The history and progress of integrating molecular cyanobacterial assays into water monitoring programs

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phytoxigene™

Toxin Biosynthesis History

- Professor Brett Neilan
  - School of Biotechnology and Biomolecular Sciences, UNSW
  - University of Newcastle
    - Microcystin
      - Tillett et al, 2000
    - Nodularin
      - Moffitt and Neilan, 2004
    - Cylindrospermopsin
      - Mihali et al, 2008
    - Saxitoxin
      - Kellmann et al, 2008
      - Jakobsen et al 2011

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CyanoDTec qPCR Test Specifics

Simultaneously identifies and quantifies the presence of total cyanobacteria along with 3 genes responsible for 4 different toxin production;

- Specific Cyanobacteria 16S rRNA gene, IAC
- Pan Microcystin & Nodularin, mcyE/ndaF gene
- Pan Cylindrospermopsin, cyrA gene
- Pan Saxitoxin, sxtA gene
- Certified gene standards (NMI) for quantitation

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A perfect storm; Toledo August 2014

Disasters motivate and result in change

Ohio's Use of qPCR as a Cyanobacteria Screening Tool

Ohio AWWA Technology Conference
September 26, 2017

Heather Raymond
Ohio EPA HAB Coordinator

Ohio Environmental Protection Agency

qPCR as Screening Tool for Microcystins

- Out of 1850 paired PWS samples:
  - 100% of microcystins >1.6 µg/L had paired mcyE gene detections.
  - 100% of microcystins >5 µg/L had mcyE detections > 5,000gc/ml
  - 90% of microcystins detections >1.6 ug/L had mcyE detections >5,000 gc/mL

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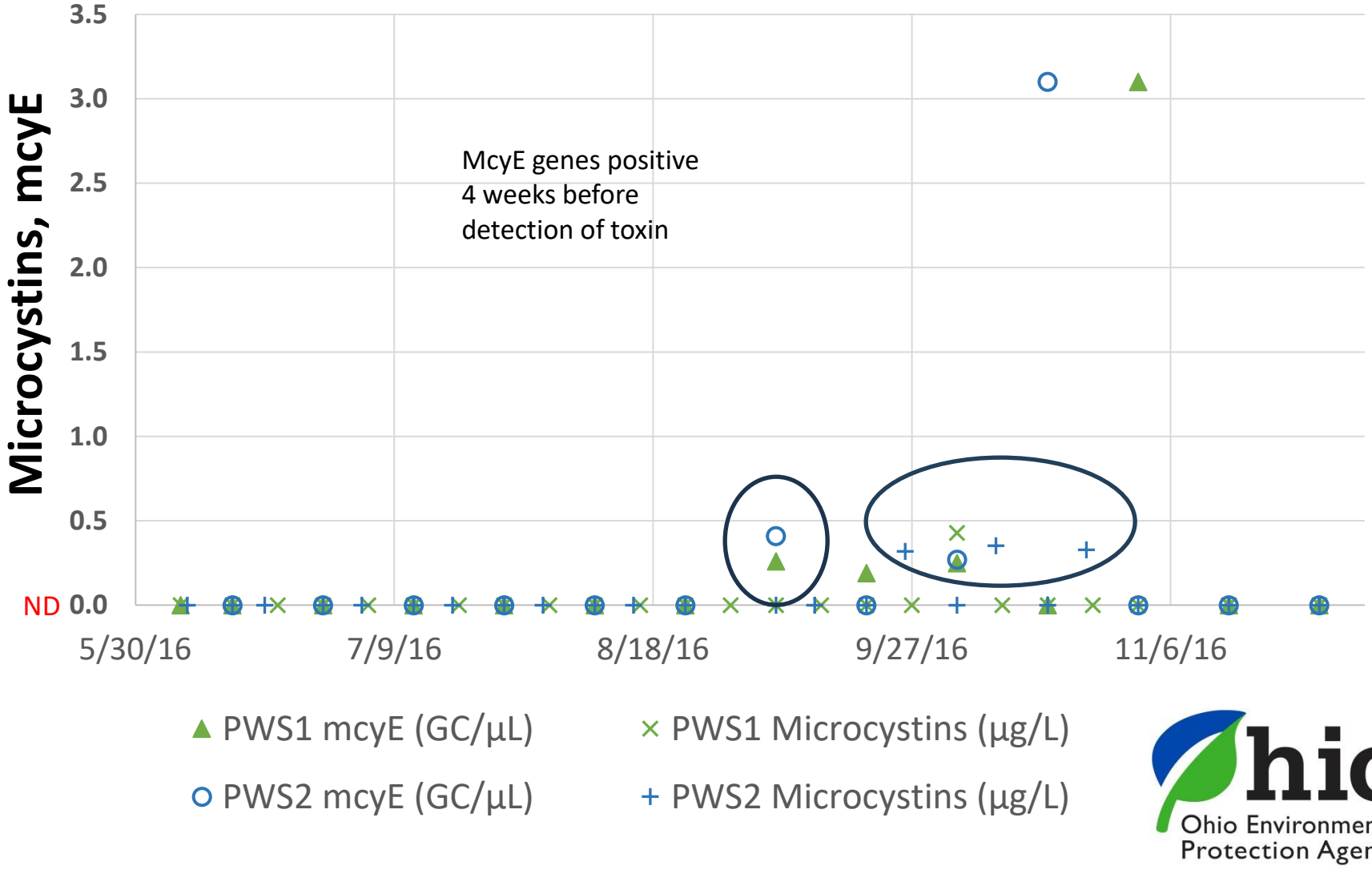
Example: mcyE Detections Precede Microcystins Detections in Central Lake Erie Basin



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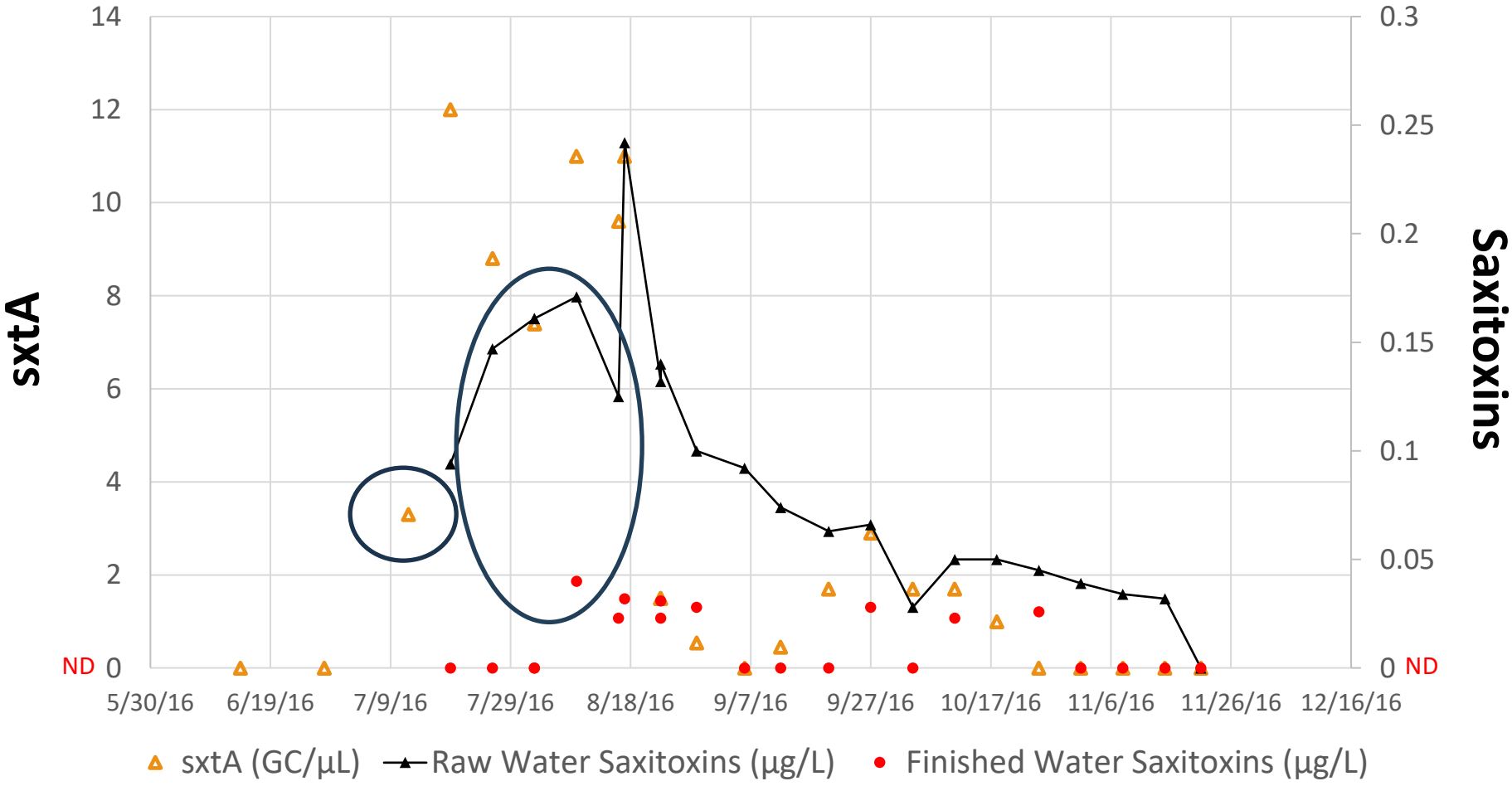
Example: sxtA Triggers Saxitoxins Sampling



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qPCR Data Interpretation

- mcyE
  - Any mcyE detection may indicate onset of a bloom, and could provide early warning.
  - More severe blooms, indicated by microcystins concentrations >5µg/L, were always associated with mcyE >5,000 GC/mL.
- sxtA
  - sxtA detections were associated with saxitoxins, but in some cases low sxtA was associated with higher saxotoxins concentrations.

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Ohio EPA quantitative Polymerase Chain Reaction (qPCR) Multi-Plex Molecular Assay for Determination of Cyanobacteria and Cyanotoxin-Producing Genes Analytical Methodology

Ohio EPA DES 705.0
Version 1.0
September 2018

1. SCOPE AND APPLICATION

This method is used as a regulatory screening procedure for cyanobacterial genes and genes capable of producing microcystins, saxitoxins, or cylindrospermopsin in surface water.

