The Lake Erie Harmful Algal Blooms Grab: High-resolution mapping of toxic and bioactive metabolites (cyanotoxins/cyanopeptides) in cyanobacterial harmful algal blooms within the western basin

Authors: Zastepa, Arthur, Westrick, Judy A., Miller, Todd R., Liang, Anqi, Szlag, David C., et al.

Source: Aquatic Ecosystem Health & Management, 27(1) : 46-63

Published By: Michigan State University Press

URL: https://doi.org/10.14321/aehm.027.01.46

AQUATIC ECOSYSTEM
HEALTH & MANAGEMENT SOCIETY

MICHIGAN STATE UNIVERSITY PRESS

The Lake Erie Harmful Algal Blooms Grab: High-resolution mapping of toxic and bioactive metabolites (cyanotoxins/cyanopeptides) in cyanobacterial harmful algal blooms within the western basin

Arthur Zastepa[1], Judy A. Westrick[2], Todd R. Miller[3], Anqi Liang[1], David C. Szlag[4], Justin D. Chaffin[5]

[1]*Environment and Climate Change Canada, Canada Centre for Inland Waters, 867 Lakeshore Road, Burlington, Ontario, Canada, L7S 1A1*
[2]*Wayne State University, 5101 Cass Ave., Detroit, Michigan 48202, United States*
[3]*Joseph J. Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA*
[4]*Oakland University, 207 Mathematics and Science Center, Rochester, Michigan, 48309 USA*
[5]*F.T. Stone Laboratory and Ohio Sea Grant, The Ohio State University, 878 Bayview Ave. P.O. Box 119, Put-In-Bay, OH 43456, USA*
Corresponding author: Arthur Zastepa, arthur.zastepa@ec.gc.ca (ORCID: 0000-0003-1292-932X)

Cyanobacterial bioactive metabolites (cyanotoxins/cyanopeptides) inhibit enzymes such as proteases, carboxypeptidases, and phosphatases that disrupt metabolic processes relevant to human and ecosystem health. From surface water samples collected in 2017 (n = 44), 2018 (n = 100), and 2019 (n = 171), we report concentrations of more than 20 biologically active cyanobacterial compounds in the western basin of Lake Erie, one of the five Laurentian Great Lakes with multinational jurisdiction. Toxic microcystins LA and LR as well as anabaenopeptin B and F were most frequently detected at >90%. Microcystins RR and YR and anabaenopeptin A were detected at relatively high frequency at >80% in 2019. There was strong correlation among arginine-containing microcystin variants (RR, YR, HtyR, LR, HilR, WR, D-Asp3-LR, Spearman's Rho > 0.80) but not with arginine-lacking LY, LW, LF. Anabaenopeptin F, with the arginine sidechain, also correlated with these same arginine-containing microcystin variants (Spearman's Rho > 0.70). In 2019, only 4% of lake water samples exceeded the recreational water guideline for total microcystins (10 μg l^{-1}) set by Health Canada with the maximum concentration ~26 μg l^{-1}. The drinking water guideline (1.5 μg l^{-1}) was exceeded in 34% of lake water samples but treated water was not tested. Although maximum total anabaenopeptins concentration was almost four times higher (almost 100 μg l^{-1}) than microcystins, no anabaenopeptin guideline values have been derived as their impact is currently considered to be ecological rather than toxic to humans/animals. These results can be used to evaluate risk from cyanobacterial bioactive metabolites, including toxins, in Lake Erie and aid binational lake management and policy development in the Great Lakes basin.

Keywords: cyanotoxins, cyanopeptides, bioactive metabolites, Lake Erie, cyanobacterial and harmful algal blooms, lake management

Aquatic Ecosystem Health & Management, 27(1): 46–63, 2024.
ISSN: 1463-4988 print / 1539-4077 online. DOI: 10.14321/aehm.027.01.46

Introduction

Cyanobacterial harmful algal blooms (cHABs) are a recurring problem in the western basin of Lake Erie, a region known for its high levels of nutrient pollution primarily from agricultural runoff (Martin et al., 2021). These excess nutrients, combined with high summertime water temperatures within this shallow basin provide an ideal environment for cyanobacteria to proliferate and manifest into cHABs. The Great Lakes Water Quality Agreement, signed in 1972, created a framework for cooperation between the United States and Canada and led to measurable reductions in cHABs during the next decade (Watson et al., 2016). However, the apparent resurgence of cHABs in the mid-1990s has re-emphasized the importance of this binational cooperation (McKindles et al., 2020). The complex nature and massive scale of these blooms requires a coordinated effort from scientists, government agencies, and other stakeholders to develop and implement effective research, monitoring, and management strategies. Cyanobacteria produce bioactive metabolites (including cyanotoxins and other cyanopeptides) that can stress metabolic processes relevant to human and ecosystem health by inhibiting proteases, carboxypeptidases, or phosphatases (Buratti et al., 2017). However, these metabolites cannot be detected using strategies and technologies commonly applied to deal with the spatial grand scale of cHABs (remote sensing, *in situ* probes, sensor networks), especially in the Great Lakes. Furthermore, correlations with cyanobacterial biomass are inconsistent and therefore, metabolite concentrations cannot be inferred reliably using cyanobacterial biomass (e.g. Chaffin et al., 2021). Even emerging technologies that can identify down to genus/species *in situ* are limited by the fact that microcystin production, as well as other metabolites, can vary at the strain level. This means sampling is required to quantify cyanotoxins and other cyanopeptides in cHABs and, due to their heterogeneous and dynamic nature, at high spatial resolution and extended coverage – making multi-institute and multinational cooperation critical to cover bodies of waters that span boundaries.

The most studied group of cyanobacterial metabolites are the hepatotoxic microcystins (Buratti et al., 2017; Harke et al., 2016). Quantitative data on microcystin variants is increasing largely due to significant advances in sensitivity and accuracy of mass spectrometry (Birbeck et al., 2019a, Jones and Janssen, 2022). The high-resolution mass spectrometry has allowed a broad suite of tentative metabolite identification including over 300 microcystin variants. However, just over ten calibration standards are available, limiting the advancement of microcystin quantitative profiling (Birbeck et al., 2019b, Xu et al., 2019). Furthermore, quantitative data on cyanopeptides beyond microcystins are limited due to their availability of calibration standards which has limited the types of institutions and funding agencies willing to support ecologically focused research and monitoring. Consequently, incomplete research is being performed on the potential negative human and animal health effects of cyanopeptides beyond microcystins. Nevertheless, recent cHAB studies have observed toxicity to grazers and allelopathic effects, both of which have implications for phytoplankton community composition and are suspected as contributing to cyanobacterial dominance. (Gademann et al., 2010, Faltermann et al., 2014, Lenz et al., 2019, Pawlik-Skowronska and Bownik, 2021). Therefore, quantitative information on concentrations of these other cyanopeptides does, albeit at least indirectly, have implications for human and animal health as many cyanobacteria co-produce cyanopeptides and other cyanotoxins with microcystins.

In this study, we quantified the chemical composition and concentration of a broad suite of cyanopeptides in Lake Erie's western basin at an unprecedented high-resolution spatial scale, including the toxic microcystin variants (12) and nodularin (1) as well as the bioactive cyanopeptides anabaenopeptins (3 + oscillamide Y), aeruginosamides (2), cyanopeptolins (2), micropeptins (1), and microginins (2). This investigation leverages the analytical methods and synthesis of new calibration standards achieved during our previous collaboration (Zastepa et al., 2023a) and the HABs Grab events, the unprecedented binational field campaigns to sample and characterize Lake Erie's blooms (Chaffin et al., 2021). Furthermore, we investigated the co-occurrence of the different cyanopeptides and tested the correlations with water chemistry and phytoplankton community composition, the latter

as indicated by accessory pigments. The findings are presented in a manner to highlight the human and environmental health risk from cyanotoxins/cyanopeptides beyond microcystins in Lake Erie.

Methods

Sample collection and processing during 2017 CCGS LIMNOS R/V *expedition*

Sample collection for 2017 was performed on board the *CCGS LIMNOS R/V* in two cruises: August 28 to September 1 and October 2 to 6 for a total of 44 samples collected. Surface water was collected at 1 m depth using a Rosette sampler or Niskin bottles at each sampling station and transferred into 20 L carboys that had been previously rinsed with station water prior to collection. The collected water was processed and preserved for nutrient analysis following standard operating procedures and quality assurance protocols and kept at 4°C before being submitted for analyses to the National Laboratory for Environmental Testing (Environment Canada,1997).

Analyses included particulate forms (1.2 µm pore size, 47 mm diameter GF/C filter) of organic nitrogen (PON), total phosphorus (TP), and particulate organic carbon (POC) as well as chlorophyll-*a* (chl-*a*). Analyses also included dissolved forms (filtrate through 0.45 µm pore size, 47 mm diameter cellulose acetate filters) of nitrate + nitrite (NO_X), ammonia + ammonium (NHX), total Kjeldahl nitrogen (TKN), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), and dissolved inorganic and organic carbon (DIC, DOC).

