Environment and Pollution; Vol. 5, No. 1; 2016
ISSN 1927-0909 E-ISSN 1927-0917
Published by Canadian Center of Science and Education

Seasonal Nearshore Occurrence of the Neurotoxin β-N-methylamino-L-alanine (BMAA) in Lake Winnipeg, Canada

Eva Pip[1], Kimber Munford[1] & Lindsay Bowman[1]

[1] Department of Biology, University of Winnipeg, Winnipeg, Manitoba, Canada

Correspondence: Eva Pip, Department of Biology, University of Winnipeg, Winnipeg, MB, R3B 2E9, Canada. Tel: 1-204-786-9319. E-mail: e.pip@uwinnipeg.ca

Received: February 10, 2016 Accepted: April 1, 2016 Online Published: April 28, 2016

doi:10.5539/ep.v5n1p110 URL: http://dx.doi.org/10.5539/ep.v5n1p110

Abstract

Seasonal fluctuation patterns of the neurotoxic amino acid β-N-methylamino-L-alanine (BMAA) were examined at four-day intervals during the ice-free season in water at three nearshore stations in the south basin of Lake Winnipeg, Canada. BMAA patterns were significantly exponentially correlated with concurrent phaeophytin, and inversely with chlorophyll *a*, indicating that free BMAA concentrations increased as blooms declined. BMAA was also significantly related to preceding microcystin concentrations, and as chlorophyll *a* declined, the proportion of BMAA relative to microcystin increased. Cross correlations identified significant relationships between BMAA and immediately *preceding* nitrate-N/inorganic phosphorus ratios, nitrate-N, rainfall, and a marginal inverse correlation with inorganic phosphorus. Total suspended solids levels were also significantly associated with BMAA, likely due to shading effects. A very high BMAA concentration was found under collapse of intense bloom conditions. These results have implications for water quality monitoring, nutrient management strategies and public health.

Keywords: BMAA, chlorophyll, microcystin, algal toxins, nitrogen, Lake Winnipeg

1. Introduction

The neurotoxic amino acid beta-N-methylamino-L-alanine (BMAA) has been shown to cause motor neuron damage and death via excitotoxic mechanisms (Chiu , Gehringer, Welch, & Neilan, 2011; Chiu et al., 2012). BMAA is transported across the blood-brain barrier (Bradley, 2008) and may be involved in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Parkinson's disease complex, with long latency times and long-term exposure before clinical impairment becomes evident (Weirich & Miller, 2014). BMAA has been found in brain tissue and hair samples of individuals who have succumbed to neurodegenerative illnesses (Cox, 2009), including Canadian Alzheimer's patients (Pablo et al., 2009). The multiple mechanisms of BMAA activity have been summarized by Chiu et al. (2011) and Bradley et al., (2013).

BMAA appears to be globally and environmentally widespread, wherever cyanobacteria are found, and all cyanobacterial taxa are believed to produce this water soluble neurotoxin (Cox et al., 2005). However eukaryotic algae, notably diatoms and dinoflagellates have also been shown to produce BMAA (Jiang et al., 2014; Lage, Annadotter, Rasmussen, & Rydberg., 2015; Lage et al., 2016).

BMAA has been found in every aquatic trophic level in ecosystems affected by cyanobacterial blooms (Jiao et al., 2014). Submerged macrophytes such as *Myriophyllum* and *Ceratophyllum* may assimilate BMAA by direct absorption (Al-Sammak, Hoagland, Cassada, & Snow, 2014), and BMAA may also be produced by algal epiphytes on plant surfaces. BMAA and epiphytic cyanobacteria on aquatic macrophytes have been implicated in avian vacuolar myelinopathy, a lethal neurological disorder of birds (Bidigare, Christensen, Wilde, & Banack, 2009). Bioaccumulation is evident in the food web as organisms at higher trophic levels have been reported to contain greater concentrations of BMAA. Jiao et al. (2014) found greater BMAA concentrations in fish muscle tissue than in invertebrates, particularly during times of cyanobacterial bloom outbreak and decline. Furthermore during bloom decline fish muscle contained higher BMAA levels than the cyanobacteria. In fish this toxin increases with age (Lage et al., 2015), and carnivorous, filter-feeding and some omnivorous species are associated with the greatest biomagnifications, as are bottom feeding species (Jiao et al., 2014). A number of workers (Caller et al.,2009, 2012; Bradley et al., 2013) have reviewed the putative links between regions subject to cyanobacterial blooms, for example proximity to lakes, agricultural lagoons or consumption of local fish, and

clusters of ALS occurrence in the human population.

