentage distributed into pore water.

Congener	Intervals where detected	Intervals where detected in pore water	% in pore water in each interval (range)[a]
Lake of the Woods			
LR	13	0	NA
LA	9	5	47-100
RR	2	0	NA
^{7dm}LR, YR, LW, WR	1	0	NA
LY, LF	0	0	NA
Baptiste Lake			
LR	55	27	20-76
LA	50	34	25-100
RR	0	0	NA
^{7dm}LR	19	8	21-59
YR	21	0	NA
LW	4	3	53-72
WR	2	2	100
LF	3	3	55-80
LY	7	7	61-100

a: excludes intervals where the microcystin congener was not detected in pore water

Table V-2: Concentrations, sedimentation rates, accumulation rates, and sediment-water diffusive fluxes of microcystin congeners (MC-LR and -LA) at three sites of Lake of the Woods. HI is Hay Island, RP is Rat Portage Bay, and BB is Bigstone Bay.

Site (m depth)	Sedimentation ($g\ cm^{-2}\ yr^{-1}$)	Sediment ($ng\ g^{-1}$ dw)		Pore water ($ng\ L^{-1}$)		Overlying water ($ng\ L^{-1}$)		Sub-surface (5 m) ($ng\ L^{-1}$)		Accumulation ($ng\ cm^{-2}\ yr^{-1}$)		Diffusion[a] ($ng\ cm^{-2}\ yr^{-1}$)	
		LA	LR	LA	LR	LA	LR	LA	LR	LA	LR	LA	LR
HI (13)	0.028	19.6	255.0	1600	1230	1048	960	1590	1260	0.54	7.05	23.6	11.1
RP (23)	0.060	2.7	13.5	1731	2044	255	1236	110	280	0.16	0.81	53.1	29.1
BB (23)	0.023	2.1	7.3	1595	1433	264	743	270	420	0.05	0.17	54.6	28.3
Mean	0.037	8.1	91.9	1642	1569	522	980	657	653	0.25	2.67	43.8	22.8
SD	0.02	9.9	141.2	77	423	455	246	812	530	0.26	3.80	17.5	10.2

a: Calculated using a microcystin diffusion coefficient of $1.4 \times 10^{-6}\ cm^2\ s^{-1}$, porosity of 0.99, and tortuosity of 1.02.

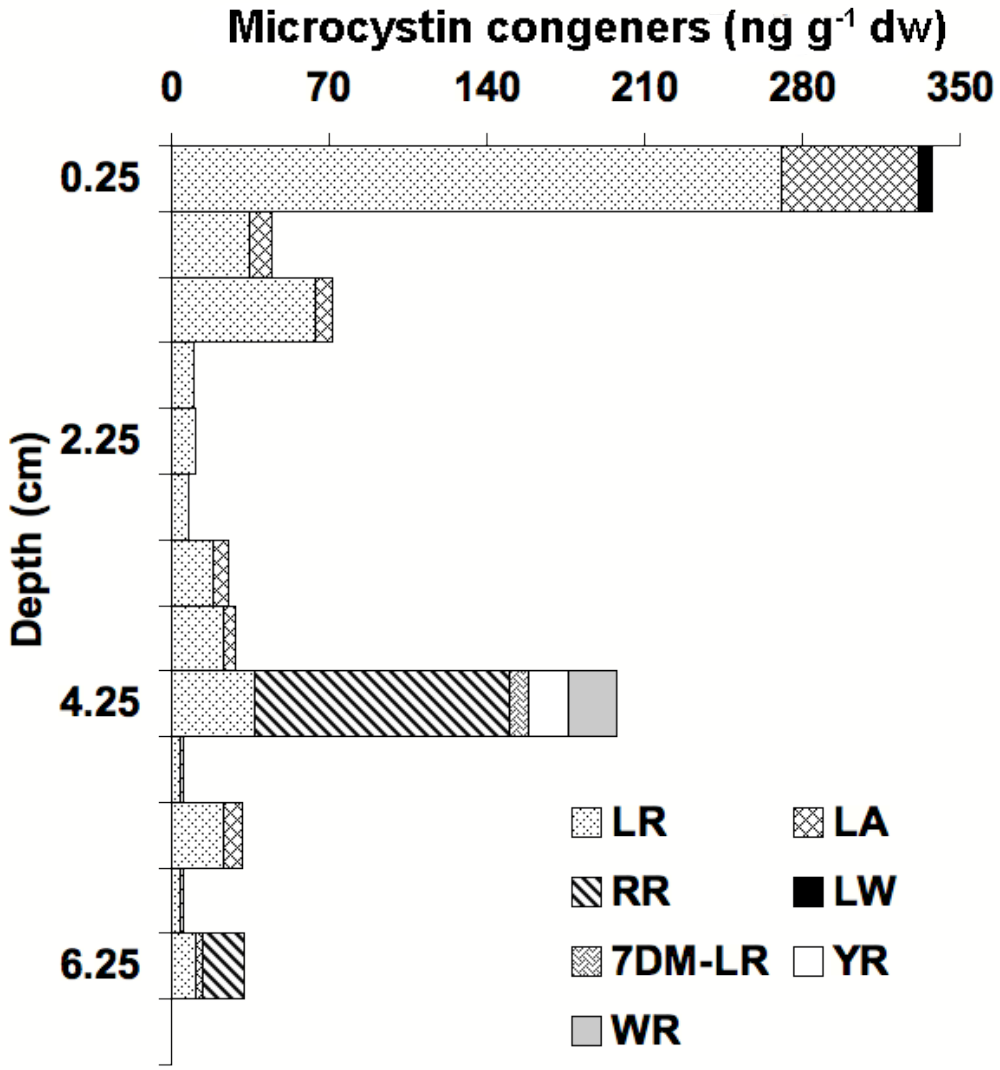


Figure V-1: Microcystin congener concentrations in a 40 cm sediment core from Lake of the Woods (Hay Island). Below 6.25 cm, measurements were below method detection limit. Interval midpoints are plotted (0.5 cm intervals from 0 to 7 cm). MC-LF and –LY were not detected in sediment.

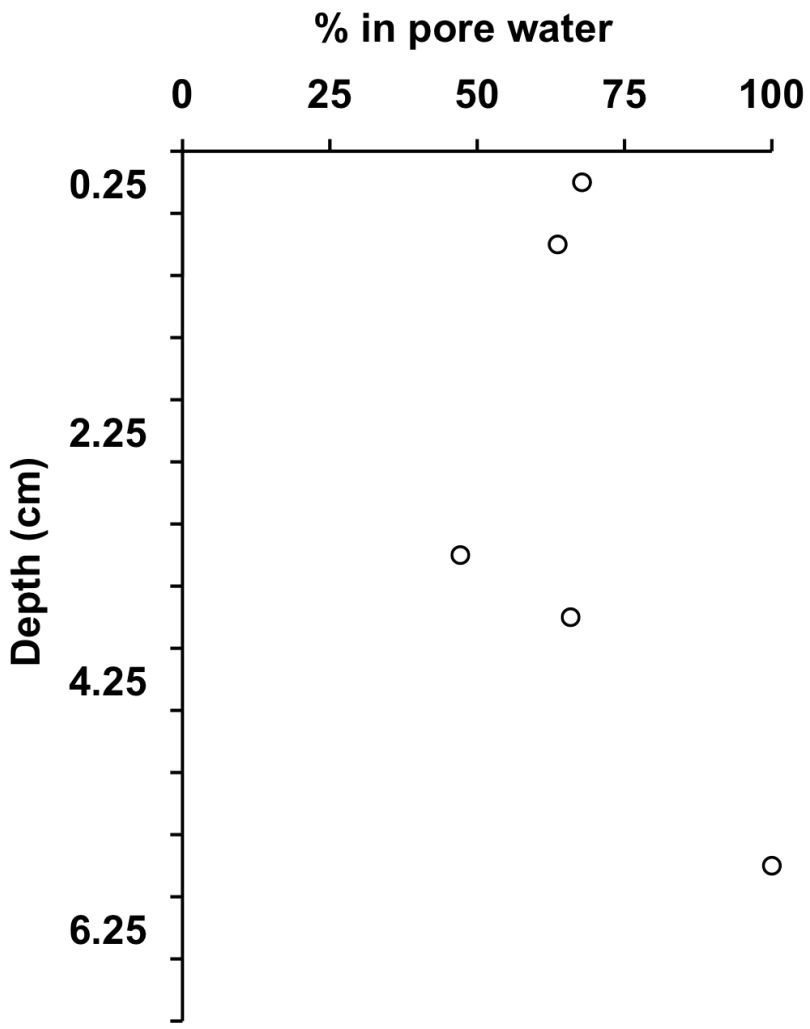


Figure V-2: Percent of microcystin congeners in the pore water of a 40 cm sediment core from Lake of the Woods (Hay Island). Interval midpoints are plotted (0.5 cm intervals from 0 to 7 cm). ○LA. LR, RR, LW, 7dmLR, YR, and WR were not detected in pore water. MC-LF and -LY were not detected in the sediment core.

