

Review

Not peer-reviewed version

Early Warning System for Toxic Cyanobacteria Blooms

[Lauren Grant](#), [Attig Rehman](#)^{*}, Diane Botelho

Posted Date: 16 October 2024

doi: 10.20944/preprints202410.1304.v1

Keywords: cyanobacteria; cyanotoxins; harmful algal blooms; ELISA; LCMS; qPCR



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Review

Early Warning System for Toxic Cyanobacteria Blooms

Lauren Grant ¹, Diane Botelho ² and Attiq Rehman ^{2,*}

¹ Department of Chemistry, Saint Mary's University, 923 Robie Street, Halifax, NS, Canada B3H 3C3; lo.grant@smu.ca

² New Brunswick Research and Productivity Council (RPC), 921 College Hill Rd, Fredericton NB Canada E3B 6Z9

* Correspondence: attiq.rehman@rpc.ca; Tel.: (01 506 452 1365)

Abstract: Harmful cyanobacterial blooms produce cyanotoxins which can adversely affect humans and animals. Without proper monitoring and detection programs, tragedies such as the loss of pets or worse are possible. Multiple factors including rising temperatures and human influence contribute to the increased likelihood of harmful cyanobacteria blooms. Current approaches to cyanobacteria and their toxins monitoring included microscopic methods, immunoassays, liquid chromatography coupled with mass spectrometry (LCMS), and molecular methods such as qPCR. This review highlights current research into early detection methods for harmful cyanobacterial blooms and the pros and cons of these methods.

Keywords: cyanobacteria; cyanotoxins; harmful algal blooms; ELISA; LCMS; qPCR

1. Introduction

Cyanobacteria blooms can have adverse health effects on humans and animals by producing various cyanotoxins. Some of the most common cyanotoxins are hepatotoxins and neurotoxins.[1] In 2018, three dogs died after ingesting anatoxins in benthic mats from the Walostaq River in New Brunswick, Canada. The dog owners said there was no water discoloration, indicating the toxic cyanobacteria were not surface-blooming species.[2] Similarly, two dogs died from anatoxin poisoning after ingesting benthic cyanobacteria mats on the shore of Shubenacadie Grand Lake in Nova Scotia, Canada in June 2021.[3]

To prevent future tragic incidents like those in New Brunswick and Nova Scotia, establishing robust protocols for assessing cyanobacteria in water is crucial. Health Canada's guideline for total microcystins in recreational water is 10 µg/L and 1.5 µg/L in drinking water.[4,5] The United States Environmental Protection Agency (EPA) health advisories for microcystins and cylindrospermopsin in drinking water for school age children and adults is 1.6 µg/L and 3.0 µg/L respectively. For infants and pre-school aged children the health advisory is 0.3 µg/L for microcystins and 0.7 µg/L for cylindrospermopsin.[6] Any methods employed for cyanotoxin detection should have a limit of detection (LOD) at or below these health advisory levels.

Cyanobacteria blooms are more likely to occur in warmer water above 25 °C with abundant nutrients such as nitrogen, phosphorus, and carbon.[7] Excessive use of fertilizers leading to run-off contributes to nutrient availability and encourages waters' eutrophication. Sewage runoff can also add to this, and combined with the impacts of climate change, these factors contribute to the rising occurrence of harmful cyanobacteria blooms.[8] In a study of Canadian lakes in Alberta, Saskatchewan, and Manitoba, Erratt et al.[9] found that greater human influence led to earlier breakpoints for increased cyanobacterial biomass. They also found that rising temperatures encouraged the increase of microcystin production potential in lakes.

With rising temperatures and other factors that increase toxic cyanobacteria bloom potential, it is important to have effective monitoring programs in place. Methods for detecting toxic cyanobacteria include traditional microscope methods, immunoassays, chromatography and mass

spectrometry, and PCR-based methods. Although each method has pros and cons, this review specifically focuses on their applicability to early warning systems.

2. Methods for Cyanotoxin Detection

2.1. ELISA

Enzyme-linked immunosorbent assay (ELISA) is a biochemical assay that detects the presence of an antigen. An enzyme-labeled antibody that specifically binds to the antigen is added before leftover unbound antibodies are washed away. A chromogenic substrate is then added to react with the enzyme and a change in colour or fluorescence can be measured to identify the presence of the antigen.[10]

ELISA results can be impacted by non-specific binding and cross-reactivity.[11] Most of the microcystin antibodies for ELISA have been made from microcystin-LR, this can impact the selectivity of the antibody for other microcystins.[11] This issue can be mitigated by using the conserved "Adda" amino group in all microcystins and nodularins.[12] However, the Adda antibody selectivity can still be impacted in environmental samples where some Adda groups may have been modified, for example to dimethyl Adda or acetylate Adda.[11] However, ELISA is a well-developed assay with low costs and is high-throughput.[13]