Lake Winnipeg, the 10th largest freshwater lake in the world, is of great importance for recreation, tourism, commercial and sport fisheries, and provides drinking water for several communities and many point-source users. This lake has experienced problem eutrophication for several decades, but studies regarding algal toxins have thus far focused on microcystins (Pip & Allegro, 2010; Pip & Bowman, 2014); other toxins such as BMAA have not been studied. Furthermore seminal data regarding seasonal fluctuations of BMAA in freshwater lakes are not available in the literature. Genera known to produce BMAA (*Anabaena, Aphanizomenon, Microcystis, Nostoc*) are present in Lake Winnipeg, which hosts both cyanobacterial and diatom blooms (Kling, 1998). The objectives of the present study were to determine whether BMAA is present in Lake Winnipeg water samples, and if so, to examine its seasonal distribution in relation to environmental, chemical and algal parameters at three nearshore sites in the south basin of Lake Winnipeg. Canada.

2. Method

2.1 Study Sites

The three study sites were sandy beaches located on the east side of the south basin of Lake Winnipeg. Site 1 (96° 37.03'W, 50° 25.43'N) was a public bathing beach at Patricia Beach Provincial Park. Site 2 (96° 34.44'W, 50° 27.55'N) was located on public reserve shoreline adjacent to a cottage development. Site 3 (96° 34.99'W, 50° 28.46'N) was located on the north side of the public marina at Balsam Harbour. All sites were bare of aquatic vegetation and were well exposed to wind and wave action.

2.2 Sampling Procedures

Each site was sampled at the same respective time of day, from spring melt to fall freeze-up, every four days between 4 May and 9 November 2011, for a total of 48 consecutive samples for each of the three sites. Water samples were collected in high-density polypropylene bottles, 10 cm below the surface and 3-10 m from the shore, by wading where water was <1 m deep. Water for chemical analyses was filtered through Whatman No. 1 filter paper. Filtrate, filter papers and whole water samples were frozen and stored at -20°C. Temperature and pH were measured *in situ* using a thermometer and portable pH meter (Radiometer, Copenhagen). For rainfall estimates, daily precipitation data from the Gimli Climate Station (WMO ID 71748) on Lake Winnipeg were pooled for the four days preceding each sampling day (Pip & Bowman, 2014).

2.3 Chemical Analyses

Orthophosphate (molybdenum reactive), nitrate-N, dissolved organic matter index, chlorophyll *a* and phaeophytin were measured according to standard methods recommended by APHA (1995). Total dissolved solids were measured using an electrode TDSTestr (Oakton, Wards Natural Science, St. Catharines, Ontario). Total suspended solids were determined using preweighed Whatman No. 1 filter papers and air-dried.

2.4 Algal Toxins

Whole water samples were subjected to four rapid freeze-thaw cycles to lyse the cells. Samples were analyzed using ELISA; standards, reagents and quality control samples for microcystins (MC) were supplied by Beacon Analytical Systems Inc. (Portland, Maine)(Pip & Allegro, 2010; Pip & Bowman, 2014). Cross reactivities for the polyclonal antibodies used to bind with MC (predominantly MC-LR) are given in Pip & Bowman (2014). For BMAA the direct competitive ELISA protocols as well as standards were provided by Abraxis (2015-2016 version) (Warminster, Pennsylvania). The latter source also provided an ELISA protocol for anatoxin-*a*, which was attempted, but discontinued because results were less consistent than for BMAA, particularly with discrepancies between results from preconcentrated vs. non-preconcentrated samples, and some inconsistencies among the three replicates. BMAA microtiter well plates were read at 450 nm using a BioTek Synergy HT Multi-Detection Microplate Reader (Winooski, Vermont).

Water sample replicates were analyzed in random sequence. A set of calibration standard replicates was read with each sample batch**,** and results were calculated from each respective calibration curve. Reproducibility among replicates averaged 94% $\pm$ 0.8% S.E for calibration standards (59 replicate sets), and 96% $\pm$ 0.3% S.E. for water samples (146 replicate sets).

There has been much discussion regarding methodologies for assessing BMAA, particularly when evaluating cellular material. Differences in reported concentrations among studies have been attributed not only to differing analytical techniques, but also within the same technique, for example selective liquid chromatography-MS/MS (Lage et al., 2016). The latter authors suggested many potential pitfalls that could contribute towards variation and underestimation for this method, for example instrumentation quality, derivatization issues, different

extraction methods and other analytical losses. ELISA assays using monoclonal or polyclonal antibodies have been developed primarily for water samples, although they can be adapted to other matrices (Moreira, Ramos, Azevedo, & Vasconcelos, 2014). For water samples, they have the advantages of minimal sample processing and extraction losses. Faassen, Beekman, and Lurling (2013) evaluated an early Abraxis protocol and found that results tended to be overestimated at higher concentrations, but reproducibility and accuracy improved at greater sample dilutions. Moreira et al. (2014) reported an optimal concentration range of 0.05-0.50 µg/L for the current Abraxis BMAA protocol.