Water samples for photosynthetic pigments analyses (phycocyanin and phycoerythrin) were collected by filtering known volumes of lake water onto GF/C Whatman™ filters (1.2 µm pore size, 47 mm diameter, Fisher Scientific, Canada) and stored in -80°C before analysis according to Zastepa et al. (2023b). Red light was used in the laboratory to avoid pigment degradation that occurs under exposure to ambient light.

Water samples for cyanotoxins and cyanopeptides extraction were collected by filtering known volumes of lake water onto GF/C Whatman™ filters and stored in -80°C until extraction.

Sample collection and processing during 2018 and 2019 HABs Grab expedition.

Complete sample collection methods for 2018 and 2019 were presented in Chaffin et al. (2021, and references within). Briefly, we collected 100 samples in the US waters of the western basin of Lake Erie on 9 August 2018 and 172 across the entire western basin on 7 August 2019. Note that it was not possible to generate data for one of the 2019 samples, so the number of samples reported in our analyses was 171. In both years, water samples were collected with a two-metre-long tube sampler to collect an integrated water sample from the surface to two metres depth. Water was stored on ice in a 2.4-L polyethylene terephthalate glycol (PETG) bottle while in transportation back to the laboratory. Upon arrival, 25 mL of unfiltered aliquots were poured into 60 mL amber glass vials for analysis of cyanotoxins/peptides and stored frozen at -20 °C until analysis.

Extraction and analyses of cyanotoxins and other cyanopeptides from 2017 CCGS LIMNOS R/V *expedition*

Cyanotoxins and cyanopeptides extraction was carried out in 10 mL of 1:1 v/v methanol: water (Fisher Chemical, Optima™, LC/MS grade) amended with 0.1% formic acid (99%, Fisher Chemical, Optima™, LC/MS grade) using the probe sonication method. Briefly, the filter was homogenized using the probe sonicator (30 s and 30 s on and off cycle, repeated three times) followed by centrifugation at 3000 rpm, 4 °C for 15 minutes. The supernatant was filtered through a PTFE syringe filter (1.0 µm pore size, 30 mm diameter) and evaporated to dryness under nitrogen gas-flow and heat at 30 °C. The sample was then reconstituted in 1 mL of 1:1 v/v methanol: water (Fisher Chemical, Optima™, LC/MS grade), and filtered through a PTFE syringe filter (0.45 µm pore size, 15 mm diameter). The final extract was collected in 2.0 mL HPLC amber glass vial and stored at −80 °C in the dark until analyses. Extracts were analyzed using LC-MS/MS with electrospray ionization operated in positive mode and using a scheduled multiple reaction monitoring (MRM) method. All toxins and peptides were analyzed by liquid chromatography tandem (MS/MS) mass

spectrometry on an ABSciex 4000 QTrap Mass Spectrometer (Framingham, MA, USA) equipped with a TurboV™ electrospray ion source and a Shimadzu HPLC Model 20A (Kyoto, Japan). Details of the LC-MS/MS method including analyte retention times, transition ions monitored, mass spectrometer settings, example chromatograms, product-ion spectra and extraction recoveries of the target analytes are published elsewhere (Beversdorf et al., 2017). The cyanotoxins and cyanopeptides standards were purchased from the National Research Council of Canada Biotoxins program (Halifax, NS, Canada), Sigma-Aldrich (Milwaukee, WI, USA), Abraxis (Warminster, PA, USA), MARBIONIC (Wilmington, NC, USA), and Tocris Bioscience (Minneapolis, MN, USA).

Extraction and analyses of cyanotoxins and other cyanopeptides from 2018 and 2019 HABs Grab expeditions.

The 25 mL of sample that was aliquoted in a 60 mL amber glass bottle and frozen at -20 °C was subjected to three freeze/thaw cycles to lyse the samples. The sample was filtered through a 0.45 um glass fiber filter to remove cellular and other debris. Sample was delivered to Wayne State University and was analyzed by liquid chromatography with tandem mass spectroscopy (LC-MS/MS) (Chaffin et al., 2021).

There was a difference in sample collection and handling between 2017 and the 2018, 2019 samplings. Cyanotoxins and cyanopeptides in the 2018, 2019 sampling were measured in whole water (which included the particulate and extracellular phases), but in 2017 they were only measured in the particulate phase (collected on filters). The 2017 methods excluded the extracellular fraction of the total analyte pool; however, this difference is likely to be minor. Extracellular microcystins were measured in the original HABs Grab study and were found to be less than 10% of total microcystins when total microcystin by ELISA was greater than 1 μg l^{-1} (Chaffin et al., 2021). Low percentage of extracellular microcystins have also been reported in previous years in other studies, including 2017 (Palagama et al., 2020).

A Thermo Scientific TSQ Altis™ triple quadrupole mass spectrometer (Waltham, MA, USA) with a TriPlus™ RSH EQuan 850 system was used to analyze samples for microcystin, nodularin, and non-microcystin cyanopeptides. A one mL sample was concentrated onto a loading column, (Thermo Scientific Hypersil GOLD aQ 2.1 x 20 nm, 12 μm). After rinsing the sample with mobile for 1 minute, the sample was eluted onto the analytical column (Hypersil GOLD™ C18 column 2.1 x 50 mm, 1.9 μm). The chromatographic gradient, mass spectrometer settings, retention times, precursors, and multiple reaction monitoring (MRM) fragments have been documented (Zastepa et al, 2023a). The method reporting limit (MRL) for the microcystins and non-microcystin cyanopeptides are less than or equal to 10 ng l^{-1} (ppt). More validation information such as standard curve, dynamic range, detection limits, minimum reporting limits, % relative standard deviation, detection limits, and % recovery were reported in Zastepa et al., 2023a. Microcystin and non-microcystin cyanopeptide standards were purchased from Enzo Life Sciences (Farmingdale, NY, USA), GreenWater Laboratories (Palatka, FL, USA), Marbionic (University of North Carolina, Wilmington) and National Research Council Canada (Ottawa, Ontario, Canada).

Thirty-seven samples from the 2019 HABs Grab were selected for saxitoxins analysis by ELISA. The 39 samples chosen (a full 96 well plate) spanned the range of chlorophyll-*a* concentrations measured. Aliquots (1 mL) from the same lysed and filtered sample used for LC-MS/MS were preserved with 0.1 mL of the Eurofins Abraxis preservative for saxitoxin (#53001L) and then saxitoxin was quantified by ELISA with Eurofins Abraxis saxitoxin kit (#52255B) following the Ohio EPA protocol (Ohio EPA 2016).

Mass estimates of cyanotoxins/peptides in western basin of Lake Erie

Chaffin et al. (2021) calculated total microcystin mass assuming that the 0–2-meter surface sample was representative of the unsampled water below 2 meters depth, and we used the same assumption for our calculation of microcystin and other cyanopeptides. The bloom spatial extent was 224.9 km^2 on 9 August 2018 and 996.5 km^2 on 7 August 2019 (Chaffin et al., 2021). To calculate the lake volume within the bloom extent, we multiplied bloom spatial extent converted to m^2 by the

average sample site depth (7.7 m in 2018, 8.62 in 2019). Total mass of the microcystins and other cyanopeptides were calculated by multiplying the average concentration by the volume of lake water within the bloom spatial extent.

Plotting, mapping, and statistics

Maps of the spatial distribution of microcystin toxin variants and other cyanobacterial bioactive metabolites (cyanopeptides) were created using the Inverse Distance Weighting (IDW) interpolation method in the Spatial Analyst extension for ESRI ArcGIS Desktop v 10.8.1. The use of IDW interpolation assumes that the value at an unknown location can be estimated by considering the values at surrounding known locations, with closer locations having a greater influence on the estimation. The IDW power parameter that determines the influence of nearby points on the interpolation, was set to the recommended value of two. A higher power gives more weight to closer points, while a lower value gives more weight to points farther away. All other IDW parameters were left at the default settings.

All data present in box plots and tables were processed and constructed with OriginPro® 2022 (OriginLab Corporation, Massachusetts, USA). For each box and whisker plot, boxes represent 25–75 % of data, horizontal line within each box is the respective median, green filled circles are respective means, unfilled red circles are outliers, and error bars represent 1.5 times the interquartile range. The box and whisker plot of total microcystin concentration from 2017 to 2019 is shown with Health Canada's guidelines for microcystin in recreational and drinking water, 10 µg l^{-1} and 1.5 µg l^{-1} respectively, as a reference (Health Canada 2018; Health Canada 2020). Note that we did not test treatment-finished drinking water.

The correlation heatmap was also created using OriginPro® 2022 and displays Spearman's correlation coefficient (Rho) between multiple variables. The Spearman's correlation is used to evaluate the monotonic relationship between two variables and no requirement for data normality. Spearman's correlation coefficient is calculated with Python using pandas library, where coefficient values range from -1 (perfect negative correlation) to +1 (perfect positive correlation). A value of 0 indicates the two variables were not correlated.