Liu et al.[13] looked at the effects of sample pre-treatment on the quantitative detection of cyanotoxins by ELISA. Liu et al.[13] performed two pretreatment methods and found that initial centrifugation and filtering of surface water samples neglected the capture of intracellular microcystins. The other method employed freeze-thaw pre-treatment for the lysis of cells allowing for both extracellular and intracellular microcystins to be quantified. The impact of dilution on sample matrices tested (tap, river, and lake water) was also investigated. Liu et al.[13] found the optimal dilution factor for reducing matrix effects was 2:1 for tap water and 4:1 for lake and river water with anti-matrix buffer containing 10x phosphate buffer solution (PBS), 1% bovine serum albumin (BSA), and 0.5% ethylene diamine tetraacetic acid (EDTA). The limit of detection (LOD) was 0.15 µg/L and the limits of quantification were between 0.27 µg/L and 1.87 µg/L for microcystin-LR.

Woodruff et al.[14] also employed ELISA for the detection of microcystins, but with a focus on water facility sludges. Cyanobacteria caught in sludges have been found to increase in numbers and release toxins into solution. Woodruff et al.[14] specifically looked at the impact of sample storage conditions, temperature and holding time, on microcystin-LR recovery efficiency using ELISA compared to LC-MS/MS. Woodruff et al.[14] found that acceptable recovery of microcystins after storage at 4 °C for 24 hours and -20 °C for 7 and 21 days was achieved. They highlighted this as adding to the accessibility of ELISA as samples can be stored inexpensively helping to minimize costs. A linear response was also found for spiked sludge samples with varying total organic carbon and turbidity. The authors conclude that although their results are promising for monitoring water facility sludges for microcystins using ELISA, LC-MS/MS is still needed in cases requiring better accuracy and precision.

ELISA has also been used for cyanotoxin identification in benthic mats. Bauer et al.[15] used ELISA to determine anatoxin-a concentrations in benthic mats from a river in Lech, Germany where previously three dogs were poisoned by benthic cyanobacteria. Bauer et al.[16] used microscopic methods to determine if the benthic cyanobacteria, *Tychonemas* sp., was present in samples. Anatoxin-a concentrations in 50 mL samples containing water, sediment, and biomass were determined. The highest concentration of anatoxin-a was 220.5 ng/L found in a sample from a bathing site along the river.

Cyanotoxins other than microcystins and anatoxins have also been measured using ELISA. Roy-Lachapelle et al.[17] evaluated an ELISA kit for the determination of anabaenopeptins, a cyanopeptide of emerging concern. Anabaenopeptins are protease inhibitors with IC₅₀ values at a nanomolar scale. They have been found in concentrations similar to microcystins in lakes in the United States and Europe.[18] Roy-Lachapelle et al.[17] tested the ELISA total anabaenopeptin kit against a SPE-UHPLC-HRMS method for quantifying anabaenopeptin A and B. They found that the

ELISA test was reproducible when proper blanks were used. However, the LCMS method had greater sensitivity and a limit of detection of 0.011 µg/L compared to 0.10 µg/L for ELISA.

2.2. Liquid Chromatography Mass Spectrometry

Chromatographic methods can have a higher level of accuracy and precision than immunoassays. However, these methods have high costs which makes them poorly suited for regular use in early detection warning systems. Regardless, liquid chromatography-mass spectrometry (LCMS) methods are regularly used for cyanotoxin detection and quantification. LCMS also acts as a valuable baseline method for comparison to other methods.[19–22]

An ultra-trace solid phase extraction (SPE) ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) method developed by Filatova et al.[23] was able to detect seven microcystins, cylindrospermopsin, anatoxin-a, and nodularin at a picogram per litre scale. Filatova et al.[23] showed good linearity for all cyanotoxins tested and acceptable inter-day and intra-day precision. The SPE-UHPLC-HRMS method was used to test water samples from three reservoirs along the Ter River in Catalonia, Spain. Only one microcystin was found, microcystin-RR, at concentrations between 1 to 2 ng/L. As the authors note, these results support that this method can be used to identify the WHO guideline value for MC-LR in real-world water samples

Reveillon et al.[24] used solid phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) to look at nine microcystins and one nodularin in cyanobacteria and extracellularly in salt water. This method was developed with estuaries in mind as cyanotoxins can potentially migrate from freshwater to salt waters and impact marine organisms. Two different SPE sorbents were compared, C18 and polymeric. The C18 SPE cartridge was found to have good recovery of extracellular microcystins and nodularins regardless of the presence of salt. This contrasts with the polymeric cartridge which was impacted by salt and was less reliable. For intracellular toxins, cell pellets were lysed using methanol before SPE extraction.