2.5 Statistical Analyses

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

3. Results

A summary of temperature and parameter values at the study sites is given in (Pip & Bowman, 2014). The seasonal free BMAA patterns at the three sites are shown in Fig. 1. Concentrations were generally low, with the highest value (2.0 µg l^{-}) observed at site 1 shortly before freeze-up. Site 1 showed significantly higher seasonal BMAA levels (mean µg l^{-} 0.96 $\pm$ 0.04 S.E.) than sites 2 (0.39 $\pm$ 0.04 S.E.) and 3 (0.45 $\pm$ 0.04 S.E.)($t = 9.14$ and 7.64 respectively, both $p < 0.0001$, separate variance estimate). Concentrations at sites 2 and 3 were not significantly different from each other. Peaks were generally higher and more sustained at site 1, and fluctuation patterns were not correlated between site 1 and either of sites 2 and 3, while fluctuations were repetitive and highly synchronized between sites 2 and 3 (Pearson $r = 0.68$, $p < 0.0001$, $n = 48$).

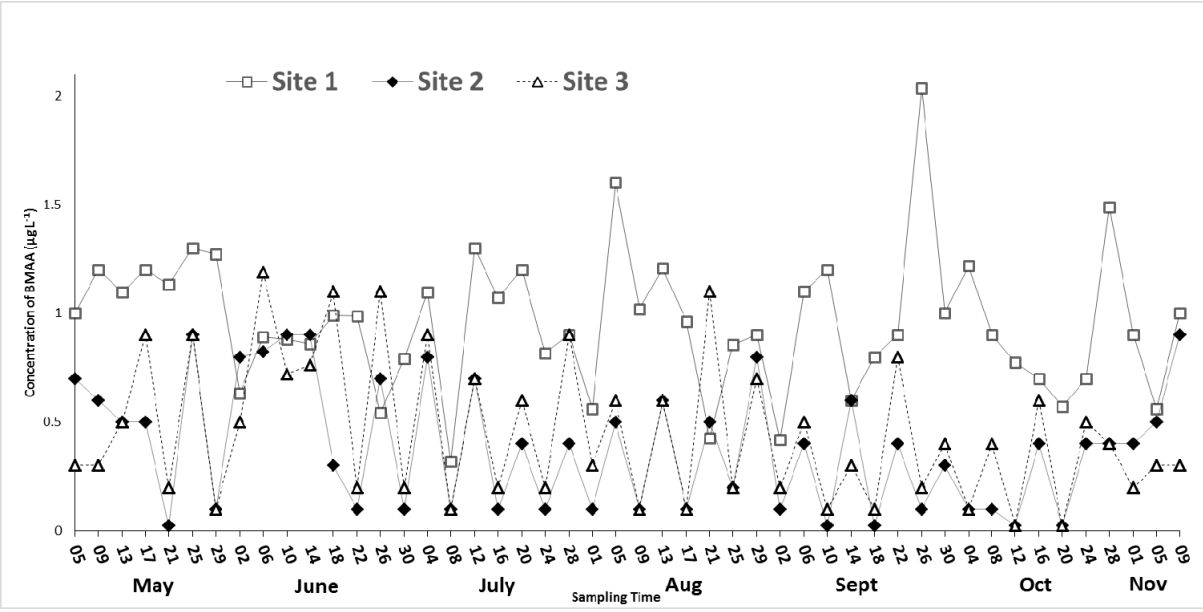

Figure 1. Seasonal fluctuation patterns of BMAA at the three sites, recorded at four-day intervals

Significant positive correlations were identified between seasonal fluctuation patterns of BMAA and each of: total suspended solids (TSS), phaeophytin, and an inverse correlation with chlorophyll *a* (chl *a*)(Table 1). Phaeophytin and chl *a* were highly inversely correlated with each other (Pip & Bowman, 2014), and therefore BMAA was also correlated with the phaeophytin/chl *a* ratio (Table 1). Seasonal BMAA patterns were not significantly correlated with *concurrent* water temperature, pH, dissolved organic matter, total dissolved solids, nitrate-N, or inorganic phosphorus.

However when cross-correlations were examined, using the immediately *preceding* environmental parameters and *delayed* BMAA, significant correlations emerged for the nitrate-N/inorganic phosphate ratio (NPR), nitrate-N and rainfall (Table 1). BMAA was marginally *inversely* correlated with inorganic phosphorus. Best-fit relationships were exponential for NPR, nitrate-N and inorganic phosphorus. Concurrent TSS showed a better fit than its lagged counterpart (Table 1).

Stepwise multiple regression analysis with ln transformed BMAA as the dependent variable, entered only

(preceding)lnNPR into Equation (1)(F = 4.97, $p = 0.027$):

$$lnBMAA = 0.16\ previous(ln[NPR]) - 1.01 \quad (1)$$

Results of microcystin (MC) analysis in relation to physical and chemical parameters are reported elsewhere (Pip & Bowman, 2014). BMAA significantly lagged linearly behind MC (Table 1). The BMAA/MC ratio was significantly correlated with concurrent TSS ($r = 0.21$, $p = 0.007$, both ratio and TSS ln transformed), and inversely with chl *a* ($r = -0.16$, $p = 0.026$, ratio ln transformed). Thus when chl *a* declined, the proportion of BMAA increased relative to MC, and BMAA fluctuations succeeded those of MC.