Results and discussion

Remote sensing is reasonably accurate in resolving the presence of cHAB biomass in the western basin of Lake Erie however, it is not currently possible to accomplish the same for cyanotoxins and other cyanopeptides (Binding et al., 2019; Binding et al., 2021; EOLakeWatch, 2023). High-resolution spatial scale sampling and analysis of cHABs in large lake systems has several benefits. It can improve the accuracy of cyanotoxin and cyanopeptide distribution as well as the strength of their concentration gradients across space and can reveal corresponding hotspots (areas of increased risk). Visualizing the temporal and spatial concentrations of cyanotoxins/cyanopeptides informs and enables scientists and policy makers on areas of increased risk to public and environmental health.

Cyanotoxins - Microcystins

Microcystins were highest along the southern shore of the western basin - at the outlet of the Maumee River/Maumee Bay area in 2019 (max. 26,529 ng l^{-1}), offshore Port Clinton (OH) in 2018 (max. 4,167 ng l^{-1}), and offshore Reno Beach (OH) (max. 934 ng l^{-1}) and north of Maumee Bay (max. 1222 ng l^{-1}) in 2017 (Fig. 1). Although Maumee River and Bay is known for annual cHABs, the offshores Port Clinton and Reno Beach have not been identified in the past as hot spots. The hotspots detected outside of Maumee Bay in 2018 (Port Clinton) was likely the result of stronger flows from the Maumee River that pushed the cHAB further east into the basin (Chaffin et al., 2021). In comparison to 2018, during the 2019 HABs Grab there was very little flow from the Maumee River and the biomass was mostly in Maumee Bay (Chaffin et al., 2021). Nevertheless, hotspots can indicate potential sources of bloom (nurseries) and guide subsequent investigations into bloom initiation and proliferation as well as targeted management and remediation efforts. This is particularly of interest if this bloom material overwinters in sediments and contributes to bloom initiation in the subsequent year. This recent data, as well as previous work, demonstrates that microcystin hotspots can have significant interannual variability and therefore, ongoing monitoring is important in the assessment of risk.

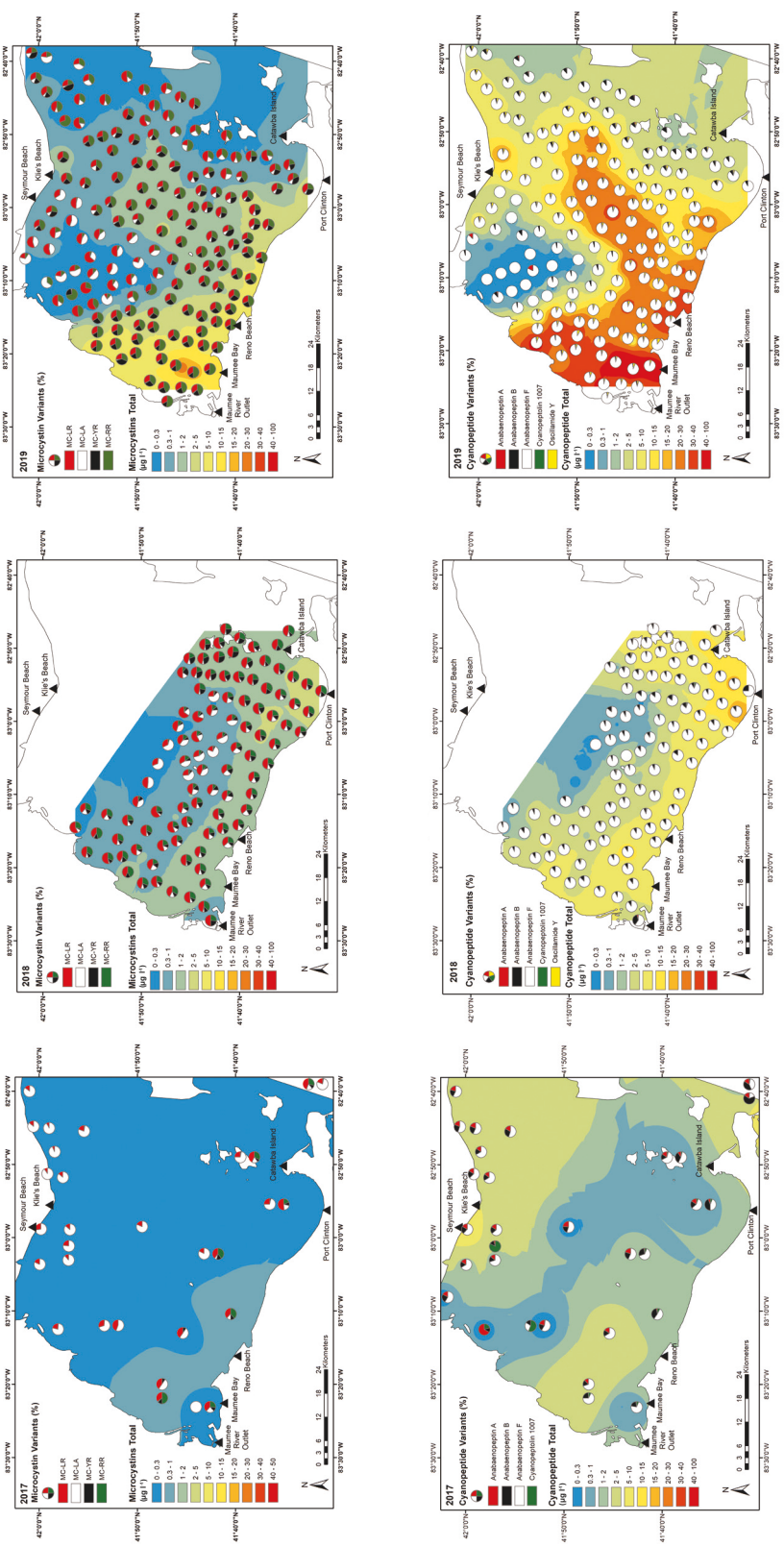

Figure 1. Spatial distribution of microcystin toxin variants and other cyanobacterial bioactive metabolites (non-microcystin cyanopeptides) in the western basin of Lake Erie in 2017 (August 28-31 and October 2-5), 2018 (August 9th), and 2019 (August 7th).

Table 1. Detection frequency (number and %) and descriptive statistics (median and maximum; ng l^{-1}) for cyanotoxins microcystins and other cyanobacterial bioactive metabolites (non-microcystin cyanopeptides) derived from sample collection in Lake Erie from 2017 to 2019. Total mass estimates (kg) was calculated for samples collected in 2019.

Compound	# of Detections n = 171 (2019)* n = 100 (2018)* n = 44 (2017)**	% Detected	Median (ng l^{-1})	Maximum (ng l^{-1})	Mass Load Estimate (kg)
	15	9	8	22	5
Aeruginosamide B	17	17	11	26	--
	NM	NM	NM	NM	--
	2	1	7	9	0
Aeruginosamide C	0	0	--	--	--
	NM	NM	NM	NM	--
	141	82	63	216	507
Anabaenopeptin A	69	70	22	107	--
	41	93	142	1,846	--
	155	91	284	1,428	2,888
Anabaenopeptin B	95	97	368	2,860	--
	42	95	353	6,780	--
	170	99	6,228	92,964	125,484
Anabaenopeptin F	96	98	3,649	19,971	--
	40	91	817	7,080	--
	15	9	59	116	35
Cyanopeptolin 1007	14	14	62	105	--
	22	50	11	115	--
	0	0	--	--	0
Cyanopeptolin 1040 MB	0	0	--	--	--
	NM	NM	NM	NM	--
	61	36	17	81	88
D-Asp3-LR	90	90	9	36	--
	NM	NM	NM	NM	--
	0	0	--	--	0
D-Asp3-RR	1	1	39	39	--
	NM	NM	NM	NM	--
	31	18	115	576	377
MC-HilR	63	63	12	38	--
	14	32	1	35	--
	54	32	130	1,152	762
MC-HtyR	22	22	14	79	--
	13	30	2	50	--