Reporting Limit (RL) for 16S, mcyE, cyrA, and sxtA genes: ≤ 0.18 Gene Copies/µL

Ohio Monitoring Guidance

http://epa.ohio.gov/Portals/28/documents/habs/2017-18HAB%20monitoring.pdf

2017-2018 HAB Monitoring at Ohio Public Water Systems (PWS)

Routine microcystins monitoring and cyanobacteria screening are required to be conducted by all surface water PWSs under Ohio Administrative Code (OAC) Rule 3745-90-03. The analytical methods and reporting requirements are described in OAC Rule 3745-90-04. The cyanobacteria screening required under OAC Rule 3745-90-03 utilizes a molecular quantitative polymerase chain reaction (qPCR) testing method to identify and quantify the presence of cyanobacteria (16s gene) and cyanotoxin-production genes (sxtA, mcyE, and cyrA) in water samples.

Currently, Ohio PWS are separated into three schedules. Schedule 1 is required to conduct biweekly screening paired with weekly raw/finished water microcystins monitoring through October 31, 2017. Schedule 2 is required to conduct biweekly screening and raw microcystins monitoring on alternating weeks of screening. Schedule 3 is required to collect screening samples biweekly. An evaluation of the 2016-2017 monitoring results indicates that the cyanobacteria screening method provides a reliable indicator for microcystins and saxitoxins. Based on occurrence and evaluation of treatment effectiveness for microcystins, Ohio EPA has revised HAB monitoring requirements starting on November 1, 2017.

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Initial Seasonal Monitoring Schedule[a]	2018 Off-Season Monitoring Requirements[b] (1/1/18 - 4/30/18)	2018 HAB Season Monitoring Requirements (5/1/18 - 10/31/18)
1, 2 or 3	**Unreduced Off-Season Schedule** • Biweekly raw water microcystin; AND • Biweekly qPCR screening (paired)	**See below**
1	**HAB Off-Season Schedule 1A** If any detections of saxitoxins, cylindrospermopsin, sxtA or cyrA genes (6/1/16-present), then; • Biweekly qPCR screening only	**Seasonal Schedule 1** • Biweekly qPCR screening • Weekly raw and finished microcystins -paired with qPCR screening
	HAB Off-Season Schedule 1B If no detections of saxitoxins, cylindrospermopsin, sxtA or cyrA genes (6/1/16-present), then; • Biweekly raw water microcystins only • PWS may elect to be on Schedule 1A with written notification by 12/15/17.	
2	**HAB Off-Season Schedule 2A** If any detections of saxitoxins, cylindrospermopsin, sxtA or cyrA genes (6/1/16-present), then; • Biweekly qPCR screening only	**Seasonal Schedule 2** • Biweekly qPCR screening • Biweekly raw water microcystins -collected on alternate week as screening sample, not paired
	HAB Off-Season Schedule 2B If no detections of saxitoxins, cylindrospermopsin, sxtA or cyrA genes (6/1/16-present), then; • Biweekly raw water microcystins only • PWS may elect to be on Schedule 2A with written notification by 12/15/17.	
3	**HAB Off-Season Schedule 3[c]** • Monthly qPCR screening; OR • Monthly raw water microcystins	**New Seasonal Schedule 3** • Monthly qPCR screening

[a] Seasonal monitoring schedules will be provided prior to 5/1/18.

[b] To be eligible for any off-season reduced monitoring, microcystins and mcyE genes must be non-detect for at least 2 consecutive weeks.

[c] PWS must notify Ohio EPA in writing their preference (qPCR screening or microcystins) by 12/15/17.

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Schedule 2 HAB Season (May 1 – October 31)

1. If microcystins are detected in the raw water:
    a. PWS must collect raw/finished water sample within 24 hours of receiving the result and complete analysis within five days. If PWS collected a paired finished water sample with their initial raw water sample, an additional raw and finished sample is not required until the following week (unless raw water was > 5ug/L or any finished water detection).
    b. PWS will continue with weekly raw/finished water monitoring.

2. If mycE genes are detected at high levels (> 5 gene counts per uL) in the raw water:
    a. PWS must collect raw/finished water microcystins sample within 24 hours of receiving the result and complete analysis within five days. Any microcystins detections in finished water or raw water > 5 ug/L will trigger additional monitoring requirements as specified in OAC Chapter 3745-90.
    b. If microcystins are not detected the PWS will remain on Schedule 2 monitoring requirements. If microcystins are detected in either the raw or finished water, the PWS will be changed to Schedule 1 monitoring requirements for the remainder of the season, through October 31.

Schedule 3 (both HAB Season and Off-Season)

1. If mcyE genes or microcystins are detected in the raw water:
    a. PWS must collect raw/finished water microcystins sample within 24 hours and complete analysis within five days.
        i. If microcystins are detected in the raw or finished water, the PWS will switch to Schedule 2B monitoring for remainder of the off-season and will be switched to Schedule 2 Seasonal Monitoring. Any microcystins detections in finished water or raw water > 5 ug/L will trigger additional monitoring requirements as specified in OAC Chapter 3745-90.
        ii. If only mcyE genes are detected, PWS transitions to Schedule 2B Off-Season Monitoring and Schedule 2 for Seasonal Monitoring.
2. If sxtA or cyrA genes are detected in the raw water;
    a. PWS must notify Ohio EPA immediately of the results so follow up sampling can be conducted by the agency.
    b. PWS will be transitioned to Schedule 2A Off-Season Monitoring and Schedule 2 Seasonal Monitoring.

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Public Water System Harmful Algal Bloom Response Strategy

Division of Drinking and Ground Waters
April 2020

Table 2. Microcystins and Cyanobacteria Screening Monitoring Requirements

Schedule	PWS Criteria	HAB Season Monitoring Requirements[a] (start first FULL week of May)	Off-season HAB Monitoring Requirements[a,b,c] (start first FULL week of November)
1	Historic microcystins detections in finished drinking water; OR High source water susceptibility (more than two historic microcystins detections greater than 1.6 µg/L in raw water since 6/15/15) and either: Pre-oxidizes with chlorine or chlorine dioxide and has limited down-stream processes to address extracellular microcystins[d] **OR** Has no advanced treatment processes in place[e]	Biweekly qPCR screening **AND** Weekly raw/finished microcystins (paired with biweekly screening sample)	Biweekly raw water microcystins **OR** Biweekly qPCR screening
2	PWSs that do not meet the criteria for Schedule 1, 3, or 4	Biweekly qPCR screening **AND** Biweekly raw water microcystins (collected on alternate week of screening sample, not paired)	Biweekly raw water microcystins **OR** Biweekly qPCR screening
3	PWSs with ground water well source considered ground water under the influence of surface water with no historic microcystins or saxitoxins detections, no sxtA or mcyE detections, and low 16S	Monthly qPCR screening	Monthly qPCR screening **OR** Monthly microcystins monitoring
4	Consecutive water systems receiving water from an out-of-state surface water source.	Weekly finished water microcystins	Biweekly finished water microcystins

[a] See monitoring requirement implementation (Table 3). Microcystins or gene detections may trigger additional sampling requirements. Systems must have two consecutive weeks with no microcystins detection in raw or finished water to be eligible for monitoring reductions. During transitions periods, eligibility will be based on microcystins results from the two weeks prior the start of HAB season (first full week of May) and off-season (first full week of November).

[b] During off-season (November through May), PWSs sampling for total microcystins may discontinue qPCR screening.

[c] Water systems must notify Ohio EPA of preference for microcystins or qPCR monitoring two weeks prior to start of off-season monitoring period and must maintain sampling for specified parameter throughout entire off-season.

[d] Some PWSs that met the criteria for Schedule 1 were moved to Schedule 2 if monitoring data demonstrated the PWS currently has the capacity to effectively remove/destroy microcystins. For example, PWSs with historic finished water microcystins detections that have since upgraded or optimized treatment and demonstrated they are capable of treating higher concentrations of microcystins (without repeat finished water detections) are on Schedule 2.

[e] Advanced treatment is treatment beyond conventional coagulation, sedimentation, filtration, and chlorine disinfection that has capacity to remove or destroy extracellular microcystins.

Table 3. HAB Monitoring Sampling Triggers and Implementation Notes

Microcystins and Cyanobacteria Screening Monitoring Requirements HAB Season (First FULL week of May through October)		
Schedule	**Monitoring Requirements**	**Additional Sampling Triggers**
1	Biweekly qPCR screening; AND Weekly raw/finished microcystins (paired with biweekly screening sample)	Increased monitoring would be triggered by finished water microcystins detections or raw water detections greater than 5 µg/L (see "All Schedules," below).
2	Biweekly qPCR screening; AND Biweekly raw water microcystins collected on alternate week of qPCR screening (not paired)	**If microcystins are detected in the raw water:** • PWS must collect raw and finished water sample within 24 hours of receiving the result and complete analysis within five days. If PWS voluntarily collected a paired finished water sample with their initial raw water sample, an additional set of raw and finished samples is not required until the following week unless raw is greater than 5 µg/L or a finished water detection triggers more immediate sampling (see "All Schedules" below). • PWS will be changed to Schedule 1 requirements for the remainder of the season. If the PWS has at least four consecutive weeks of non-detect microcystins sampling results, and mcyE is less than 5 gene counts/µL during that same time period, the PWS can send an e-mail to their HAB coordinator requesting a transition back to schedule 2 monitoring. **If mcyE are detected in raw water greater than 5 gene copies/µL:**
		• PWS must collect raw/finished water microcystins sample within 24 hours of receiving the result and complete analysis within five days. • If microcystins are not detected, the PWS will remain on Schedule 2 monitoring requirements. • If microcystins are detected in either the raw or finished water, the PWS will be changed to Schedule 1 monitoring requirements for the remainder of the season. If the PWS has at least four consecutive weeks of non-detect microcystins sampling results, and mcyE is less than 5 gene counts/µL during that same time period, the PWS can send an e-mail to their HAB coordinator requesting a transition back to schedule 2 monitoring.

3	Monthly qPCR screening	**If mcyE genes are detected:** • PWS must collect raw and finished water microcystins sample within 24 hours and complete analysis within five days. • If microcystins are not detected (only mcyE genes are detected), PWS transitions to Schedule 2 monitoring. • If microcystins are detected in the raw or finished water, the PWS will switch to Schedule 1 monitoring for remainder of the season. If the PWS has at least four consecutive weeks of non-detect microcystins sampling results, and mcyE is less than 5 gene counts/µL during that same time period, the PWS can send an e-mail to their HAB coordinator requesting a transition to schedule 2 monitoring.
4	Weekly finished water microcystins	Increased monitoring would be triggered by finished water microcystins detections (see "All Schedules," below).
	If sxtA or cyrA genes are detected in the raw water: • PWS must notify Ohio EPA no later than the end of the next business day per OAC Rule 3745-89-08. Ohio EPA also recommends written or verbal results be communicated as soon as possible to ensure timely Ohio EPA follow up.	