Table 1. Correlations between BMAA concentrations vs. concurrent and immediately preceding (cross-correlated at four-day intervals) environmental parameters. NS = not significant

Parameter	Concurrent ($n = 144$)	Previous (cross-correlation)($n = 141$)
Nitrate-N	NS	$r = 0.16$, $p = 0.027$ (both ln transformed)
Inorganic phosphorus	NS	$r = -0.14$, $p = 0.05$ (both ln transformed)
Nitrate-N/ Inorganic P ratio	NS	$r = 0.19$, $p = 0.014$ (both ln transformed)
Total suspended solids	$r = 0.22$, $p = 0.007$ (both ln transformed)	$r = 0.15$, $p = 0.037$
Rainfall	NS	$r = 0.16$, $p = 0.027$
Chlorophyll *a*	$r = -0.15$, $p = 0.040$ (chlorophyll *a* ln transformed)	NS
Phaeophytin	$r = 0.16$, $p = 0.030$	NS
Phaeophytin/ Chlorophyll *a* ratio	$r = 0.17$, $p = 0.019$	NS
Microcystin	NS	$r = 0.15$, $p = 0.036$

4. Discussion

Large variations in the intensity and character of blooms as well as MC concentrations have been observed in Lake Winnipeg (Pip & Bowman, 2014), which may be dominated by cyanobacteria or diatoms in different years (Kling, 1998). While only cyanobacteria are known to produce MC, both cyanobacteria and a number of diatoms and some dinoflagellates have been reported to produce BMAA (Jiang et al., 2014; Lage et al., 2015, 2016). BMAA concentrations in the present study were lower than those reported for water samples from Nebraska reservoirs (Al-Sammak et al., 2014). The highest seasonal values were found at site 1 (Patricia Beach), a popular family bathing beach with a high density of users, and the seasonal BMAA pattern at this site differed significantly from sites 2 and 3, whose patterns were mutually similar. However BMAA levels on collapse of visible blooms in Lake Winnipeg may achieve much higher local concentrations: a water sample we collected adjacent to a senescent bloom washed up on shore between sites 2 and 3 on 11 August 2015 yielded a BMAA concentration of 306 μg l^{-1}.

The present study found significant exponential correlations between BMAA and the *preceding* nitrate-N/inorganic phosphorus ratio (NPR) as well as nitrate-N concentrations (Table 1). Thus BMAA levels in the water followed fluctuations of these parameters. BMAA also showed a marginal inverse correlation with previous inorganic phosphorus, suggesting that phosphorus decline and incipient limitation may also be linked with BMAA release and bloom decline. NPR appeared to be the most important predictive parameter (of those examined); however its significance was due not only to proportional phosphorus decline, but also to absolute increase of nitrate-N, as seen in the positive correlation of BMAA with preceding nitrate-N concentrations. This field result differed from the experimental findings of Downing, Banack, Metcalf, Cox, and Downing (2011), who reported that nitrogen starvation of axenic cultures of strains of (non-nitrogen fixing) *Microcystis* and *Synechocystis* resulted in increases of free cellular BMAA. The latter represented *de novo* synthesis, as the amount of protein-associated BMAA did not decrease. Additional studies are needed for nitrogen fixers and

eukaryotic algae.

BMAA concentrations were also significantly correlated with total suspended solids, which reflected algal density as well as turbidity arising from turbulence and runoff. Possibly stress of crowding and reduced light penetration may be associated with greater BMAA production and/or release. Indeed BMAA was significantly associated with phaeophytin, a breakdown product of chlorophyll, and also with the phaeophytin/chl *a* ratio (Table 1). Therefore free BMAA increased with bloom decline. Jiao et al. (2014) sampled cyanobacterial blooms three times during the season in eutrophic Lake Taihu, China, and reported that total (free and protein-associated) BMAA concentrations in cyanobacterial fractions were higher during bloom outbreak and decline than during bloom emergence. These authors suggested that *Microcystis* spp. may biosynthesize more BMAA at these times. From our results particularly, it appears that more free BMAA is apparent during algal decline and lysis, supporting the suggestion of Berntzon et al. (2013) that release of intracellular BMAA occurs largely at this time. The latter authors also noted the chlorotic state of cells experimentally treated with BMAA and surmised that this was due to breakdown of chl *a*; our field results clearly supported this observation. This may suggest a contributing mechanism for rapid bloom collapse as is typical of many eutrophic systems: as a bloom builds, and cells start to senesce and lyse as a consequence of short generation times and environmental stressors such as light (or nutrient, e.g. phosphorus) limitation, BMAA release may in turn initiate a chain reaction causing other cells to cease growth and die. Berntzon et al. (2013) have suggested that BMAA above threshold levels may instigate programmed cell death and ensuing bloom failure.