The LC-MS/MS methods used a C18 column with a gradient elution consisting of mobile phase A, water and B, acetonitrile both with 0.1% formic acid. Reveillon et al.[24] found that the limit of detection (LOD) was between 1.0 and 22 pg and the limit of quantification (LOQ) was between 5.5 and 124 pg for the intracellular matrix. The extracellular matrix had a LOD between 0.59 and 11 pg and a LOQ between 1.8 and 34 pg. Reveillon et al.[24] did not use an internal standard or matrix-matched calibration curve. However, they were able to achieve excellent accuracy using a correction factor. The authors note using a correction factor achieved similar accuracy to methods which used an internal standard or matrix-matched calibration curves. Although, methods using isotopically labelled microcystins as internal standards were even more accurate.

The authors also tested the impacts of increasing salinity on the growth of *Microcystis* spp. and the presence of extracellular cyanotoxins. They found that *Microcystis* spp. could survive increases in salinity while the concentration of extracellular cyanotoxins also increased. This shows that methods for saltwater analysis of cyanotoxins could be crucial for monitoring estuaries which are connected to freshwater bodies containing harmful cyanoblooms.

In a method developed for both fresh and salt water, Vo Duy et al.[25] used an on-line SPE hydrophilic interaction liquid chromatography (HILIC) MS method to quantify saxitoxin, neosaxitoxin, and their decarbamoyl analogues. Five filters were tested for filtration recovery, including glass fibre, nitrocellulose, nylon, polyethersulfone, and regenerated cellulose. All filters except glass fibre provided acceptable recovery of the cyanotoxins tested. The optimal chromatographic conditions using an Accucore 150 Amide HILIC column were 5.0 mM ammonium formate and 0.05% formic acid in HPLC-water for mobile phase A and 0.05% formic acid in acetonitrile for mobile phase B.

Vo Duy et al.[25] also evaluated four different methods to mitigate matrix effects. These included an external calibration curve in water, an internal calibration curve in water, an external matrix-matched calibration curve, and an internal matrix-matched calibration curve. Absolute matrix effects were between -62% and -91% for brackish water and between -1.5% and -54% for freshwater using an external calibration curve in water. Absolute matrix effects were greatly improved when matrix-

matched isotopically labelled internal standards were used. Brackish water absolute matrix effects were between -11.5% and 1.8% while freshwater absolute matrix effects were between -6.2% and 22.2% respectively. The LOD ranged from 0.72-3.9 ng/L for freshwater samples and 6.9 to 30 ng/L for brackish water. These results highlight the sensitivity of LCMS compared to other methods used for cyanotoxin detection.

2.3. Polymerase Chain Reaction (PCR) Methods

Many studies have used PCR-based techniques to determine the presence of cyanotoxin biosynthetic gene clusters in water samples. Using PCR to evaluate the cyanotoxin-producing potential of water sources could be an invaluable tool for early warning systems. However, some considerations for using PCR for these warning systems include the genes targeted, assumptions on how gene copies correlate to toxin concentrations, and the exact method used such as quantitative, real-time, or digital duplex PCR.

In a study by Ribeiro et al.,[26] multiple primers were used for the detection of cylindrospermopsin (*cyrA*, *cyrB*, *cyrC*, and *cyrJ*), microcystins (*mcyE*), and saxitoxin (*sxtA*, *sxtB*, and *sxtI*). Samples were collected from the Billings Reservoir in São Paulo, Brazil during a rainy and dry period. The *mcyE* gene was found in all samples and of the cylindrospermopsin genes tested, only *cyrB* and *cyrC* were found. The only sample positive for *cyrB* also tested positive for *cyrC*. Interestingly, for saxitoxin, some samples tested positive only for *sxtB* while other samples tested positive only for *sxtI* and no samples were positive for *sxtA*.

From these results, Ribeiro et al.[26] concluded that using only one gene from a cyanotoxin gene cluster can lead to false negatives and false positives. False negatives can occur if there is a silent point mutation at the primer binding site and false positives can occur when other genes necessary for the biosynthesis of the targeted cyanotoxin are absent. Ribeiro et al.[26] note that other studies have found similar results where differential amplification between genes in the same gene cluster was observed.