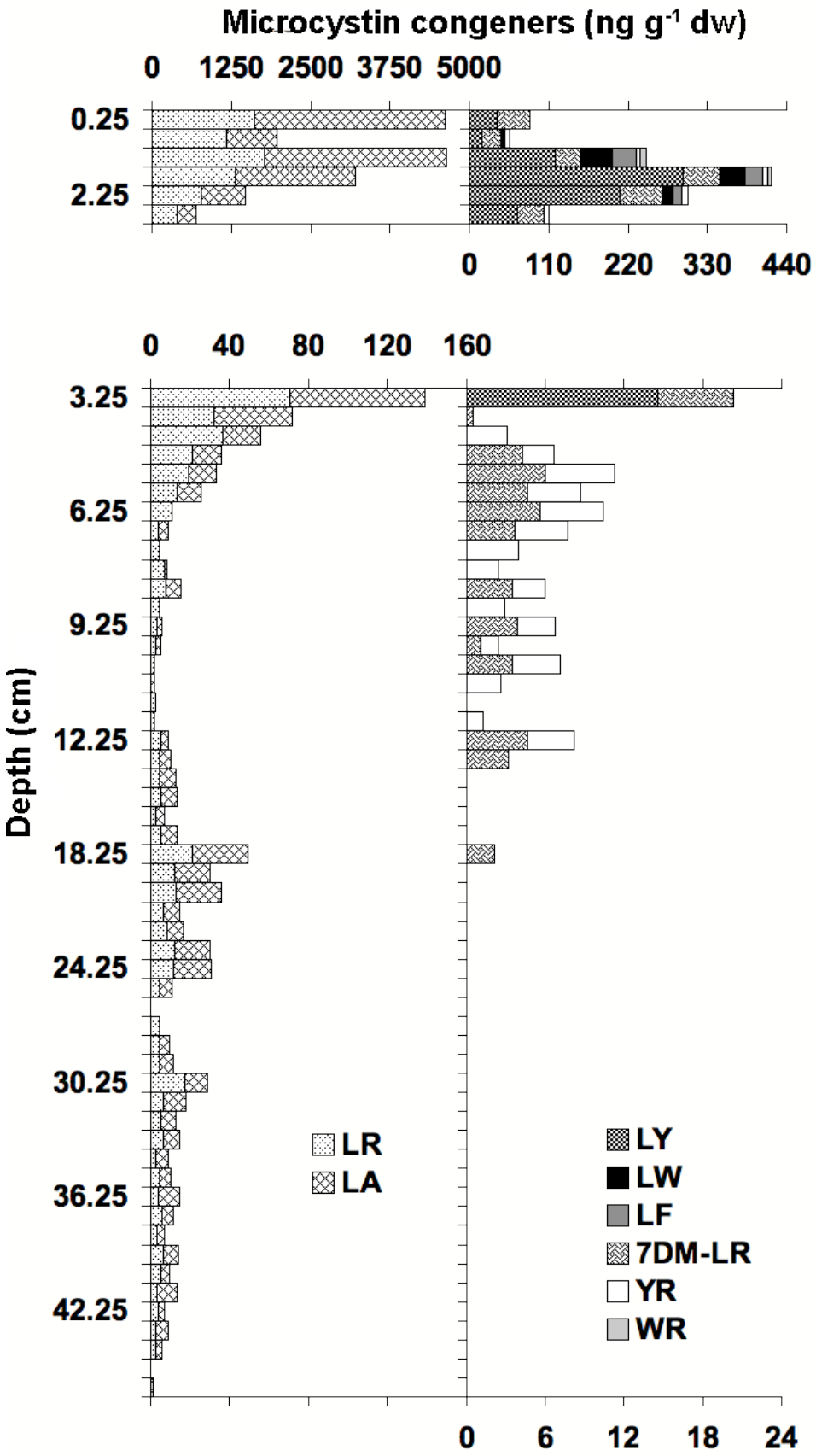

Microcystin congeners (ng g-1 dw)
0 1250 2500 3750 5000
0.25
2.25
0 110 220 330 440
0 40 80 120 160
3.25
6.25
9.25
12.25
18.25
24.25
30.25
36.25
42.25
Depth (cm)
LR
LA
LY
LW
LF
7DM-LR
YR
WR
0 6 12 18 24

Figure V-3: Microcystin congener concentrations in a 46 cm sediment core from Baptiste Lake. Interval midpoints are plotted (0.5 cm intervals from 0 to 12 cm with every other interval analyzed starting at 12 cm). MC-RR was not detected in the sediment core.

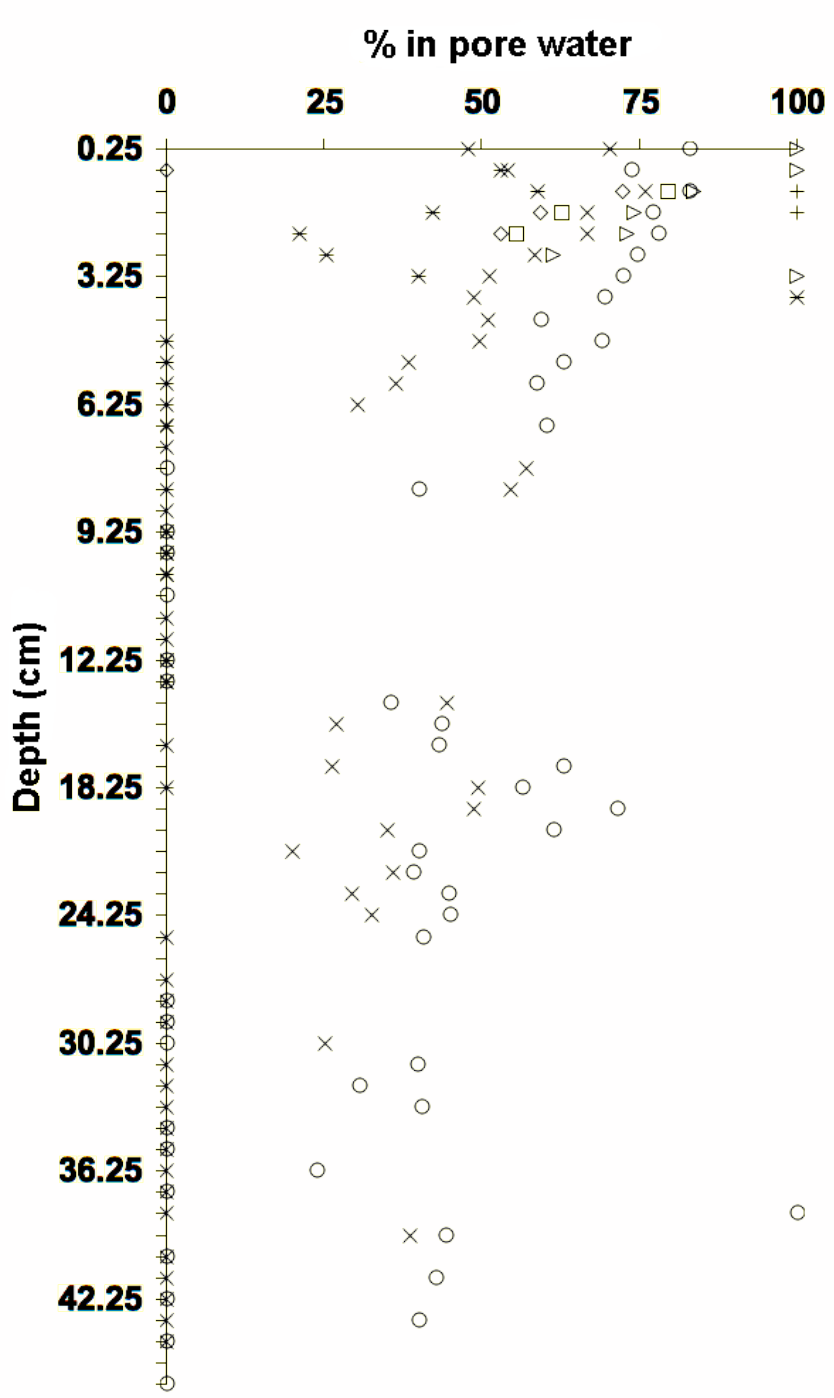


Figure V-4: Percent of microcystin congeners in the pore water of a 46 cm sediment core from Baptiste Lake. Interval midpoints are plotted (0.5 cm intervals from 0 to 12 cm with every other interval analyzed starting at 12 cm). ×LR, ○LA, △LY, ◇LW, □ LF, ✳7dmLR, + WR. MC-YR was not detected in pore water even though it was detected throughout the sediment core (Figure V-3). MC-RR was not detected in the sediment core.

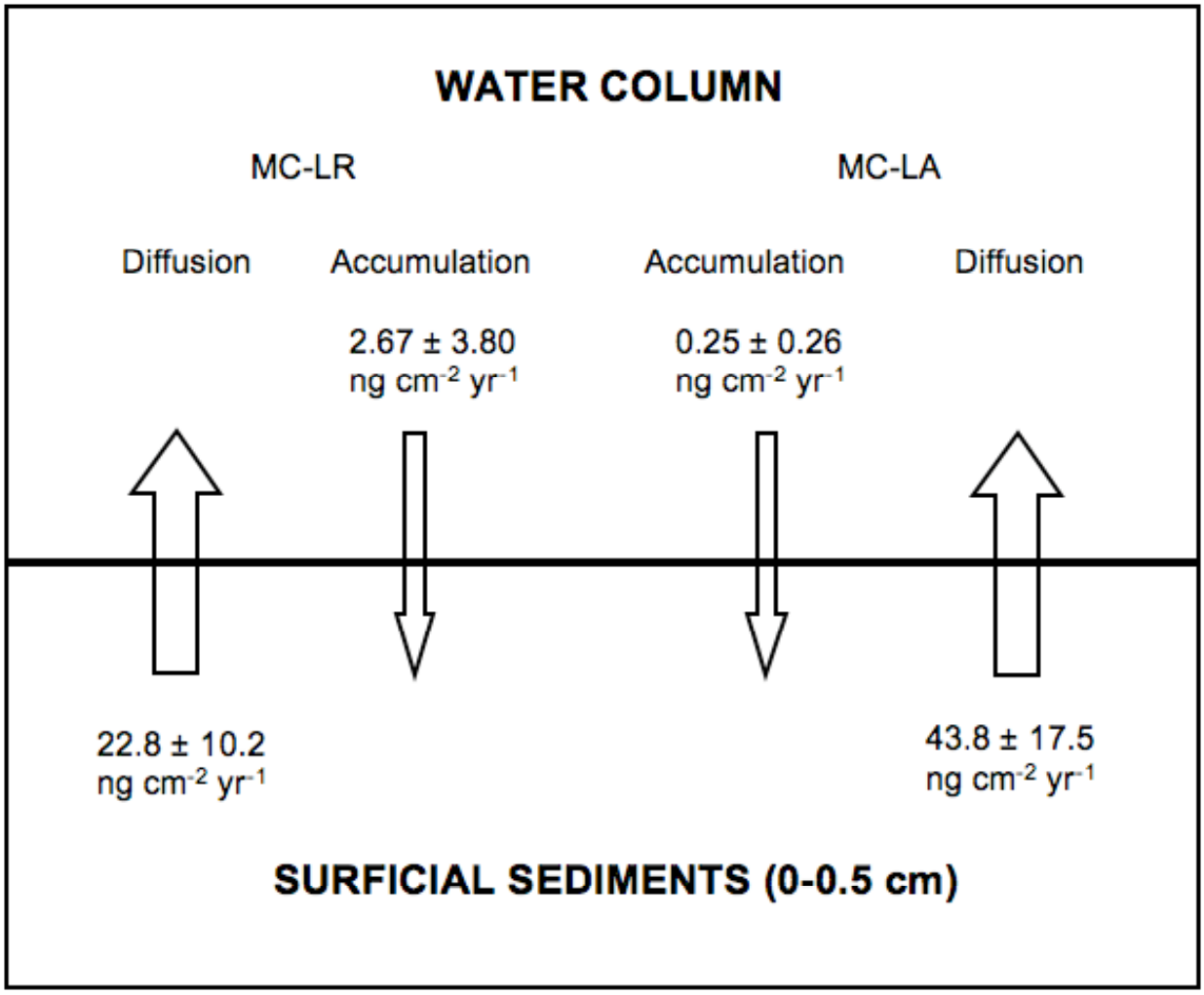


Figure V-5: A schematic summary of the accumulation rates and diffusive fluxes of microcystin congeners (MC-LR and -LA) (mean ± SD) in surficial sediments (0-0.5 cm) and at the sediment-water interface at three sites at Lake of the Woods (Hay Island, Rat Portage Bay, Bigstone Bay).

General Conclusion

Toxigenic cyanobacteria blooms are thought to be increasing globally due to anthropogenically increased nutrients and climate change. However, most contemporary datasets cannot determine whether the apparent increase is real or simply attributable to better reporting. Nevertheless, increased scientific, public, and media attention to toxigenic cyanobacterial blooms has raised concerns among water management authorities and health officials about associated health and ecological risks. The aim of the thesis was to improve the understanding of the fate and persistence of microcystin cyanotoxins in the environment, assess associated risks of exposure, and examine the history of toxigenic cyanobacterial occurrence.