Compound	# of Detections n = 171 (2019)* n = 100 (2018)* n = 44 (2017)**	% Detected	Median (ng l^{-1})	Maximum (ng l^{-1})	Mass Load Estimate (kg)
MC-LA	170	100	57	331	642
	100	100	102	425	--
	42	95	31	139	--
MC-LF	0	0	--	--	0
	0	0	--	--	--
	0	0	--	--	--
MC-LR	168	99	262	8,504	8,060
	100	100	441	1,825	--
	41	93	9	386	--
MC-LW	5	3	6	9	0
	0	0	--	--	--
	2	5	2	3	--
MC-LY	10	6	7	18	0
	1	1	17	17	--
	14	32	0	1	--
MC-RR	169	99	252	12,886	10,877
	98	98	242	1,543	--
	15	34	18	548	--
MC-WR	26	15	99	354	253
	2	2	17	23	--
	7	16	4	30	--
MC-YR	144	85	240	3,050	4,410
	98	98	150	572	--
	23	52	5	145	--
MCs Total	170	100	718	26,529	25,470
	100	100	978	4,167	--
	23	52	5	145	--
Microginin 690 methyl ester	0	0	--	--	0
	0	0	--	--	--
	0	0	--	--	--
Micropeptin 1106	1	1	10	10	1
	0	0	--	--	--
	NM	NM	NM	NM	--
Nodularin	0	0	--	--	0
	0	0	--	--	--
	0	0	--	--	--

Compound	# of Detections n = 171 (2019)* n = 100 (2018)* n = 44 (2017)**	% Detected	Median (ng l^{-1})	Maximum (ng l^{-1})	Mass Load Estimate (kg)
Oscillaginin A	0	0	--	--	0
	0	0	--	--	--
	NM	NM	NM	NM	--
Oscillamide Y	123	72	891	4,143	7,062
	47	48	70	193	--
	NM	NM	NM	NM	--

*In 2019 microcystins were analyzed in 170 samples while the other cyanopeptides were analyzed in 171 samples. In 2018 microcystins were analyzed in 100 samples while the other non-microcystin cyanopeptides were analyzed in 98 samples.
**For 2017, microcystins were analyzed in 44 samples except the D-Asp3 MC-LR and D-Asp3 MC-LR variants and six non-microcystin cyanopeptides (Oscillamide Y, Oscillaginin A, Aeruginosamide B, Aeruginosamide C, Micropeptin 1106, and Cyanopeptolin 1040 MB) were excluded from the analysis due to a lack of calibration standards for mass spectroscopy quantitation.

Primarily, three microcystin variants (MC-RR, -LR, and –YR) were frequently found and at elevated concentrations (e.g. 2019 median almost 300 ng l^{-1} for each and maximum almost 13,000 ng l^{-1} of RR) (Fig. 2, Table 1). In fact, several of the arginine-containing microcystin variants were co-occurring (Fig. 3, Spearman's Rho > 0.70). MC-LA was also frequently present, e.g. in all samples in 2019, but concentrations were generally lower (median ~ 60 ng l^{-1}; maximum ~ 300 ng l^{-1}) (Table 1). When total microcystins exceeded 1,000 ng l^{-1}, MC-RR and -LR were the variants in highest concentration. On the other hand, when total microcystins were less than 1,000 ng l^{-1}, the dominant variant was MC-LA and -LR (Fig. 2, Table 1). Specifically, MC-LA was the dominant variant towards the centre of the basin in 2018 and in the north-west region of the basin (near Detroit River outflow) in 2019 - in each case consistent with a decreasing gradient of total microcystin concentration (Fig. 1). MC-LA is more than twice as toxic as MC-LR (Chernoff et al., 2021), and therefore, low total microcystin concentrations could underreport toxicity. Several other arginine-containing variants were detected - MC-D-Asp3-LR and MC-HtyR were detected about a third of the time while MC-HilR and MC-WR were detected about a sixth of the time (Table 1). Farther offshore and along the northern shores of the western basin, total microcystin concentrations were less than 1,000 ng l^{-1}.

While the HABs Grabs showed microcystins in high spatial resolution, the HABs Grab dataset does not include temporal patterns. Recently, Chaffin et al. (2023) documented changes in the microcystin congener profile during bloom seasons 2018 and 2019. They showed that MC-LR and MC-RR made up the majority of all microcystins in July and early August and then MC-LR and MC-LA dominated during late August and September. The shift from MC-RR to MC-LA co-dominance with MC-LR was attributed to depletion of bioavailable nitrogen throughout the bloom season. MC-RR is 17.5% nitrogen by mass, whereas MC-LA is 10.8% nitrogen by mass.

Cyanotoxins - Anatoxins, Cylindrospermopsins, and Saxitoxins

In 2019, 39 samples were tested for saxitoxins, and all 39 samples analyzed for saxitoxin were below the detectable limit (15 ng l^{-1}). However, one of the biosynthetase genes for saxitoxin, *sxtA*, has been found sporadically and ephemerally throughout the Maumee River (Laiveling et al., 2022), in the western basin (Nauman et al., 2024), and the central basin of Lake Erie (Chaffin et al., 2019). Additionally, saxitoxin has been detected in many inland lakes in northern Ohio with the ELISA method (Chaffin unpublished data). While we did not detect saxitoxin during the HABs Grab, we do recommend continued and routine monitoring of the *sxtA* gene and saxitoxins, with confirmation by LC-MS/MS of the latter. The monitoring of saxitoxins is especially relevant in the presence of cyanobacteria with the potential to produce saxitoxins (as indicated by either taxonomic identification and enumeration and/or PCR of the *sxtA* gene). Unfortunately, analyses of other

cyanotoxins (e.g. anatoxins, cylindrospermopsins) was not done due to a lack of sufficient sample volume and improper handling methods (i.e. anatoxins quickly degrade).

Anabaenopeptins and beyond

Like microcystins, anabaenopeptins were also prevalent along the southern shore (close to 100,000 ng l^{-1} in Maumee Bay in 2019). The spatial pattern of anabaenopeptins in the southern half of the basin followed that of microcystins (Fig. 1) and chl-*a* concentration (Fig. SM1, available on-line at the publisher's website). However, the pattern was not the same along the Canadian (north) shore - there were higher non-microcystin cyanopeptide concentrations (and mostly anabaenopeptin F) (2,000 to 5,000 ng l^{-1}) compared to the south and also the center of the basin during 2017 (< 1,000 ng l^{-1}; Fig. 1). Again, during the 2019 HABs Grab anabaenopeptins were higher in samples closest to the Canadian shore (2,000 to 15,000 ng l^{-1}) than the adjacent samples collected further from shore (1,000 to 2,000 ng l^{-1}; Fig. 1). In 2019, the elevated anabaenopeptins appeared to be due to the northern extension of the cHAB initiated at the south end, near the Maumee River outlet while in 2017 the non-microcystin cyanopeptides were isolated at the north, nearshore, and appeared only during the expedition in autumn. The higher anabaenopeptins along the Canadian shore in 2017 may be from a different producer and reiterates the heterogeneity of cHABs in large systems. Anabaenopeptin-F was almost always detected (2019; 99%, n = 171)

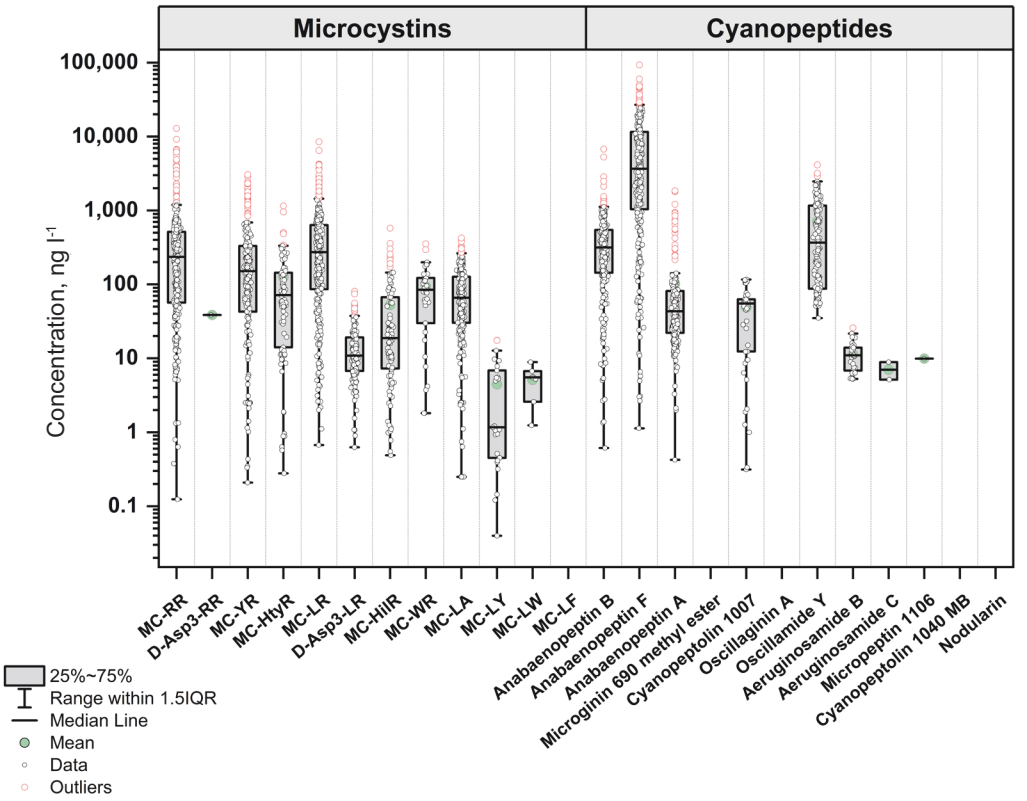

Figure 2. Box and whisker plots of concentrations (ng l^{-1}) of bioactive metabolites (cyanotoxins and non-microcystin cyanopeptides) found in phytoplankton samples from Lake Erie (Canada and USA) – data from 2017 (top panel: August 28-31 and October 2-5), 2018 (middle panel: August 9th), and 2019 (bottom panel: August 7th) as detailed in Materials and Methods. Boxes represent 25–75 % of data, horizontal line within each box is the respective median, green filled circles are respective means, unfilled red circles are outliers, and error bars represent 1.5 times the interquartile range. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

and at relatively high concentrations (median ~6,000 ng l^{-1}, maximum ~93,000 ng l^{-1}) with anabaenopeptin-B and –A also very common (>80%) albeit at lower concentrations (maximum < 1,500 ng l^{-1}) (Table 1). Oscillamide Y was detected less often (72%) but when present, it was generally at relatively high concentrations (median ~900 ng l^{-1}, maximum ~4,100 ng l^{-1}). Cyanopeptolin 1007 and aeruginosamide B were detected about a tenth of the time but at very low concentrations.