Ohio Environmental Protection Agency

Public Water System Harmful Algal Bloom Response Strategy

Division of Drinking and Ground Waters
November 2022

Table 2. Monitoring requirements based on revised HAB Rule

Microcystins and Cyanobacteria Screening Monitoring Requirements Based on Revised HAB Rule in 2022 HAB Season (begins first full week of June) -- Off-season (begins first full week of December)	
Monitoring Requirements	**Additional Sampling Triggers**
Routine HAB Monitoring for all PWS with a surface water source (Former Schedule 1 and 2): HAB SEASON Biweekly cyanobacteria screening AND Biweekly **raw** water microcystins (alternate week of cyanobacteria screening) OFF-SEASON Biweekly **FINISHED** water microcystins	**If microcystins are detected in the raw water:** • PWS must collect raw and finished water samples within 24 hours of receiving the result and complete analysis within five days. If PWS voluntarily collected a paired finished water sample with their initial raw water sample, an additional set of raw and finished samples is not required until the following week unless raw is greater than 5 µg/L or a finished water detection triggers more immediate sampling (see "All Schedules" below). • **PWS will continue with weekly paired raw and finished water microcystins monitoring until non-detect for at least two consecutive weeks**. **If mcyE are detected in raw water greater than 5 gene copies/µL:** • PWS must collect raw/finished water microcystins sample within 24 hours of receiving the result and complete analysis within five days. • If microcystins are not detected, the PWS will resume biweekly monitoring. • If microcystins are detected in either the raw or finished water, the PWS continues with weekly raw/finished microcystins monitoring and biweekly qPCR screening until microcystins are non-detect for at least two consecutive weeks.
Reduced Monitoring (Former Schedule 3): HAB SEASON: Monthly cyanobacteria screening OFF-SEASON: Monthly FINISHED water microcystins	If cyanobacteria screening detects presence of cyanotoxins or cyanotoxin-producing genes, the PWS will transition to routine HAB monitoring and must collect raw and finished water samples within 24 hours of receiving the result and complete analysis within five days.

Microcystins and Cyanobacteria Screening Monitoring Requirements Based on Revised HAB Rule in 2022 HAB Season (begins first full week of June) -- Off-season (begins first full week of December)	
Monitoring Requirements	**Additional Sampling Triggers**
All PWS	**If cyanobacteria screening indicates the presence of saxitoxins, cylindrospermopsin or genes (sxtA or cyrA):** • PWS must notify Ohio EPA no later than the end of the next business day per OAC Rule 3745-89-08. Ohio EPA also recommends written or verbal results be communicated as soon as possible to ensure timely response by Ohio EPA. **If microcystins are detected in finished water greater than 0.3 µg/L (reported value ≥0.35 µg/L):** • PWS must collect one **resample** of raw/finished water within 24 hours of action level exceedance and collect an additional **repeat** sample of raw/finished water within 24 hours of resample. Analysis must be completed within 24 hours in each case. • If microcystins are greater than 0.3 µg/L in either resample or repeat, PWS must notify all consecutive systems within three hours of receiving results. • PWS and consecutive systems ***may*** collect samples from representative distribution points established in the contingency plan. • PWS will sample raw water once per week and increase sampling to three times per week at finished water sampling point. • PWS can resume routine monitoring when microcystins are non-detect in finished water for two consecutive samples.

Toxic algae blooms found in parts of Detroit Lake again, Oregon health officials warn

by KATU Staff | Thu, June 14th 2018, 6:41 AM GMT+10

Detroit Lake. (KATU Photo)

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EQ's quality assurance review.
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ted here. The data are released on
neither Oregon DEQ, nor the State
held liable for any damages
r authorized or unauthorized use."

Lessons Learned: qPCR Projects for Toxin Producing Genes in Oreg Waterbodies

Kale Clauson

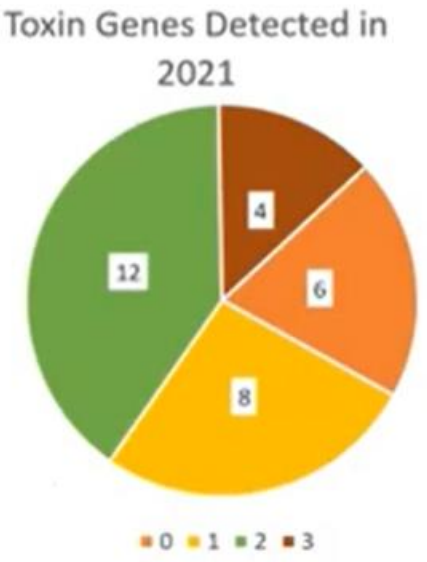
ODEQ Laboratory (Hillsboro)

(OLA 3-2022)

State of Oregon
DEQ Department of Environm

Public Water System	Samples With Gene Detected			
	#	cyrA	mcyE	sxtA
Anglers Cove/SCHWC	6	6		6
Ashland Water Department	6			
Buell-Red Prairie Water Association	8	6	6	
Canby Utility/Veolia Water	6	1	1	2
City of Albany	4			
City of Creswell	3			2
City of Estacada	6			3
City of Gates	6	5		5
City of Gold Hill	7	5		4
City of Grants Pass	6	5		2
City of Jefferson	6	5		3
City of Rogue River	6	5		2
City of Roseburg	6			
Eugene Water & Electric Board	6	3		5
Glide Water Association	6			5
Hiland WC - Shady Cove	6	5		5
Jackson Co Pks Emigrant Lake	3			
Josephine Co Pks Lake Selmac 1	7	5	5	3
Josephine Co Pks Lake Selmac 2	6	4	6	3
Lake Oswego - Tigard Water Supply	6			
Lyons Mehama Water District	6			
Medford Water Commission	6	4		5
North Clackamas County Water Commission	6	1	1	2
PP&L-Toketee Village	6	2		
Salem Public Works	6	6		6
South Fork Water Board - Oregon City	6			1
Stayton Water Supply	6	6		6
Umpqua Basin Water Association	6			2
Umpqua Ranch Co-op	6			6
USFS Steamboat Work Center	6			6

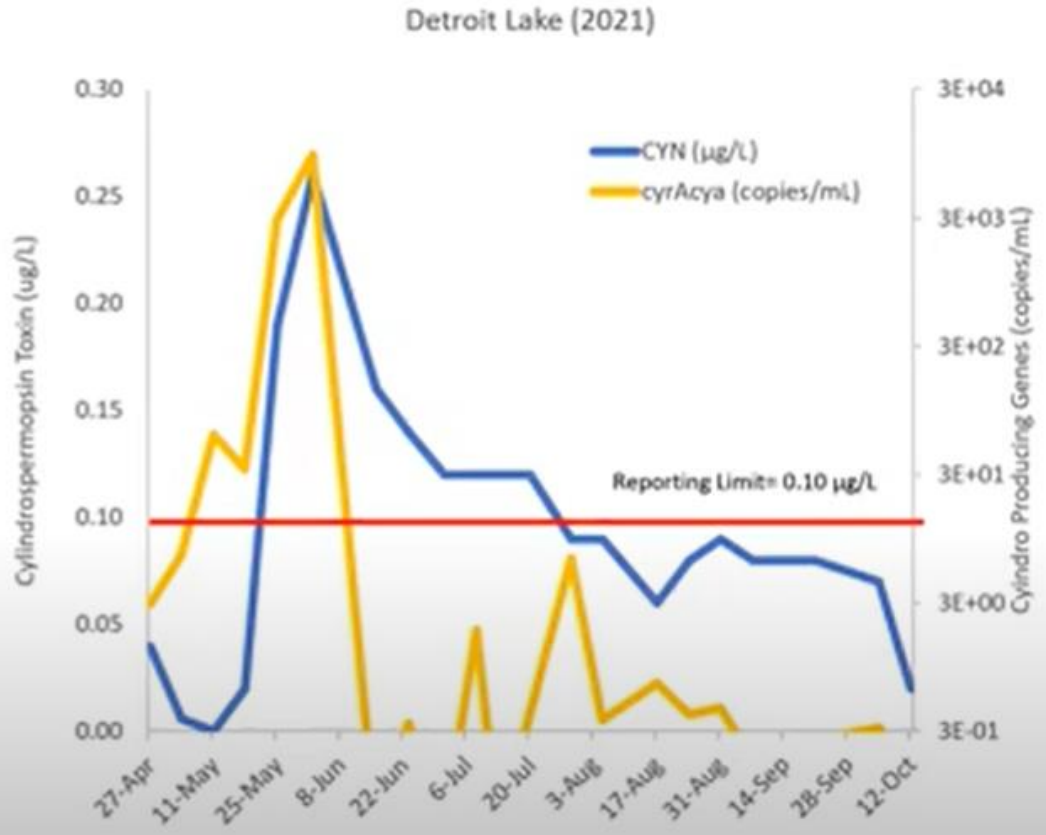
	16Scya	cyrA	mcyE	sxtA
Amplified in Sample	176 (100%)	75 (43%)	19 (11%)	84 (48%)
Mean Amplified copies/mL	110000	230	520	500



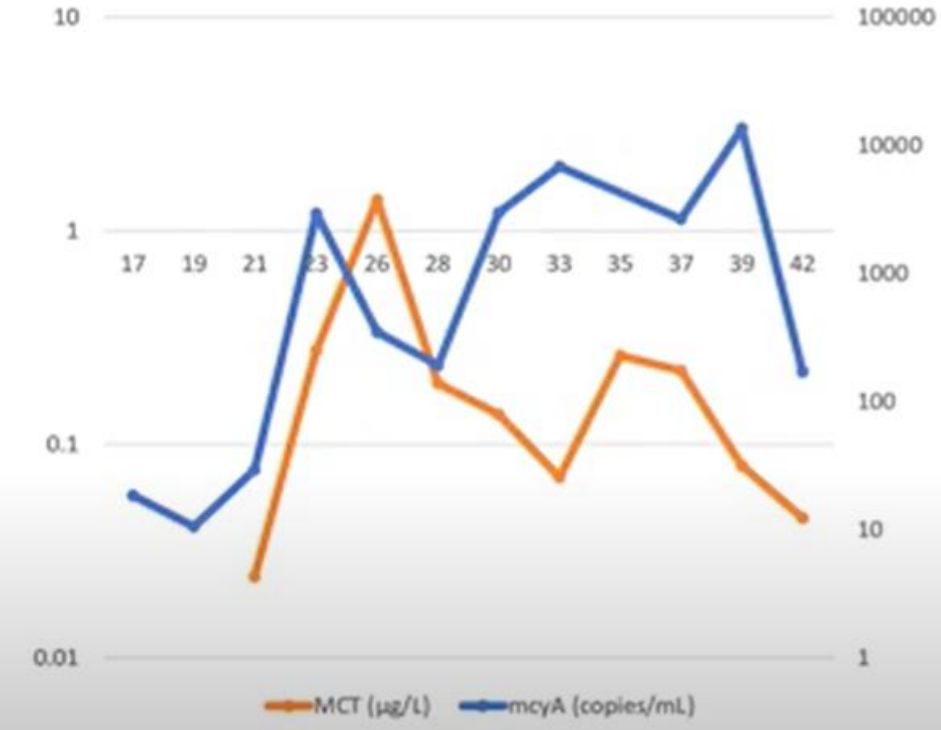
Timing is everything, sadly 2021 was quiet year But!!