The fate and persistence of microcystin cyanotoxins in aquatic ecosystems remains poorly understood in part because of a lack of analytical methods for microcystins in sediments. The analytical method developed in Chapter II addressed significant challenges faced by existing analytical methods that utilize traditional extraction and quantitation techniques. The new analytical method significantly expands the range of microcystin congeners that can be successfully recovered from sediments and unambiguously quantified in sediment extracts. Furthermore, the efficacy of recovering intracellular cyanotoxins from sediments was demonstrated. The thesis provides the first measurements of microcystins in sediment pore waters, which afforded the first estimates of diffusive flux of microcystin congeners from sediments. Furthermore, improvements were made in reducing the MDL in sediments, affording trace level quantitation of nine microcystin congeners representing the chemical diversity of this group of cyanotoxins. The marine analogue nodularin was also included in the analytical method.

Utilizing the new analytical method in a multi-year, whole lake study provided a rare assessment of the fate and persistence of the understudied MC-LA congener, in both water and sediments following the natural senescence of *Microcystis* blooms (decomposition rate, persistence, and sedimentary deposition). Concurrent *in vitro* experiments enabled a comparison of half-lives estimated *in situ*. *In situ* half-lives were shorter due to additional loss processes in the lake including dispersion, UV radiation, and sorption. *In vitro* experiments demonstrated the important role of temperature and light in the decomposition of particulate and dissolved microcystins, respectively. MC-LA appeared to persist longer in surface waters than the equally toxic MC-LR, requiring almost the entire recreational season (9.5 weeks) in one year to reach guideline concentrations (20 µg/L). Both *in vitro* and *in situ* results suggest that the dominant congener MC-LA has a longer half-life and greater persistence than MC-LR and related -YR and -RR. Recreational policies and management practices should be re-visited in light of these findings given that MC-LA is as toxic as MC-LR.

Measurements of sedimentary deposition from the whole lake study and separate estimates of sediment-pore water distribution coefficients, sediment accumulation rates, and diffusive flux from sediments revealed dramatic differences in the fate of microcystin congeners in sediments. MC-LA preferentially distributed into pore water and had a tendency to remobilize (by diffusion) from sediments into overlying water to a greater extent than MC-LR. In contrast, MC-RR adsorbed more strongly to sediment particles. In order to assess human and ecological health risks, the environmental fate of different microcystin congeners needs to be specifically

considered. Remobilization of microcystins from sediments would represent a health risk in reservoirs that withdraw water at depth for human consumption. In shallow systems used for recreation, this source of dissolved microcystins means a new route of exposure particularly important in the absence of blooms. Testing for microcystins may thus be necessary even in the absence of bloom episodes particularly in lakes with a history of toxigenic blooms.

To address the question of whether the apparent increase in toxigenic cyanobacterial blooms is real or simply attributable to better reporting, we used sedimentary microcystins to reconstruct the history of toxigenic cyanobacteria in a deep, eutrophic lake of major recreational importance. The microcystin record indicates that toxigenic cyanobacteria are part of the history of this ecosystem as they were detected prior to the first permanent settlements in the 1880s. This should be considered in efforts to restore the ecosystem to a state prior to significant anthropogenic disturbance. Based on the analysis of fossil diatoms, phosphorus and nitrogen were the likely drivers of toxigenic cyanobacteria in this lake however, a divergence in these relationships in the last few years suggests there may be important factors other than nutrients, although no correlations with simple climate-related variables were detected. Additionally, analysis of congener composition throughout the core suggested changes in cyanobacterial species or strain succession possibly due to recent changes in environmental conditions. However, this changing profile may simply be due to differential congener preservation throughout the sediment core.

Accurate reconstruction and interpretation of the sediment microcystin record and thus the history of toxigenic cyanobacteria requires a clearer understanding of the preservation of microcystins. The preferential deposition of microcystins within intact cyanobacterial cells reported by several studies indicates a likelihood of good preservation. Intracellular microcystins have even been measured more than 25 cm deep in sediment core layers (Latour et al. 2007). The detection of microcystins in sediments over 100 years old (Lake Baptiste, chapter IV) is a testament to this good preservation. Still, little is known about the post-depositional decomposition processes. Holst et al. (2003) and Chen et al. (2010) observed decomposition in both aerobic and anaerobic conditions, however experiments were conducted under elevated temperatures and nutrient supplementation. Thus, the significance of natural processes remains unclear. To improve accuracy and provide a clearer interpretation of the sediment microcystin record, quantitative studies on the natural, post-depositional decomposition of microcystins in sediments should be the next research step. Estimates of congener-specific decomposition in sediment as well as additional studies on the mobility through the sediment pore waters are required.

The high chemical diversity of microcystin congeners in this group of cyanotoxins makes analytical method development challenging. Although the microcystin congeners evaluated in sediments using the developed analytical method encompassed a range of chemistries representative of this group of cyanotoxins, two types of congeners could not be included due to lack of available commercial standards. These were congeners containing glutamic acid and methysulfanyl groups (methionine and methionine S-oxide).

The thesis results clearly demonstrate differences in environmental fate among microcystin congeners and underscore the need for more comparative studies *in situ*. However, the thesis did not examine the fate and persistence of different congeners in biota and this remains poorly understood particularly in animals. It is becoming clear that the risk of exposure to microcystins varies depending on which congener is present. Most dramatically, Miller et al. (2010) discovered that sea otters, an endangered species, died off the coast of California as a result of consuming shellfish tainted with MC-LA that originated in a lake several kilometres upstream of the ocean. This incident was likely due to the relatively high mammalian toxicity of this congener but also its relatively high persistence as reported in this thesis (see also Zastepa et al. 2013 in press). Future work should focus on bioaccumulation and biomagnification of different congeners as there is debate as to their occurrence (Ibelings and Havens 2008).

The prevalence of toxigenic cyanobacterial blooms and anticipated increase in conditions favourable for their dominance (increased temperatures, nutrient loading, stratification, water residence time, and salinization in part due to climate change), will put increased pressure on drinking and recreational waters. Comprehensive and congener-specific risk assessment and management strategies are of critical importance in identifying significant routes of exposure and providing a safe and cost-effective enjoyment of water resources. An understanding of the history of these toxigenic blooms can provide reference conditions in efforts to restore the ecosystem to a state prior to significant anthropogenic disturbance thus, affording a measured response.

References

Adams, K., Z. Taranu, R. Zurawell, and I. Gregory-Eaves. 2013. Insights for lake management gained when paleolimnological and water column monitoring studies are combined: A case study from Baptiste Lake. Lake and Reservoir Management Submitted.

An, J. S., and W. W. Carmichael. 1994. Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. Toxicon 32: 1495–1507.

Anderson, D. M., P. M. Glibert, and J. M. Burkholder. 2007. Harmful Algal Blooms and Eutrophication Nutrient Sources, Composition, and Consequences. Estuaries 25: 704–726.

Appleby, P. G. 2001. Chronostratigraphic techniques in recent sediments. In Last, W.M. and Smol, J.P. (eds.), Tracking Environmental Change using Lake Sediments, Volume 1, Basin Analysis, Coring, and Chronological Techniques. Kluwer Academic Publishers, Dordrecht 171–203.

Appleby, P. G., and F. Oldfield. 1978. The calculation of lead-210 dates assuming a constant rate of supply of unsupported lead-210 to the sediment. Catena 5: 1–8.

Aranda-Rodriguez, R., A. Tillmanns, F. Benoit, F. R. Pick, J. Harvie, and L. Solenaia. 2005. Pressurized liquid extraction of toxins from cyanobacterial cells. Environmental Toxicology 20: 390–396.

Babica, P., J. Kohoutek, L. Bláha, O. Adamovský, and B. Marsalek. 2006. Evaluation of extraction approaches linked to ELISA and HPLC for analyses of microcystin-LR, -RR and -YR in freshwater sediments with different organic

material contents. Analytical and Bioanalytical Chemistry 385: 1545–1551.

Barco, M., L. Lawton, J. Rivera, and J. Caixach. 2005. Optimization of intracellular microcystin extraction for their subsequent analysis by high-performance liquid chromatography. Journal of Chromatography A 1074: 23–30.

Bartram, J. 2003. Guidelines for safe recreational water environments. Volume 1: Coastal and fresh waters. World Health Organization Press Malta.

Beaulieu, M., F. Pick, and I. Gregory-Eaves. 2013. Nutrients and water temperature are significant predictors of cyanobacterial biomass in a 1147 lakes dataset. Limnology and Oceanography 58: 1736–1746.

Bianchi, T. S., E. Engelhaupt, P. Westman, T. Andren, C. Rolff, and R. Elmgren. 2000. Cyanobacterial blooms in the Baltic Sea: Natural or human-induced? Limnology and Oceanography 45: 716–726.

Binford, M. W. 2013. Calculation and uncertainty analysis of Pb-210 dates for PIRLA project lake sediment cores. Journal of Paleolimnology 3: 253–267.

Blais, J. M., J. Kalff, R. J. Cornett, and R. D. Evans. 1995. Evaluation of Pb-210 dating in lake sediments using stable Pb, Ambrosia pollen, and Cs-137. Journal of Paleolimnology 13: 169–178.

Boudreau, B. P. 1996. The diffusive tortuosity of fine-grained unlithified sediments. Geochimica et Cosmochimica Acta 60: 3139–3142.

Bourne, D. G., G. J. Jones, R. L. Blakeley, and A. Jones. 1996. Enzymatic Pathway for the Bacterial Degradation of the Cyanobacterial Cyclic Peptide Toxin Microcystin LR. Applied and Environmental Microbiology 62: 4086–4094.

Bowling, L. C. 2010. Observations on the management of cyanobacterial blooms in

Canada. NSW Office of Water, Sydney, Australia

Boyer, G. L. 2007. The occurrence of cyanobacterial toxins in New York lakes: Lessons from the MERHAB-Lower Great Lakes program. Lake and Reservoir Management 23: 153–160.

Brady, N. C., and R. R. Weil. 2002. The Nature and Properties of Soils, 13th ed. Prentice Hall, New York 960.

Braun, A., and T. Pfeiffer. 2002. Cyanobacterial blooms as the cause of a Pleistocene large mammmal assemblage. Paleobiology 28: 139–154.

Butterwick, C., S. Heaney, and J. Talling. 2004. Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. Freshwater Biology 50: 291–300.

Caldwell-Eldridge, S., T. Wood, K. Echols, and B. Topping. 2013. Microcystins, nutrient dynamics, and other environmental factors during blooms of non-microcystin-producing Aphanizomenon flos-aquaein Upper Klamath Lake, Oregon, 2009. Lake and Reservoir Management 29: 68–81.

Carlson, M. 2008. State of the Baptiste Lake Watershed. Terra Ecological Consulting, ALCES Group. Prepared for the Baptiste Lake Watershed Stewardship Group

Carmichael, W. W., S. M. Azevedo, J. S. An, R. J. R. Molica, E. M. Jochimsen, S. Lau, K. L. Rinehart, G. R. Shaw, and G. Eaglesham. 2001. Human fatalities from cyanobacteria: Chemical and biological evidence for cyanotoxins. Environmental Health Perspectives 109: 663–668.

Carpenter, S. R., and J. F. Kitchell. 1988. Consumer Control of Lake Productivity.

BioScience 38: 764–769.

Cerasino, L., and N. Salmaso. 2013. Diversity and distribution of cyanobacterial toxins in the Italian subalpine lacustrine district. International Journal of Oceanography and Hydrobiology 41: 54–63.

Chen, H., J. M. Burke, W. P. Dinsmore, E. E. Prepas, and P. M. Fedorak. 2007. First assessment of cyanobacterial blooms and microcystin-LR in the Canadian portion of Lake of the Woods. Lake and Reservoir Management 23: 169–178.

