



Cyanotoxins and Drinking Water Quality: Treatment Options

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Overview Drinking Water Treatment

- Treatment to remove intracellular algal toxins
 - Conventional treatment
 - Filtration
 - Membrane technologies
- Treatment to remove extracellular algal toxins
 - Oxidation
 - Physical removal
 - Biologically active filters



Understanding microorganism and chemical removal/inactivation

- Living organisms
 - Nonviable
 - Removal
- Chemical Contaminants
 - Adsorption
 - High Pressure Membrane Filtration
 - Degradation/Biodegradation

Source Water

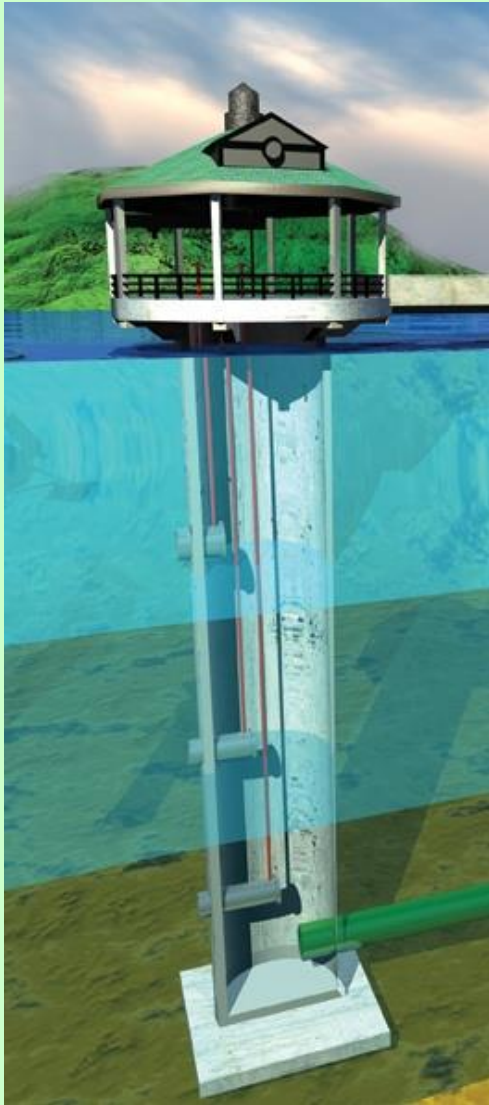


Photo courtesy of John Lehman, University of Michigan

- Intracellular Toxin
 - Flushing
 - Harvesting
 - Diversion
 - Flocculants
 - Algaecides (low levels)
 - Ultrasound

- Extracellular Toxin
 - Awareness and get ready to treat

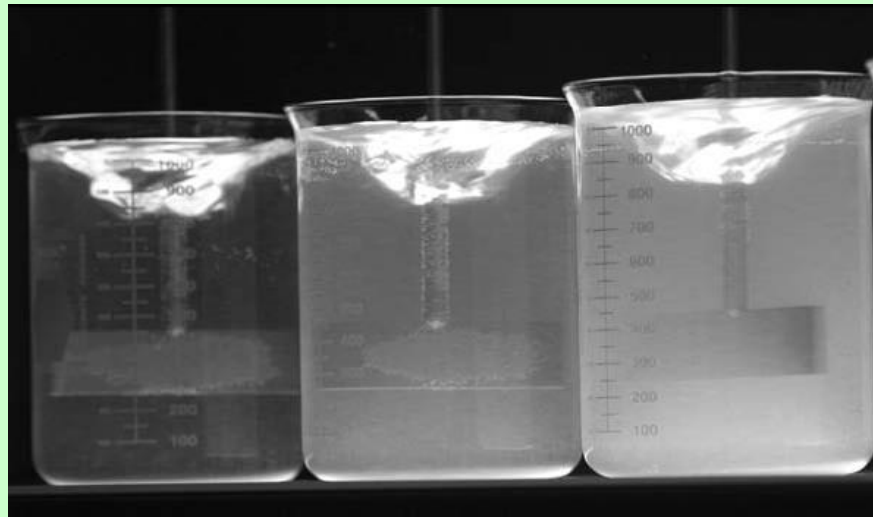
Intake



- Intracellular Toxin
 - Adjustable Intake
 - Night vs Day
- Extracellular Toxin
 - Oxidants
 - Inline Powdered Activated Carbon (PAC)
- A conventional treatment plant will want to keep the cells intact.

Powdered Activated Carbon

- Wood-based PAC is more effective than coconut based and bituminous PACS in the removal of microcystins
- Jar Test
- Pre-chlorination is not recommended before the use of PAC

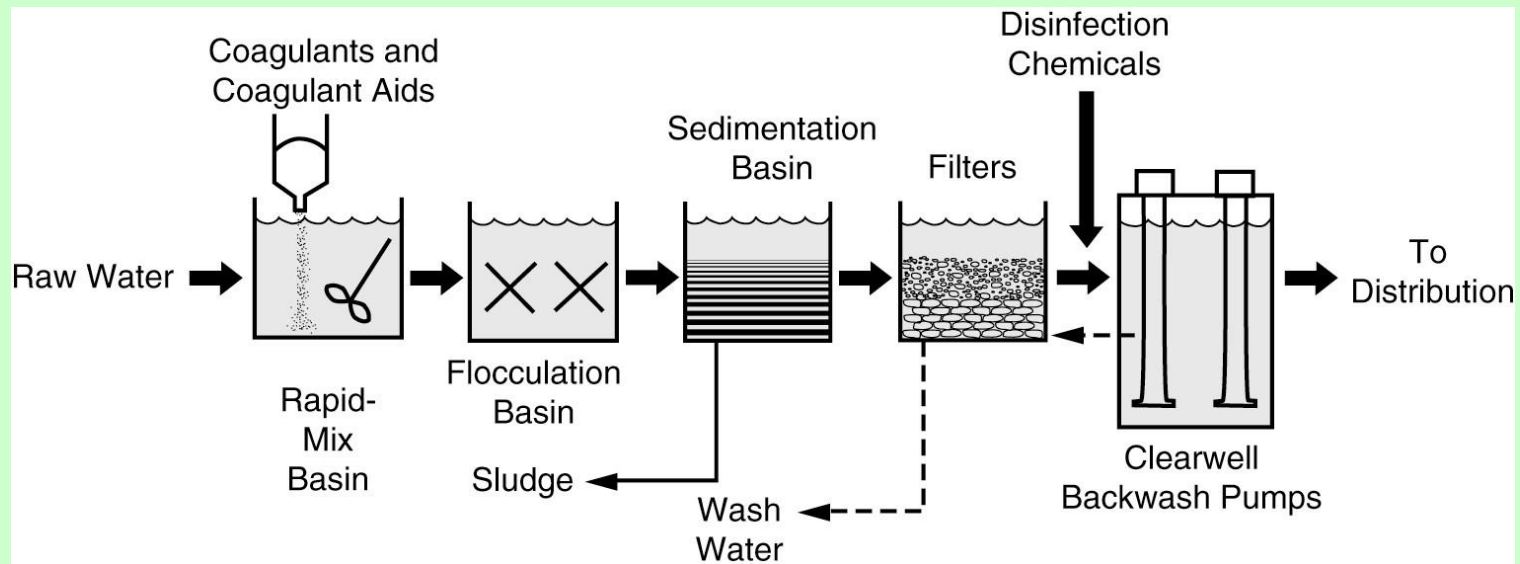


Summary of Intact Algal Cell Removal Performance.

Treatment	Intact Cell Removal
Coagulation/sedimentation or dissolve air flotation /rapid sand filtration	> 99.5% auxiliary
Lime precipitation/sedimentation/rapid sand filtration	> 99.5 % ancillary
Microfiltration/Ultrafiltration	> 75% (becoming auxiliary)

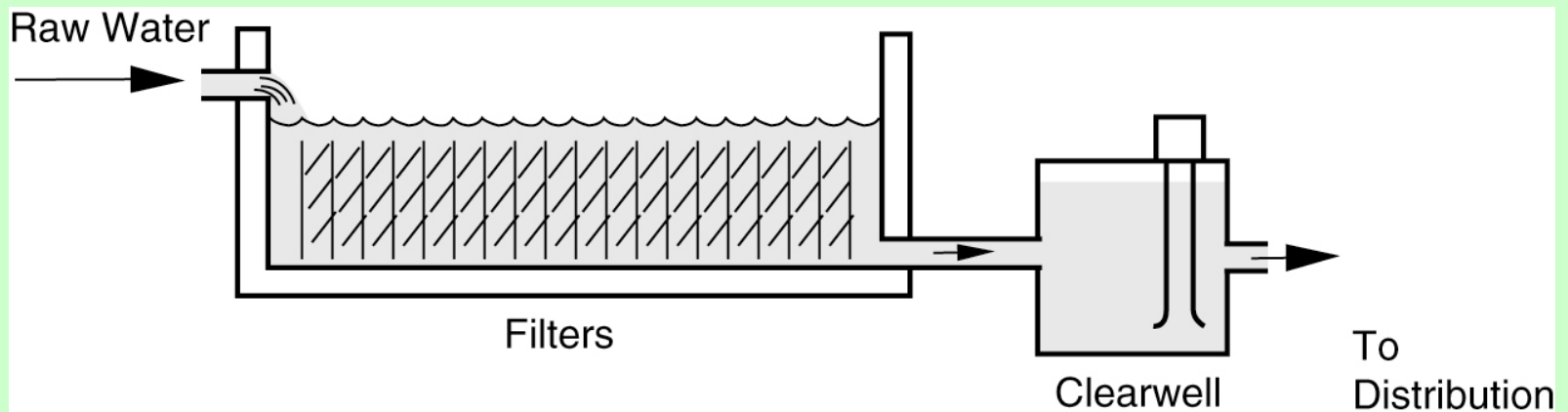
Coagulation/Sedimentation

- Intracellular Toxin
 - Oxidants (not often used, afraid of lysing cell)
 - Flocculent aides
 - Settled water with less than 100 units algae/mL
- Extracellular Toxin
 - Activated Carbon
 - Powder (PAC)
 - Granular (GAC)
 - Filtration
 - Conventional
 - Biologically Active
- Monitoring Techniques to determine treatment
 - Turbidimeter
 - Streaming current detector
 - Particle Counter
 - Chlorophyll-a
 - Cell counts
 - ELISA
 - Saxitoxin, Anatoxin-a, Cylindrospermopsin, Microcystin
 - Plate, Test tube kit, Dip Stick