The significant correlation between BMAA and preceding rainfall (Table 1) was likely related to runoff of nitrogen and elevated TSS, and indeed both nitrate-N and TSS (but not phosphorus) were significantly correlated with precipitation ($p = 0.003$, and <0.0001 respectively)(Pip & Bowman, 2014).

Typically, several cyanobacterial toxins occur in the same environmental sample (Metcalf & Codd, 2009). Metcalf et al. (2008) identified MC, anatoxin-*a*, nodularin and saxitoxin in a number of British surface waters in addition to BMAA. Al-Sammak et al. (2014) reported an association of BMAA with MC in Nebraska reservoirs. In the present study BMAA fluctuations in the water samples were generally correlated with previous, but not concurrent MC. BMAA and MC appeared to show different dynamic characteristics: while MC was significantly positively correlated with chl *a* and inversely with phaeophytin (Pip & Bowman, 2014), the opposite was observed for BMAA. Thus while MC in water samples was most associated with vigorous blooms, BMAA increased with bloom decline.

Berntzon et al. (2013) found that BMAA is persistent under laboratory conditions and "was not spontaneously degraded or significantly altered with time." Its fate in aquatic ecosystems therefore requires investigation: while some is absorbed by macrophytes and other biota, little is known regarding its eventual degradation by microbial or photolytic processes, or its persistence in bottom sediments. It is suggestive that benthivorous fish typically show higher BMAA levels (Jonasson et al., 2010; Lage et al., 2015).

Much less persistent in freshwater environments is anatoxin-*a,* a potent rapid paralytic cyanobacterial neurotoxin (Weirich & Miller, 2014) that is responsible for many animal fatalities (Osswald, Rellan, Gago, & Vasconcelos, 2007). Unlike BMAA, this toxin degrades readily in the environment in sunlight and alkaline pH, as well as by microbial action, and decomposition is independent of oxygen (Osswald et al., 2007). Accordingly detectable levels of anatoxin-a in water would be expected primarily during bloom maxima, and its relative instability would likely result in underestimation on analysis. In addition to high levels of BMAA, we detected anatoxin-*a* in the range of 25-125 $\mu g\ l^{-1}$ in water adjacent to a large senescing bloom that had washed up on shore between sites 2 and 3 on 11 August 2015. Therefore such blooms present significant immediate health risks, yet children were observed playing with the algal mats and families were splashing and swimming in the algal-infested water. Clearly public education and timely public health warnings are required. However, unlike BMAA, anatoxin-*a* does not appear to be associated with risks in fish (Al-Sammak et al., 2014).

The functional role of BMAA in cyanobacteria remains unclear. While its toxicity may confer obvious advantages against grazers, biosynthesis of this molecule evolved well before appearance of animal neurons (Berntzon et al., 2013) and therefore this property is unrelated to its original purpose. External BMAA is a strong inhibitor of nitrogenase activity and causes growth arrest of *Nostoc,* but BMAA is apparently not itself utilized as a nitrogen source (Berntzon et al., 2013). Possibly BMAA may be implicated in diminution of nitrogen-fixation when externally available inorganic nitrogen (as suggested in our study) has increased.

The ubiquitous distribution of BMAA provides a multiplicity of potential routes for human exposure from drinking water (Metcalf et al., 2008; Bradley et al., 2013), bathing and showering, and consumption of aquatic foodstuffs, as well as dietary supplements that incorporate certain "edible" cyanobacteria (e.g. *Nostoc*

flagelliforme)(Jiao et al., 2014). When livestock drink water from farm dugouts or ditches containing algal blooms, a possible risk of tainted meat and milk may ensue, and BMAA may promote chronic animal intoxication (Metcalf et al., 2008). Consumption of waterfowl such as Canada geese and ducks from BMAA tainted wetlands has been identified as another possible human health risk (Bidigare et al., 2009). Crops may be contaminated by irrigation with water harboring algae. Inhalation of aerosols containing BMAA has been identified as another route of exposure (Stommel, Field, & Caller, 2013), similar to that of aerosolized MC (Backer et al., 2008, 2010). Aerosols can arise for example through wave action, splashing, boating, showering, water hose mists, humidifier use as well as bubble formation (Schlichting, 1974) and exposure to biofilms in cooling towers (Hauer, Capek, & Bohmova, 2015). Caller et al. (2009) reported higher ALS incidence for residents within 0.5 mile of, and especially downwind of, eutrophic New Hampshire lakes; similarly Bradley et al. (2013) postulated that degree of ALS risk may be related to distance from water bodies which harbor cyanobacterial blooms. Such observations have implications for lakefront communities and cottage owners, as well as commercial fishers. Wind-borne mists and dusts from cropland application of animal waste from lagoons associated with intensive livestock production facilities may present a hazard in agricultural areas.