Chen, W., L. Li, N. Gan, and L. Song. 2006. Optimization of an effective extraction procedure for the analysis of microcystins in soils and lake sediments. Environmental Pollution 143: 241–246.

Chen, W., L. Song, L. Peng, N. Wan, X. Zhang, and N. Gan. 2008. Reduction in microcystin concentrations in large and shallow lakes: Water and sediment-interface contributions. Water Research 42: 763–773.

Chen, X., X. Yang, L. Yang, B. Xiao, X. Wu, J. Wang, and H. Wan. 2010. An effective pathway for the removal of microcystin LR via anoxic biodegradation in lake sediments. Water Research 44: 1884–1892.

Chorus, I., and J. Bartram. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. World Health Organization. E & FN Spon. London and New York.

Chorus, I., I. R. Falconer, H. J. Salas, and J. Bartram. 2000. Health risks caused by freshwater cyanobacteria in recreational waters. Journal of Toxicology and Environmental Health, Part B 3: 323–347.

Christoffersen, K., S. Lyck, and A. Winding. 2002. Microbial activity and bacterial community structure during degradation of microcystins. Aquatic Microbial Ecology 27: 125–136.

Codd, G. A. 2007. Cyanobacterial Toxins: Occurrence, Properties and Biological Significance. Water Science and Technology 32: 149–156.

Codd, G. A., L. Morrison, and J. Metcalf. 2005. Cyanobacterial toxins: risk management for health protection. Toxicology and Applied Pharmacology 203: 264–272.

Cook, D., and G. Newcombe. 2008. Comparison and modeling of the adsorption of two microcystin analogues onto powdered activated carbon. Environmental Technology 29: 234–525.

Cooke, S. E., and E. E. Prepas. 1998. Stream phosphorus and nitrogen export from agricultural and forested watersheds on the Boreal Plain. Canadian Journal of Fisheries and Aquatic Sciences 55: 2292–2299.

Cousins, I., D. Bealing, H. A. James, and A. Sutton. 1996. Biodegradation of microcystin-LR by indigenous mixed bacterial populations. Water Research 30: 481–485.

Delongchamp, T., J. Ridal, D. Lean, L. Poissant, and J. M. Blais. 2010. Mercury transport between sediments and the overlying water of the St. Lawrence River area of concern near Cornwall, Ontario. Environmental Pollution 158: 1487–1493.

DeMaagd, P. G., A. J. Hendriks, W. Seinen, and D. Sijm. 1999. pH-Dependent hydrophobicity of the cyanobacteria toxin microcystin-LR. Water Research 33:

677–680.

Dittmann, E., B. A. Neilan, M. Erhard, H. Dohren, and T. Borner. 1997. Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatotoxin production in the cyanobacterium Microcystis aeruginosa PCC 7806. Molecular Microbiology 26: 779–787.

Dokulil, M., and K. Teubner. 2005. Do phytoplankton communities correctly track trophic changes? An assessment using directly measured and palaeolimnological data. Freshwater Biology 50: 1594–1604.

Dolman, A., J. Rücker, F. R. Pick, J. Fastner, T. Rohrlack, U. Mischke, and C. Wiedner. 2012. Cyanobacteria and Cyanotoxins: The Influence of Nitrogen versus Phosphorus. Plos ONE 7: e38757.

Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting Cyanobacteria dominance in lakes. Canadian Journal of Fisheries and Aquatic Sciences 58: 1905–1908.

Duy, T. N., P. K. Lam, G. R. Shaw, and D. W. Connell. 2000. Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. Rev Environ Contam Toxicol 163: 113–185.

Edwards, C., D. Graham, N. Fowler, and L. A. Lawton. 2008. Biodegradation of microcystins and nodularin in freshwaters. Chemosphere 73: 1315–1321.

Efting, A., D. Snow, and S. Fritz. 2011. Cyanobacteria and microcystin in the Nebraska (USA) Sand Hills Lakes before and after modern agriculture. Journal of Paleolimnology 46: 17–27.

Falconer, I. R., M. D. Burch, D. A. Steffensen, M. Choice, and R. O. Coverdale.

1994. Toxicity of the blue-green alga (Cyanobacterium) Microcystis aeruginosa in drinking water to growing pigs, as animal model for human injury and risk assessment. Journal of Environmental Toxicology and Water Quality 9: 131–139.

Fastner, J., I. Flieger, and U. Neumann. 1998. Optimised Extraction of Microcystins from Field Samples - A Comparison of Different Solvents and Procedures. Water Research 32: 3177–3181.

Fawell, J. K., C. P. James, and H. A. James. 1994. Toxins from Blue-Green Algae: Toxicological Assessment of microcystin-LR and a Method for its Determination in Water: FR0359/2/DoE 3. Foundation for Water Research, UK

Finni, T., K. Kononen, R. Olsonen, and K. Wallström. 2001. The History of Cyanobacterial Blooms in the Baltic Sea. Ambio 30: 172–178.

Frank, C. 2002. Microcystin-producing cyanobacteria in recreational waters in southwestern Germany. Environmental Toxicology 17: 361–366.

Giani, A., D. F. Bird, Y. Prairie, and J. Lawrence. 2005. Empirical study of cyanobacterial toxicity along a trophic gradient of lakes. Canadian Journal of Fisheries and Aquatic Sciences 62: 2100–2109.

Glew, J. R. 1989. A new trigger mechanism for sediment samplers. Journal of Paleolimnology 2: 241–243.

Gorham, E., J. W. G. Lund, J. E. Sanger, and W. E. Dean. 1974. Some Relationships Between Algal Standing Crop, Water Chemistry, and Sediment Chemistry in the English Lakes. Limnology and Oceanography 19: 601–617.

Graham, J. L., J. R. Jones, S. B. Jones, J. A. Downing, and T. E. Clevenger. 2004. Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. Water Research 38: 4395–4404.

Graham, J. L., A. C. Ziegler, B. L. Loving, and K. Loftin. 2012. Fate and Transport of Cyanobacteria and Associated Toxins and Taste-and-Odor Compounds from Upstream Reservoir Releases in the Kansas River, Kansas, September and October 2011. USGS Scientific Investigations Report 5129.

Gurbuz, F., J. S. Metcalf, A. G. Karahan, and G. A. Codd. 2009. Analysis of dissolved microcystins in surface water samples from Kovada Lake, Turkey. Science of the Total Environment 407: 4038–4046.

Hakanson, L., A. C. Bryhn, and J. K. Hytteborn. 2007. On the issue of limiting nutrient and predictions of cyanobacteria in aquatic systems. Science of the Total Environment 379: 89–108.

Hall, R., P. R. Leavitt, J. P. Smol, and N. Zirnhelt. 1997. Comparison of diatoms, fossil pigments and historical records as measures of lake eutrophication. Freshwater Biology 38: 401–417.

Hallegraeff, G. 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: A formidable predictive challenge. Journal of Phycology 46: 220–235.

Hamilton, P. B., L. Ley, S. Dean, and F. R. Pick. 2008. The occurrence of the cyanobacterium Cylindrospermopsis raciborskii in Constance Lake: an exotic cyanoprokaryote. Phycologia 44: 17–25.

Harada, K. I., and K. Tsuji. 1998. Persistence and Decomposition of Hepatotoxic

Microcystins Produced by Cyanobacteria in Natural Environment. Toxin Reviews 17: 385–403.

Health, and Canada. 2012. Guidelines for Canadian Recreational Water Quality. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario H129-15/2012E.

Heiri, O., A. F. Lotter, and G. Lemcke. 2000. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of Paleolimnology 25: 101–110.

Heisler, J., P. M. Gilbert, J. M. Burkholder, D. M. Anderson, W. Cochlan, W. C. Dennison, Q. Dortch, C. J. Gobler, C. A. Heil, E. Humphries, A. Lewitus, R. Magnien, H. G. Marshall, K. Sellner, D. A. Stockwell, D. K. Stoecker, and M. Suddleson. 2008. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Alage 8: 3–13.

Hickman, M., C. Schweger, and D. M. Klarer. 1990. Baptiste Lake, Alberta - A late Holocene history of changes in a lake and its catchment in the southern Boreal forest. Journal of Paleolimnology 4: 253–267.

Hitzfeld, B. C., C. Lampert, N. Spaeth, D. Mountfort, K. Kaspar, and D. Dietrich. 2000. Toxin production in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. Toxicon 38: 1731–1748.

Ho, L., T. Tang, D. Hoefel, and B. Vigneswaran. 2012. Determination of rate constants and half-lives for the simultaneous biodegradation of several cyanobacterial metabolites in Australian source waters. Water Research 46: 5735–5746.

Holst, T., N. Jorgensen, C. Jorgensen, and A. Johansen. 2003. Degradation of microcystin in sediments at oxic and anoxic, denitrifying conditions. Water Research 37: 4748–4760.

Huisman, J., H. C. P. Matthijs, and P. M. Visser. 2005. Harmful Cyanobacteria. Springer Dordrecht.

Ibelings, B.W., and K.E. Havens. 2008. Cyanobacterial toxins: a qualitative meta-analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota (Chapter 32). In: Hudnell, K. (Ed.), Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Advances in Experimental Medicine and Biology 619: 675-732.

Ihle, T., S. Jahnichen, and J. Benndorf. 2005. Wax and Wane of Microcystis (cyanophyceae) and Microcystins in Lake Sediments: a case study in Quitzdorf Reservoir (Germany). Journal of Phycology 41: 479–488.

Imanishi, S., H. Kato, M. Mizuno, a Kiyomi Tsuji, and K. Harada. 2005. Bacterial Degradation of Microcystins and Nodularin. Chemical Research in Toxicology 18: 591–598.

Ishii, H., M. Nishijima, and T. Abe. 2004. Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. Water Research 38: 2667–2676.

Izaguirre, G., A. Jungblut, and B. A. Neilan. 2007. Benthic cyanobacteria (Oscillatoriaceae) that produce microcystin-LR, isolated from four reservoirs in southern California. Water Research 41: 492–498.

Jacoby, J. M., D. C. Collier, E. B. Welch, and F. J. Hardy. 2000. Environmental

factors associated with a toxic bloom of Microcystis aeruginosa. Canadian Journal of Fisheries and Aquatic Sciences 57: 231–240.

Jöhnk, K., J. Huisman, J. Sharples, B. Sommeijer, P. M. Visser, and J. Stroom. 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Global Change Biology 14: 495–512.

Johnston, W. A. 1915. Rainy River District, Ontario: Surficial Geology and Soils. Survey of Canada, Geological Series Report No.

Jones, G. J., D. G. Bourne, R. L. Blakeley, and H. Doelle. 1994. Degradation of the cyanobacterial hepatotoxin microcystin by aquatic bacteria. Natural Toxins 2: 228–235.

Jones, G. J., and P. Orr. 1994. Release and degradation of microcystin following algicide treatment of a Microcystis aeruginosa bloom in a recreational lake, as determined by HPLC and Protein Phosphatase Inhibition Assay. Water Research 28: 817–876.

Kalff, J. 2001. Limnology: inland water ecosystems. Prentice Hall 2nd Edition.

