# Cyanotoxins and Drinking Water Quality: Treatment Options

Judy Westrick, PhD

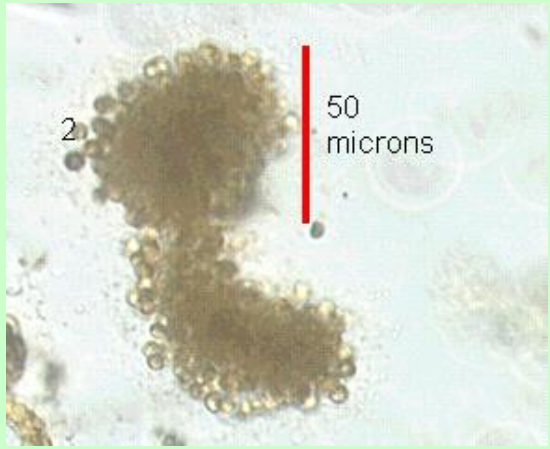
Paul Zimba, PhD

David Szlag, PhD

Benjamin Southwell, MS

# 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

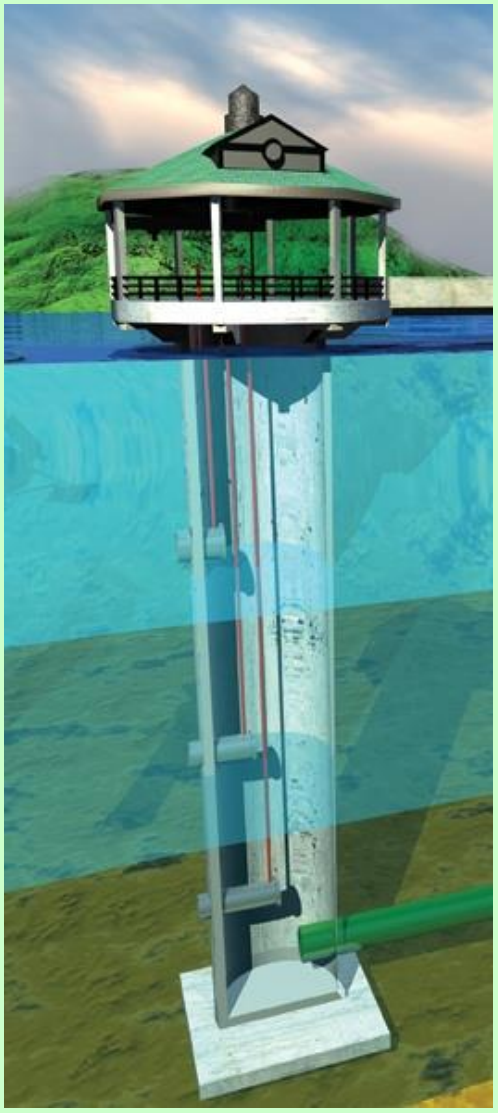
# Source Water

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

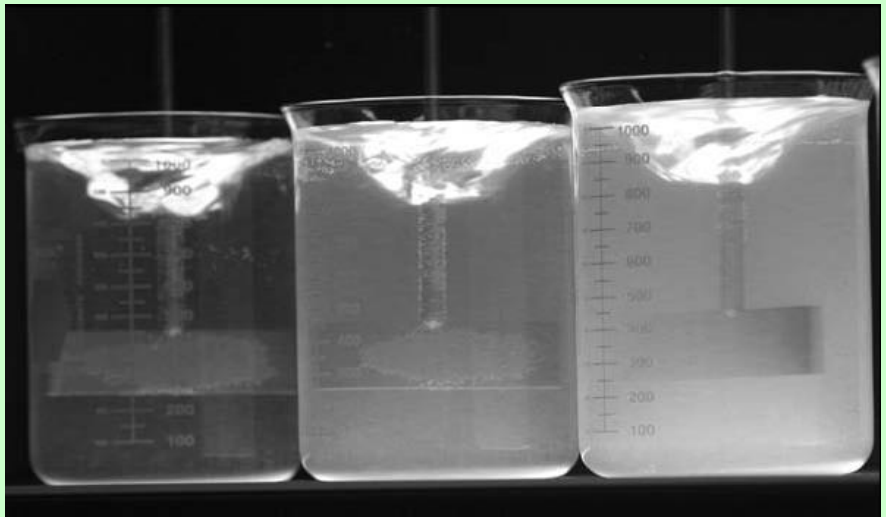
# Intake



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