Given the widespread occurrence of BMAA in the environment and the multiple routes of human exposure, it is important to commence monitoring programs for this and related toxins in drinking water and food. The difficulty of obtaining clinical data for cyanobacterial-linked illness is overshadowed and confounded by the frequency of mis- and under-diagnosis (Stewart, Webb, Schluter, & Shaw, 2006). The effects of exposure to low levels of combined MC and BMAA, or/and other algal toxins such as anatoxin, are not known (Metcalf & Codd, 2009). Of particular concern is the known synergistic toxic effect of BMAA on methylmercury, which are both neurotoxins (Rush, Liu, & Lobner, 2012). Similarly, a greater understanding is needed of the efficacy of drinking water treatment methods in removing, reducing or mitigating toxins (Metcalf & Codd, 2009). In view of ever-increasing eutrophication of surface waters in Canada and globally, and the anticipated adverse effects of climate change, exposure to cyanobacterial and other algal toxins is expected to escalate. Therefore strategies to reduce nitrogen and phosphorus input to surface waters must remain primary objectives in dealing with the proliferation of algal toxins in our environment.

In conclusion this study showed that BMAA can be detected in nearshore Lake Winnipeg water throughout most of the ice-free season, and of the three sites examined, BMAA values were greatest at the public bathing beach. BMAA concentrations were significantly correlated with phaeophytin (a breakdown product of chlorophyll) and inversely with chlorophyll *a,* indicating that BMAA increased during bloom decline. Seasonal fluctuation patterns were significantly exponentially correlated with preceding (in this study, 4 day intervals) nitrate-N/inorganic phosphorus ratio and nitrate-N concentrations. The significant association with environmental nitrogen suggests yet another reason for nitrogen removal by waste treatment plants in lake refurbishment strategies (Pip & Bowman, 2014), and the association of nitrogen with precipitation shows the importance of addressing point-source and overland runoff.

While BMAA and MC were often both present in the samples, they were not concurrently synchronized with each other: while inorganic phosphorus was of greater importance than nitrogen for MC (Pip & Bowman, 2014), the reverse was true for BMAA, and BMAA fluctuations lagged behind those of MC. These observations have implications for water quality monitoring, as presently water is not monitored for BMAA. Currently the only cyanobacterial toxin for which a Canadian water quality guideline exists is MC, at 1 µg/L. We found particularly high levels of BMAA and anatoxin-*a* in water associated with a nuisance bloom, emphasizing the need for public education and timely posting of public health warnings, perhaps even patrolling and enforcement, during bloom outbreaks. Ultimately, the major underlying cause of algal blooms must be addressed in any refurbishment strategy: nutrient reduction to Lake Winnipeg presents an urgent need for concerted action.

References

Al-Sammak, M. A., Hoagland, K. D., Cassada, D., & Snow, D. D. (2014). Co-occurrence of the cyanotoxins BMAA, DABA and anatoxin-*a* in Nebraska reservoirs, fish, and aquatic plants. *Toxins, 6,* 488-508.

APHA. (1995). *Standard Methods for the Examination of Water and Wastewater.* American Public Health Association, Washington DC.

Backer, L. C. et al. (2008). Recreational exposure to low concentrations of microcystins during an algal bloom in a small lake. *Marine Drugs, 6,* 389-406. http://dx.doi.org/10.1016/j.toxicon.2009.07.006

Backer, L. C. et al. (2010). Recreational exposure to microcystins during algal blooms in two California lakes. *Toxicon, 55,* 909-921. http://dx.doi.org/ 10.3390/md20080018

Berntzon, L., Erasmie, S., Celepli, N, Eriksson, J., Rasmussen, U., & Bergman, B. (2013). BMAA inhibits nitrogen fixation in the cyanobacterium *Nostoc* sp. PCC 7120. *Marine Drugs, 11,* 3091-3108. http://dx.doi.org/10.3390/md11083091

Bidigare, R. R., Christensen, S. J., Wilde, S. B., & Banack, S. A. (2009). Cyanobacteria and BMAA: possible linkage with avian vacuolar myelinopathy (AVM) in the southeastern United States. *Amyotrophic Lateral Sclerosis, 10, Suppl. 2,* 71-73. http://dx.doi.org/10.3109/17482960903273056

Bradley, W. (2008). New aspects of the cyanobacteria/BMAA hypothesis and future directions. *Amyotrophic Lateral Sclerosis, 9, Suppl. 1,* 38-40, C69. http://dx.doi.org/10.1080/17482960802444618

Bradley, W. G., Borenstein, A. R., Nelson, L. M., Codd, G. A., Rosen, B. H., Stommel, E. W., & Cox, P. A. (2013). Is exposure to cyanobacteria an environmental risk for amyotrophic lateral sclerosis and other neurodegenerative diseases? *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 14,* 325-333. http://dx.doi.org/10.3109/21678421.2012.750364