Kankaanpaa, H. T., O. Sjovall, M. Huttunen, M. Olin, K. Karlsson, K. Hyvarinen, L. Sneitz, J. Harkonen, V. O. Sipia, and J. A. Meriluoto. 2009. Production and sedimentation of peptide toxins nodularin-R and microcystin-LR in the northern Baltic Sea. Environmental Pollution 157: 1301–1309.

Kosten, S., V. Huszar, E. Bécares, L. Costa, E. Donk, L. Hansson, E. Jeppesen, C. Kruk, G. Lacerot, N. Mazzeo, L. Meester, B. Moss, M. Lürling, T. Nõges, S. Romo, and M. Scheffer. 2011. Warmer climates boost cyanobacterial dominance in shallow lakes. Global Change Biology 18: 118–126.

Kotak, B. G., and R. W. Z. Zurawell. 2007. Cyanobacterial toxins in Canadian freshwaters: A review. Lake and Reservoir Management 23: 109–122.

Kuiper-Goodman, T., I. R. Falconer, and J. Fitzgerald. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management, in: Chorus, I., Bartram, J. (Eds.). World Health Organization. E & FN Spon. London and New York. 113–153.

Lahti, K., J. Rapala, M. Färdig, M. Niemelä, and K. Sivonen. 1997. Persistence of cyanobacterial hepatotoxin, microcystin-LR in particulate material and dissolved in lake water. Water Research 31: 1005–1012.

Lam, A., P. M. Fedorak, and E. E. Prepas. 1995. Biotransformation of the Cyanobacterial Hepatotoxin Microcystin-LR, as Determined by HPLC and Protein Phosphatase Bioassay. Environmental Science & Technology 29: 242–246.

Lam, A., E. E. Prepas, D. Spink, and S. Hrudey. 2008. Chemical control of Hepatotoxic Phytoplankton Blooms: Implications for Human Health. Water Research 29: 1845–1854.

Latour, D., H. Giraudet, and J. L. Berthon. 2004a. Frequency of dividing cells and viability of Microcystis aeruginosa in sediment of a eutrophic reservoir. Aquatic Microbial Ecology 36: 117–122.

Latour, D., O. Sabido, M. Solencon, and H. Giraudet. 2004b. Dynamics and metabolic activity of the benthic cyanobacterium Microcystis aeruginosa in the Grangent reservoir (France). Journal of Plankton Research 26: 719–726.

Latour, D., M. Salencon, J. Reyss, and H. Giraudet. 2007. Sedimentary imprint of

Microcystis aeruginosa (cyanobacteria) blooms in Grangent reservoir (Loire, France). Journal of Phycology 43: 417–425.

Leandro, C. C., P. Hancock, R. J. Fussell, and B. Keely. 2006. Comparison of ultra-performance liquid chromatography and high-performance liquid chromatography for the determination of priority pesticides in baby foods by tandem quadrupole mass spectrometry. Journal of Chromatography A 1103: 94–101.

Leavitt, P. R. 1993. A review of factors that regulate carotenoid and chlorophyll deposition and fossil pigment abundance. Journal of Paleolimnology 9: 109–127.

Leavitt, P. R., and D. L. Findlay. 2013. Comparison of Fossil Pigments with 20 Years of Phytoplankton Data from Eutrophic Lake 227, Experiments Lakes Area, Ontario. Canadian Journal of Fisheries and Aquatic Sciences 51: 2286–2299.

LeBlanc, S., F. R. Pick, and P. B. Hamilton. 2008. Fall cyanobacterial blooms in oligotrophic-to-mesotrophic temperate lakes and the role of climate change. Verh. Internat. Verein. Limnol. 30: 90–94.

Macdonald, G., E. Bennett, and Z. Taranu. 2012. The influence of time, soil characteristics, and land-use history on soil phosphorus legacies: a global meta-analysis. Global Change Biology 18: 1904–1917.

Makarewicz, J. C., G. L. Boyer, T. W. Lewis, W. Guenther, J. Atkinson, and M. Arnold. 2009. Spatial and Temporal Distribution of the Cyanotoxin Microcystin-LR in the Lake Ontario Ecosystem: Coastal Embayments, Rivers,

Nearshore and Offshore, and Upland Lakes. Journal of Great Lakes Research 35: 83–89.

MDDEP. 2012. Portrait de la qualité des eaux de surface au Québec 1999 – 2008. Ministère du Développement durable de l'Environnement et des Parcs. Québec, Direction du suivi de l'état de l'environnement. http://www.mddefp.gouv.qc.ca/eau/portrait/eaux-surface1999-2008/index.htm: 1–6.

Meissner, K., E. Dittmann, and T. Borner. 1996. Toxic and non-toxic strains of cyanobacterium Microcystis aeruginosa contain sequences homologous to peptide sythetase genes. FEMS Microbiology Letters 135: 295–303.

Mez, K., K. A. Beattie, G. A. Codd, K. Hanselmann, B. Hauser, H. Naegeli, and H. Preisig. 1997. Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. European Journal of Phycology 32: 111–117.

Miller, M. A., R. M. Kudela, A. Mekebri, D. Crane, S. C. Oates, M. T. Tinker, M. Staedler, W. A. Miller, S. Toy-Choutka, C. Dominik, D. Hardin, G. Langlois, M. Murray, K. Ward, and D. A. Jessup. 2010. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. Plos ONE 5: e12576.

Miller, M. J., M. Critchley, J. Hutson, and H. Fallowfield. 2001. The adsorption of cyanobacterial hepatotoxins from water onto soil during batch experiments. Water Research 35: 1461–1468.

Miller, M. J., J. Hutson, and H. J. Fallowfield. 2005. The adsorption of cyanobacterial

hepatoxins as a function of soil properties. Journal of Water and Health 3: 339–347.

Misson, B., M. Sabart, C. Amblard, and D. Latour. 2012. Benthic survival of Microcystis: Long-term viability and ability to transcribe microcystin genes. Harmful Algae 13: 20–25.

Moisander, P., E. Mcclinton, and H. W. Paerl. 2002. Salinity Effects on Growth, Photosynthetic Parameters, and Nitrogenase Activity in Estuarine Planktonic Cyanobacteria. Microbial Ecology 43: 432–442.

Moore, R. E., J. L. Chen, B. S. Moore, G. M. L. Patterson, and W. W. Carmichael. 1991. Biosynthesis of microcystin-LR. Origins of carbons in the Adda and Masp units. Journal of the American Chemical Society 113: 5083–5084.

Morris, R., D. Williams, H. Luu, C. F. B. Holmes, R. Andersen, and S. Calvert. 2000. The adsorption of microcystin-LR by natural clay particles. Toxicon 38: 303–308.

Murphy, T., K. Irvine, J. Guo, J. Davies, H. Murkin, M. Charlton, and S. B. Watson. 2008. New Microcystin Concerns in the Lower Great Lakes. Water Quality Research Journal of Canada 38: 127–140.

NationalCapitalCommision. Meech Lake beach closed due to blue-green algae bloom. CBC Available at http://www.cbc.ca/news/canada/ottawa/story/2009/08/14/ottawa–gatineau.html. Accessed August 2009.

Newcombe, G., D. Cook, S. Brooke, L. Ho, and N. Sylman. 2003. Treatment options for microcystin toxins: Similarities and differences between variants.

Environmental Technology 24: 299–308.

O'Donoghue, J. G., and G. S. Wilton. 1951. Algal Poisoning in Alberta. Canadian Journal of Comparative Medicine 15: 193–198.

Orihel, D., D. F. Bird, M. Brylinsky, H. Chen, D. Donald, D. Huang, A. Giani, D. Kinniburgh, H. Kling, B. Kotak, P. Leavitt, C. Nielsen, S. Reedyk, R. Rooney, S. Watson, R. Zurawell, R. Vinebrooke, and R. Smith. 2012. High microcystin concentrations occur only at low nitrogen-to-phosphorus ratios in nutrient-rich Canadian lakes. Canadian Journal of Fisheries and Aquatic Sciences 69: 1457–1462.

Paerl, H. W., R. S. Fulton, E. Graneli, and J. Turner. 2006. Ecology of Harmful Marine Algae. Springer Berlin: 95–107.

Paerl, H. W., and J. Huisman. 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environmental Microbiology Reports 1: 27–37.

Paerl, H. W., and V. J. Paul. 2012. Climate change: Links to global expansion of harmful cyanobacteria. Water Research 46: 1349–1363.

Park, H., Y. Sasaki, T. Maruyama, E. Yanagisawa, A. Hiraishi, and K. Kato. 2001. Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertrophic lake. Environmental Toxicology 16: 337–343.

Pawlik-Skowronska, B., R. Kornijow, and J. Pirszel. 2011. Sedimentary imprint of cyanobacterial blooms - a new tool for insight into recent history of lakes. Polish Journal of Ecology 58: 663–670.

Perez, S., and D. Aga. 2005. Recent advances in the sample preparation, liquid

chromatography tandem mass spectrometric analysis and environmental fate of microcystins in water. Trends in Analytical Chemistry 24: 658–670.

Petrauskas, A. A., and E. Kolovanov. 2000. ACD/Log P method description. Perspectives in Drug Discovery and Design 19: 99–116.

Pick, F. R., and D. Lean. 1987. The role of macronutrients (C, N, P) in controlling cyanobacterial dominance in temperate lakes. New Zealand Journal of Marine and Freshwater Research 21: 425–434.

Polis, G. A. 1994. Food webs, trophic cascades and community structure. Australian Journal of Ecology 19: 121–136.

Pouria, S., A. de Andrade, J. Barbosa, R. L. Cavalcanti, V. T. Barreto, C. J. Ward, W. Preiser, G. K. Poon, G. H. Neild, and G. A. Codd. 1998. Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. Lancet 352: 21–26.

Prepas, E. E., and P. Mitchell. 1990. Atlas of Alberta Lakes. University of Alberta Press Available at: http://alberta–lakes.sunsite.ualberta.ca/. Accessed March 2013.

Rapala, J., K. Lahti, and K. S. Niemela. 1994. Biodegradability and adsorption on lake sediments of cyanobacterial hepatotoxins and anatoxin-a. Letters in Applied Microbiology 19: 423–428.

Reynolds, C. S. 2006. Ecology of Phytoplankton. Cambridge University Press

Riedinger-Whitmore, M. A., T. J. Whitmore, J. M. Smoak, M. Brenner, A. Moore, J. Curtis, and C. L. Schelske. 2005. Cyanobacterial Proliferation is a Recent Response to Eutrophication in Many Florida Lakes: A Paleolimnological Assessment. Lake and Reservoir Management 21: 423–435.

Rinta-Kanto, J. M., M. A. Saxton, J. Debruyn, J. Smith, C. Marvin, K. Krieger, G. Sayler, G. L. Boyer, and S. W. Wilhelm. 2009. The diversity and distribution of toxigenic Microcystis spp. in present day and archived pelagic and sediment samples from Lake Erie. Harmful Algae 8: 385–394.

Rivasseau, C., S. Martins, and M. Hennion. 1998. Determination of some physicochemical parameters of microcystins (cyanobacterial toxins) and trace level analysis in environmental samples using liquid chromatography. Journal of Chromatography A 799: 155–169.

Rouhiainen, L., T. Vakkilainen, B. Lumbye, W. Buikema, R. Haselkorn, and K. Sivonen. 2004. Genes coding for hepatotoxic heptapeptides (microcystins) in the cyanobacterium Anabaena strain 90. Applied and Environmental Microbiology 70: 686–692.