Cylindrospermopsin in Detroit Lake - 2021

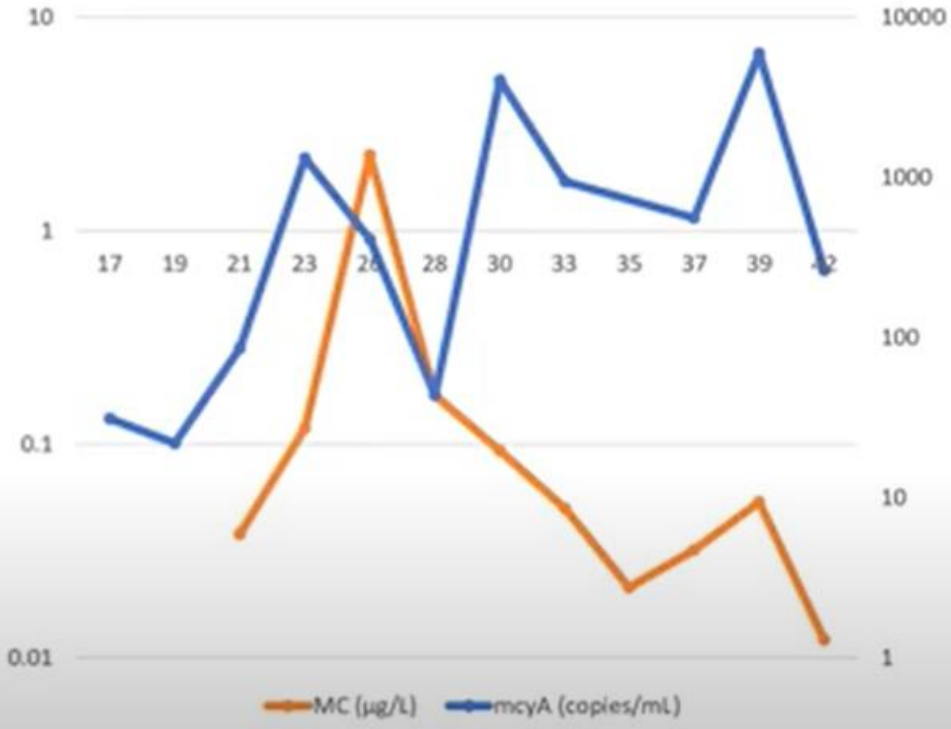
- Very low levels – essentially non-detect for toxins.
- qPCR is sensitive enough to detect the presence of genes prior to toxin detections.
- Filtering 100 mL source water should provide low enough detection limit when purifying samples.



Lake Billy Chinook – 2021 Microcystins and Genes

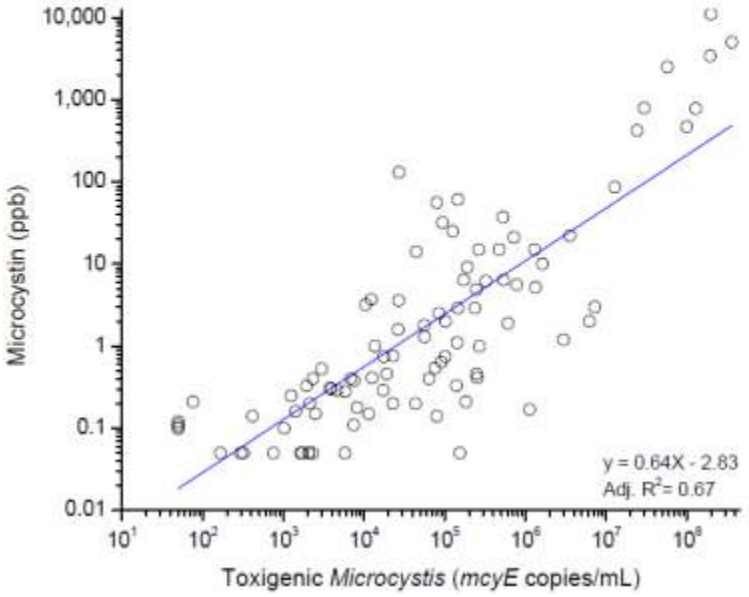


Cove Palisades Resort



Selective Water Withdrawal Tower

Figure 5. Relationship between microcystin-producing *Microcystis* (*mcyE*) and microcystin concentration. Concentrations below the reporting limits were set at half the RL (0.05 µg/L for microcystin and 50 *mcyE* cell equivalents/mLfor QPCR).

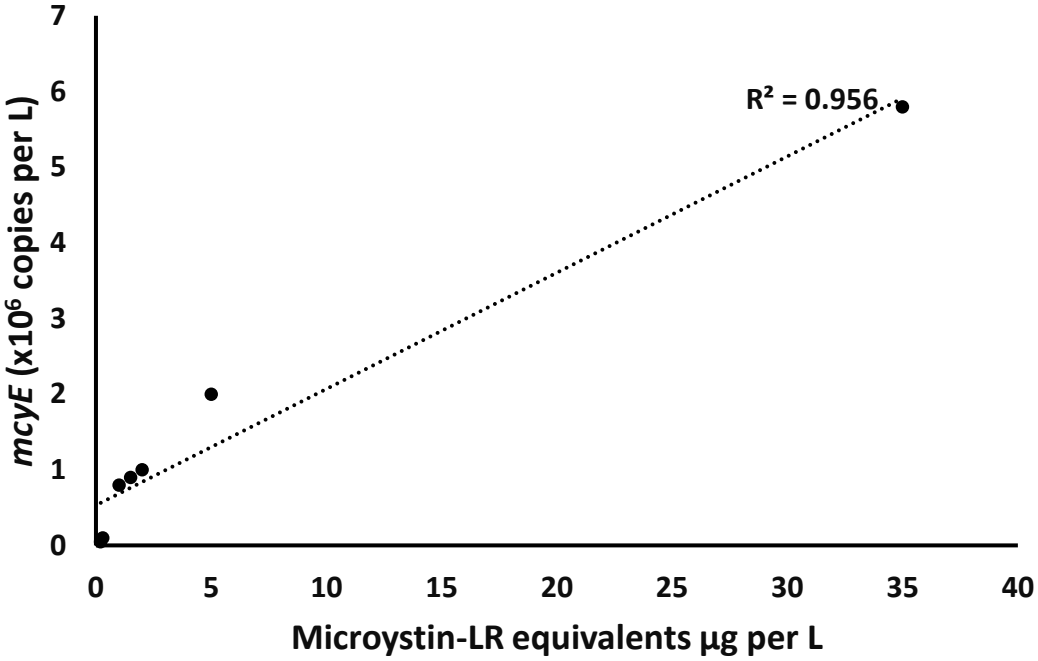


Application of genetic tools for improved cyanobacterial bloom monitoring in the Klamath River system: Implications for public health monitoring

Pacific Corp funded study Oregon 2017

Prepared by: Tim Otten, PHD, MPH
Bend Genetics, LLC
4/12/17

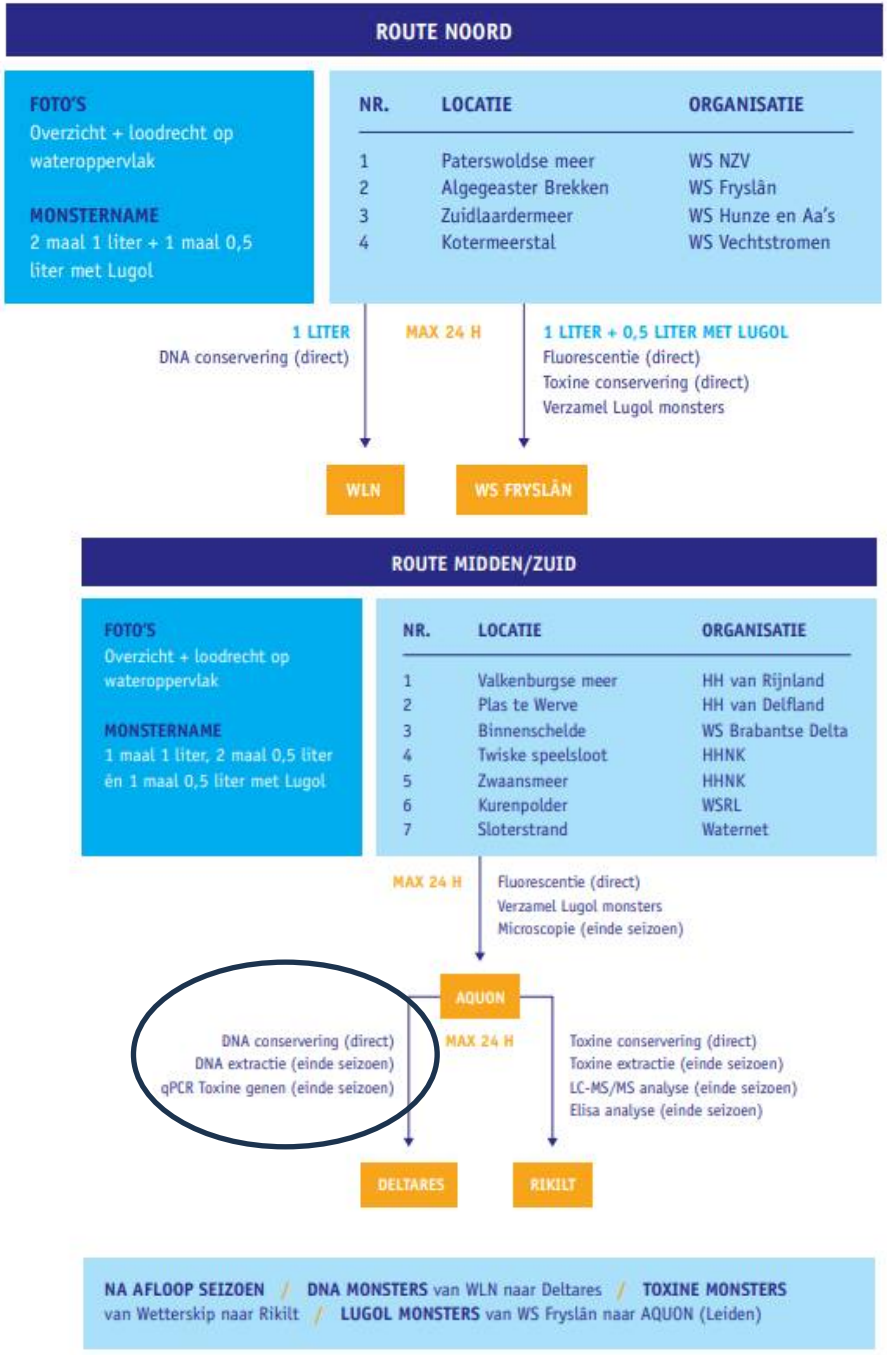
mcyE copy number correlates with MCs and total N