Filtration

- Conventional
- Biologically Active
- GAC
- Low Pressure Membrane



Ultrasonic Technology Treatment

Before Ultrasound



After Ultrasound



Low power ultrasound

Commercial
Sonic Solutions
LG Sonic

Typical operating
parameters

average 18 W
28 kHz

George Hutchinson, Opflow April 2008

Low power ultrasound

Tunable (79 frequencies)

Critical resonance (gas vesicles)

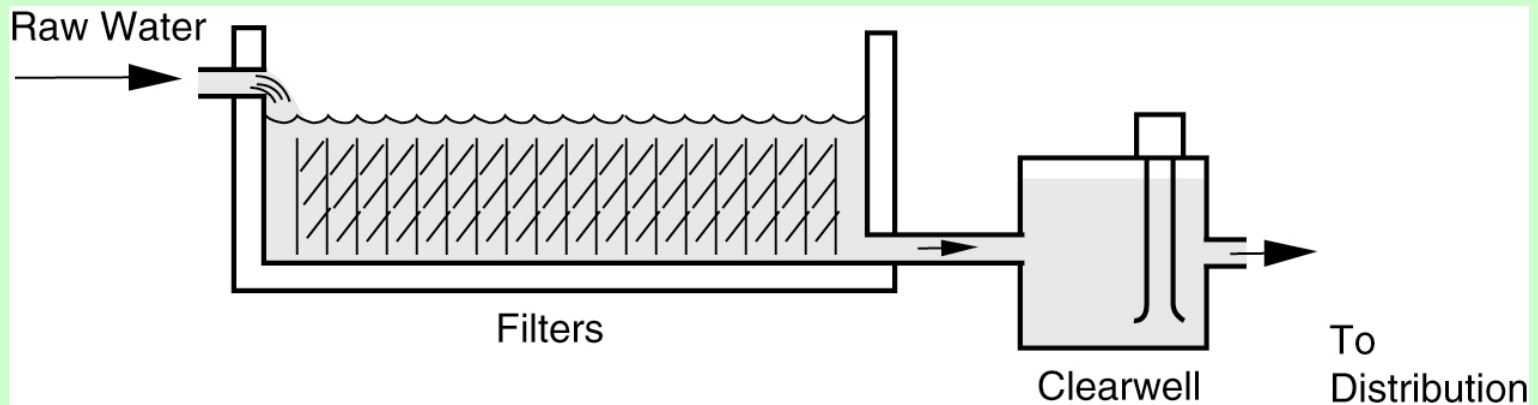
Cyanobacteria – *Microcystis*, *Anabaena*, *Lyngba* (Sonic Solutions)

Biologically active filters

- INTRACELLULAR TOXIN
- MCY-LR, MCY-LA, cylindrospermopsin, and anatoxin-a can be removed by biologically active sand and GAC filters
- Empty bed contact times-- 5 to 15 minutes.
 - Slow filtration
 - Rapid filtration
- Saxitoxin - not removed

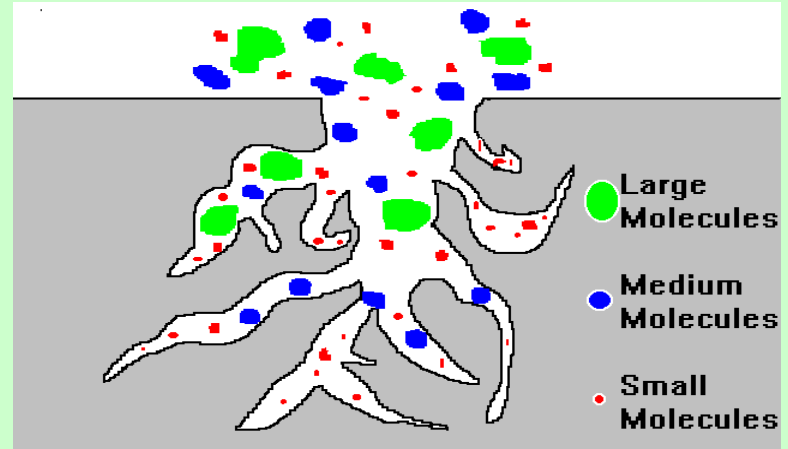
GAC filtration

- Effectiveness of GAC filtration against cyanotoxins is source water dependent
- Significant differences in adsorption between LA and LR
- Saxitoxins and anatoxin-a are more readily adsorbed than microcystins



Pore Size

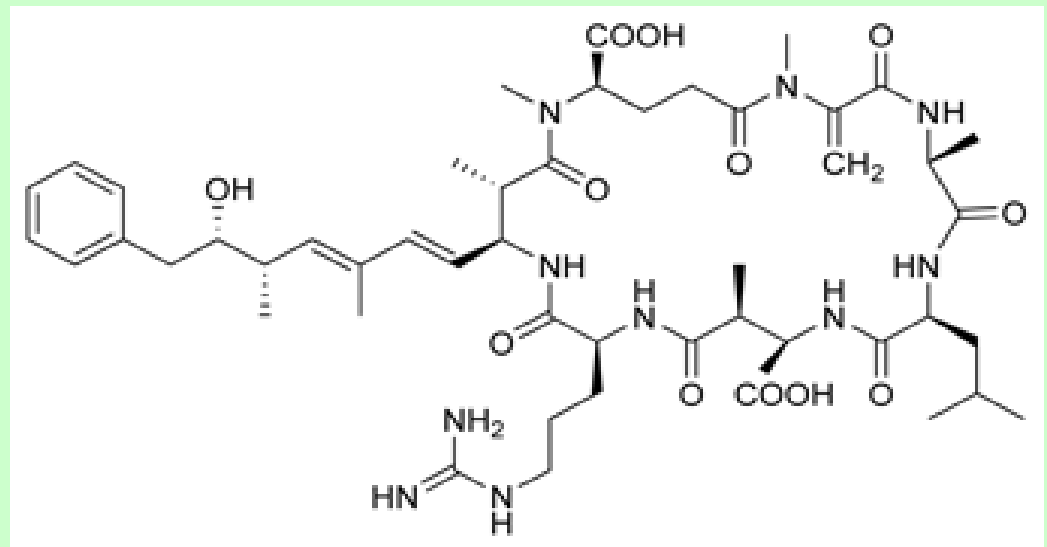
- Equilibrium
 - Micropore
 - Taste and odor
 - Industry spills, solvents
 - Anatoxin-a
 - Mesopore
 - Microcystins
RR>YR>LR>LA
 - Cylindrospermopsin
 - Saxitoxin



- Kinetic <1 hour contact time
- Large pore volume seems to be more effective

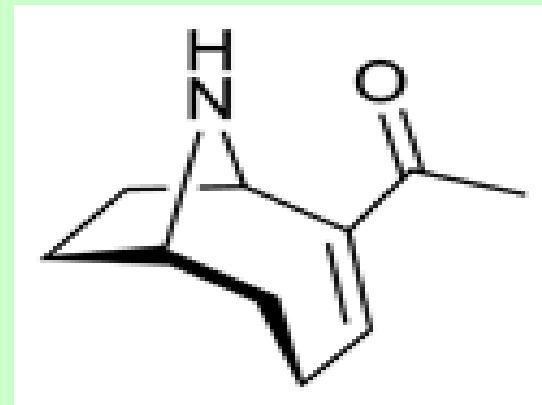
Summary of Oxidation Treatment Processes Extracellular Toxins

	Microcystin
Chlorine	Yes
Ozone	Yes
Chloramine	No
Chlorine dioxide	No
Hydroxyl radical	Yes
Potassium permanganate	Yes



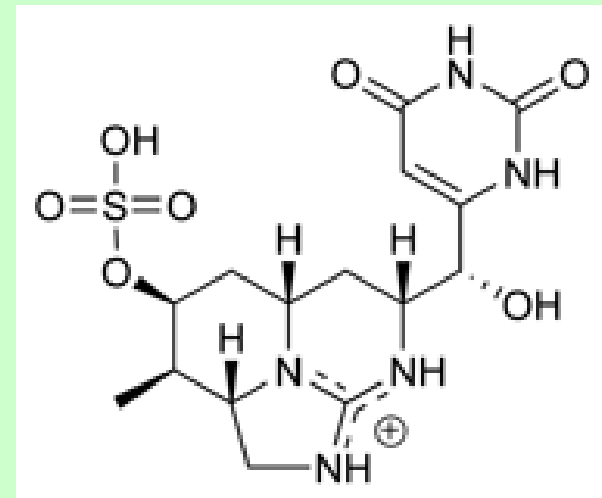
Summary of Oxidation Treatment Processes Extracellular Toxins