Data on non-microcystins cyanopeptides is scarce relative to microcystins, and some studies did not include the most abundant congener anabaenopeptin-F (Roy-Lachapelle et al., 2019; Skafi et al., 2021). Beversdorf et al. (2017, 2018) presented anabaenopeptins data for Lake Winnebago and several smaller nearby lakes and showed most samples had less than 10,000 ng l^{-1} of total anabaenopeptins. One sample from Lake Koshkonong had 24,050 ng l^{-1} and 3,400 ng l^{-1} of anabaenopeptins B and F, respectively, during a bloom that reached 75 µg l^{-1} of chl-*a* (Beversdorf et al., 2017). In comparison, during the 2019 HABs Grab, the maximum non-microcystin cyanopeptides peaked at 96,509 ng l^{-1} (and anabaenopeptin F accounted for 92,964 ng l^{-1}), but also chl-*a* concentrations were more than double (196 µg l^{-1}) that of Lake Koshkonong. A study from Greece lakes also highlighted the importance of including anabaenopeptin-F in analysis (Zervou et al., 2022). Collectively, these studies highlight the importance of monitoring for the full suite of anabaenopeptins because not including all congeners might significantly underestimate the concentration of anabaenopeptins (Roy-Lachapelle et al., 2019; Skafi et al., 2021).

Co-occurrence of cyanotoxins and non-microcystin cyanopeptides

Total microcystin and total anabaenopeptin concentrations correlated statistically over the entire dataset (2017-2019) (Fig. 3, Spearman's Rho > 0.70). These correlations were reinforced

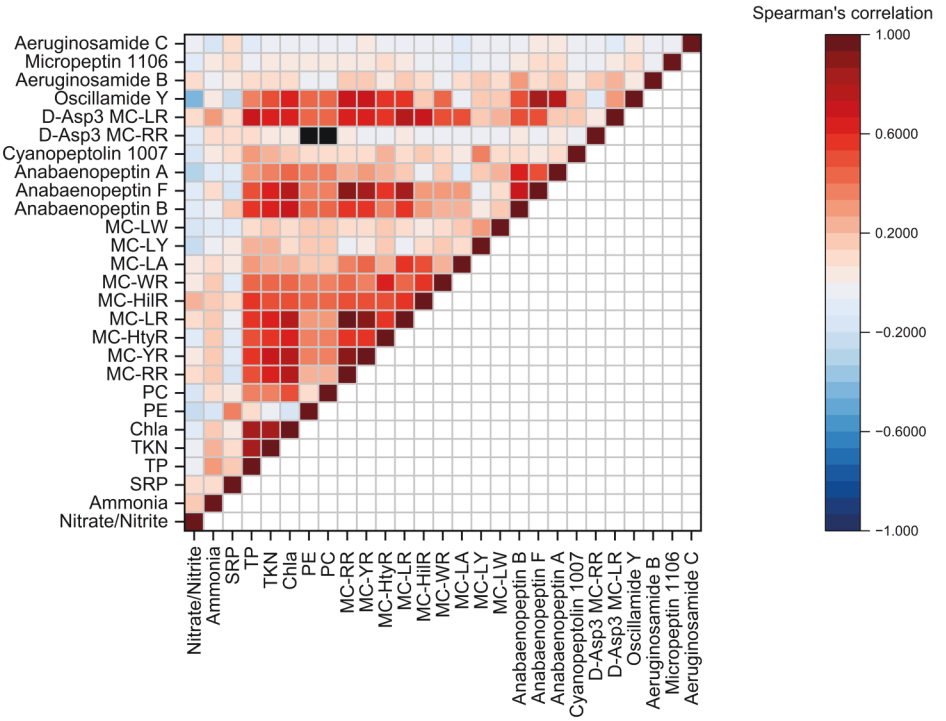

Figure 3. Spearman's correlation matrix with correlation coefficients between pairs of variables measured from 2017 to 2019. Black squares mean null values (no data available for correlation coefficient). MC, microcystin; PC, phycocyanin; PE, phycoerythrin; TKN, total kjeldahl nitrogen; TP, total phosphorus; SRP, soluble reactive phosphorus. For optimal interpretation with respect to colours in this figure legend, the reader is referred to the web version of this article.

when visualizing the data spatially (Fig. 1). For example, in 2018, peaks/hotspots in microcystins and anabaenopeptins were in the vicinity of, or even overlapped with, one another such as near Port Clinton. In 2019, the gradients of each of microcystins and anabaenopeptins were aligned and over greater spatial extent, with relatively higher concentrations in the southwest, extending east along the south shore and then decreasing north into the centre of the western basin. Furthermore, there was a separate, localized hotspot for both microcystins and anabaenopeptins on the north shore, east of Klie's Beach. However, there were clear spatial differences in 2017. In 2017 peaks in microcystins and anabaenopeptins appeared on opposite sides of the western basin - south and north, respectively (Fig. 1) – and during different times, with microcystins more prevalent in summer and anabaenopeptins in autumn (seasonal data not show separately). Although in 2018 and 2019 microcystins and anabaenopeptins co-occurred spatially, the 2017 patterns demonstrate that this is not always the case and highlight the need to always visualize data rather than simply relying on statistical results.

Environmental conditions

Collection and analyses of corresponding water quality data during high-resolution spatial scale cyanotoxin/cyanopeptide characterization and quantitation can indicate environmental conditions for their production. Variants within the microcystin (generally the arginine-containing) and anabaenopeptin chemical groups correlated strongly with chl-*a*, TP, and TKN, indicating an association with eutrophication and the autocorrelation among total nutrients and biomass metrics (i.e. cells contain a lot of the total P and N) (Fig. 3, Spearman's Rho > 0.70). However, it

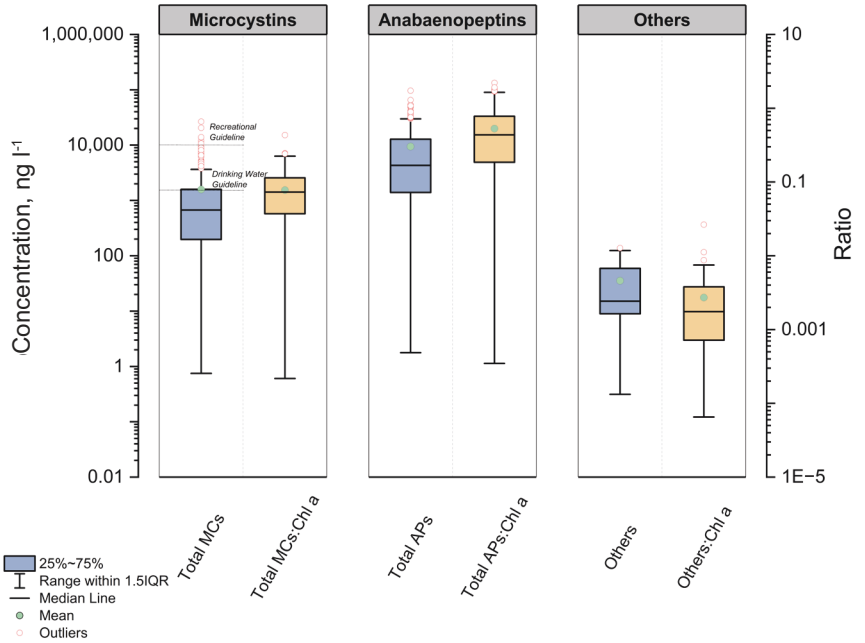

Figure 4. Box and whisker plots of concentrations (ng l^{-1}) of bioactive metabolites (cyanotoxins and non-microcystin cyanopeptides) (top) and its ratio to chlorophyll-*a* (Chl-*a*) concentration (ng l^{-1}) (bottom) found in phytoplankton samples from Lake Erie (Canada and USA) – data from 2017 (August 28-31 and October 2-5), 2018 (August 9th), and 2019 (August 7th) as detailed in Materials and Methods. For the 2017 samples, microcystin (MC) D-Asp3-RR and D-Asp3-LR were excluded from "Total MCs", Oscillamide Y was excluded from "Total APs" (anabaenopeptins), and Oscillaginin A, Aeruginosamide B, Aeruginosamide C, Micropeptin 1106 and Cyanopeptolin 1040 MB were excluded from "Others" metabolites due to the unavailability of calibration standards quantitation by mass spectroscopy. Boxes represent 25–75 % of data, horizontal line within each box is the respective median, green filled circles are respective means, unfilled red circles are outliers, and error bars represent 1.5 times the interquartile range. Regulatory guidelines for microcystins were obtained from Health Canada (Health Canada, 2020). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