Kramer BJ, Davis TW, Meyer KA, Rosen BH, Goleski JA, et al. (2018) Nitrogen limitation, toxin synthesis potential, and toxicity of cyanobacterial populations in Lake Okeechobee and the St. Lucie River Estuary, Florida, during the 2016 state of emergency event. PLOS ONE 13(5): e0196278. https://doi.org/10.1371/journal.pone.0196278
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0196278

The Netherlands

Risk assessment blue-green algae in bathing water



TABEL 2.1 BLAUWALGGEVOELIGE PLASSEN DIE BEMONSTERD ZIJN TIJDENS ZWEMSEIZOEN 2019

Nr.	Naam	Organisatie	Contactpersoon
1	Paterswoldse meer	WS Noorderzijlvest	Jannes Schenkel
2	Plas te Werve	HH van Delfland	Rob Bovelander
3	Binnenschelde	WS Brabantse Delta	Guido Waajen
4	Twiske Speelsloot	HH Hollands Noorderkwartier	Saskia Zierfuss
5	Zwaansmeer	HH Hollands Noorderkwartier	Saskia Zierfuss
6	Oudegaasterbrekken	Wetterskip Fryslân	Richard Feenstra
7	Zuidlaardermeer	WS Hunze en Aa's	Hermen Klomp
8	Kurenpolder	WS Rivierenland	Arnold Osté
9	Valkenburgse meer	HH van Rijnland	Johan Oosterbaan
10	Kotermeerstal	WS Vechtstromen	Alberta Groteboer
11	Sloterstrand	Waternet	Joost Stoffels

De protocollen die gevolgd zijn in dit project zijn:

- **Fluoroprobe**: AQUON B HYB 009 - FluoroProbe - v-001.a
- **Filtratieprotocol tbv toxinebepaling**: RIKILT Filtration water samples for Stowa/Aquon
- **Biovolume**: AQUON V HYB 008 - Kwantitatieve bepaling van de celdichtheid en biovolume van potentieel toxische blauwalgen en AQUON F V HYB 008-06 - Kwantitatieve analyse telaf-spraken potentieel toxische blauwalgen - v-001
- **DNA extractie**: 250 ml oppervlaktewater is gefiltreerd over een 0,22uM membraanfilter, waar-van microbieel DNA is geïsoleerd met behulp van DNeasy PowerBiofilm van Qiagen (Conform NEN6254)
- **DNA analyse**: De aanwezige toxine genen in het geïsoleerde DNA zijn gekwantificeerd met Phytoxigene™ CyanoDTec kit (Diagnostic Technology, Australia) volgens het meegeleverde protocol
- **Toxine extractie**: De filters zijn geëxtraheerd zoals beschreven in de genoemde SOP's (zie Toxine analyse LC-MS/MS)
- **Toxine analyse LC-MS/MS**: LC-MS/MS conform SOP A1167 voor microcystines en nodularine. LC-MS/MS conform SOP A1282 voor anatoxines en cylindrospermopsines
- **Toxine analyse ELISA**: microcystine-ADDA ELISA kit van Enzo en Anatoxin-a ELISA kit van Abraxis

Risk assessment blue-green algae in bathing water; Stowa, Sept 2020

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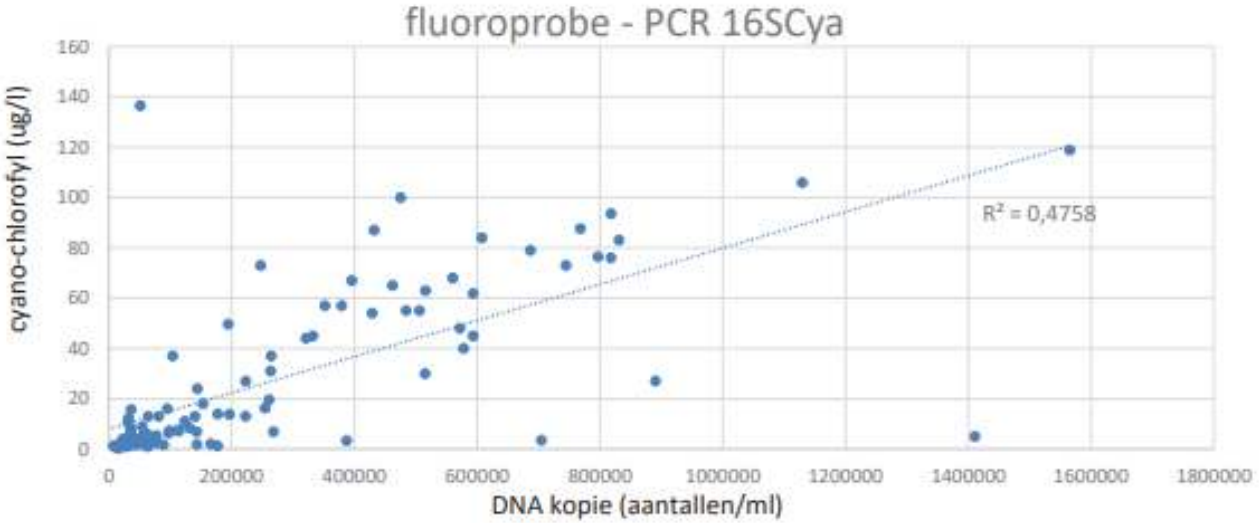
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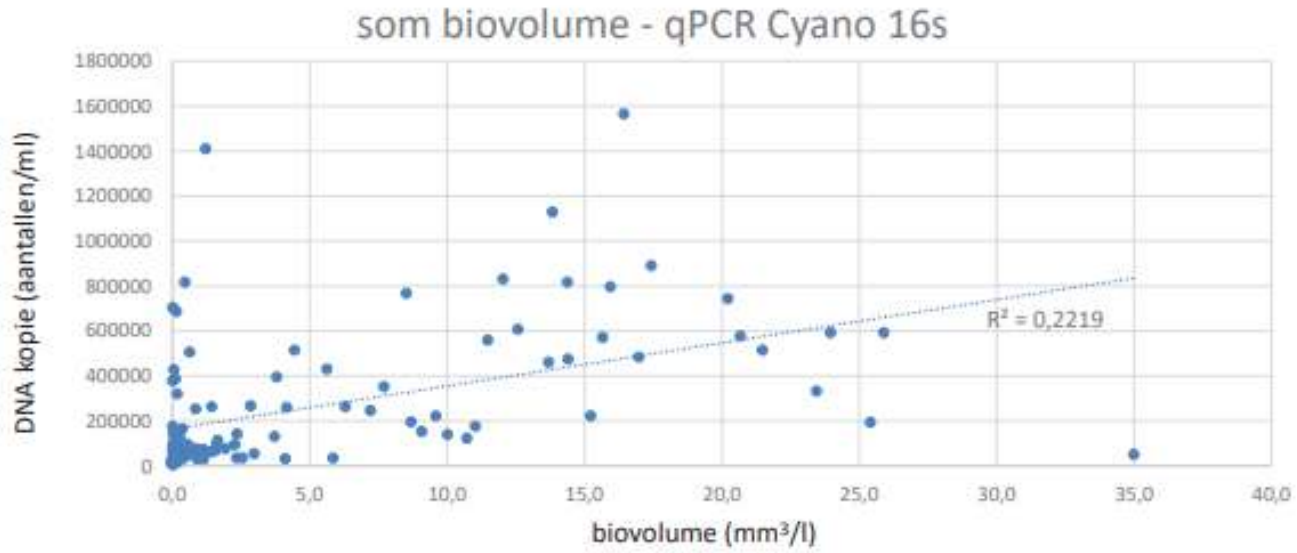
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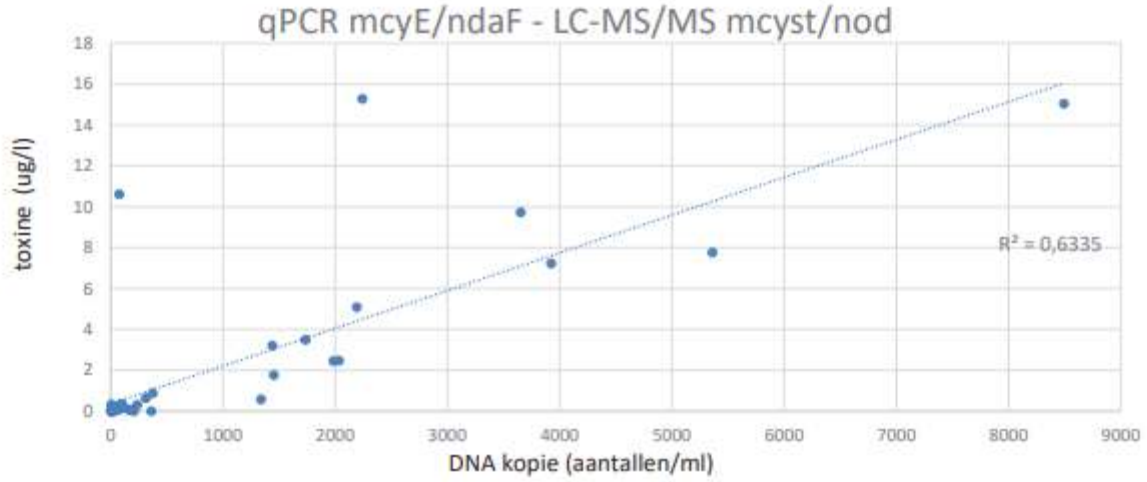