Brand, L. E. (2009). Human exposure to cyanobacteria and BMAA. *Amyotrophic Lateral Sclerosis, 10, Suppl. 2,* 85-95. http://dx.doi.org/10.3109/17482960903273585

Brand, L. E., Pablo, J., Compton, A., Hammerschlag, N., & Mash, D.C. (2010). *Harmful Algae, 9,* 620-635. http://dx.doi.org/10.1016/j.hal.2010.05.002

Caller, T. A. et al. (2009). A cluster of amyotrophic lateral sclerosis in New Hampshire: a possible role for toxic cyanobacteria blooms. *Amyotrophic Lateral Sclerosis, 10, Suppl. 2,* 101-108. http://dx.doi.org/10.3109/17482960903278485

Caller, T. A., Field, N. C., Chipman, J. W., Shi, X., Harris, B. T., & Stommel, E. W. (2012). Spatial clustering of amyotrophic lateral sclerosis and the potential role of BMAA. *Amyotrophic Lateral Sclerosis, 13,* 25-32. http://dx.doi.org/10.3109/17482968.2011.62436

Chiu, A. S., Gehringer, M. M., Braidy, N., Guillemin, G. J., Welch, J. H., & Neilan, B. A. (2012). Excitotoxic potential of the cyanotoxin β-N-methylamino-L-alanine (BMAA) in primary human neurons. *Toxicon, 60,* 1159-1165. http://dx.doi.org/10.1016/j.toxicon.2012.07.169

Chiu, A. S., Gehringer, M. M., Welch, J. H., & Neilan, B. A. (2011). Does α-amino-β-methylaminopropionic acid (BMAA) play a role in neurodegeneration? *International Journal of Environmental Research and Public Health, 8,* 3728-3746. http://dx.doi.org/10.3390/ijerph8093728

Cox, P. A. (2009). Conclusion to the Symposium: The seven pillars of the cyanobacteria/BMAA hypothesis. *Amyotrophic Lateral Sclerosis, 10, Suppl. 2,* 124-126. http://dx.doi.org/10.3109/17482960903312912

Cox, P. A., Banack, S. A., Murch, S. J., Rasmussen, U., Tien, G., Bidigare, R. R., … Bergman, B. (2005). Diverse taxa of cyanobacteria produce β-N-methylamino-L-alanine, a neurotoxic amino acid. *Proceedings of the National Academy of Sciences, 102,* 5074-5078. http://dx.doi.org/10.1073/pnas.0501526102

Downing, S., Banack, S. A., Metcalf, J. S., Cox, P. A., & Downing, T. G. (2011). Nitrogen starvation of cyanobacteria results in the production of β-N-methylamino-L-alanine. *Toxicon, 58,* 187-194. http://dx.doi.org/10.1016/j.toxicon.2011.05.017

Esterhuizen, M., Pflugmacher, S., & Downing, T. G. (2011). β-N-methylamino-L-alanine (BMAA) uptake by the aquatic macrophyte *Ceratophyllum demersum. Ecotoxicology and Environmental Safety, 74,* 74-77. http://dx.doi.org/10.1016/j.ecoenv.2010.04.005

Faassen, E. J. (2014). Presence of the neurotoxin BMAA in aquatic ecosystems: What do we really know? *Toxins, 6,* 1109-1138. http://dx.doi.org/ 10.3390/toxins6031109

Faassen, E. J., Beekman, W., & Lurling, M. (2013). Evalustion of a commercial enzyme linked immunosorbent assay (ELISA) for the determination of the neurotoxin BMAA in surface waters. *PlosONE, 8,* e65260.

Hauer, T., Capek, P., & Bohmova, P. (2015). Main photoautotrophic components of biofilms in natural draft cooling towers. *Folia Microbiologica.*

Holtcamp, W. (2012). The emerging science of BMAA. *Environmental Health Perspectives, 120,* A110-A116.

Jiang, L., Eriksson, J., Lage, S., Jonasson, S., Shams, S., Mehine, M., Ilag, L. L., & Rasmussen, U. (2014). Diatoms: a novel source for the neurotoxin BMAA in aquatic environments. *PlosONE, 9*(8), e106695.

Jiao, Y., Chen, Q., Chen, X., Wang, X., Liao, X., Jiang, L., Wu, J., & Yang, L. (2014). Occurrence and transfer of a cyanobacterial neurotoxin β-N-methylamino-L-alanine within the aquatic food webs of Gonghu Bay

(Lake Taihu, China) to evaluate the potential human health risk. *Science of the Total Environment, 468-469,* 457-463.