is important to note that associations do not equate to causation and that real-time measurements of activity (e.g. gene transcription) are more appropriate to elucidate drivers of cyanotoxin/cyanopeptide production. Although there is significant correlation between chl-*a* and both the cyanotoxins and other cyanopeptides, it is important to remember that there can be some aberrations depending on the phytoplankton community composition in time and space. For example, visualization of our 2018 spatial distribution data demonstrated some variation in the peaks of these parameters - chl-*a* peaked at the Maumee River outlet while the peak in total microcystins and total non-microcystin cyanopeptides were north/northwest of Port Clinton (also northwest of Reno Beach for the latter) (Fig. SM1 available on-line at the publisher's website; Fig. 1). Similarly, in 2017, an increase in chl-*a* levels was observed near Seymour Beach however, there was no corresponding rise in the total concentration of microcystins. Instead, an increase in anabaenopeptins was observed, suggesting two different producers of each class of compounds that year.

Normalizing concentrations

By considering cyanotoxins/cyanopeptides normalized to chl-*a*, we can better evaluate the relative and potential toxicity of cHABs and make informed decisions regarding management actions for drinking and recreational water and public and ecological health. In 2017, despite lower absolute concentrations of chl-*a* and microcystins near the southeastern shores (near Catawba Island) (Fig. SM1 available on-line at the publisher's website; Fig. 1), relative microcystins (microcystins to chl-*a* ratio) were similar to the westward peak observed off Reno Beach (Fig. SM3 available on-line at the publisher's website), indicating a secondary area of high toxicity that was not visible by simply looking at absolute concentrations. There was a similar west versus east discrepancy along the southern shore in 2019 - relative microcystin concentrations were similar with at least two hotspots north of Port Clinton (Fig. SM3), which were not evident by simply looking at absolute concentrations (Fig. 1). Reflecting on these two observations we conclude that there is an area of higher potential toxicity on a relative scale (microcystins to chl-*a* ratio) on the southeast end of the western basin despite lower absolute microcystin and chl-*a* concentrations. We observed a very similar pattern in 2017 for anabaenopeptins along the northern shore, namely peaks in anabaenopeptins relative to chl-*a* in the north-east part of the basin as well as north of Catawba Island that were not visible by looking at absolute concentrations (Fig. 1, Fig. SM4 available on-line at the publisher's website). These measures are useful as an early warning indicator of higher risk areas possible under changing environmental conditions. This provides a measure of the ecological dynamics and potential risks associated with cyanotoxin/cyanopeptide production within a given phytoplankton community, highlighting the potential for cyanotoxin/cyanopeptide production even in areas with lower overall chl-*a* concentrations. By normalizing cyanotoxin/cyanopeptide concentrations to chl-*a*, we can account for variations in phytoplankton community composition and gain insights into the relative production or persistence of harmful compounds. An important nuance, however, is that the biomass of non-cyanobacteria phytoplankton will affect the interpretation of the cyanotoxin, or cyanopeptide, to chl-*a* ratio. Ultimately, knowing the relative production of cyanotoxins and cyanopeptides in relation to chl-*a*, or more specifically cyanobacterial biomass, provides a more comprehensive understanding of cHAB dynamics and drivers and aids in the development of effective strategies for cHAB monitoring, prevention, and mitigation.

Insight for public health

In 2019, only 4% of lake water samples exceeded the recreational guideline for total MCs of 10,000 ng l^{-1} (Health Canada 2020) (Fig. 4, Table SM1 available on-line at the publisher's website). Drinking water guideline of 1,500 ng l^{-1} (Health Canada 2018) was exceeded in 34% of lake water samples and the maximum total microcystin concentration was ~26,000 ng l^{-1} albeit treated water was not tested. This suggests that a significant portion of the lake water samples may pose a risk to human consumption if treatment fails to remove cyanotoxins from the water. The lack of testing of treated water in this study makes it challenging to assess the actual risk to human health from consuming water that has undergone treatment

processes. Breakthroughs of microcystins have occurred in surrounding municipalities (Steffen et al., 2017) therefore, it is crucial to conduct comprehensive testing on treated water to ensure the effectiveness of the treatment methods in removing or reducing the concentrations of cyanotoxins.

While microcystins are commonly tested for and encountered in Lake Erie, other cyanotoxins and cyanopeptides are not tested by resource managers. For example, the Ohio Environmental Protection Agency requires public drinking water systems monitor for microcystins in raw water weekly (Ohio EPA, 2022), however, no other cyanotoxins/cyanopeptides are monitored. This is potentially concerning because we found that the maximum total anabaenopeptin concentration was about four times higher (almost 100,000 ng l^{-1}) than total microcystins (~26,000 ng l^{-1}). Toxicological studies of anabaenopeptins are scarce but some evidence exists of potential toxicity. In one study that investigated the survival of the model organism the nematode *Caenorhabditis elegans*, anabaenopeptin-A, -B, and -F were two to three times more toxic than MC-RR (Lenz et al., 2019). In another study with *Daphnia magna*, anabaenopeptin-B had similar toxicological effects on *Daphnia* swimming behavior as MC-LR, but there was a synergistic effect when both oligopeptides were administered together (Pawlik-Skowronska and Bownik, 2021). A recent meta-analysis showed that the lower range of anabaenopeptin IC_{50} (half maximal inhibitory concentration) for human serine protease (~2 µg l^{-1}) overlaps with microcystins IC_{50} for phosphatases (Fig. 4 in Janssen 2019). Although no guideline values are currently available specifically for anabaenopeptins, their significantly higher concentration and their potentially similar toxicity compared to total microcystins (Lenz et al., 2019, Janssen 2019, Pawlik-Skowronska and Bownik 2021) suggests a potentially greater ecological impact. While the impact on human health may not be well-defined, it highlights the need for further research and understanding of the potential risks associated with these specific cyanopeptides. It is crucial to recognize that the risk associated with cHABs extends beyond the production of microcystins - cHABs produce a diverse array of bioactive compounds, of which microcystins represent only a small subset.

Reflection on HABs Grab approach, the high-resolution spatial scale characterization of cHABs and cyanotoxins/cyanopeptides.

The systematic sampling during the HABs Grab ensured representative coverage of different areas regardless of bloom history and provided a comprehensive assessment, including spatial variability, of the overall distribution and magnitude of cHABs and associated cyanotoxins/cyanopeptides. A subsequent targeted sampling (bloom chasing) of specific areas where hotspots are identified is useful for capturing the highest cyanotoxin/cyanopeptide concentrations and studying specific bloom dynamics in detail. A combination of both approaches would provide a holistic understanding of cHABs and their associated compounds.

The standardization of methods for sample collection, processing, and analysis was critical for ensuring consistency and comparability of results. across regions and facilitating robust correlations. However, data visualization still revealed important nuances despite these relationships (see above). Standardization also supports the development of comprehensive databases and meta-analyses, which contribute to a broader understanding of cHAB dynamics. By adhering to standardized methods, it becomes easier to identify trends, patterns, and commonalities across different systems and enables the establishment of more reliable cause-effect relationships.

The availability of high-resolution spatial data on water quality combined with comprehensive analysis of cHABs and cyanotoxins/cyanopeptides contributes to the development of more accurate and reliable models for predicting bloom occurrence, duration, and intensity. Furthermore, the inclusion of cyanotoxin/cyanopeptide measurements will contribute significantly to a cyanotoxin/cyanopeptide forecast model (Zhou et al, 2023). These models aid in early warning systems that can enable proactive management actions and reduce the spread of cHABs and the potential impacts on water resources and human health.