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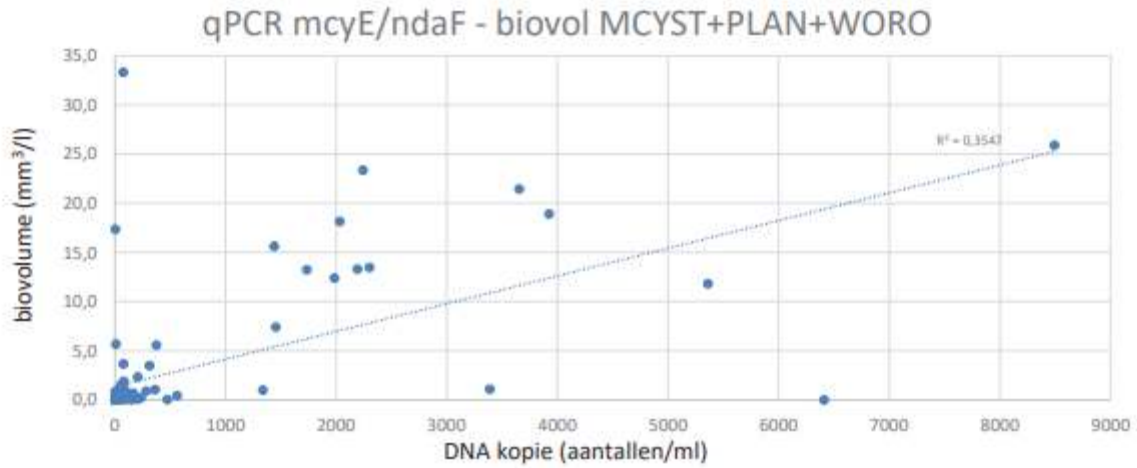

Risk assessment blue-green algae in bathing water; Stowa, Sept 2020

TABEL 3.2 *Pearson's correlaties (r) en significantie van de correlatie (p) tussen verschillende technieken. De hoge en zeer hoge correlaties zijn dikgedrukt. Een correlatie is significant wanneer p<0,05.*

	Biovolume	Celaantal	cyanobacteriën PCR Cyano	toxinegenen PCR mcyE	toxine Elisa Mcyst	toxine LC-MS/MS Mcyst
Cyanochlorofyl (fluoroprobe)	0,64* (p=0,00)	0,67* (p=0,00)	0,69 (p=0,00)	0,18 (p=0,07)	0,21 (p=0,15)	0,26** (p=0,07)
Biovolume (microscopie mcyst prod)		**0,95*** (p=0,00)	0,44* (p=0,00)	0,60 (p=0,00)	**0,73** (p=0,00)	**0,91** (p=0,00)
Celaantal (microscopie mcyst prod)			0,44* (p=0,00)	0,59 (p=0,00)	**0,72** (p=0,00)	**0,90** (p=0,00)
Cyanobacteriën (PCR Cyano)				0,35 (p=0,00)	0,21 (p=0,14)	0,20** (p=0,16)
Toxinegenen (PCR mcyE)					**0,86** (p=0,00)	**0,80** (p=0,00)
Toxines (Elisa Mcyst)						**0,84** (p=0,00)

*0.00 < r < 0.30: nauwelijks of geen correlatie; 0.30 < r < 0.50: lage correlatie; 0.50< r < 0.70: middelmatige correlatie; 0.70 < r < 0.90: hoge correlatie; 0.90 < r < 1.00: zeer hoge correlatie. * voor deze relatie is gebruik gemaakt van de som van de biovolumes en celaantallen van alle potentieel toxische blauwalgen, ++ relatie met alle gemeten toxines.*

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Cyanotoxin-encoding genes as powerful predictors of cyanotoxin production during harmful cyanobacterial blooms in an inland freshwater lake: Evaluating a novel early-warning system

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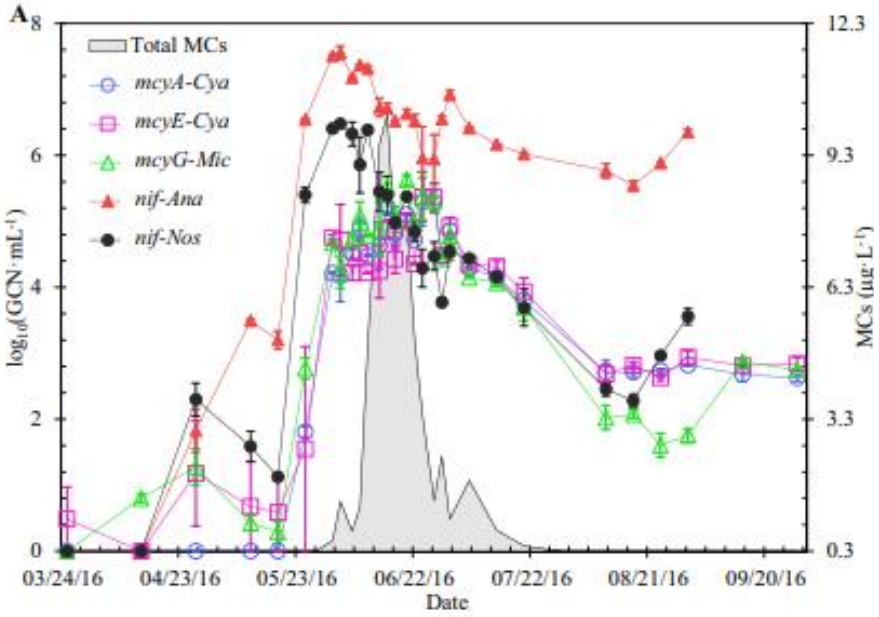
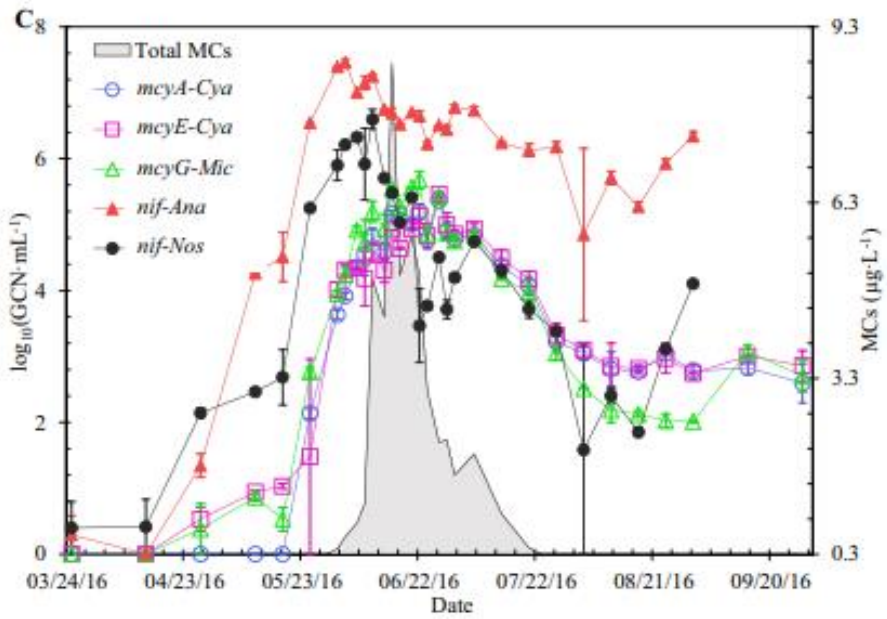
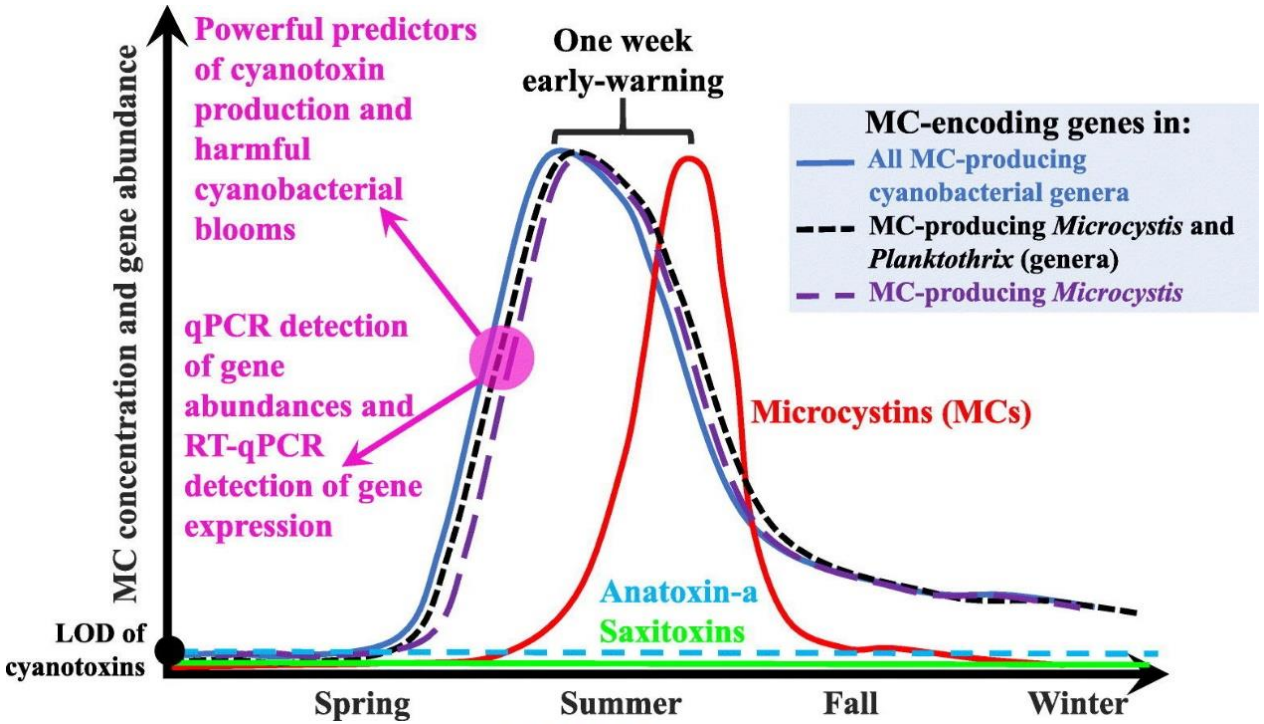




2022



ABSTRACT

Freshwater harmful cyanobacterial blooms (HCBs) potentially produce excessive cyanotoxins, mainly microcystins (MCs), significantly threatening aquatic ecosystems and public health. Accurately predicting HCBs is thus essential to developing effective HCB mitigation and prevention strategies. We previously developed a novel early-warning system that uses cyanotoxin-encoding genes to predict cyanotoxin production in Harsha Lake, Ohio, USA, in 2015. In this study, we evaluated the efficacy of the early-warning system in forecasting the 2016 HCB in the same lake. We also examined potential HCB drivers and cyanobacterial community composition. Our results revealed that the cyanobacterial community was stable at the phylum level but changed dynamically at the genus level over time. *Microcystis* and *Planktothrix* were the major MC-producing genera that thrived in June and July and produced high concentrations of MCs (peak level 10.22 $\mu g \cdot L^{-1}$). The abundances of the MC-encoding gene cluster *mcy* and its transcript levels significantly correlated with total MC concentrations (before the MC concentrations peaked) and accurately predicted MC production as revealed by logistic equations. When the *Microcystis*-specific gene *mcyG* reached approximately 1.5×10^3 copies$\cdot mL^{-1}$ or when its transcript level reached approximately 2.4 copies$\cdot mL^{-1}$, total MC level exceeded 0.3 $\mu g\ L^{-1}$ (a health advisory limit) approximately one week later (weekly sampling scheme). This study suggested that cyanotoxin-encoding genes are promising predictors of MC production in inland freshwater lakes, such as Harsha Lake. The evaluated early-warning system can be a useful tool to assist lake managers in predicting, mitigating, and/or preventing HCBs.