	Anatoxin-a
Chlorine	No
Ozone	Yes
Chloramine	No
Chlorine dioxide	No
Hydroxyl radical	Yes
Potassium permanganate	Yes



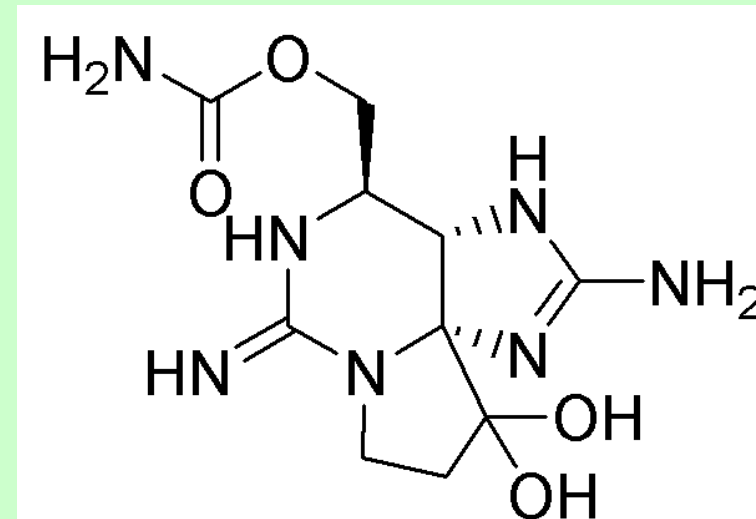
Summary of Oxidation Treatment Processes Extracellular Toxins

	Cylindrospermopsin
Chlorine	Yes
Ozone	Yes
Chloramine	No
Chlorine dioxide	No
Hydroxyl radical	Yes
Potassium permanganate	No



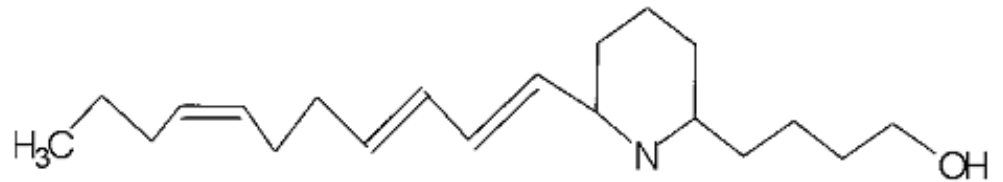
Summary of Oxidation Treatment Processes Extracellular Toxins

	Saxitoxin
Chlorine	Yes
Ozone	No
Chloramine	Has not been investigated.
Chlorine dioxide	Has not been investigated.
Hydroxyl radical	Has not been investigated.
Potassium permanganate	No



PREDICITON of Oxidation Treatment Processes Extracellular Toxins

	Euglenophycin
Chlorine	Yes?
Ozone	Yes?
Chloramine	No?
Chlorine dioxide	No?
Hydroxyl radical	Yes?
Potassium permanganate	Yes?



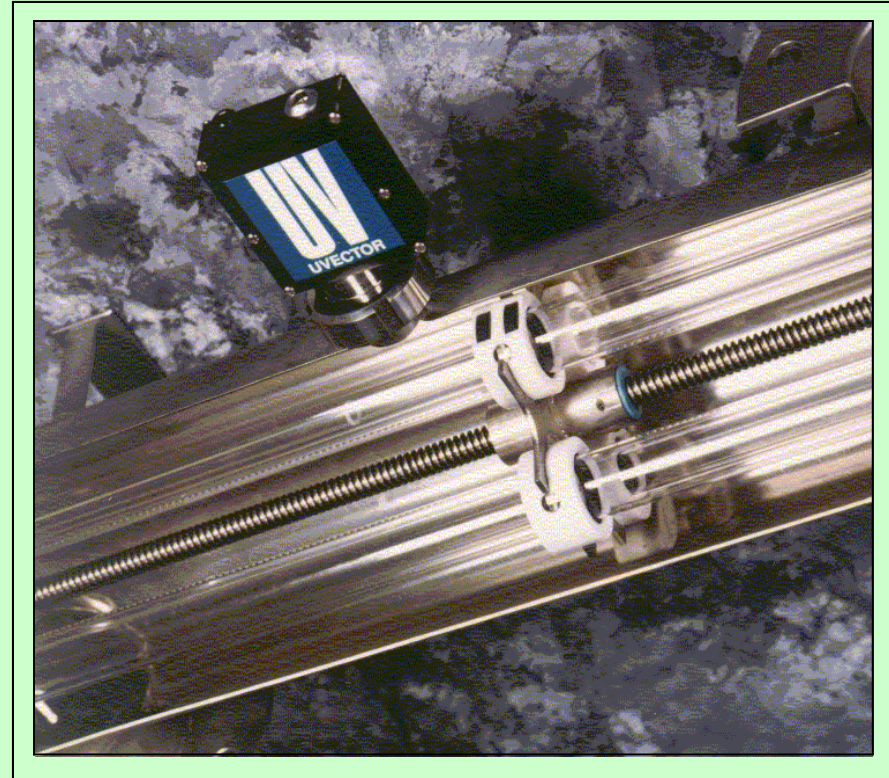
Chlorine CT values for reducing microcystin concentration to 1 $\mu\text{g l}^{-1}$ (Acero et al 2005)

pH	[MCLR] ₀ $\mu\text{g l}^{-1}$	CT-values, $\text{mg l}^{-1}\text{min}$			
		10°C	15°C	20°C	25°C
6	50	46.6	40.2	34.8	30.3
	10	27.4	23.6	20.5	17.8
7	50	67.7	58.4	50.6	44.0
	10	39.8	34.4	29.8	25.9
8	50	187.2	161.3	139.8	121.8
	10	110.3	94.9	82.3	71.7
9	50	617.2	526.0	458.6	399.1
	10	363.3	306.6	269.8	234.9

Compared to CT Values for Disinfectants to inactivate 99.9 (3-logs) of *Giardia Lamblia* cysts.

UV Treatment

- UV inactivation dose is about 40 mJ/cm^2 – inactivation of *Cryptosporidium parvum*.
- Photolytic destruction dose for microcystin, cylindrospermospin, anatoxin-a and saxitoxin is 1530 to 20,000 mJ/cm^2 .



Photolysis and Advanced Oxidation Processes

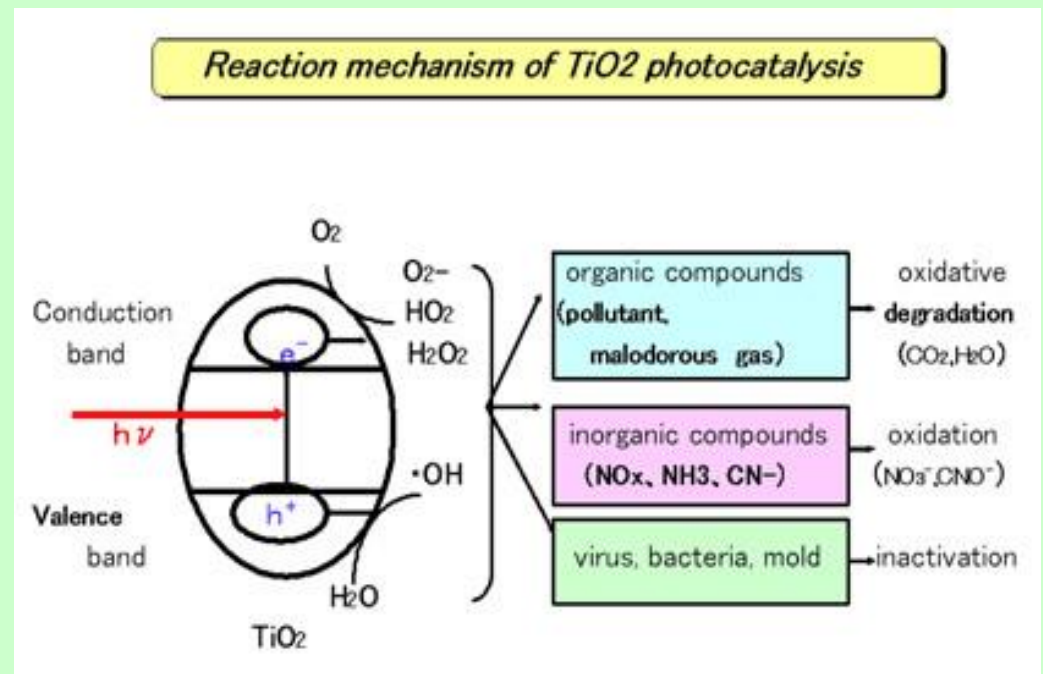
- Photolysis
- UV/H₂O₂
- Fenton Reagent
- Radiolysis
- Ultrasonic degradation
- TiO₂ photocatalysis
- Ferrate