The high level of effort required to generate such high-resolution spatial characterization of cHABs and cyanotoxins/cyanopeptides limits the

approach to short-term, grab sampling initiatives as described in our study and in more detail by Chaffin et al. (2021). A HABs Grab approach would be challenging to implement in routine, long-term monitoring programs. However, the success of the HABs Grab demonstrated the level of detail possible and the application of such data. The HABs Grab can be used as a proof of concept of what is possible, and with advancements in sampling techniques (e.g. automation) and sensor technologies (e.g. remote sensing) it can be applied to routine, long-term monitoring. However, to enable near real-time monitoring and quicker decision-making rapid, on-site detection tools for cyanotoxins/cyanopeptides need to be developed and implemented (e.g. Watson et al., 2017). Collaborative research efforts, interdisciplinary approaches, and increased funding support are necessary to drive these improvements and effectively address the existing challenges in cHAB monitoring and management.

Conclusions

The results of this study can be used to evaluate risk from bioactive cyanopeptides (including cyanotoxins) in Lake Erie and aid binational lake management and policy development in the Great Lakes basin. By analyzing the presence, distribution, and concentration of these metabolites, stakeholders and water managers can assess the potential harm posed to aquatic ecosystems and human health. Such information is crucial for informing binational lake management and policy development under the Great Lakes Water Quality Agreement (2012). Understanding the extent and severity of cyanobacterial blooms and their associated bioactive metabolites at a high-resolution spatial scale allows for targeted and effective mitigation strategies to be implemented. These results provide valuable insights into the specific risks and challenges posed by cyanobacterial toxins and other bioactive metabolites in Lake Erie, enabling policymakers to make informed decisions and develop robust management approaches to protect the lake's ecological integrity and safeguard public well-being.

Declaration of competing interest

The authors affirm that they do not have any known financial interests or personal relationships that could have potentially influenced the findings presented in this paper.

Acknowledgements and funding

This research received funding from multiple sources: The National Oceanic and Atmospheric Administration's (NOAA) National Centers for Coastal Ocean Science provided funding under award NA17NOS4780186 to the Ohio State University; the Bowling Green State University Great Lakes Center for Fresh Waters and Human Health received support from the National Institutes of Health (NIH) (1P01ES028939-01) and the National Science Foundation (OCE-1840715). Wayne State University's Center for Urban Responses to Environmental Stressors also received NIH funding (P30 ES020957). Canadian faculty received support from the Natural Sciences and Engineering Research Council of Canada Discovery grants. The operation of the R.V. Cisco and Captain Robert D. Linley was funded by the Great Lakes Program of Fisheries and Oceans. Environment and Climate Change Canada contributed use of the CCGS LIMNOS and funding from the Great Lakes Protection Initiative for post-sampling analyses. We thank the Cooperative Institute for Great Lakes Research, the U.S. Environmental Protection Agency and the NOAA Great Lakes Environmental Research Laboratory for their assistance in sampling and vessel use. We are grateful to the laboratory technicians, students, and boat captains from all the organizations who helped with collection and sample processing during the HABs Grabs. Satellite data used in the study originates from the Copernicus Sentinel-3 program. This contribution is numbered #1094 under NOAA NCCOS awards.

Any opinions expressed do not necessarily reflect those of the agencies/departments, and it is not intended that any endorsement be assumed. Mentioning brand names or commercial goods does not imply support or endorsement. The manuscript has been significantly enhanced based on the valuable feedback and suggestions provided by the reviewers. We recognize the importance of

their insights in ensuring the publication of high-quality scientific research. We sincerely appreciate the time and effort they devoted to reviewing the manuscript, as their contributions have been instrumental in its refinement.

Author Contributions

Conceptualization, J.D.C, J.A.W, and A.Z.; methodology, J.D.C., J.A.W, A.Z., T.R.M., A.L., and D.C.S.; validation, A.Z. and A.L; formal analysis, J.D.C., J.A.W, A.Z., T.R.M., and A.L.; investigation, J.D.C., J.A.W, and A.Z.; resources, J.D.C., J.A.W, A.Z., T.R.M.; data curation, J.D.C., J.A.W, A.Z., T.R.M., and A.L.; writing—original draft preparation, A.Z.; writing—review and editing, J.D.C., J.A.W, A.Z., T.R.M., A.L., and D.C.S.; visualization, A.Z., A.L., and D.C.S.; supervision, A.Z.; project administration, J.D.C, J.A.W, and A.Z.; funding acquisition, J.D.C, J.A.W, and A.Z. All authors have read and agreed to the published version of the manuscript.

Supplementary material

Supplementary material for this article is available on-line at the publisher's website.

References

Beversdorf, L.J., Weirich, C.A., Bartlett, S.L., Miller, T.R. 2017. Variable cyanobacterial toxin and metabolite profiles across six eutrophic lakes of differing physiochemical characteristics. Toxins, 9 (2), art. no. 62. DOI: 10.3390/toxins9020062

Lucas J. Beversdorf, L.J., Rude, K., Weirich, C.A., Bartlett, S.L., Seaman, M., Kozik, C., Biese, P., Gosz, T., Suha, M., Stempa, C., Shaw, C., Hedman, C.J., Piatt, J.J., Miller, T.R. 2018. Analysis of cyanobacterial metabolites in surface and raw drinking waters reveals more than microcystin. Water Research, 140 280-290.DOI: 10.1016/j.watres.2018.04.032

Binding, C.E., Pizzolato, L., Zeng, C. 2021. EOLakeWatch; delivering a comprehensive suite of remote sensing algal bloom indices for enhanced monitoring of Canadian eutrophic lakes. Ecological Indicators, 121, art. no. 106999. DOI: 10.1016/j.ecolind.2020.106999

Binding, C.E., Zastepa, A., Zeng, C. 2019. The impact of phytoplankton community composition on optical properties and satellite observations of the 2017 western Lake Erie algal bloom. Journal of Great Lakes Research, 45 (3), 573-586. DOI: 10.1016/j.jglr.2018.11.015

Birbeck, J.A., Westrick, J.A., O'neill, G.M., Spies, B., Szlag, D.C. 2019a. Comparative analysis of microcystin prevalence in michigan lakes by online concentration LC/MS/MS and ELISA. Toxins, 11 (1), art. no. 13. DOI: 10.3390/toxins11010013

Birbeck, J.A., Peraino, N.J., ONeill, G.M., Coady, J., Westrick, J.A. 2019b. Dhb microcystins discovered in usa using an online concentration LC/MS/MS platform. Toxins, 11 (11), art. no. 653. DOI: 10.3390/toxins11110653

Buratti, F.M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., Funari, E. 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Archives of Toxicology, 91 (3), 1049-1130. DOI: 10.1007/s00204-016-1913-6

Chaffin, J.D., Mishra, S., Kane, D.D., Bade, D.L., Stanislawczyk, K., Slodysko, K.N., Jones, K.W., Parker, E.M., Fox, E.L. 2019. Cyanobacterial blooms in the central basin of Lake Erie: Potentials for cyanotoxins and environmental drivers. Journal of Great Lakes Research, 45 (2), 277-289. DOI: 10.1016/j.jglr.2018.12.006

Chaffin, J.D., Bratton, J.F., Verhamme, E.M., Bair, H.B., Beecher, A.A., Binding, C.E., Birbeck, J.A., Bridgeman, T.B., Chang, X., Crossman, J., Currie, W.J.S., Davis, T.W., Dick, G.J., Drouillard, K.G., Errera, R.M., Frenken, T., MacIsaac, H.J., McClure, A., McKay, R.M., Reitz, L.A., Domingo, J.W.S., Stanislawczyk, K., Stumpf, R.P., Swan, Z.D., Snyder, B.K., Westrick, J.A., Xue, P., Yancey, C.E., Zastepa, A., Zhou, X. 2021. The Lake Erie HABs Grab: A binational collaboration to characterize the western basin cyanobacterial harmful algal blooms at an unprecedented high-resolution spatial scale. Harmful Algae, 108, art. no. 102080. DOI: 10.1016/j.hal.2021.102080

Chaffin, J.D., Westrick, J.A., Reitz, L.A., Bridgeman, T.B. 2023. Microcystin congeners in Lake Erie follow the seasonal pattern of nitrogen availability. Harmful Algae, 127, 102466 DOI: 10.1016/j.hal.2023.102466

Chernoff, N., Hill, D., Lang, J., Schmid, J., Farthing, A., Huang, H. 2021. Dose–Response Study of Microcystin Congeners MCLA, MCLR, MCLY, MCRR, and MCYR Administered Orally to Mice. Toxins, 13 (2), art. no. 86. DOI: 10.3390/TOXINS13020086

Environment Canada. 1997. Manual of analytical methods, major ions and nutrients. National Laboratory for Environmental Testing (NLET), Canadian Center for Inland Waters.

EOLakeWatch, 2023. https://www.canada.ca/en/environment-climate-change/services/water-overview/satellite-earth-observations-lake-monitoring.html (accessed March 2023).