Ruhland, K., A. M. Paterson, K. Hargan, A. Jenkin, B. J. Clark, and J. P. Smol. 2010. Reorganization of algal communities in the Lake of the Woods (Ontario, Canada) in response to turn-of-the-century damming and recent warming. Limnology and Oceanography 55: 2433–2451.

Rusak, J. A., and T. Mosindy. 1997. Seasonal movements of lake sturgeon in Lake of the Woods and the Rainy River, Ontario. Canadian Journal of Zoology 74: 383–395.

Schindler, D. W. 2006. Recent advances in the understanding and management of eutrophication. Limnology and Oceanography 51: 356–363.

Schmidtkunz, C., H. B. Stich, and T. Welsch. 2009. Improving the Selectivity and Confidence in the HPLC Analysis of Microcystins in Lake Sediments. Journal

of Liquid Chromatography & Related Technologies 32: 801–821.

Schupp, D. H., and V. Macins. 1977. Trends in percid yields from Lake of the Woods, 1888-1973. Journal of the Fisheries Research Board of Canada 34: 1784–1791.

Schwarzenbach, R. P., P. M. Gschwend, and D. M. Imboden. 2003. Environmental Organic Chemistry, 2nd ed. John Wiley & Sons, Inc., New York

Sedmak, B., and G. Kosi. 1998. The role of microcystins in heavy cyanobacterial bloom formation. Journal of Plankton Research 20: 691–708.

Serieyssol, C., M. Edlund, and L. Kallemeyn. 2009. Impacts of settlement, damming, and hydromanagement in two boreal lakes: a comparative paleolimnological study. Journal of Paleolimnology 42: 497–513.

Serieyssol-Bleser, C. 2010. Determining the Impacts of Damming, Water-Level Fluctuations, Climate, and Landscape Changes in Voyageurs National Park and Vicinity. University of Minnesota, Minneapolis Ph.D. thes.

Sivonen, K., and G. J. Jones. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management, in: Chorus, I., Bartram, J. (Eds.) . World Health Organization. E & FN Spon. London and New York. 41–111.

Smith, J. L., and G. L. Boyer. 2009. Standardization of microcystin extraction from fish tissues: A novel internal standard as a surrogate for polar and non-polar variants. Toxicon 53: 238–245.

Smith, V. H. 2003. Eutrophication of Freshwater and Coastal Marine Ecosystems. Environmental Science and Pollution Research 10: 126–139.

Smith, V. H. 2008. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221: 669–671.

Smol, J. P., H. J. B. Birks, and W. M. Last. 2001. Tracking Environmental Change Using Lake Sediments. Terrestrial, Algal, and Silaceous Indicators.,.

Song, L., W. Chen, L. Peng, N. Wan, N. Gan, and X. Zhang. 2007. Distribution and bioaccumulation of microcystins in water columns: A systematic investigation into the environmental fate and the risks associated with microcystins in Meiliang Bay, Lake Taihu. Water Research 41: 2853–2864.

Soranno, P. A. 1997. Factors affecting the timing of surface scums and epilimnetic blooms of blue-green algae in a eutrophic lake. Canadian Journal of Fisheries and Aquatic Sciences 54: 1965–1975.

Swain, E. B. 2005. Measurement and interpretation of sedimentary pigments. Freshwater Biology 15: 53–75.

Takenaka, S., and M. F. Watanabec. 2003. Microcystin LR Degradation by Pseudomonas Aeruginosa Alkaline Proteases. Chemosphere 34: 749–757.

Taranu, Z., R. Zurawell, F. Pick, and I. Gregory-Eaves. 2012. Predicting cyanobacterial dynamics in the face of global change: the importance of scale and environmental context. Global Change Biology 18: 3477–3490.

Tillett, D., E. Dittmann, M. Erhard, and H. von Döhren. 2000. Structural organization of microcystin biosynthesis in Microcystis aeruginosa PCC7806: an integrated peptide-polyketide synthetase system. Chemistry and Biology 7: 753–764.

Tillmanns, A., F. R. Pick, and R. Aranda-Rodriguez. 2007. Sampling and analysis of microcystins: Implications for the development of standardized methods.

Environmental Toxicology 22: 132–143.

Tonk, L., K. Bosch, P. M. Visser, and J. Huisman. 2008. Salt tolerance of the harmful cyanobaterium Microcystis aeruginosa. Aquatic Microbial Ecology 46: 117–123.

Trew, D. O., D. J. Beliveau, and E. I. Yonge. 1978. The Baptiste Lake study summary report. Alberta Environment, Pollution Control Division, Water Quality Control Branch

Tsuji, K., S. Nalto, F. Kondo, N. Ishikawa, M. Suzuki, M. Watanabe, and K. I. Harada. 1994. Stability of Microcystins from Cyanobacteria: Effect of Light on Decomposition and Isomerization. Environmental Science & Technology 28: 173–177.

UKWIR. 1997. Algal Toxins, Occurrence and Treatability of Anatoxin a and Microcystins. United Kingdom Water Industry Research Limited 97/DW/07/05.

Verspagen, J., E. Snelder, P. M. Visser, K. Johnk, B. Ibelings, L. Mur, and J. Huisman. 2005. Benthic-pelagic coupling in the population dynamics of the harmful cyanobacterium Microcystis. Freshwater Biology 50: 854–867.

Visser, P. M., B. Ibelings, and L. Mur. 1995. Autumnal sedimentation of Microcystis spp. as result of an increase in carbohydrate ballast at reduced temperature. Journal of Plankton Research 17: 919–933.

Watanabe, M. F., K. Tsuji, Y. Watanabe, K. Haradab, and M. Suzuki. 1992. Release of Heptapeptide Toxin (Microcystin) During the Decomposition Process of Microcystis aeruginosa. Natural Toxins 1: 48–53.

Welker, M., L. Sejnohova, D. Nemethova, H. Dohren, J. Jarkovsky, and B.

Marsalek. 2007. Seasonal shifts in chemotype composition of Microcystis sp. communities in the pelagial and the sediment of a shallow reservoir. Limnology and Oceanography 52: 609–619.

Welker, M., and C. Steinberg. 1999. Indirect photolysis of cyanotoxins: one possible mechanism for their low persistence. Water Research 35: 1159–1164.

Welker, M., and C. Steinberg. 2000. Rates of humic substance photosensitized degradation of microcystin-LR in natural waters. Environmental Science & Technology 34: 3415–3419.

Whitton, B. A., and M. Potts. 2000. The Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer Academic Publishers. Netherlands 37–59.

WHO. 2006. Guidelines for drinking-water quality. World Health Organization Press Vol. 1: 3rd ed.

Winter, J., A. DeSellas, R. Fletcher, and et al. 2011. Algal blooms in Ontario, Canada: Increases in reports since 1994. Lake and Reservoir Management 27: 105–112.

Winter, J., L. Heintsch, and A. Morley. 2008. Blooms of Cyanobacteria in Ontario: Incidences and Response. Quebec City Symposium on Blue-Green Algae 35.

Wolf, H. U., and C. Frank. 2002. Toxicity Assessment of Cyanobacterial Toxin Mixtures. Environmental Toxicology 17: 395–399.

Wörmer, L., S. Cires, and A. Quesada. 2011. Importance of natural sedimentation in the fate of microcystins. Chemosphere 82: 1141–1146.

Wu, X., B. Xiao, R. Li, C. Wang, J. Huang, and Z. Wang. 2011. Mechanisms and Factors Affecting Sorption of Microcystins onto Natural Sediments.

Environmental Science & Technology 45: 2641–2647.

Yang, Z., and J. Teller. 2005. Modeling the history of Lake of the Woods since 11,000 cal yr B.P. using GIS. Journal of Paleolimnology 33: 483–497.

Zakaria, M. A., E. M. Hassan, and A. S. Wafaa. 2007. Microcystin Concentrations in the Nile River Sediments and Removal of Microcystin-LR by Sediments During Batch Experiments. Archives of Environmental Contamination and Toxicology 52: 489–495.

Zapata, M., F. Rodriguez, and J. L. Garrido. 2000. Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reverse phase C8 column and pyridine-containing mobile phases. Marine Ecology Progress Series 195: 29–45.

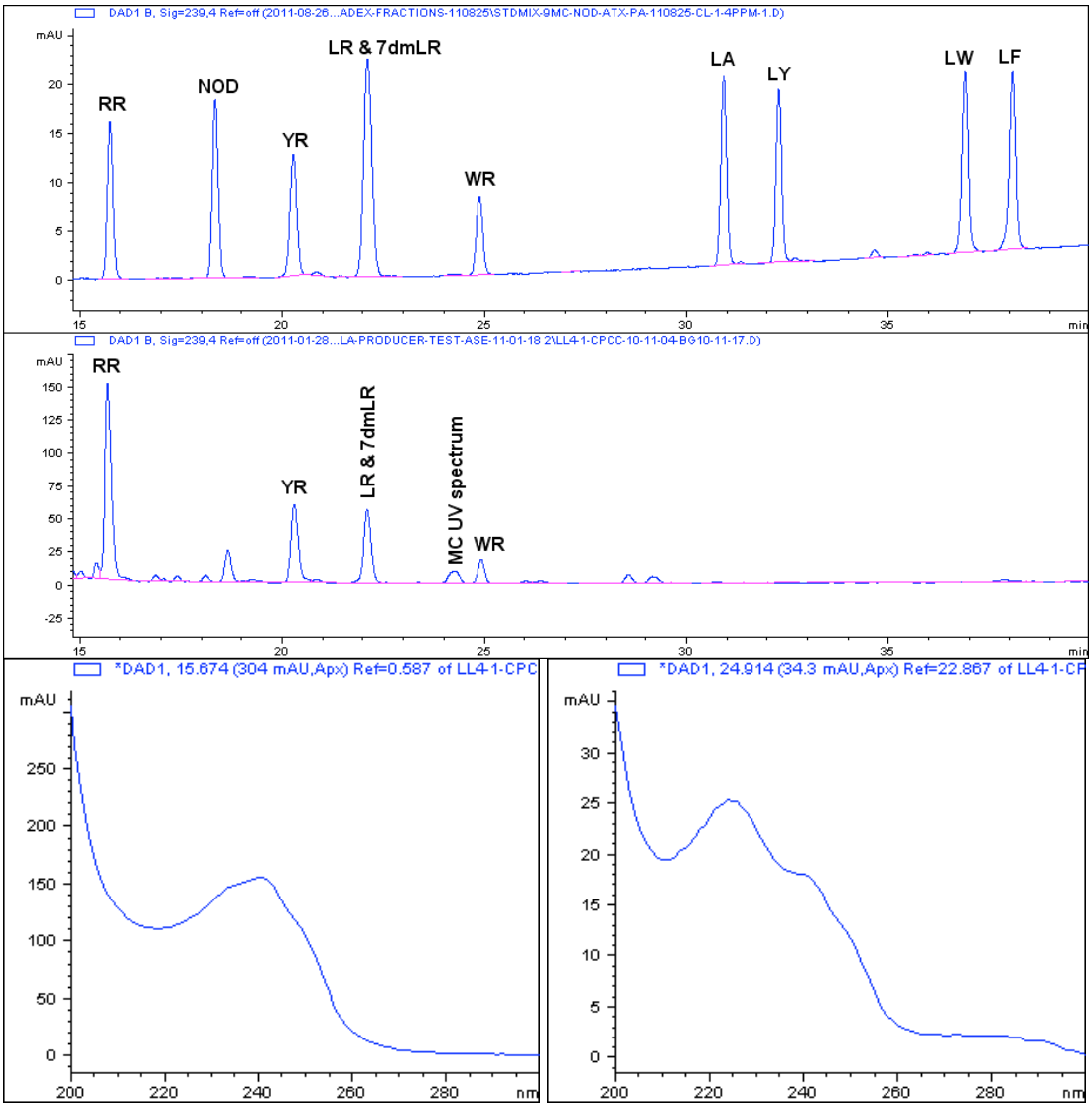
Zastepa, A., F. R. Pick, and J. M. Blais. 2013. Fate and persistence of particulate and dissolved microcystin-LA from Microcystis blooms. International Journal of Human and Ecological Risk Assessment In press.