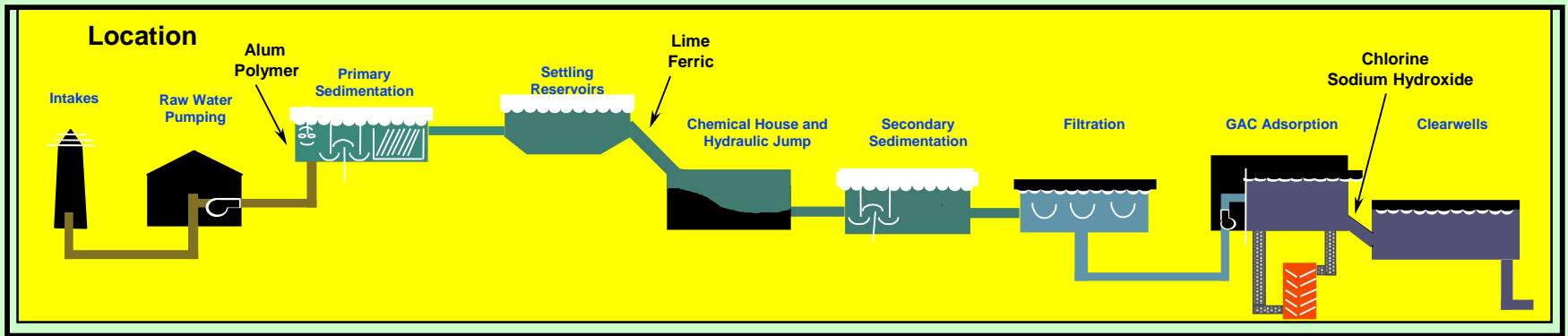
Ultrasonic Degradation

- Acoustic Cavitation
 - Formation and collapse of microbubbles
 - Transient high temperature (>5000 K) and pressure (>1000 atm)
- Generates reactive species (radicals)
 - Hydroxyl
 - Hydrogen
 - Oxygen
 - And more

TiO₂ Photocatalysis

- Generates reactive species
 - Hydroxyl
 - Oxygen
- 254 UV light
- pH dependent
 - Surface pH
 - Toxin pI





- Intake
- Inline Chemical
- Coagulation/Flocculation/Sedimentation
- Storage Reservoir
- Filtration
- Carbon Adsorber
- Chlorine

Pilot and Plant Studies

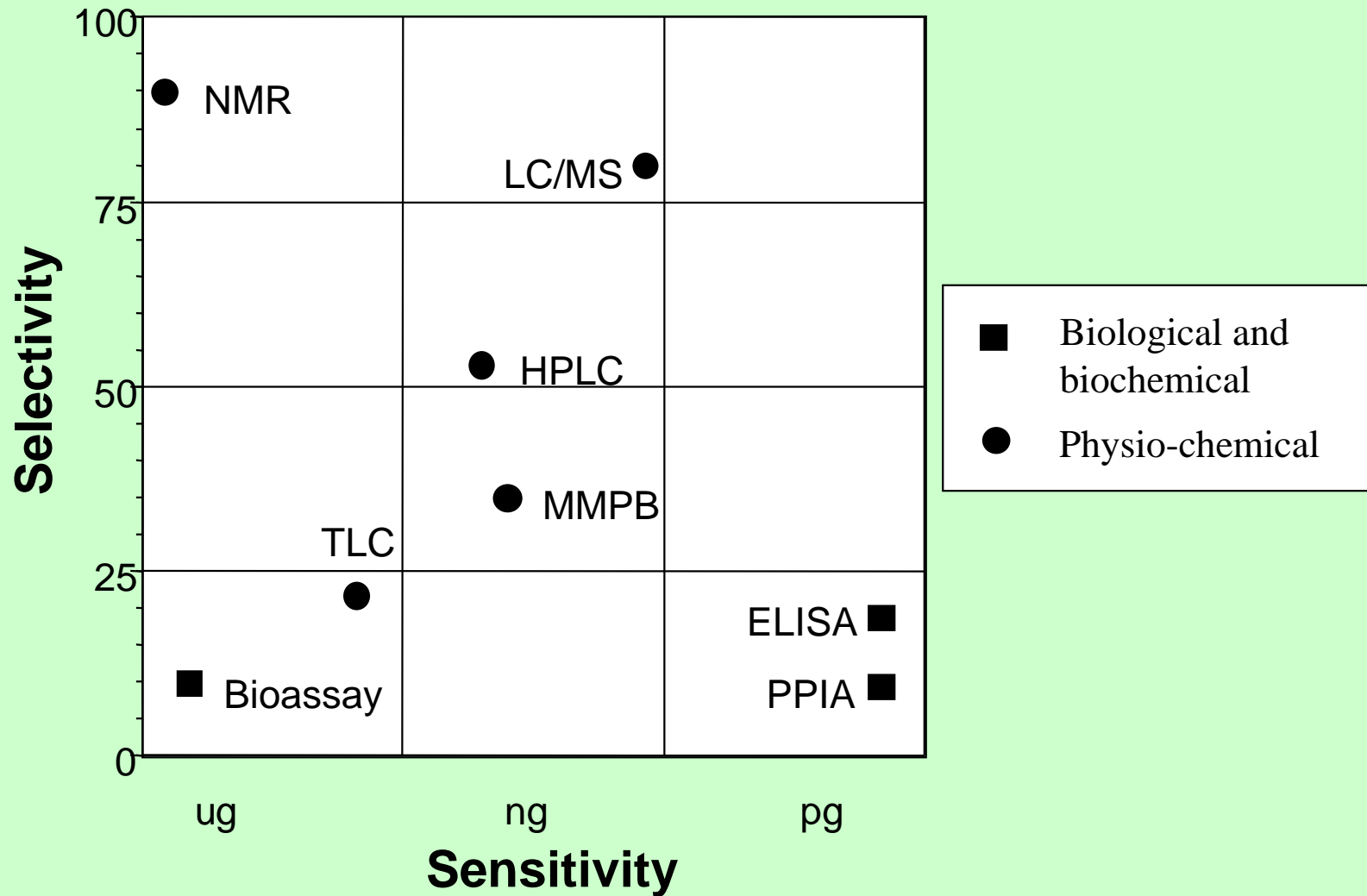
- Early studies focused on the removal of intracellular toxins only
- Complete treatment gives 31%-99% removal
- Most of the published studies are microcystin removal
 - Algae: Source to Treatment 2010
 - Cyanobacterial Harmful Algal Blooms 2008
 - Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management 1999
 - Lambert et al 1996
 - Karner et al 2001
 - Schmidt et al 2002
 - Hoeger et al 2005

Assay and Analytical Methodologies

Sample Preparation

- *In situ*
 - No sample modification
 - Potential for greater matrix interference
- Cell Lyse
 - Sonication
 - Chemical
 - Freeze/thaw
- Filtration
 - Vacuum
 - Centrifugation
- Lyophilization
 - Sample Concentration
 - Analyte volatilization?
- Immunocolumn
 - Retention
 - Specificity
- Solid Phase Extraction
 - Analyte retention

Selectivity and Sensitivity Relationships between Analytical Methods for Microcystins



Bioassay Based Detection

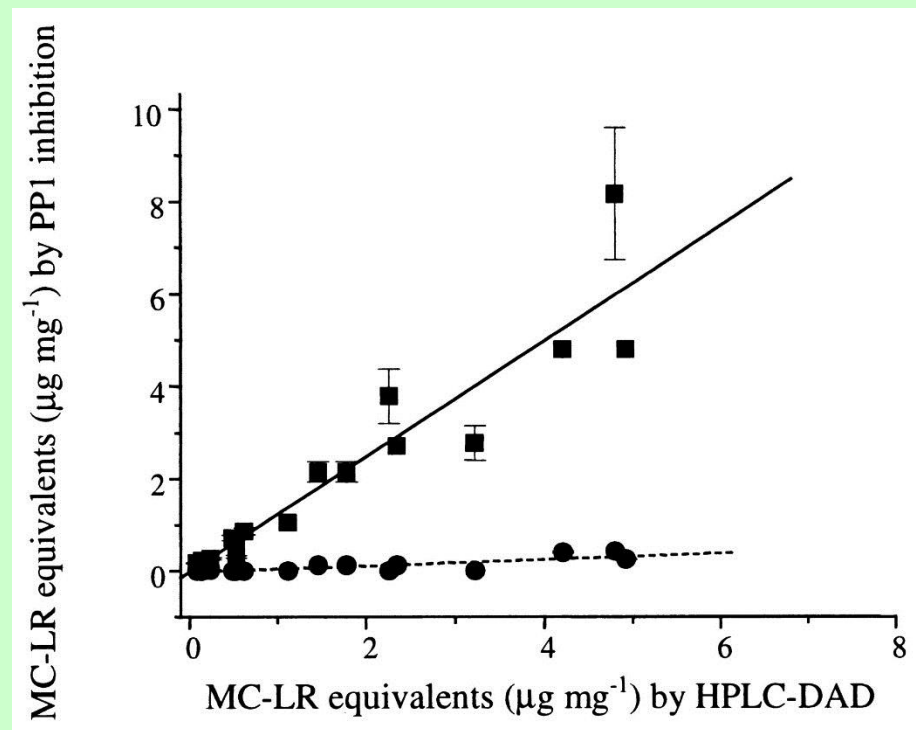
- Protein

Phosphatase

Inhibition Assay
(PPIA)