Whatever gene clusters are used for PCR-based analysis of cyanotoxins, threshold values for corresponding gene copies to cyanotoxin concentrations need to be established. However, multiple factors can impact PCR results for cyanotoxins, such as primers potentially leading to false negatives or positives as mentioned previously, the assumption that gene copy numbers accurately represent toxicity, and other considerations more extensively covered by Pacheco et al.[27]

Given these complexities and potential sources of error, it is important to assess how reliably a given gene copy value corresponds to a cyanotoxin exceeding guideline values. Lu et al. (2019)[28] found values for gene copy numbers of *mcyB* (microcystin), and *pks* (cylindrospermopsin) which had a 10%, 25%, and 50% probability of corresponding to exceeding alert levels for microcystin and cylindrospermopsin. Lu et al. (2019)[28] tested a response-level model based on the 10% probability gene copy numbers and compared the results to ELISA. The level of agreement between qPCR and ELISA for the response level of thirty-six samples was calculated. The authors found excellent agreement between cyanotoxin gene copy numbers and ELISA results. They conclude that the proposed bio-marker model could be a reliable reference for response levels to toxic cyanobacteria blooms.

In another study, Lu et al. (2020)[29] developed an early warning system for toxic cyanobacteria blooms based on gene copy numbers. In this method, genes targeting general cyanobacteria, *Microcystis*, *Planktothrix*, *Cylindrospermopsis*, and *Nostoc*. Microcystin producers were also targeted by the genes *mcyE* and *mcyA*. This study found gene copy numbers corresponding to total microcystin levels of 0.3, 1.6, and 4 µg/L. Gene copy number values were determined using pooled ELISA data from four sites from Harsha Lake in Ohio, USA.

Lu et al. (2020)[29] tested both qPCR and reverse transcriptase (RT) qPCR. RT-qPCR was included to evaluate toxin gene expression in comparison to toxin levels. The authors found that *mcyA* determination by qPCR or RT-qPCR concentrations spiked approximately 3 weeks before LC-MS or ELISA observed an increase in microcystins. These results show that qPCR could be valuable for monitoring the possibility of future harmful cyanobacteria blooms.

Zupančič et al.[30] collected planktonic water samples and biofilms mostly from rocks from multiple lakes and rivers in Slovenia. They compared qPCR testing for the *mcyE*, *cyrJ*, and *sxtA* genes to microscopic analysis and LC-MS/MS quantification of microcystins, cylindrospermopsin, and saxitoxin. Three *mcyE* assays, *cyrJ*, *sxtA*, and 16S cyano assays were tested.

The authors found non-target binding occurred for the 16S cyano assay and two microcystin assays. These results highlight the importance of the chosen qPCR assay for cyanotoxin detection. Ribeiro et al.[26] found some cyanotoxin genes may not be detected and lead to false negatives and results by Zupančič et al.[30] show qPCRs potential for false negatives.

An important finding by Zupančič et al.[30] was the presence of cylindrospermopsin and saxitoxin genes in benthic mats while neither cyanotoxin was detected by LC-MS/MS. This means these water bodies have future cyanotoxin production potential, an important finding for future water management strategies.

3. Comparison of Methods for Early Warning Systems

Few studies directly compare multiple methods for their applicability in early warning systems for harmful cyanobacteria bloom detection. Table 1 summarizes results of papers which tested various methods for cyanotoxin detection and lists their pros and cons.

Immunoassays such as ELISA excel at being easily accessible, fast, and easy. However, it is not as sensitive as LC-MS methods and can have issues due to cross-reactivity and matrix effects. LC-MS methods are excellent for cross-comparing and validating other methods due to their sensitivity and specificity. LC-MS may not be an ideal choice for early detection warning systems as they can have a high cost and would not be ideal for routine testing. Lastly, PCR-based methods have the potential to indicate future risk of harmful cyanobacteria blooms before cyanotoxins are produced. However, few studies have attempted to correlate cyanotoxin gene copy numbers to cyanotoxin concentrations. More studies such as that by Lu et al. (2019)[28] would be needed to determine appropriate threshold values of gene-copy numbers associated with a notable risk for cyanotoxin production.

Table 1. Comparison of Methods for Cyanotoxin Detection.