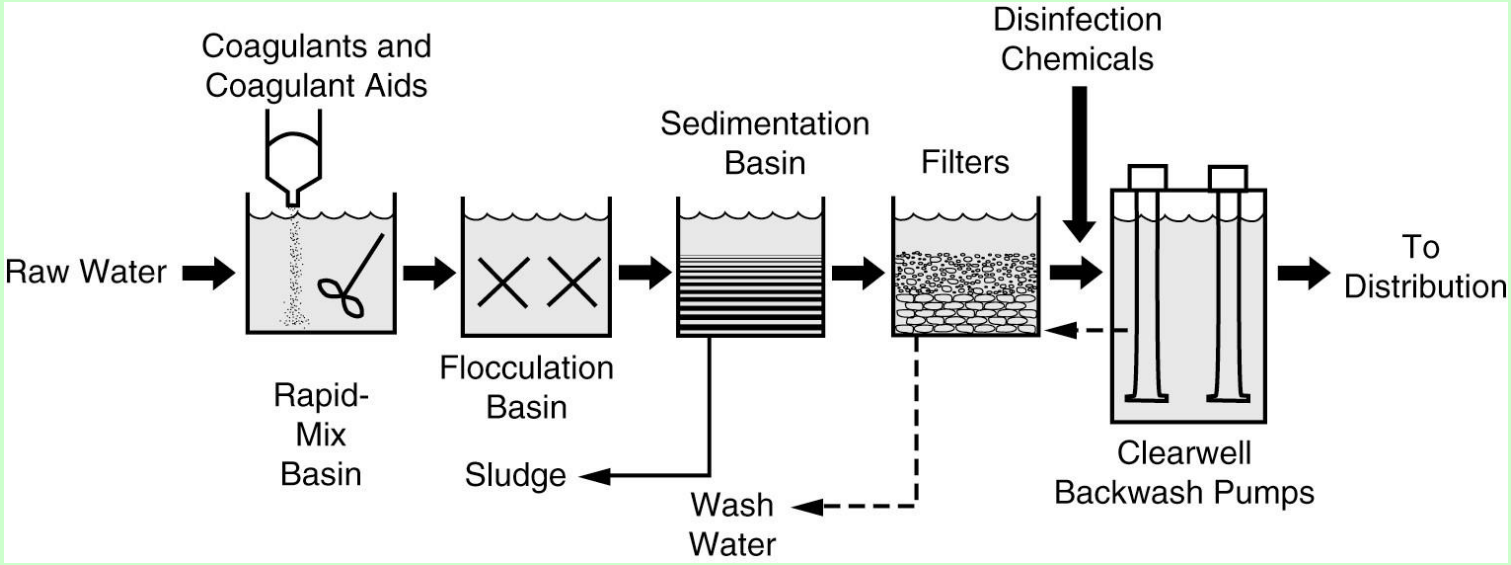
# Summary of Intact Algal Cell Removal Performance,

| 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

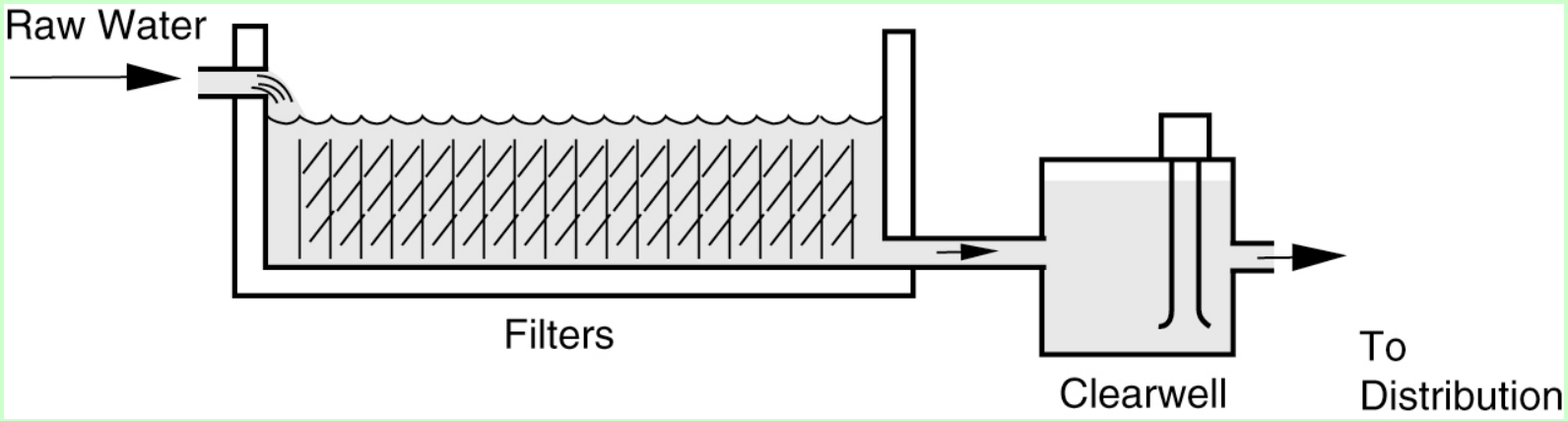
- Intracellular Toxin
  - Oxidants (not often used, afraid of lysing cell)
  - Flocculent aides
  - Settled water with less than 100 units algae/mL
- Extracellular Toxin
  - Activated Carbon
    - Powder (PAC)
    - Granular (GAC)
  - Filtration
    - Conventional
    - Biologically Active