Zurawell, R. 2010. Cyanotoxin Program Status Report. Alberta Environment Available at: www.environment.gov.ab.ca/info/library/8296.pdf. Accessed March 2013.

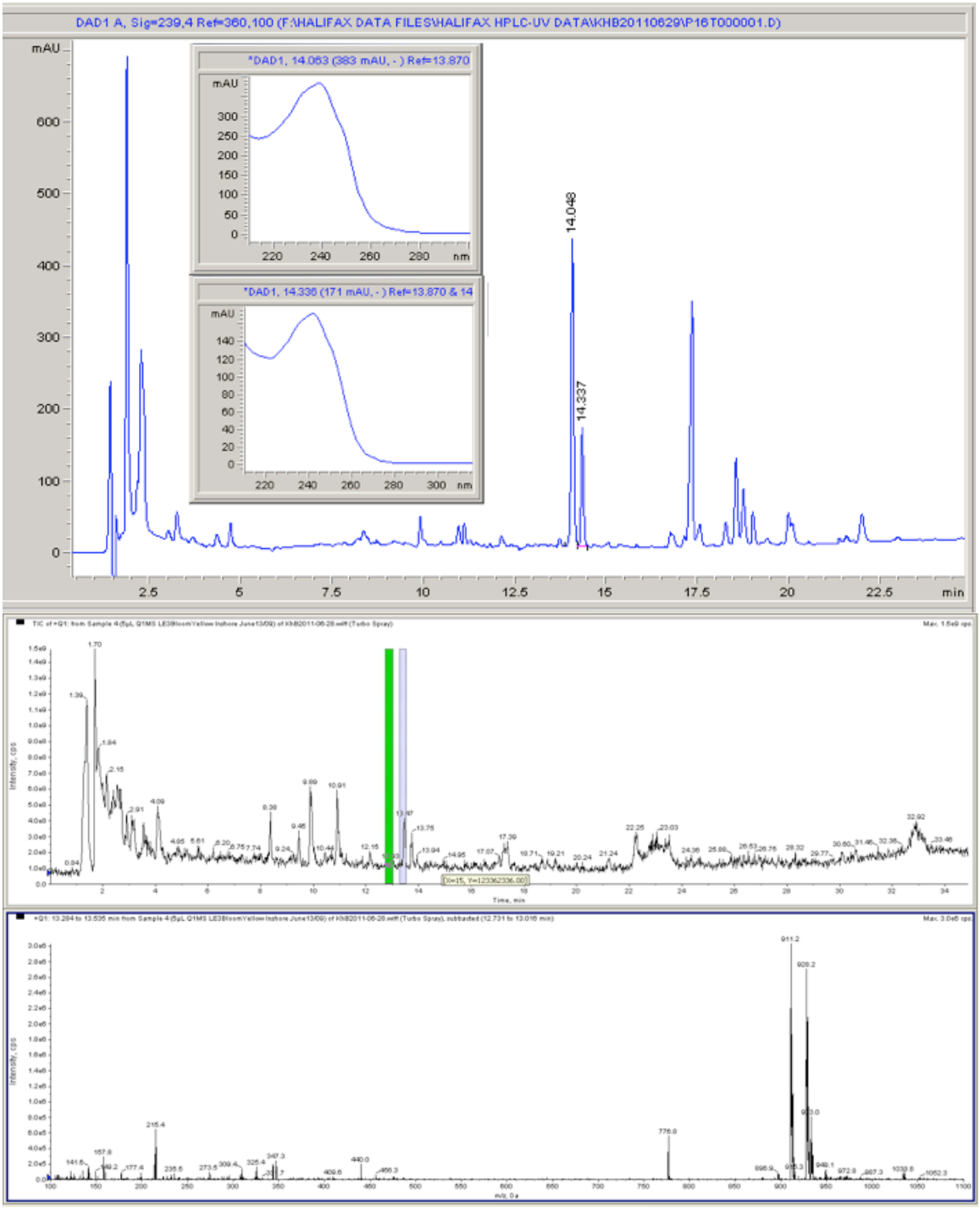
Zurawell, R. W., H. Chen, J. M. Burke, and E. E. Prepas. 2005. Hepatotoxic Cyanobacteria: A Review of the Biological Importance of Microcystins in Freshwater Environments. Journal of Toxicology and Environmental Health, Part B 8: 1–37.

Zurawell, R. W. Z. 2008. Alberta's Experience with Cyanobateria and Cyanotoxins. Quebec City Symposium on Blue-Green Algae

Appendix



A1: HPLC-UV chromatograms of a standard mix of microcystins and nodularin (marine analogue) as well as an extract of the CPCC 702 culture used for spike and recovery experiments in the development of the analytical method for microcystins in sediment (top two panels). Bottom left panel shows a characteristic UV spectrum of microcystins (peak 239 nm), shown here MC-LR. Bottom right panel shows a characteristic UV spectrum of microcystins containing a tryptophan substituent, shown here MC-WR. Column: Zorbax Eclipse XDB-C18 column (3.0 x 150 mm, 5 micron).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mAU
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14.048
14.337
min
Time, min
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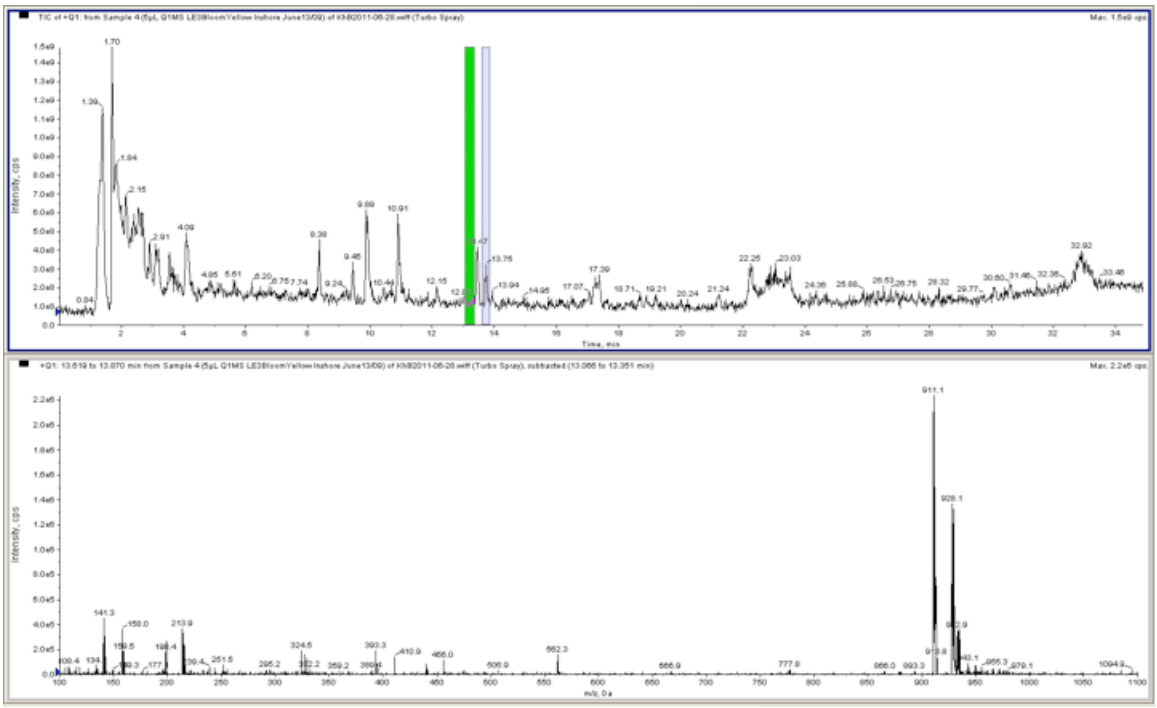
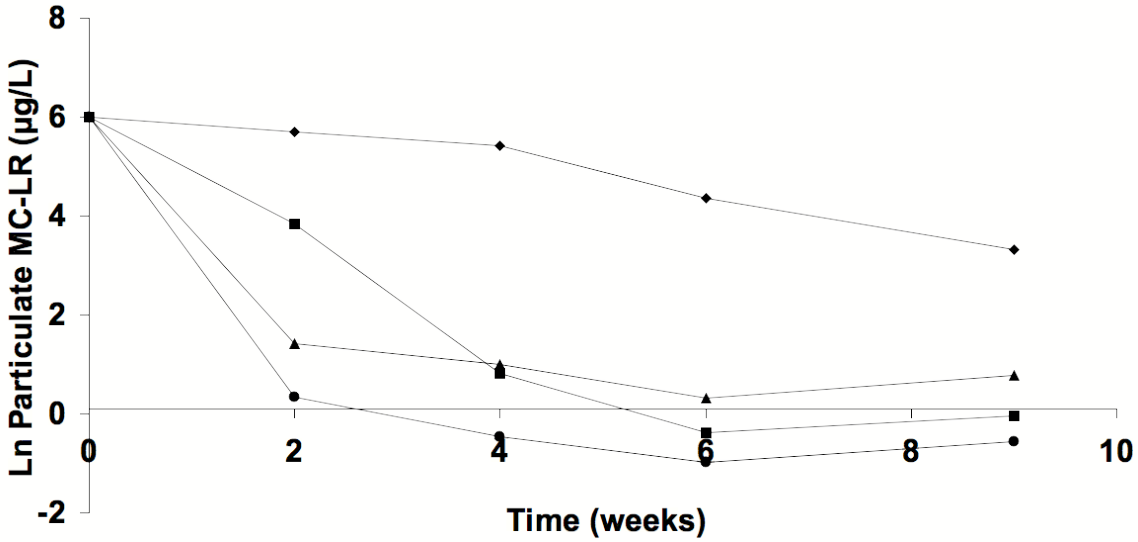
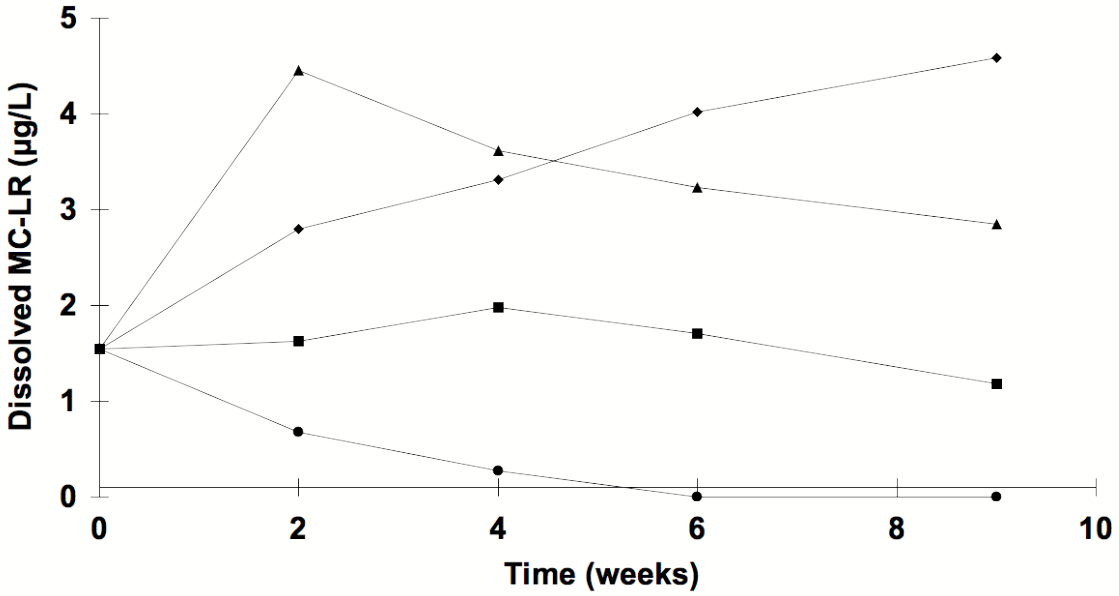