Methods	Cyanotoxins	Pros	Cons	Reference
LC-MS/MS (LOD 0.001 µg/L)	12 MC congeners, NOD, ATX, and CYN	High sensitivity High specificity	Lack of MC reference standards Requires more advanced laboratory set-up	Hammoud et al.[20]
ELISA (LOD 0.10 µg/L)	MCs	Possible to detect MC congeners not targeted by LCMS Relatively fast and easy	Matrix effects Cross-reactivity of MCs Possible overestimation due to degradation or transformation of products	
PPIA (LOD 0.25 µg/L)	MCs	Possible to detect MC congeners not targeted by LCMS Relatively fast and easy	Lacks sensitivity Possible over or underestimation of MCs	

qPCR	MC, CYN, and STX	Samples with gene <i>mcyE</i> align with samples found to contain MCs in LCMS, ELISA, and PPIA	Only used to confirm presence of cyanobacteria and genes. Not evaluated for use in monitoring programs.	
Fluorometry	Estimated chlorophyll-a associated with cyanobacteria	Cheaper and more easily accessible than methods such as LC-MS/MS	Weak correlation with LC-MS/MS Less accurate than direct measurements of cyanotoxins Tended to overestimate risk to the public	Schürmann et al.[31]
Microscopy	Looked for the presence of 11 potentially toxic cyanobacteria	Cheaper and more easily accessible than methods such as LC-MS/MS	Less accurate than direct measurements of cyanotoxins Tended to overestimate risk to the public	
qPCR	16s, <i>mcyE</i>	Strong correlation between <i>mcyE</i> qPCR and LC-MS/MS	16S qPCR had a weak correlation with LC-MS/MS Some sample with high MC levels had low <i>mcyE</i> copy numbers No current guideline values for qPCR risk assessment	
ELISA	MCs, and ATX	Strong correlation with LC-MS/MS	Overestimated MCs in comparison with LC-MS/MS	
LC-MS/MS	MCs, ATX, CYN,	Assumed to be the “gold standard” for cyanotoxin detection	More expensive Longer sample processing times	
Shotgun metagenomics	Characterization of multiple cyanobacteria genera and gene abundance	Bloom vs non-bloom samples could be differentiated by the abundance of various genes such as those for nitrogen and	Cyanotoxin genes not detected Not as effective as ELISA and qPCR for real-time toxigenic potential analysis	Saleem et al.[32]

		phosphorus metabolism		
qPCR	16S RNA, <i>mycE</i> , <i>stxA</i>	Relatively fast compared to shotgun sequencing <i>mycE</i> gene copies correlated with MC/NOD levels Better for real-time toxigenic potential analysis than shotgun sequencing	Purified DNA found fewer gene copies for cyanotoxins than crude DNA. Purified DNA may underrepresent cyanotoxin potential while crude DNA has more PCR inhibition. Does not provide as much information on the cyanobacteria community present as shot-gun sequencing	
ELISA	MCs/NODs	Relatively fast compared to shotgun sequencing Better for real-time toxigenic potential analysis than shotgun sequencing	Does not provide as much information on the cyanobacteria community present as shot-gun sequencing	
LC-MS/MS	MCs, NOD, CYN, ATX	Analysis of microbial mats possible More reliable than ELISA	Unable to determine an MC structure in one sample based on fragments	Khomutovska et al.[33]
qPCR	<i>mycA</i> , <i>mycD</i> , <i>mycE</i> , <i>mycE/ndaF</i> , <i>sxtA</i> , and <i>anaC</i>	Analysis of microbial mats possible More information on the microbial community present	Primers may not be universal for both planktonic and benthic mat cyanobacteria	
ELISA	MCs (LOD 0.5 ng/L), CYN (LOD 0.5 ng/L), ATX (LOD 0.4 ng/L)	Rapid, results in ~30 mins	ATX was found in 6 samples, but not by LC-MS/MS or qPCR. False positives.	
Mass spectrometry multiple reaction	MCs, STX, CYN	More sensitive than ELISA	Same samples where MCs were detected by ELISA and <i>mycE</i> genes were detected	McKindles et al.[34]

monitoring (MS-MRM)	by qPCR were not positive for MCs by MS-MRM		
qPCR	<i>mcyE</i> , <i>stxA</i> , and <i>cyrA</i>	Potential as a valuable early warning tool, especially in the summer	Some samples which had MCs or CYN did not have detectable <i>mcyE</i> or <i>cyr</i> genes
ELISA	MCs	Lower sensitivity compared to HPLC methods	

4. Conclusions

Early warning systems for harmful cyanobacteria blooms are important for the health and safety of humans and animals. With the rise in harmful cyanobacteria blooms [8] effective monitoring is becoming increasingly important. As reviewed here, several methods for cyanobacteria toxin detection exist, each with pros and cons.

Tiered approaches have been suggested by Schürmann et al.[31] and Almuhtaram et al.[35] for cyanobacteria monitoring. Tier one would consist of rapid, cheaper, but less reliable methods compared to higher tiers. These may include methods such as microscopy and fluorometry to measure the biomass of a water body. A second-tier response would be prompted if tier-one measures exceeded a pre-defined threshold value. Second-tier or third-tier methods would include more accurate and sensitive methods that have a greater cost or require more specialized equipment. These can include LC-MS/MS, qPCR, or immunoassays.