- No commercial kit available
- End Point Kinetics

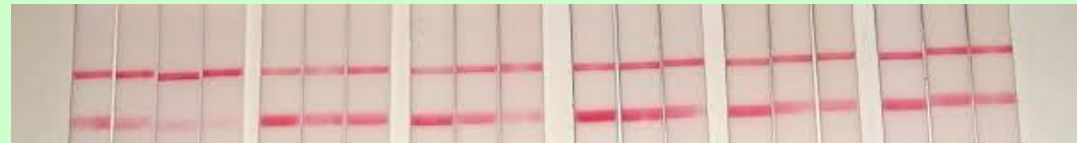
Comparison of microcystin-LR (MC-LR) equivalents determined by HPLC with DAD and PP1 inhibition



Metcalf J S et al. Appl. Environ. Microbiol. 2001;67:904-909

Bioassay Based Detection

- Enzyme Linked Immunosorbent Assay (ELISA)
 - Inexpensive to setup and to run
 - Screening (Care must be taken as to the data's use)
 - Antibody Based
 - Microcystin
 - Cylindrospermopsin
 - Saxitoxin
 - Receptor Based
 - Anatoxin-a



Liquid Chromatography

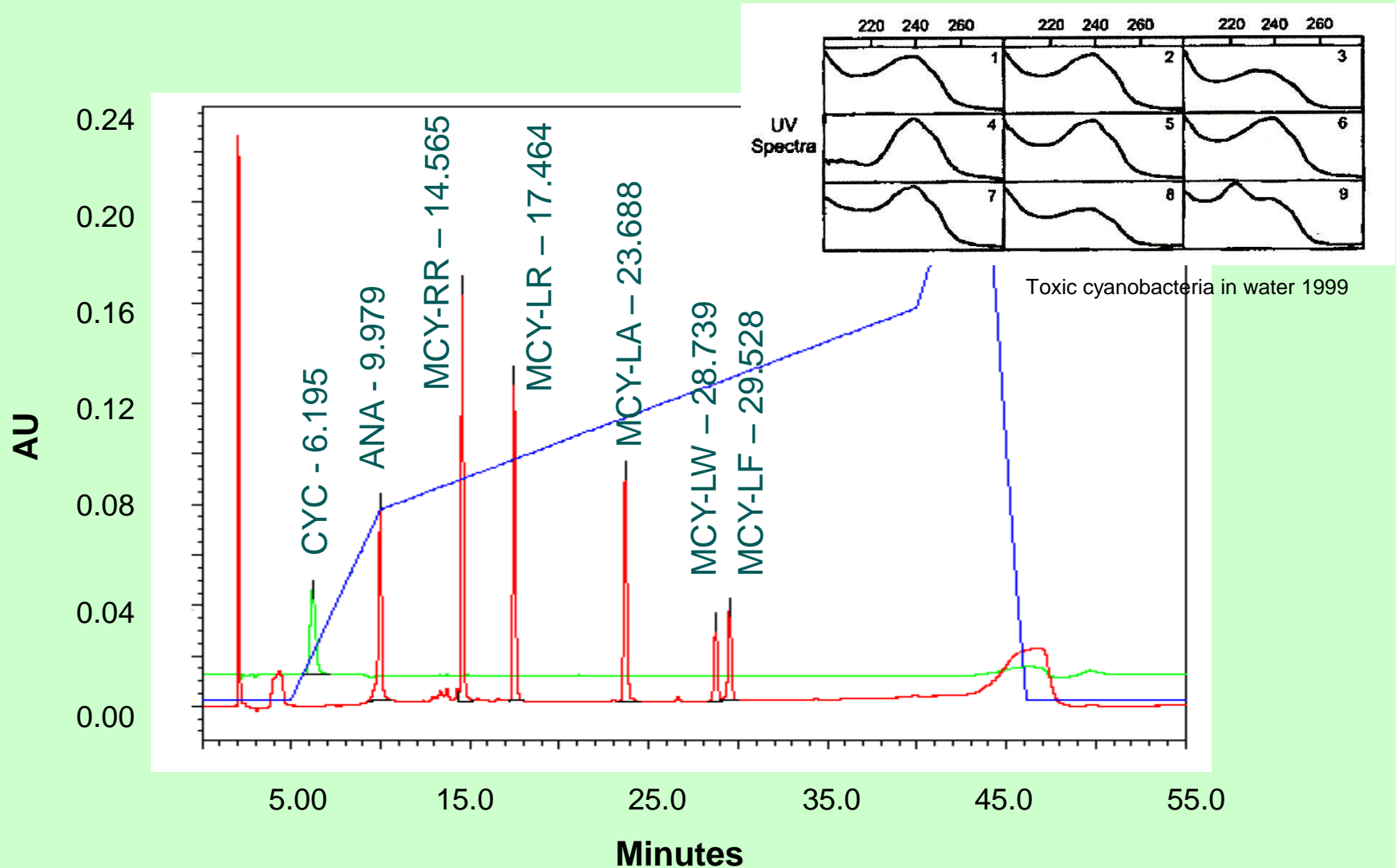
- High Performance Liquid Chromatography (UPLC and HPLC) with various detectors
- Liquid Chromatography Mass Spectrometry (UPLC and HPLC)
- Analyte Verification
 - Standards
 - Surrogates

UPLC and HPLC

- Various detectors available (PDA and fluorescence most common)
- Variable analysis time
- Only approved method for Microcystin LR (ISO 20179:2005)
- Limitations include relying solely on retention time for identification and the inability to differentiate co-eluting peaks



Separation of the Cyanotoxins by HPLC-PDA

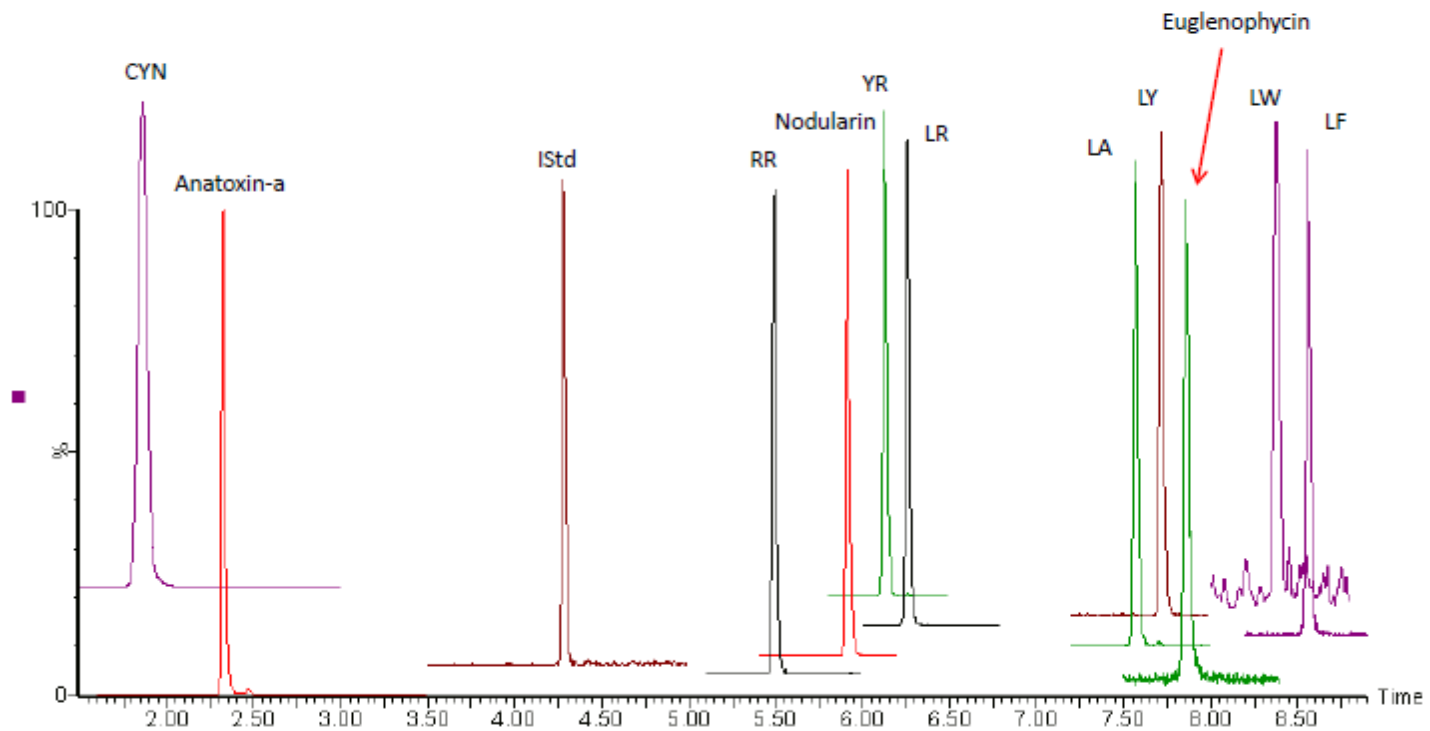


LC/MS(MS)