A2: HPLC-UV (top panel) and HPLC-MS/MS (bottom four panels) chromatograms used to identify a suspected microcystin peak as an isomer of MC-LA in a sample from Lakeland Estates (Chapter III). Top panel: Retention time of 14.063 min with UV peak at 239 nm corresponds to MC-LA. Bottom four panels: Retention time of 13.47 min corresponds to MC-LA with m/z 911. Column: Poroshell 120 SB-C18 (2.1 x 150 mm, 2.7 micron).



A3: Degradation of particulate (cell-bound) microcystin-LR (µg L^{-1}) in culture (CPCC 300) under conditions of 4°C darkness (diamonds), 25°C darkness (triangles), 25°C low light (squares), and 25°C high light (circles). Solid and dashed lines represent independent culture replicates. Natural log of data has been plotted.

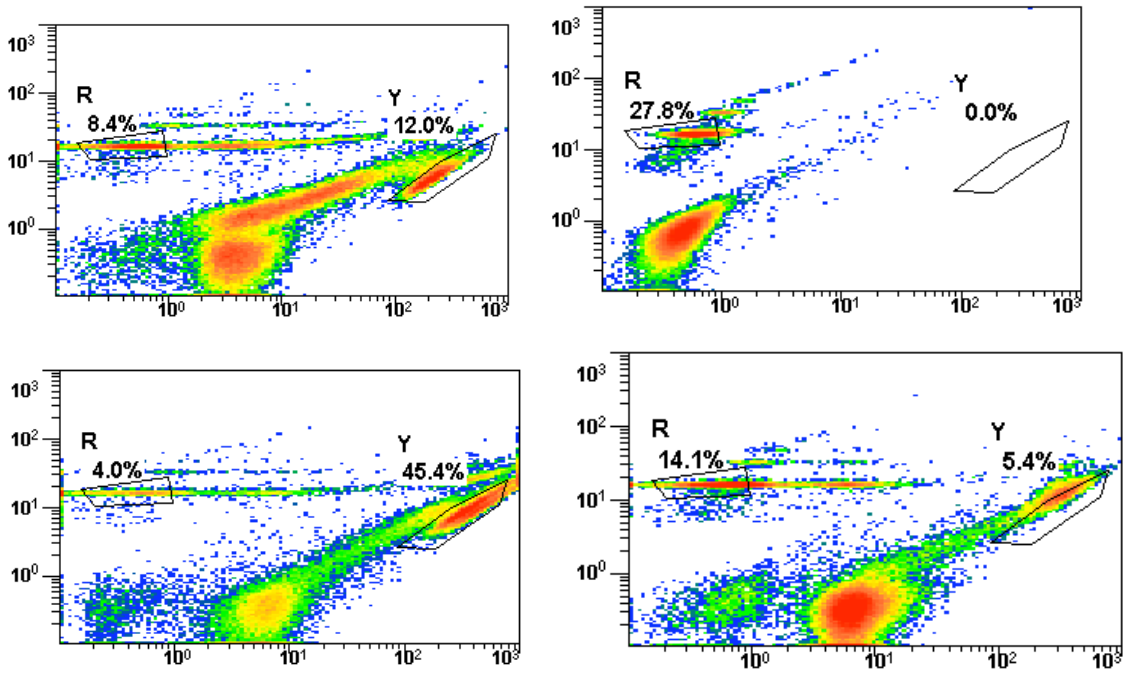


A4: Degradation of dissolved microcystin-LR (µg L^{-1}) in culture (CPCC 300)under conditions of 4°C darkness (diamonds), 25°C darkness (triangles), 25°C low light (squares), and 25°C high light (circles). Solid and dashed lines represent independent culture replicates.

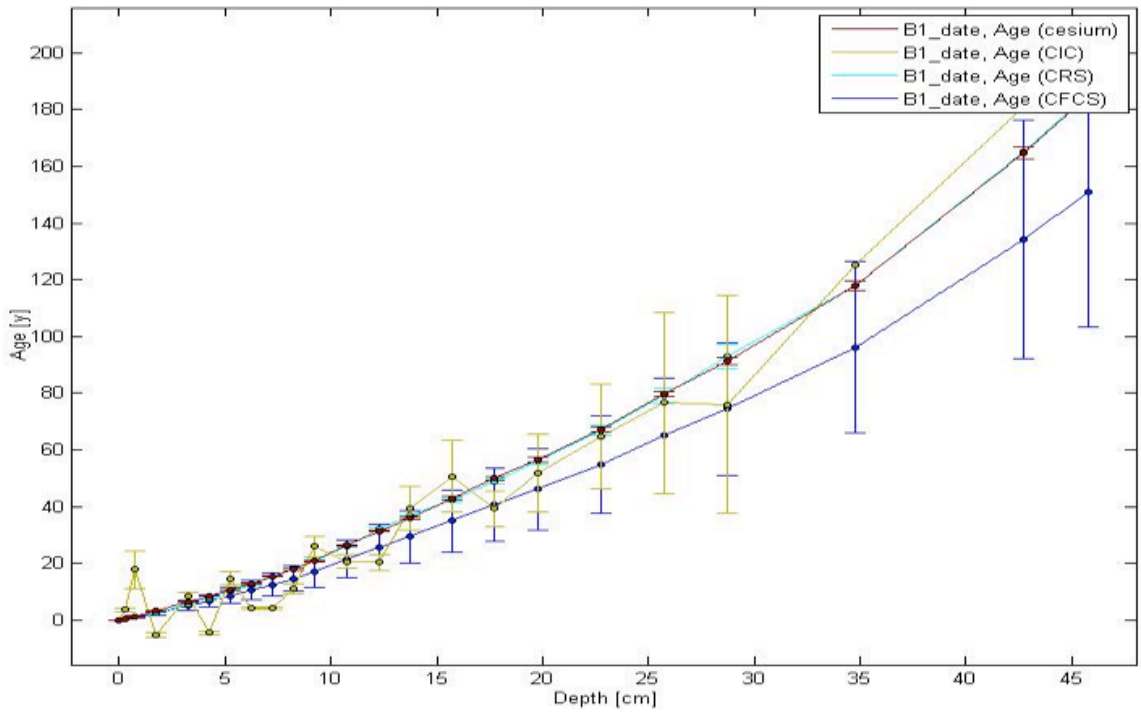
A5: Half-life of particulate (cell-bound), dissolved, and total microcystin-LR under four degradation conditions.

Conditions	Half-life of microcystin-LR (days)		
	Particulate	**Dissolved**	**Total**
25 ^{o}C (260 µE/m^{2}s)	1.7 +/- 0.04	21.5 +/- 0.6	1.8 +/- 0.01
25 ^{o}C (45 µE/m^{2}s)	3.8 +/- 0.2	35.0 +/- 6.9	4.3 +/-0.08
25 ^{o}C (µE/m^{2}s)	2.1 +/- 0.0	70.9 +/- 2.3	2.5 +/- 0.02
4 ^{o}C (Dark)	32.5 +/- 0.9	NA*	32.9 +/- 0.9

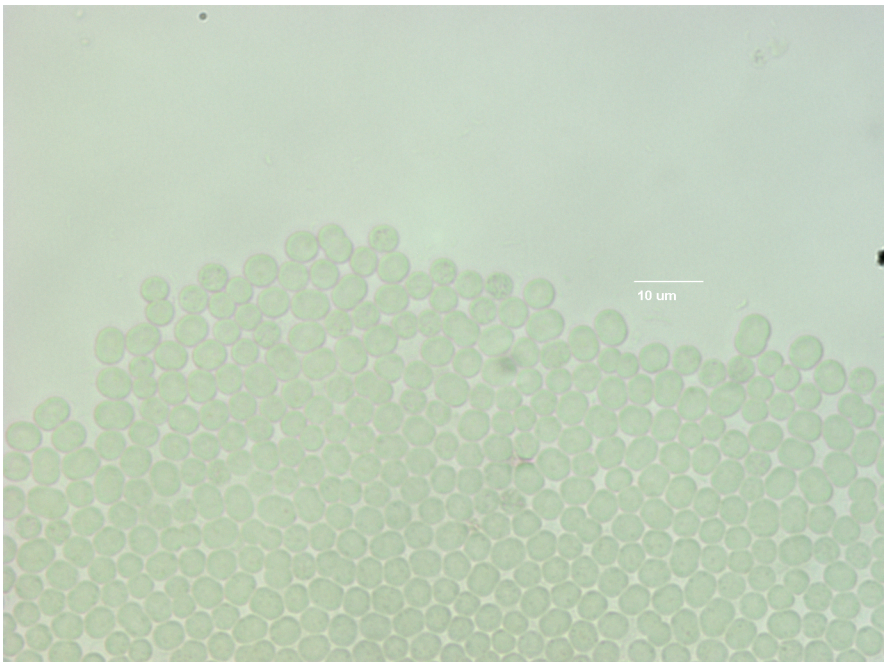
*MC-LR concentration was actually increasing at 0.33 µg/L per week. Live/intact cells still present.



A6: Dot density diagrams showing live cyanobacterial cell counts represented by gate Y at 2 weeks (left) and 9 weeks (right) from the start of the degradation experiment under conditions of 25°C low light (top) and 4°C darkness (bottom). Cyanobacterial cells were identified using FL4 (Exc: 488, 635 nm and Em: 675+/-25 nm) on the x-axis and FL3 (Exc: 488 nm and Em: 620+/-20 nm) on the y-axis.



A7: Example of a comparison of the age-depth relationship obtained using different models of Pb-210 dating in a sediment core obtained from Baptiste Lake (south basin), Alberta. CIC, constant initial concentration. CRS, constant rate of supply. CFCS, constant flux constant sedimentation.



A8: Digital photo of CPCC 702 culture used for spike and recovery experiments in the development of the analytical method for microcystins in lake sediments. Microscopic analysis and recording was done on a Zeiss AXIO A1 inverted microscope at 63X magnification.