Jonasson, S., Eriksson, J., Berntzon, L., Rasmussen, U., & Bergman, B. (2008). A novel cyanobacterial toxin (BMAA) with potential neurodegenerative effects. *Plant Biotechnology, 25,* 227-232. http://dx.doi.org/10.5511/plantbiotechnology.25.227

Jonasson, S., Eriksson, J., Berntzon, L., Spacil, Z., Ilag, L. L., Ronnevi, L., Rasmussen, U., & Bergman, B. (2010). Transfer of a cyanobacterial neurotoxin within a temperate aquatic ecosystem suggests pathways for human exposure. *Proceedings of the National Academy of Sciences, 107,* 9252-9257. http://dx.doi.org/10.1073/pnas.0914417107

Kling, H. J. (1998). A summary of past and recent plankton of Lake Winnipeg, Canada using algal fossil remains. *Journal of Paleolimnology, 19,* 297-307. http://dx.doi.org/10.1023/A:1007990217568

Lage, S., Annadotter, H., Rasmussen, U., & Rydberg, S. (2015). Biotransfer of β-N-methylamino-L-alanine (BMAA) in a eutrophicated freshwater lake. *Marine Drugs, 13,* 1185-1201. http://dx.doi.org/10.3390/md3031185

Lage, S., Burian, A., Rasmussen, U., Costa, P. R., Annadotter, H., Godhe, A., & Rydberg, S. (2016). BMAA extraction of cyanobacteria samples: which method to choose? *Environmental Science and Pollution Research, 23,* 338-350. http://dx.doi.org/10.1007/s11356-015-5266-0

Metcalf, J. S., & Codd, G. A. (2009). Cyanobacteria, neurotoxins and water resources: Are there implications for human neurodegenerative disease? *Amyotrophic Lateral Sclerosis, 10, Suppl. 2,* 74-78. http://dx.doi.org/10.3109/17482960903272942

Metcalf, J. S., Banack, S. A., Lindsay, J., Morrison, L. F., Cox, P. A., & Codd, G. A. (2008). Co-occurrence of β-N-methylamino-L-alanine, a neurotoxic amino acid with other cyanobacterial toxins in British waterbodies, 1990-2004. *Environmental Microbiology, 10,* 702-708. http://dx.doi.org/10.1111/j.1462-2920.2007.01492.x

Moreira, C., Ramos, V., Azevedo, J., & Vasconcelos, V. (2014). Methods to detect cyanobacteria and their toxins in the environment. *Applied Microbiology and Biotechnology, 98,* 8073-8082. http://dx.doi.org/10.1007/s00253-014-5951-9

Osswald, J., Rellan, S., Gago, A., & Vasconcelos, V. (2007). Toxicology and detection methods of the alkaloid neurotoxin produced by cyanobacteria, anatoxin-a. *Environment International, 33,* 1070-1089. http://dx.doi.org/10.1016/j.envint.2007.06.003

Pablo, J. et al. (2009). Cyanobacterial neurotoxin BMAA in ALS and Alzheimer's disease. *Acta Neurologica Scandinavica, 120,* 216-225. http://dx.doi.org/10.1111/j.1600-0404.2008.01150x

Pip, E., & Allegro, E. (2010). Nearshore fluctuations in water chemistry, microcystins and coliform bacteria during the ice-free season in Lake Winnipeg, Manitoba, Canada. *Ecohydrology & Hydrobiology, 10,* 35-43. http://dx.doi.org/10.2478/v10104-010-0020-1

Pip, E., & Bowman, L. (2014). Microcystin and algal chlorophyll in relation to nearshore nutrient concentrations in Lake Winnipeg, Canada. *Environment and Pollution*, *3,* 36-47. http://dx.doi.org/10.5539/epv3n2p36

Rush, T., Liu, X., & Lobner, D. (2012). Synergistic toxicity of the environmental neurotoxins methymercury and beta-N-methylamino-L-alanine. *Neuroreport, 23,* 216-219. http://dx.doi.org/10.1097/WNR.0b013e32834fe6d6

Schlichting Jr., H. E. (1974). Ejection of microalgae into the air via bursting bubbles. *Journal of Allergy and Clinical Immunology, 53,* 185-188.

Sokal, R. R., & Rohlf, F. J. (1981). *Biometry*. New York, N.Y.: W.H. Freeman and Co.

Stewart, I., Webb, P. M., Schluter, P. J., & Shaw, G. R. (2006). Recreational and occupational field exposure to freshwater cyanobacteria – a review of anecdotal and case reports, epidemiological studies and the challenges for epidemiologic assessment. *Environmental Health: A Global Access Science Source, 5,* 6-18. http://dx.doi.org/10.1186/1476-069X-5-6

Stommel, E. W., Field, N. C., & Caller, T. A. (2013). Aerosolization of cyanobacteria as a risk factor for amyotrophic lateral sclerosis. *Medical Hypotheses, 80,* 142-145. http://dx.doi.org/10.1016/j.mehy.2012.11.012

Weirich, C. A., & Miller, T.R. (2014). Freshwater harmful algal blooms: toxins and children's health. *Current Problems in Pediatric and Adolescent Health Care, 44,* 2-24. http://dx.doi.org/10.1016/j.cppeds.2013.10.007