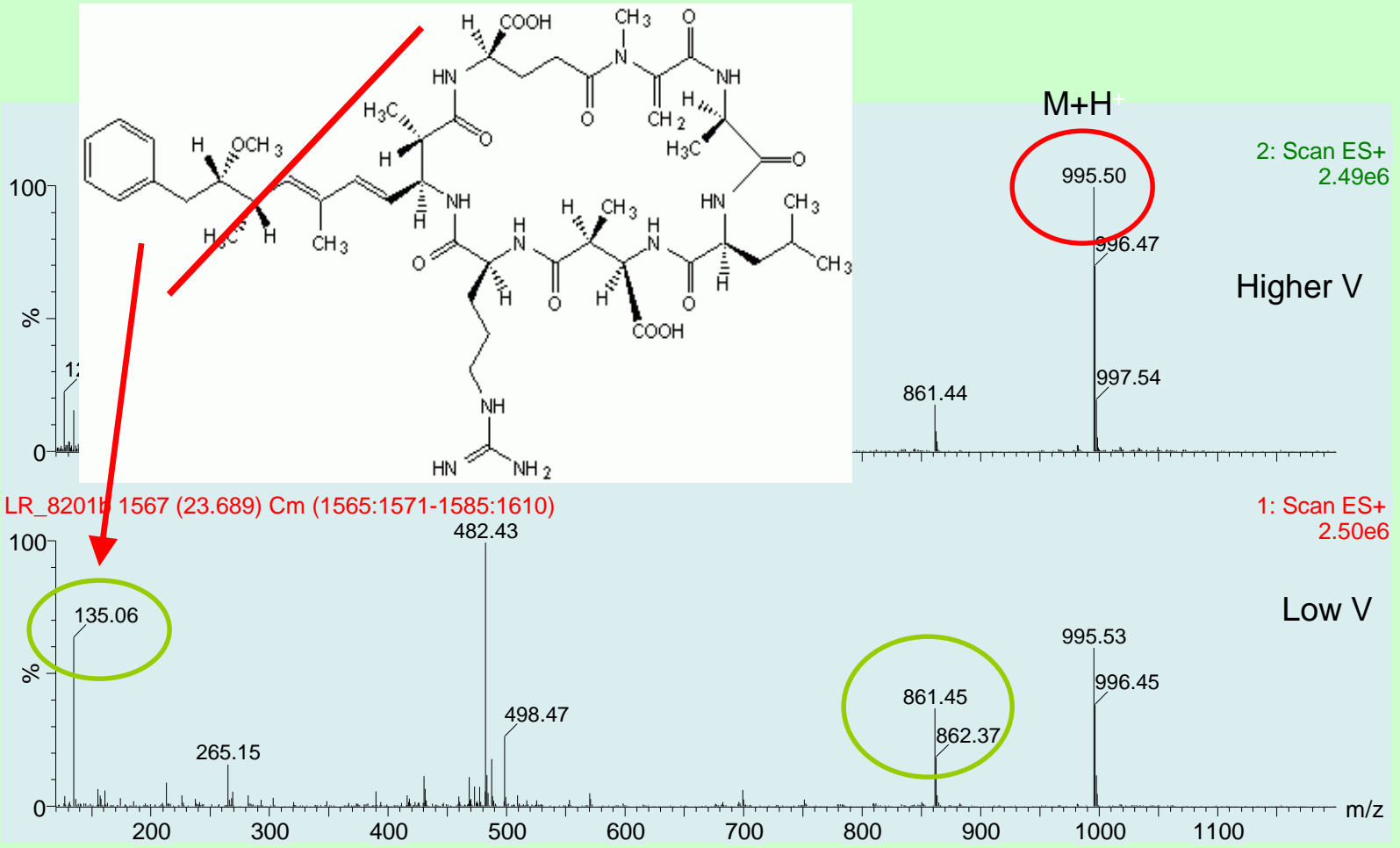
- Analysis can be performed by both single and triple quadrupole instruments
- Allows for positive conformation of compound
- Variable analysis time
- No approved method
- Limitations can include variable analyte response and non-linear standard curves