- 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

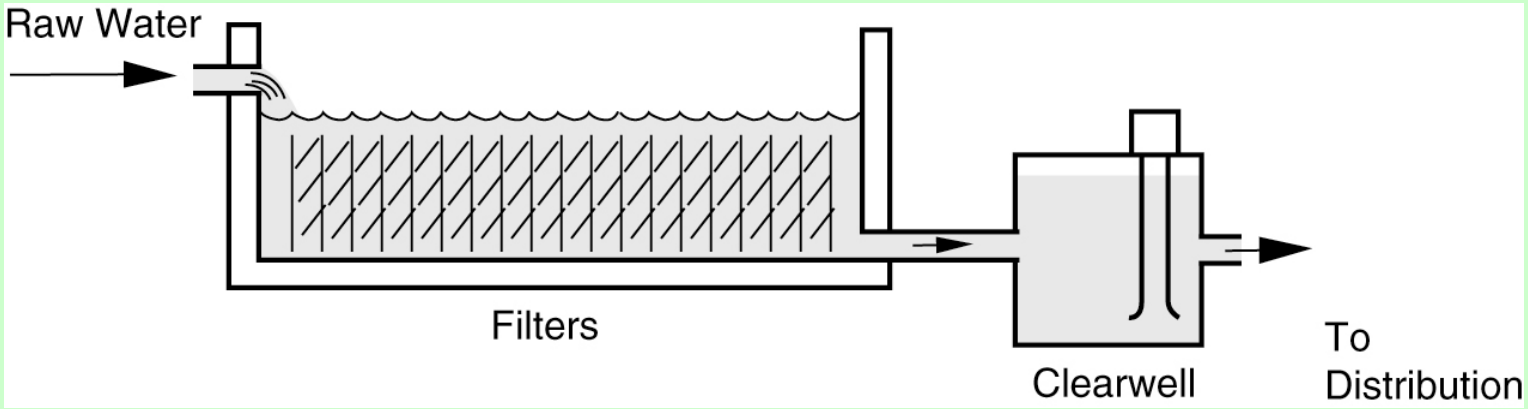
Low power ultrasound
Tunable (79 frequencies)
Critical resonance (gas vesicles)
Cyanobacteria – *Microcystis*, *Anabaena, Lyngba (Sonic Solutions)*