Duan et al; Sci Total Env, 2022

Proactively Monitoring Algal Blooms, Taste & Odor, and Cyanotoxins

J. Hunter Adams, M.S.
hunter.adams@wichitafallstx.gov

Wichita Falls TEXAS
Blue Skies. Golden Opportunities.

Integrated Toolkit

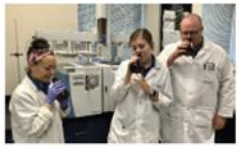
Cypress Environmental Laboratory Bloom Detection Workflow

Field testing

YSI EXO1 sondes: Thermal profiling measuring pH, DO, temperature, and phycocyanin

Lab analyses

On a need basis based on qPCR



TON/FPA: Classify odor and intensity

FlowCam: Identify organisms, plus count, and concentration

Thermo GC–MS/ECD: Quantify MIB/geosmin and others

Phytoxigene qPCR: Quantify 16S rRNA gene and toxin-producing genes

DO—dissolved oxygen, ECD—electron capture detector, FPA—flavor profile analysis, GC—gas chromatography, MIB—2-methylisoborneol, MS—mass spectrometry, qPCR—quantitative polymerase chain reaction, rRNA—ribosomal RNA, TON—threshold odor number, WTP—water treatment plant

Hunter Water

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Monitoring Cyanobacteria in Grahamstown Dam

Evaluating Hunter Water's actions to ensure drinking water remains safe

V Shah, D Turner, C Hancock, P O'Donoghue, A Sneddon, J Stanmore, A Morrow, A Lundmark, J Bates, A Hanson

INTRODUCTION

Grahamstown Dam (capacity about 182,000 ML), Hunter Water's largest dam, is a broad, relatively shallow, man-made, off-river storage that is primarily used to store water extracted from the Williams River. The Dam also receives runoff from its own small 73 km^2 catchment area and direct rainfall on its 28 km^2 surface area. The key components of the Grahamstown Dam supply scheme are Seaham Weir (limits the upstream movement of tidal saltwater), Balickera Canal and pumping station (transfer water from the Williams River to Grahamstown Dam), Campvale Pumping Station (pumps run off from the developing Medowie area located on the eastern margins of the Dam), George Schroder Pumping Station and delivery mains (delivers water from the Dam to water treatment plant), and Grahamstown Water Treatment Plant (WTP).

Routine algae monitoring locations within Grahamstown Dam catchment are the Dam's key northern (R2), middle (R12) and southern (R6) locations, Campvale Pump Station (R9) and Boags Hill at Seaham Weir (R1) (Figure 1). The raw water offtake is located near the Dam's key southern monitoring location R6. To protect drinking water quality, public access to Grahamstown Dam is minimised for recreational purposes.

Keywords: Grahamstown Dam, Dolichospermum, Microcystis, algae toxins, PhytoxigeneTM, geosmin, PAC dosing, water quality incident, operational response

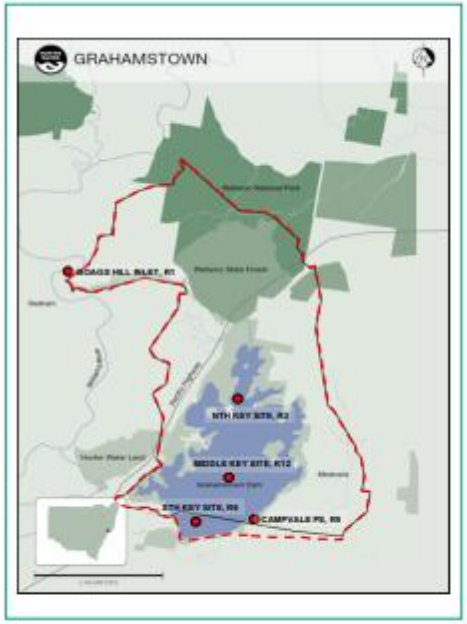


Figure 1: Key Routine Monitoring Sites in Grahamstown Dam Catchment

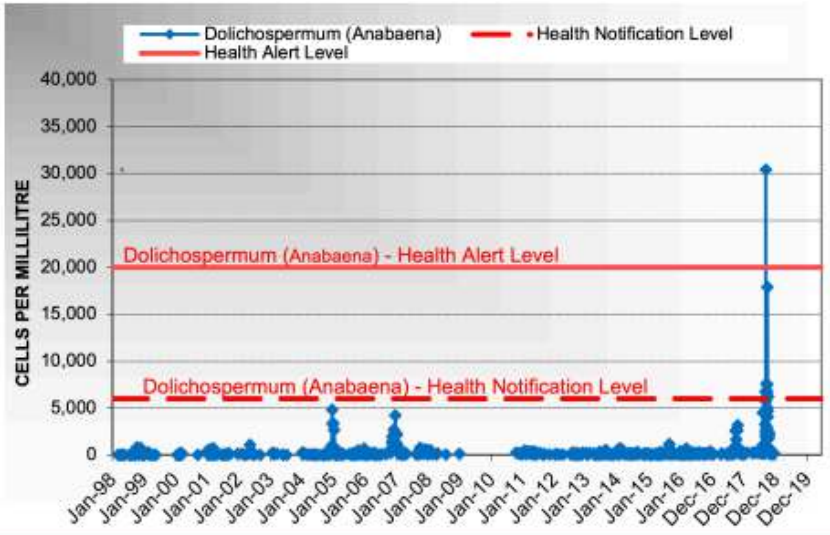
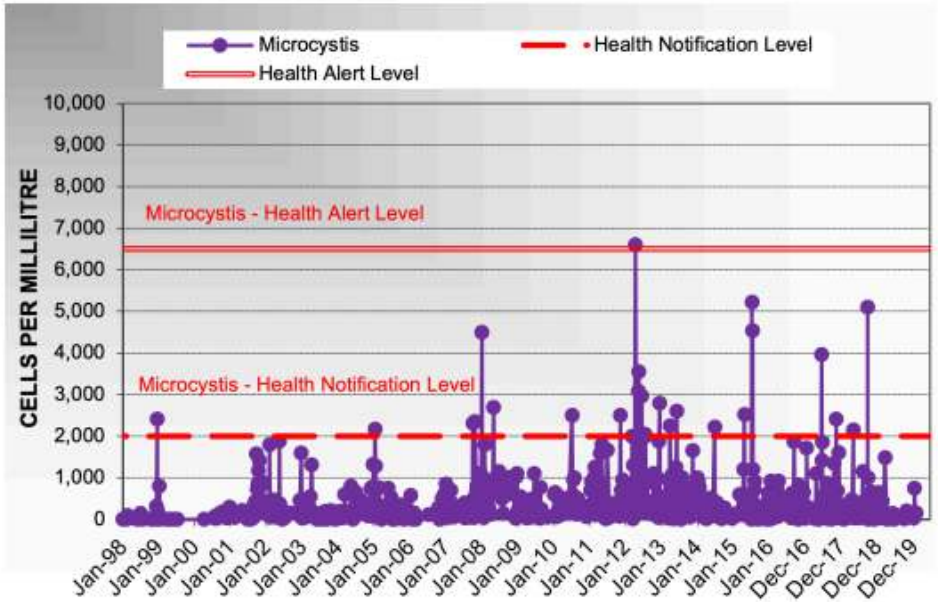


Figure 4: Historical Dolichospermum Levels in Grahamstown Raw Water

A total of 46 algae scum and water samples collected from Grahamstown Dam (three routine key monitoring locations and Schroder Pump Station at raw water offtake area) and Grahamstown WTP raw water, backwash recovery and treated water were analysed for algae toxins (Anatoxin-a, Cylindrospermopsin, Deoxycylindrospemopsin, Microcystin-RR, Microcystin-YR, Microcystin-LR, Microcystin-Total, Nodularin, Saxitoxin, Neosaxitoxin, Saxitoxin GTX2 & 3, Saxitoxin GTX1 & 4, Saxitoxin C1 & C2, and Saxitoxin dcGTX2&3) throughout the incident. No toxins were detected in any of these samples. Potential for productions of Cylindrospermopsin gene cyrA and Saxitoxin gene sxtA was not detected by Phytoxigene™ in any of the 10 samples analysed (including the 'worst' sample i.e. highest algae counts for algae scum sample during this incident).



However, 2200 gene copies/mL of Microcystin/Nodularin gene mcyE were detected in the 'worst' algae scum sample collected from southern shoreline of dam. This sample had *Dolichospermum* and *Microcystis* counts (cells/mL) of 2,903,700 and 6500 respectively. This sample was also analysed for algae toxins but none were detected. Together with decreasing algae levels in the raw water, this indicated a low likelihood of toxin production for the remainder of the incident. Hunter Water then adopted the approach of conducting algae toxin testing on an ad-hoc basis in any samples over the ADWG health notification and alert levels and from any algae scums observed.