Another consideration for early warning systems is that many methods only detect the presence of cyanotoxins and not the possibility of future toxic events. The only methods shown to have some capacity to indicate a future toxic algal bloom are PCR-based methods such as qPCR. Duan et al.[36] determined a threshold value for the *mcyG* microcystin encoding gene cluster which indicated the health advisory level for microcystins, 0.3 µg/L, would be exceeded in approximately one week. If implemented, this method could allow for preventative action to be taken.

Author Contributions: Conceptualization, A.R. and L.G.; methodology, A.R and L.G.; writing—original draft preparation, L.G and A.R; writing—review and editing, A.R and D.B.; supervision, A.R.; project administration, A.R.; funding acquisition, A.R and D.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by **Environmental Trust Fund (ETF)** under the Priority Area - Protecting our Environment, grant number 220129.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Cathy Hay, Corrie Maston and Micro team members both at Fredericton and Moncton locations for their contributions to this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Thawabteh, A.M.; Naseef, H.A.; Karaman, D.; Bufo, S.A.; Scrano, L.; Karaman, R. Understanding the Risks of Diffusion of Cyanobacteria Toxins in Rivers, Lakes, and Potable Water. *Toxins* **2023**, *15*, 582, doi:10.3390/toxins15090582.
2. McCarron, P.; Rafuse, C.; Scott, S.; Lawrence, J.; Bruce, M.R.; Douthwright, E.; Murphy, C.; Reith, M.; Beach, D.G. Anatoxins from Benthic Cyanobacteria Responsible for Dog Mortalities in New Brunswick, Canada. *Toxicon* **2023**, *227*, 107086, doi:10.1016/j.toxicon.2023.107086.