Single analytical method discerns common MYC and other toxins



Microcystin-LR Mass Spectrum

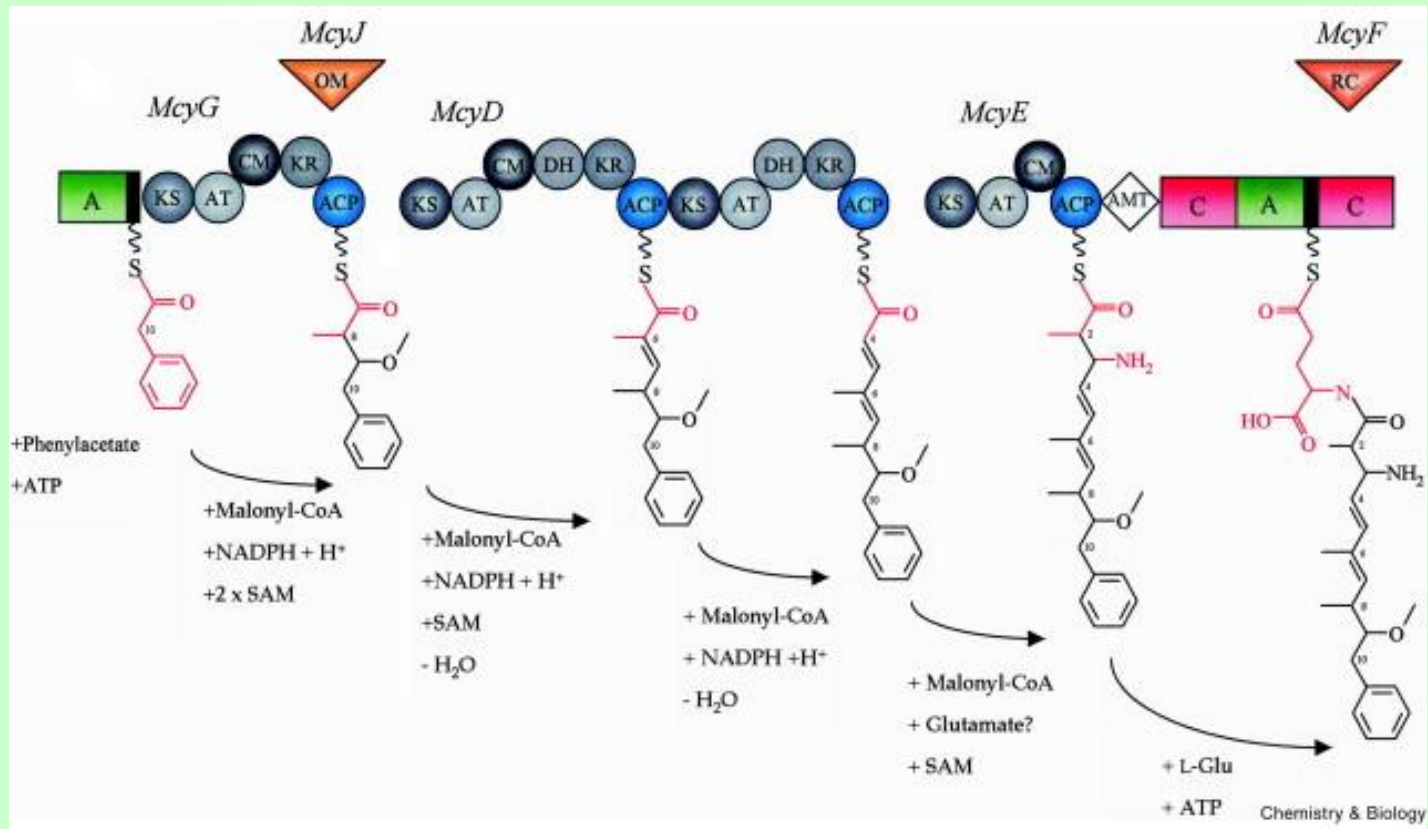


DNA Based Technologies

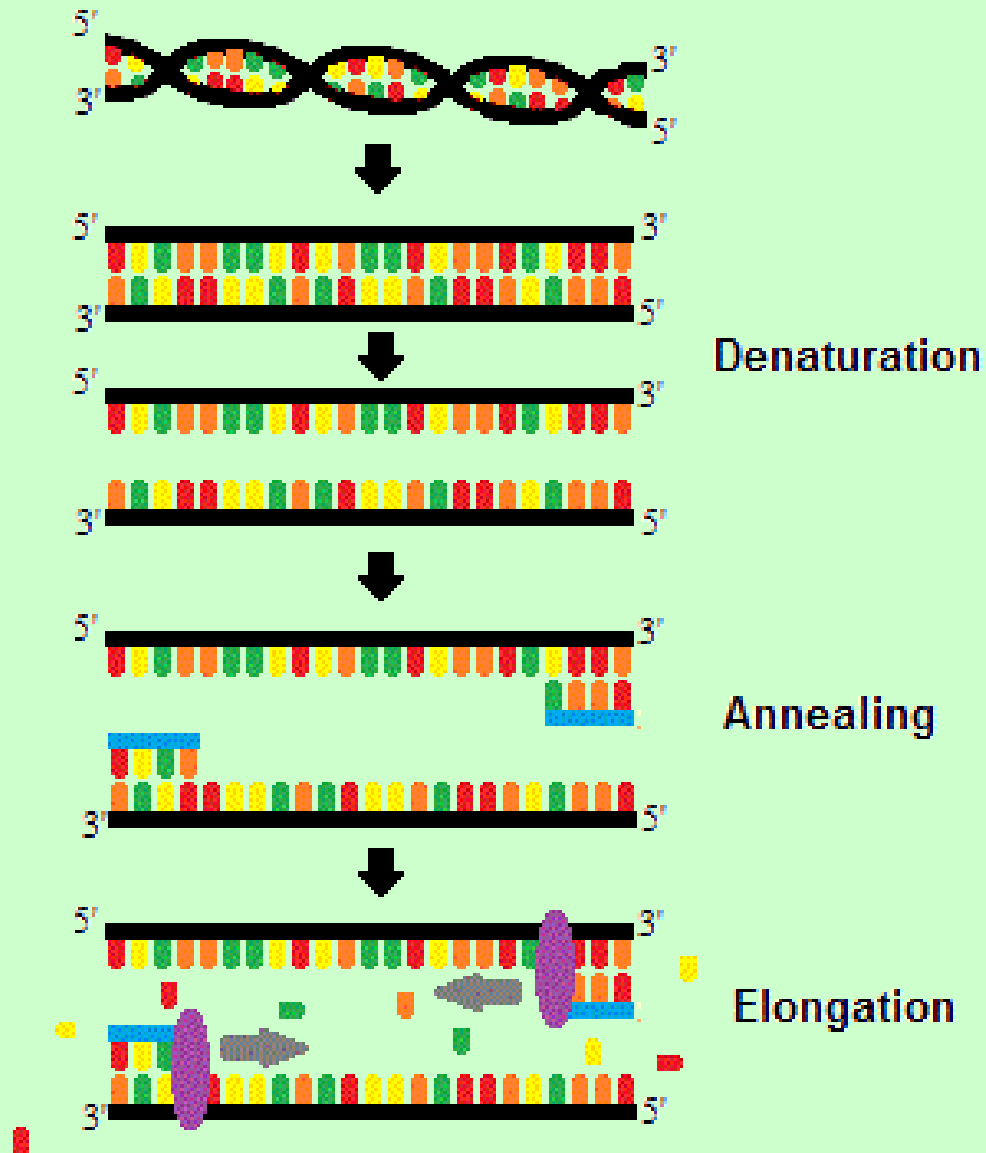
- Methodology allows for presence determination of a species and the presence of the toxin gene.
- Appropriate gene clusters have been determined for the toxins microcystin, cylindrospermopsin, anatoxin, and saxitoxin.
- Does not determine toxin concentrations.



Microcystin Gene



Cycling



Summary

- Intra vs extra cellular toxin
- Multi-barrier approach for each toxins
- One species can make multiple toxins
- More than one toxin may be present
- Understand the different analyses
 - Surrogate measurement
 - Semi-quantitative
 - Quantitative
- Have a contingency plan with in-house analyses to guide treatment

Development of a multiplex freshwater and marine method for cyanotoxin and euglenophycin detection

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Paul Zimba, Texas A&M University Corpus Christi

Collaborators

Brett Nielan

Tim Davis

Benjamin Southwell

David Szlag

Toxins (multiple!) can be produced by the same species

e.g. microcystin and saxitoxin

cylindrospermopsin and saxitoxin

anatoxin and microcystin

This cocktail of toxins likely causes synergistic effects when present at levels below that known to cause mortality/visible impacts in low level exposures

Detection methods

Specific:

toxin measurement analytically (HPLC, MS, MS/MS, NMR)

toxin activity assessment (ELISA, aptamer)

genomic analyses (PCR, multiplex possible?)

Indirect/ambiguous:

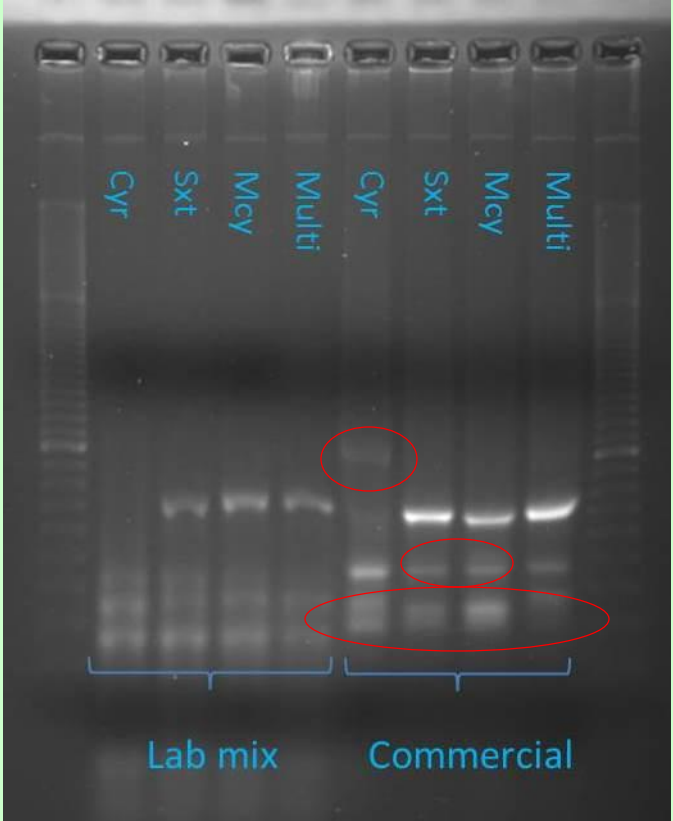
pigments

cell counts

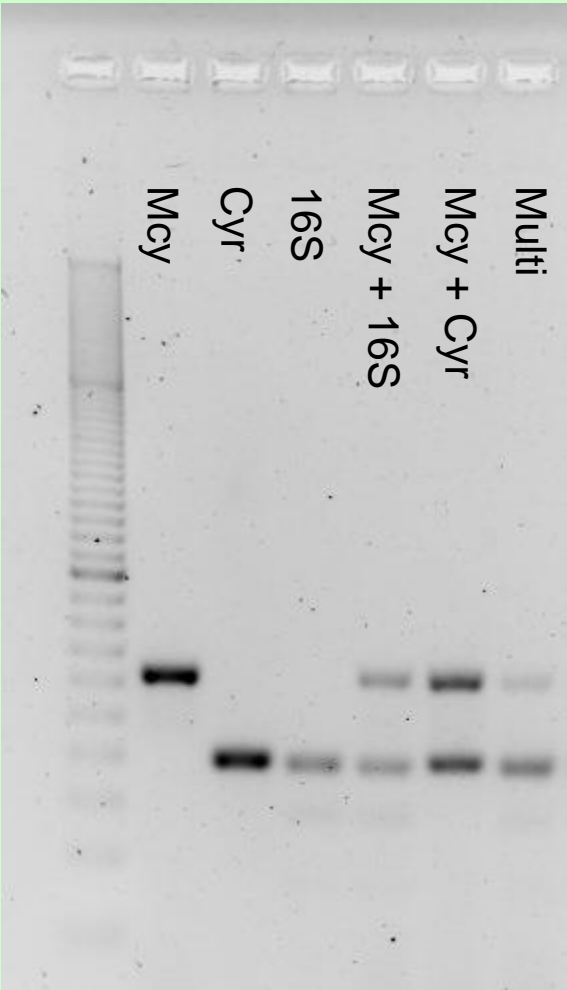
Project Aims:

- 1) Develop multiplex PCR method patterned after published research
- 2) Alter PCR method to detect anatoxin-a –replacing saxitoxin
- 3) Turn off euglenophycin production, RNA sequencing comparison to wild type
- 4) Add euglenophycin toxin-specific primer to PCR multiplex method
- 5) Apply new methods to field samples in several locales

Project partially funded in September 2012, fully funded January 2013



Demonstration a novel multiplex assay successfully generating specific products



Correlations of Microcystin analytical/PCR Mcy

$R= 0.71, p. < 0.20$

Correlations of CYL with CYR:

$R=0.72, p. < 0.065$

Correlations of Saxitoxin require more (+) samples

Microcystin: 34 positive by qPCR, 37 positive by LC/MS-MS
(borderline qPCR concentrations)