Figure 5: Historical Microcystis Levels in Grahamstown Raw Water

Environ Monit Assess (2017) 189: 202
DOI 10.1007/s10661-017-5916-4

Use of three monitoring approaches to manage a major *Chrysosporum ovalisporum* bloom in the Murray River, Australia, 2016

Adam Crawford · Jon Holliday · Chester Merrick · John Brayan · Mark van Asten · Lee Bowling

Received: 25 October 2016 / Accepted: 23 March 2017 / Published online: 31 March 2017

qPCR results

Seventy-one samples from sites located throughout the river systems were tested by qPCR. Although 36 of these samples gave a positive result for *cyrA* gene presence, all but two of these were below the level of quantification (mostly 450 copies ml^{-1}). Most of the positive samples came from the Murray River between Albury and Moama collected in February and March, and from sites on the Edward-Wakool-Niemur River

Toxin analysis

Toxin analysis was undertaken on samples collected from nine Murray River sites between Albury and Barham and five sites on the Edward-Wakool-Niemur River system in early March and again in early April. The concentrations of microcystin, saxitoxins and cylindrospermopsin were all below the level of detection in all samples, these being 0.01, 1.0 and 0.01 $\mu g\ l^{-1}$ for MCY, STX and CYN, respectively.

measure of the abundance of toxin-producing cyanobacteria present (Pacheco et al. 2016), so the general low occurrence of *cyrA* genes in the Murray River samples tested indicated that the bloom was generally incapable of the cylindrospermopsin production typical of many other *Chrysosporum ovalisporum* blooms (Cirés and Ballot 2016). Likewise, the absence or low copy numbers of *mcyE* and *stxA* genes indicated that the bloom was also unlikely to produce microcystins or saxitoxins, although Lee et al. (2015)

Management Implications

The three monitoring methods used for the response management during the 2016 Murray River bloom can complement each other when high biovolume blooms occur which are not necessarily toxic and can be combined in a three tiered monitoring strategy. Firstly routine monitoring using microscopic identification and enumeration will provide information on bloom development, including species composition and abundance or biovolume, and is unlikely to be replaced. Once this monitoring detects blooms of high biovolume, especially of potentially toxic species, **qPCR can then be employed to assess the potentially toxigenicity of the bloom. Thirdly, if high copy numbers of cyanotoxin biosynthesis genes are detected**, analysis of water samples for toxin presence can then be conducted. Such a strategy would mean that some relatively expensive analyses, such as toxin analysis, can be reduced, lowering overall bloom management costs

Crawford et al, Environ Monit Assess 2017

Harmful Algae 127 (2023) 102476

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journal homepage: www.elsevier.com/locate/hal

A microcystin synthesis *mcyE/ndaF* gene assay enables early detection of microcystin production in a tropical wastewater pond

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[b] Power and Water Corporation, Water Services, P.O. Box 37471, Winnellie, NT, Australia

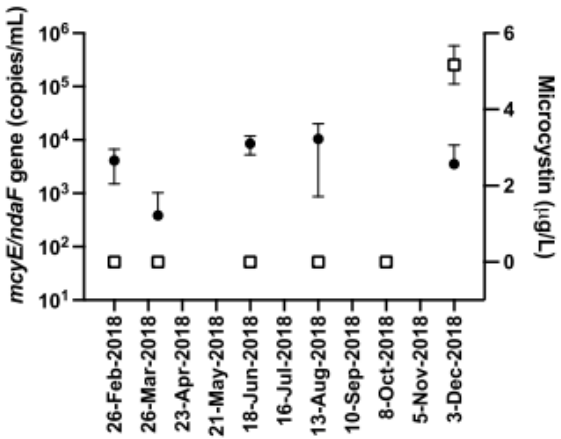


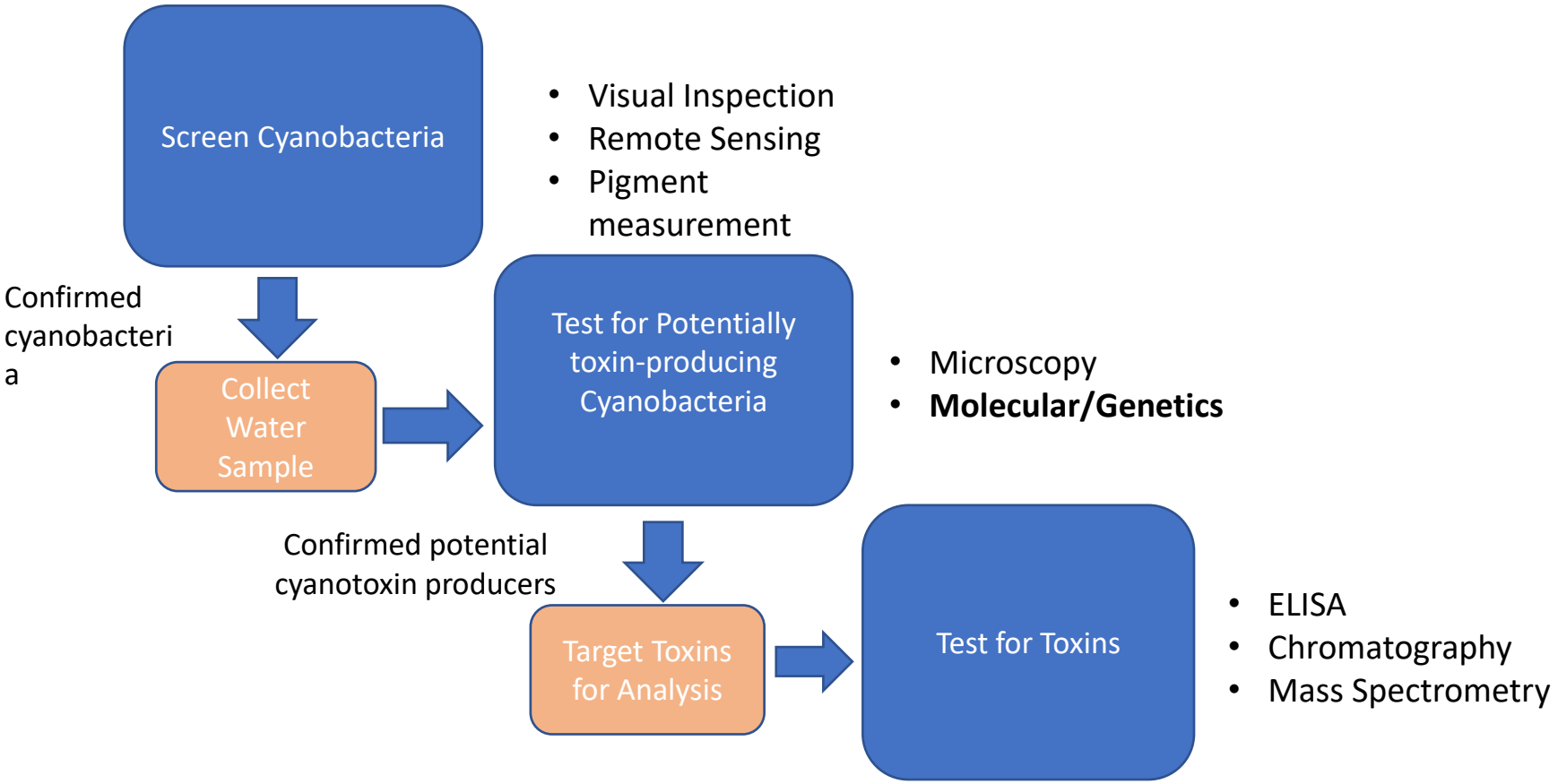
Fig. 2. Concentration of the *mcyE/ndaF* gene (●) and microcystin toxin (£) in effluent in 2018.

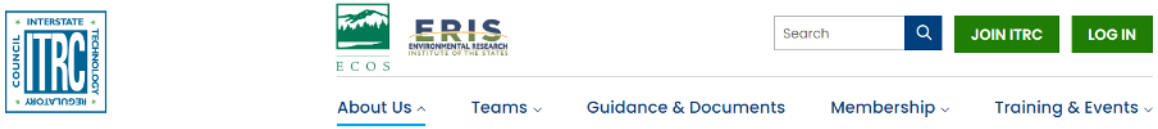
ABSTRACT

Cyanobacteria can dominate the algal community in wastewater ponds, which can lead to the production of cyanotoxins and their release into the environment. We applied traditional and molecular techniques to identify cyanotoxin hazards and high-risk periods in a tropical wastewater treatment system. Potentially toxic cyanobacteria were identified by microscopy and amplicon sequencing over the course of a year. Toxin gene levels were monitored and compared to toxin production to identify likely toxin producing species and high-risk periods. Cyanobacteria were persistent in the effluent year-round, with *Planktothrix* and *Microcystis* the most abundant genera; *Microcystis* could not be resolved beyond genus using amplicon sequencing, but *M. flos-aquae* was identified as a dominant species by microscopy. Microcystin toxin was detected for the first time in treated effluent at the beginning of the wet season (December 2018), which correlated with an increase in *Microcystis* amplicon sequence abundance and elevated microcystin toxin gene (*mcyE/ndaF*) levels. Concomitantly, microscopy data showed an increase in *M. flos-aquae* but not *M. aeruginosa*. These data informed a refined sampling campaign in 2019 and results showed a strong correlation between *mcyE/ndaF* gene abundance, microcystin toxin levels and *Microcystis* amplicon sequence abundance. Microscopy data showed that in addition to *M. flos-aquae*, *M. aeruginosa* was also abundant in February and March 2019, with highest levels coinciding with toxin detection and toxin gene levels. *M. aeruginosa* was the most abundant *Microcystis* species detected in selected treated effluent samples by metagenomics analysis, and elevated levels coincided with toxin production. All microcystin genes in the biosynthesis pathway were detected, but microcystin genes from *Planktothrix agardhii* were not detected.

Gene toxin assays were successfully used to predict microcystin production in this wastewater system. Changes in amplicon sequence relative abundance were a useful indicator of changes in the cyanobacterial community. We found that metagenomics was useful not just for identifying the most abundant *Microcystis* species, but the detection of microcystin biosynthesis genes helped confirm this genus as the most likely toxin producer in this system. We recommend toxin gene testing for the early detection of potential toxin producing cyanobacteria to manage the risk of toxicity and allow the implementation of risk management strategies.

Generalised monitoring steps used to evaluate risk from cyanobacteria exposure; Source ITRC





The Interstate Technology and Regulatory Council (ITRC) is a state-led environmental coalition working to create innovative solutions and best management practices. ITRC produces documents and training that broaden and deepen technical knowledge and expedite quality regulatory decision making while protecting human health and the environment

Summary

- There’s good data across the globe, reproducible
- Technology is referenced in guidance documents as an option
- But there is a lack consensus for use and interpretation
  - Public Water Systems
  - Recreational Water
- Lack of momentum for change (only when a crisis happens)
- Conversion as principle monitoring test some time away