3. Johnston, L.H.; Huang, Y.; Bermarija, T.D.; Rafuse, C.; Zamlynnny, L.; Bruce, M.R.; Graham, C.; Comeau, A.M.; Valadez-Cano, C.; Lawrence, J.E.; et al. Proliferation and Anatoxin Production of Benthic Cyanobacteria Associated with Canine Mortalities along a Stream-Lake Continuum. *Sci. Total Environ.* **2024**, *917*, 170476, doi:10.1016/j.scitotenv.2024.170476.
4. Guidelines for Canadian Drinking Water: Guideline Technical Document - Cyanobacterial Toxins; Health Canada: Ottawa, 2017; ISBN 978-0-660-07494-8.
5. Guidelines for Canadian Recreational Water Quality: Cyanobacteria and Their Toxins : Guideline Technical Document; Health Canada = Santé Canada: Ottawa, ON, 2022; ISBN 978-0-660-41541-3.
6. 2015 Drinking Water Health Advisories for Two Cyanobacterial Toxins 2015.
7. Merel, S.; Walker, D.; Chicana, R.; Snyder, S.; Baurès, E.; Thomas, O. State of Knowledge and Concerns on Cyanobacterial Blooms and Cyanotoxins. *Environ. Int.* **2013**, *59*, 303–327, doi:10.1016/j.envint.2013.06.013.
8. Ibelings, B.W.; Backer, L.C.; Kardinaal, W.E.A.; Chorus, I. Current Approaches to Cyanotoxin Risk Assessment and Risk Management around the Globe. *Harmful Algae* **2014**, *40*, 63–74, doi:10.1016/j.hal.2014.10.002.
9. Erratt, K.J.; Creed, I.F.; Lobb, D.A.; Smol, J.P.; Trick, C.G. Climate Change Amplifies the Risk of Potentially Toxigenic Cyanobacteria. *Glob. Change Biol.* **2023**, *29*, 5240–5249, doi:10.1111/gcb.16838.
10. Gan, S.D.; Patel, K.R. Enzyme Immunoassay and Enzyme-Linked Immunosorbent Assay. *J. Invest. Dermatol.* **2013**, *133*, 1–3, doi:10.1038/jid.2013.287.
11. He, X.; Liu, Y.-L.; Conklin, A.; Westrick, J.; Weavers, L.K.; Dionysiou, D.D.; Lenhart, J.J.; Mouser, P.J.; Szlag, D.; Walker, H.W. Toxic Cyanobacteria and Drinking Water: Impacts, Detection, and Treatment. *Harmful Algae* **2016**, *54*, 174–193, doi:10.1016/j.hal.2016.01.001.
12. Weller, M. Immunoassays and Biosensors for the Detection of Cyanobacterial Toxins in Water. *Sensors* **2013**, *13*, 15085–15112, doi:10.3390/s131115085.
13. Liu, J.; Xing, Y.; Lin, Y.; Xie, Y.; Zhou, X. Effect of Pretreatment Approach on the ELISA-Based Detection of Cyanotoxins in Water: Analysis and Application. *Sci. Total Environ.* **2023**, *871*, 161988, doi:10.1016/j.scitotenv.2023.161988.
14. Woodruff, P.; McNeely, M.; Sanan, T.; Dugan, N. Analysis of Microcystins in Alum Water Treatment Sludges: Holding Times, Temperatures, Linearity of Response, and Sensitivity to Pre-Coagulation Cell Titers. *Environ. Technol.* **2024**, 1–14, doi:10.1080/09593330.2024.2349263.
15. Bauer, F.; Wolfschlaeger, I.; Geist, J.; Fastner, J.; Schmalz, C.W.; Raeder, U. Occurrence, Distribution and Toxins of Benthic Cyanobacteria in German Lakes. *Toxics* **2023**, *11*, 643, doi:10.3390/toxics11080643.
16. Bauer, F.; Stix, M.; Bartha-Dima, B.; Geist, J.; Raeder, U. Spatio-Temporal Monitoring of Benthic Anatoxin-a-Producing Tychonema Sp. in the River Lech, Germany. *Toxins* **2022**, *14*, 357, doi:10.3390/toxins14050357.
17. Roy-Lachapelle, A.; Sollic, M.; Sauvé, S.; Gagnon, C. Evaluation of ELISA-Based Method for Total Anabaenopeptins Determination and Comparative Analysis with on-Line SPE-UHPLC-HRMS in Freshwater Cyanobacterial Blooms. *Talanta* **2021**, *223*, 121802, doi:10.1016/j.talanta.2020.121802.
18. Janssen, E.M.-L. Cyanobacterial Peptides beyond Microcystins – A Review on Co-Occurrence, Toxicity, and Challenges for Risk Assessment. *Water Res.* **2019**, *151*, 488–499, doi:10.1016/j.watres.2018.12.048.
19. Sanseverino, I.; Loos, R.; Lettieri, T.; Conduto António, D. Cyanotoxins: Methods and Approaches for Their Analysis and Detection. **2017**, 28624.
20. Hammoud, N.A.; Zervou, S.-K.; Kaloudis, T.; Christophoridis, C.; Paraskevopoulou, A.; Triantis, T.M.; Slim, K.; Szpunar, J.; Fadel, A.; Lobinski, R.; et al. Investigation of the Occurrence of Cyanotoxins in Lake Karaoun (Lebanon) by Mass Spectrometry, Bioassays and Molecular Methods. *Toxins* **2021**, *13*, 716, doi:10.3390/toxins13100716.
21. Yang, J.; Si, R.; Wu, G.; Wang, Y.; Fang, R.; Liu, F.; Wang, F.; Lei, H.; Shen, Y.; Zhang, Q.; et al. Preparation of Specific Nanobodies and Their Application in the Rapid Detection of Nodularin-R in Water Samples. *Foods* **2021**, *10*, 2758, doi:10.3390/foods10112758.
22. Roy-Lachapelle, A.; Sollic, M.; Sauvé, S.; Gagnon, C. Evaluation of ELISA-Based Method for Total Anabaenopeptins Determination and Comparative Analysis with on-Line SPE-UHPLC-HRMS in Freshwater Cyanobacterial Blooms. *Talanta* **2021**, *223*, 121802, doi:10.1016/j.talanta.2020.121802.
23. Filatova, D.; Núñez, O.; Farré, M. Ultra-Trace Analysis of Cyanotoxins by Liquid Chromatography Coupled to High-Resolution Mass Spectrometry. *Toxins* **2020**, *12*, 247, doi:10.3390/toxins12040247.
24. Réveillon, D.; Georges Des Aulnois, M.; Savar, V.; Robert, E.; Caruana, A.M.N.; Briand, E.; Bormans, M. Extraction and Analysis by Liquid Chromatography – Tandem Mass Spectrometry of Intra- and Extracellular Microcystins and Nodularin to Study the Fate of Cyanobacteria and Cyanotoxins across the Freshwater-Marine Continuum. *Toxicon* **2024**, *237*, 107551, doi:10.1016/j.toxicon.2023.107551.
25. Vo Duy, S.; Munoz, G.; Dinh, Q.T.; Zhang, Y.; Simon, D.F.; Sauvé, S. Fast Screening of Saxitoxin, Neosaxitoxin, and Decarbamoyl Analogues in Fresh and Brackish Surface Waters by on-Line Enrichment Coupled to HILIC-HRMS. *Talanta* **2022**, *241*, 123267, doi:10.1016/j.talanta.2022.123267.