Faltermann, S., Zucchi, S., Kohler, E., Blom, J.F., Pernthaler, J., Fent, K. 2014. Molecular effects of the cyanobacterial toxin cyanopeptolin (CP1020) occurring in algal blooms:

Global transcriptome analysis in zebrafish embryos. Aquatic Toxicology, 149, 33-39. DOI: 10.1016/j.aquatox.2014.01.018

Gademann, K., Portmann, C., Blom, J.F., Zeder, M., Jüttner, F. 2010. Multiple toxin production in the cyanobacterium Microcystis: Isolation of the toxic protease inhibitor cyanopeptolin 1020. Journal of Natural Products, 73 (5), 980-984. DOI: 10.1021/np900818c

Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A., Paerl, H.W. 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, Microcystis spp. Harmful Algae, 54, 4-20. DOI: 10.1016/j.hal.2015.12.007

Health Canada. 2018. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Cyanobacterial Toxins. Accessed April 17, 2022. https://www.canada.ca/content/dam/canada/health-canada/migration/healthy-canadians/publications/healthy-living-vie-saine/water-cyanobacteria-cyanobacterie-eau/alt/water-cyanobacteria-cyanobacterie-eau-eng.pdf

Health Canada. 2020. Guidelines for Canadian Recreational Water Quality Cyanobacteria and their Toxins. Accessed April 17, 2022. https://www.canada.ca/content/dam/hc-sc/documents/programs/consultation-cyanobacteria-toxins-recreational-water/consultation-cyanobacteria-toxins-recreational-water.pdf

Janssen, E.M.L., 2019. Cyanobacterial peptides beyond microcystins – A review on co-occurrence, toxicity, and challenges for risk assessment. Water Research, 151, 488-499. DOI: 10.1016/j.watres.2018.12.048

Jones, M.R., Janssen, E.M.L., 2022. Quantification of Multi-class Cyanopeptides in Swiss Lakes with Automated Extraction, Enrichment and Analysis by Online-SPE HPLC-HRMS/MS. Chimia, 76 (1-2), 133-144. DOI: 10.2533/chimia.2022.133

Laiveling, A., Nauman, C., Stanislawczyk, K., Bair, H.B., Kane, D.D., Chaffin, J.D., 2022. Potamoplankton of the Maumee River during 2018 and 2019: The relationship between cyanobacterial toxins and environmental factors. Journal of Great Lakes Research, 48 (6), 1587-1598. DOI: 10.1016/j.jglr.2022.08.015

Lenz, K.A., Miller, T.R., Ma, H., 2019. Anabaenopeptins and cyanopeptolins induce systemic toxicity effects in a model organism the nematode Caenorhabditis elegans. Chemosphere, 214, 60-69. DOI: 10.1016/j.chemosphere.2018.09.076

Martin, J.F., Kalcic, M.M., Aloysius, N., Apostel, A.M., Brooker, M.R., Evenson, G., Kast, J.B., Kujawa, H., Murumkar, A., Becker, R., Boles, C., Confesor, R., Dagnew, A., Guo, T., Long, C.M., Muenich, R.L., Scavia, D., Redder, T., Robertson, D.M., Wang, Y.-C., 2021. Evaluating management options to reduce Lake Erie algal blooms using an ensemble of watershed models. Journal of Environmental Management, 280, art. no. 111710. DOI: 10.1016/j.jenvman.2020.111710

McKindles, K., Frenken, T., McKay, R.M.L., Bullerjahn, G.S., 2020. Binational efforts addressing cyanobacterial harmful algal blooms in the great lakes. Handbook of Environmental Chemistry, 101, 109-133. DOI: 10.1007/698_2020_513

Nauman, C., K. Stanislawczyk, L. A. Reitz, and J. D. Chaffin. 2024. The spatiotemporal distribution of potential saxitoxin-producing cyanobacteria in western Lake Erie. Journal of Great Lakes Research 50: 102342. doi:10.1016/j.jglr.2024.102342

Ohio Environmental Protection Agency, 2022. Public water system harmful algal bloom response strategy. Division of Drinking and Ground Waters November 2022. Accessed June 1, 2023. https://epa.ohio.gov/divisions-and-offices/drinking-and-ground-waters/public-water-systems/harmful-algal-blooms

Ohio Environmental Protection Agency, 2016. Ohio EPA total (extracellular and intracellular) saxitoxin by ELISA analytical methodology Ohio EPA DES 702.0 Version 2.0.

Palagama, D.S.W., Baliu-Rodriguez, D., Snyder, B.K., Thornburg, J.A., Bridgeman, T.B., Isailovic, D. 2020. Identification and quantification of microcystins in western Lake Erie during 2016 and 2017 harmful algal blooms. Journal of Great Lakes Research, 46, 389-301. DOI: 10.1016/j.jglr.2020.01.002.

Pawlik-Skowrońska, B., Bownik, A., 2021. Cyanobacterial anabaenopeptin-B, microcystins and their mixture cause toxic effects on the behavior of the freshwater crustacean Daphnia magna (Cladocera). Toxicon, 198, 1-11. DOI: 10.1016/j.toxicon.2021.04.023

Roy-Lachapelle, A., Vo Duy, S., Munoz, G., Dinh, Q.T., Bahl, E., Simon, D.F., Sauvé, S., 2019. Analysis of multiclass cyanotoxins (microcystins, anabaenopeptins, cylindrospermopsin and anatoxins) in lake waters using on-line SPE liquid chromatography high-resolution Orbitrap mass spectrometry. Analytical Methods, 11 (41), 5289-5300. DOI: 10.1039/c9ay01132c

Skafi, M., Duy, S V., Munoz, G., Dinh, Q. T., Simon, D.F., Juneau, P., Sauve, S., 2021. Occurrence of microcystins, anabaenopeptins and other cyanotoxins in fish from a freshwater wildlife reserve impacted by harmful cyanobacterial blooms. Toxicon, 194, 44-52. DOI: 10.1016/j.toxicon.2021.02.004

Steffen, M.M., Davis, T.W., McKay, R.M.L., Bullerjahn, G.S., Krausfeldt, L.E., Stough, J.M.A., Neitzey, M.L., Gilbert, N.E., Boyer, G.L., Johengen, T.H., Gossiaux, D.C., Burtner, A.M., Palladino, D., Rowe, M.D., Dick, G.J., Meyer, K.A., Levy, S., Boone, B.E., Stumpf, R.P., Wynne, T.T., Zimba, P.V., Gutierrez, D., Wilhelm, S.W., 2017. Ecophysiological Examination of the Lake Erie Microcystis Bloom in 2014:

Linkages between Biology and the Water Supply Shutdown of Toledo, OH. Environmental Science and Technology, 51 (12), 6745-6755. DOI: 10.1021/acs.est.7b00856

Watson, S.B., Zastepa, A., Boyer, G.L., Matthews, E., 2017. Algal bloom response and risk management: On-site response tools. Toxicon, 129, 144-152. DOI: 10.1016/j.toxicon.2017.02.005

Watson, S.B., Miller, C., Arhonditsis, G., Boyer, G.L., Carmichael, W., Charlton, M.N., Confesor, R., Depew, D.C., Höök, T.O., Ludsin, S.A., Matisoff, G., McElmurry, S.P., Murray, M.W., Peter Richards, R., Rao, Y.R., Steffen, M.M., Wilhelm, S.W., 2016. The re-eutrophication of Lake Erie: Harmful algal blooms and hypoxia. Harmful Algae, 56, 44-66. DOI: 10.1016/j.hal.2016.04.010

Xu, X., Zhu, B., Liu, Z., Wang, F., Liang, J., 2019. Simultaneous determination of eight microcystins in fish by PRiME pass-through cleanup and online solid phase extraction coupled to ultra high performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1125, art. no. 121709. DOI: 10.1016/j.jchromb.2019.06.036

Sistema, A., Westrick, J.A., Liang, A., Birbeck, J.A., Furr, E., Watson, L.C., Stockdill, J.L., Ramakrishna, B.S., Crevecoeur, S., 2023a. Broad screening of toxic and bioactive metabolites in cyanobacterial and harmful algal blooms in Lake of the Woods (Canada and USA), 2016–2019. Journal of Great Lakes Research, 49 (1), 134-146. DOI: 10.1016/j.jglr.2022.12.006

Zastepa, A., Comte, J., Crevecoeur, S., 2023b. Prevalence and ecological features of deep chlorophyll layers in Lake of the Woods, a complex hydrological system with strong trophic, physical, and chemical gradients. Journal of Great Lakes Research, 49 (1), 122-133. DOI: 10.1016/j.jglr.2022.09.007

Zervou, S., Kaloudis, T., Gkelis, S., Hiskia, A., Mazur-Marzec, H., 2022. Anabaenopeptins from cyanobacteria in freshwater bodies of Greece. Toxins, 14(1), 4. DOI: 10.3390/toxins14010004

Zhou, X., Chaffin, J.D., Bratton J.F., Verhamme, E.M., Xue, P., 2023. Forecasting microcystin concentrations in Lake Erie using an Eulerian tracer model, Journal of Great Lakes Research, Volume 49, Issue 5, 1029-1044. DOI: 10.1016/j.jglr.2023.06.006.