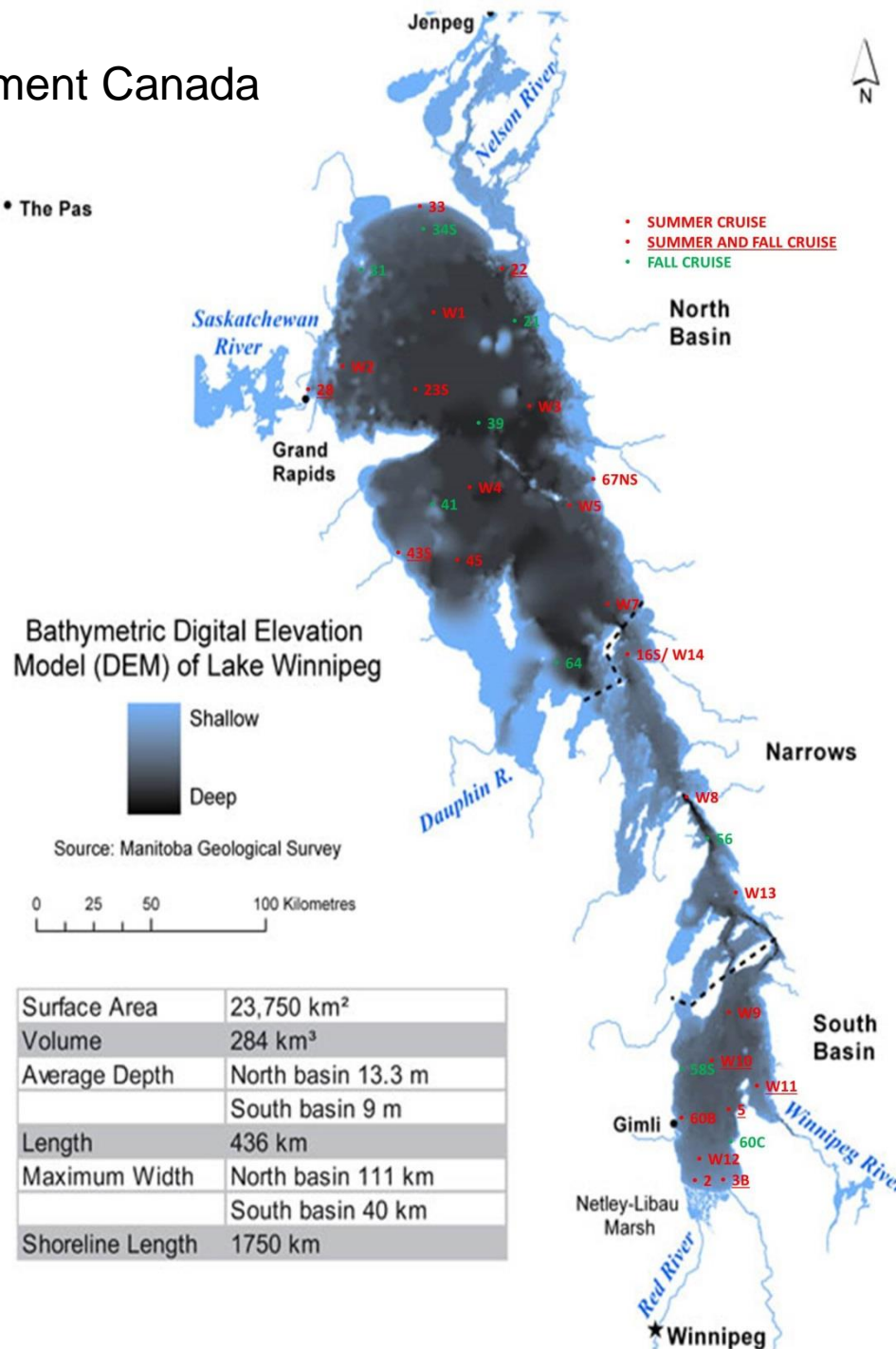
Cylindrospermopsin: 9 positive by qPCR, 7 positive by LC/MS-MS

With a working q-PCR multiplex, we will

- Compare summer vs winter differences (3 sites).
 - Parameters- CqPCR, cyanotoxins, cyanobacteria identification/enumeration
- Multivariate toxin/evaluation (5 systems).
 - Parameters-CqPCR, cyanotoxins, cyanobacteria identification/enumeration, nutrients, water chemistries, trace metals for 9 weeks during summer-fall
- Drinking Water study (10 plants).
 - Parameters – CqPCR, cyanotoxins, cyanobacteria identification/enumeration

Work with Environment Canada On 10 largest lake

• The Pas

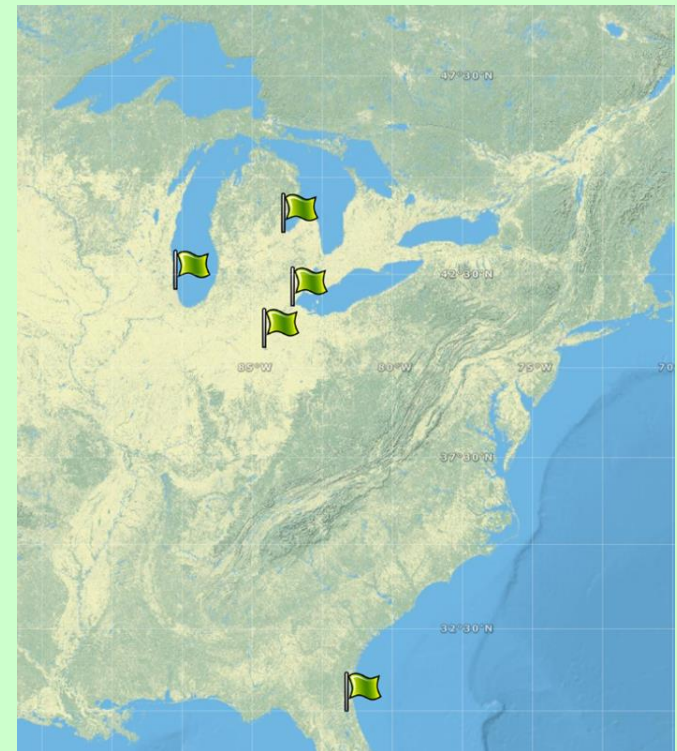


Lake Winnipeg toxin load, Summer, Fall 2013

Site	Collection date	Myc genes/ml	Cyr genes/ml	Sxt genes/ml		Site	Collection date	Mcy genes/ml	Cyr genes/ml	Sxt genes/ml
SUMMER CRUISE						FALL CRUISE				
W9	23-Jul-13	.	.	.						
W13	24-Jul-13	2222.181	.	.						
W8	25-Jul-13	74.592	0.103	.						
W14	25-Jul-13	129.168	.	.						
W7	26-Jul-13	1740.019	6.307	0.002						
W5	26-Jul-13	7731.941	26.495	15.665						
67NS	27-Jul-13	2461.026	3.910	0.080						
W3	27-Jul-13	44091.565	46.668	0.110						
W1	27-Jul-13	74.541	4.600	.						
22	28-Jul-13	1050.822	112.868	4.016		22	28-Sep-13	102.886	0.399	.
33	29-Jul-13	170.002	4.216	.						
W2	29-Jul-13	197.488	.	.						
28	29-Jul-13	240.953	1.615	.		28	25-Sep-13	3358.431	7.211	.
W4	30-Jul-13	1696.903	.	.						
43S	31-Jul-13	164.712	6.655	.		43S	29-Sep-13	193.300	.	.
45	31-Jul-13	658.671	1.206	.						
W10	1-Aug-13	7.950	.	0.137		W10	2-Oct-13	123.309	0.287	.
2	6-Aug-13	0.435	0.010	.						
3B	6-Aug-13	1404.106	0.360	.		3B	3-Oct-13	234.452	0.490	.
5	6-Aug-13	40.375	0.047	.		5	2-Oct-13	206.460	25.243	17.420
W12	7-Aug-13	0.012	.	.						
60B	7-Aug-13	0.004	0.179	.						
W11	8-Aug-13	0	0.061	.		W11	2-Oct-13	770.927	2.435	0.031
						64	30-Sep-13	138.787	0.315	.
						31	25-Sep-13	6411.982	11.299	0.151
						56	30-Sep-13	476.475	1.518	0.288
						23S	25-Sep-13	225.899	5.850	.
						60C	3-Oct-13	165.123	0.986	.
						21	28-Sep-13	114.177	4.828	1.002
						34S	25-Sep-13	208.471	.	.
						58S	1-Oct-13	225.989	0.551	.
						39	28-Sep-13	257.933	1.115	.
						41S	29-Sep-13	776.116	.	.
						2M	28-Sep-13	103.308	0.747	.

Natural Water Study: Causality

- Source water evaluated at 5 known cyanosites. (qPCR, cyanotoxins, cyanobacteria ID)
- Sampling requested weekly for nine weeks (7/8– 9/19/13)
- Water Quality Parameters



Location	Cyanobacteria Species present	ANA	CYL	MC-RR	MC-YR	MC-LR	MC-LA	MC-LW	MC-LF
St. Johns River, FL Shand Pier	<i>Pseudanabaena</i> <i>Anabaena</i> <i>Aphanizomenon</i> <i>Microcystis</i>			0.05 (1)					0.07 (1)
Lake Huron Saginaw Bay Bay City, MI	<i>Pseudanabaena</i> <i>Anabaena</i> <i>Aphanizomenon</i> <i>Microcystis</i>	0.38 (1)		0.05 (1)		0.02 (1)	0.05- 0.07 (2)	0.02- 0.03 (2)	0.03- 0.06 (4)
Grand Lake St. Marys Celina City, OH	<i>Pseudanabaena</i> <i>Planktothrix</i> <i>Anabaena</i> <i>Aphanizomenon</i> <i>Microcystis</i>	0.05 (1)	0.07- 0.05 (2)	0.19- 0.07 (6)	0.19- 0.05 (7)	0.07- 0.04 (6)	0.02- 0.07 (7)		0.04- 0.05 (5)
Western Basin, Lake Erie Toledo, OH	<i>Pseudanabaena</i> <i>Anabaena</i> <i>Microcystis</i>	0.04- 0.06 (3)		.06 (1)	0.03 (1)	0.02 (4)	0.08 (1)	0.02- 0.03 (2)	0.03 (1)
Lake Michigan Winnetka, IL	<i>Aphanizomenon</i>	0.04- 0.1 (4)		1.33- 0.3 (3)		0.02 (1)			0.03 (1)

Questions?



Acknowledgements

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