26. Ribeiro, M.S.F.; Tucci, A.; Matarazzo, M.P.; Viana-Niero, C.; Nordi, C.S.F. Detection of Cyanotoxin-Producing Genes in a Eutrophic Reservoir (Billings Reservoir, São Paulo, Brazil). *Water* **2020**, *12*, 903, doi:10.3390/w12030903.
27. Pacheco, A.; Guedes, I.; Azevedo, S. Is qPCR a Reliable Indicator of Cyanotoxin Risk in Freshwater? *Toxins* **2016**, *8*, 172, doi:10.3390/toxins8060172.
28. Lu, K.-Y.; Chiu, Y.-T.; Burch, M.; Senoro, D.; Lin, T.-F. A Molecular-Based Method to Estimate the Risk Associated with Cyanotoxins and Odor Compounds in Drinking Water Sources. *Water Res.* **2019**, *164*, 114938, doi:10.1016/j.watres.2019.114938.
29. Lu, J.; Struewing, I.; Wymer, L.; Tettenhorst, D.R.; Shoemaker, J.; Allen, J. Use of qPCR and RT-qPCR for Monitoring Variations of Microcystin Producers and as an Early Warning System to Predict Toxin Production in an Ohio Inland Lake. *Water Res.* **2020**, *170*, 115262, doi:10.1016/j.watres.2019.115262.
30. Zupančič, M.; Kogovšek, P.; Šter, T.; Remec Rekar, Š.; Cerasino, L.; Baebler, Š.; Krivograd Klemenčič, A.; Eleršek, T. Potentially Toxic Planktic and Benthic Cyanobacteria in Slovenian Freshwater Bodies: Detection by Quantitative PCR. *Toxins* **2021**, *13*, 133, doi:10.3390/toxins13020133.
31. Schürmann, Q.J.F.; Visser, P.M.; Sollié, S.; Kardinaal, W.E.A.; Faassen, E.J.; Lokmani, R.; Van Der Oost, R.; Van De Waal, D.B. Risk Assessment of Toxic Cyanobacterial Blooms in Recreational Waters: A Comparative Study of Monitoring Methods. *Harmful Algae* **2024**, *138*, 102683, doi:10.1016/j.hal.2024.102683.
32. Saleem, F.; Atrache, R.; Jiang, J.L.; Tran, K.L.; Li, E.; Paschos, A.; Edge, T.A.; Schellhorn, H.E. Characterization of Taxonomic and Functional Dynamics Associated with Harmful Algal Bloom Formation in Recreational Water Ecosystems. *Toxins* **2024**, *16*, 263, doi:10.3390/toxins16060263.
33. Khomutovska, N.; Sandzewicz, M.; Lach, L.; Suska-Malawska, M.; Chmielewska, M.; Mazur-Marzec, H.; Ceglowska, M.; Niyatbekov, T.; Wood, S.A.; Puddick, J.; et al. Limited Microcystin, Anatoxin and Cylindrospermopsin Production by Cyanobacteria from Microbial Mats in Cold Deserts. *Toxins* **2020**, *12*, 244, doi:10.3390/toxins12040244.
34. McKindles, K.M.; Zimba, P.V.; Chiu, A.S.; Watson, S.B.; Gutierrez, D.B.; Westrick, J.; Kling, H.; Davis, T.W. A Multiplex Analysis of Potentially Toxic Cyanobacteria in Lake Winnipeg during the 2013 Bloom Season. *Toxins* **2019**, *11*, 587, doi:10.3390/toxins11100587.
35. Almuhtaram, H.; Kibuye, F.A.; Ajampur, S.; Glover, C.M.; Hofmann, R.; Gaget, V.; Owen, C.; Wert, E.C.; Zamyadi, A. State of Knowledge on Early Warning Tools for Cyanobacteria Detection. *Ecol. Indic.* **2021**, *133*, 108442, doi:10.1016/j.ecolind.2021.108442.
36. Duan, X.; Zhang, C.; Struewing, I.; Li, X.; Allen, J.; Lu, J. Cyanotoxin-Encoding Genes as Powerful Predictors of Cyanotoxin Production during Harmful Cyanobacterial Blooms in an Inland Freshwater Lake: Evaluating a Novel Early-Warning System. *Sci. Total Environ.* **2022**, *830*, 154568, doi:10.1016/j.scitotenv.2022.154568.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.