# 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

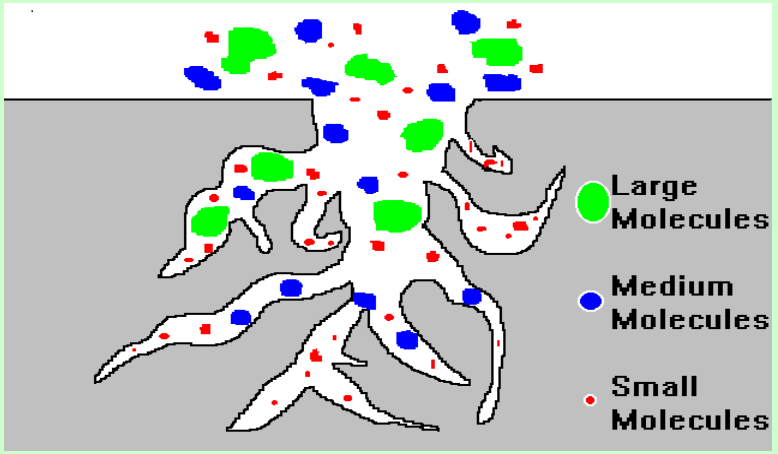
# GAC filtration

- 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

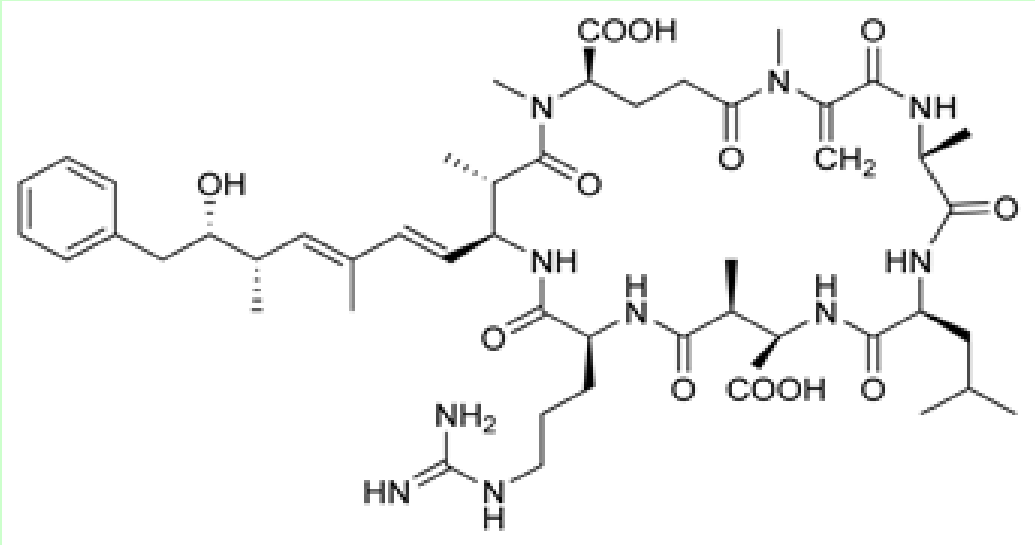
- Equilibrium
  - Micropore
    - Taste and odor
    - Industry spills, solvents
    - Anatoxin-a
  - Mesopore
    - Microcystins RR>YR>LR>LA
    - Cylindrospermopsin
    - Saxitoxin




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

# Summary of Oxidation Treatment Processes Extracellular Toxins

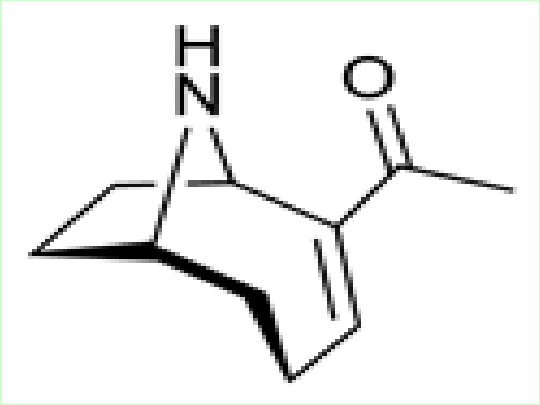
| | Microcystin |
|---|---|
| Chlorine | Yes |
| Ozone | Yes |
| Chloramine | No |
| Chlorine dioxide | No |
| Hydroxyl radical | Yes |
| Potassium permanganate | Yes |




Westrick et. al. Anal. Bioanal. Chem. (2010) 397:1705–1714

# Summary of Oxidation Treatment Processes Extracellular Toxins

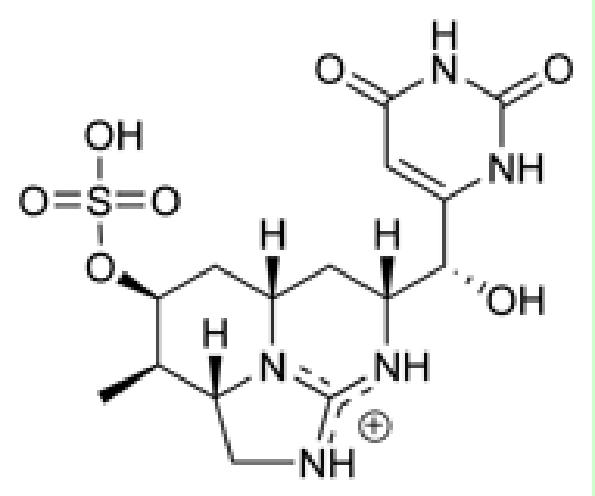
| | Anatoxin-a |
|---|---|
| Chlorine | No |
| Ozone | Yes |
| Chloramine | No |
| Chlorine dioxide | No |
| Hydroxyl radical | Yes |
| Potassium permanganate | Yes |




Westrick et. al. Anal. Bioanal. Chem. (2010) 397:1705–1714

# Summary of Oxidation Treatment Processes Extracellular Toxins

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

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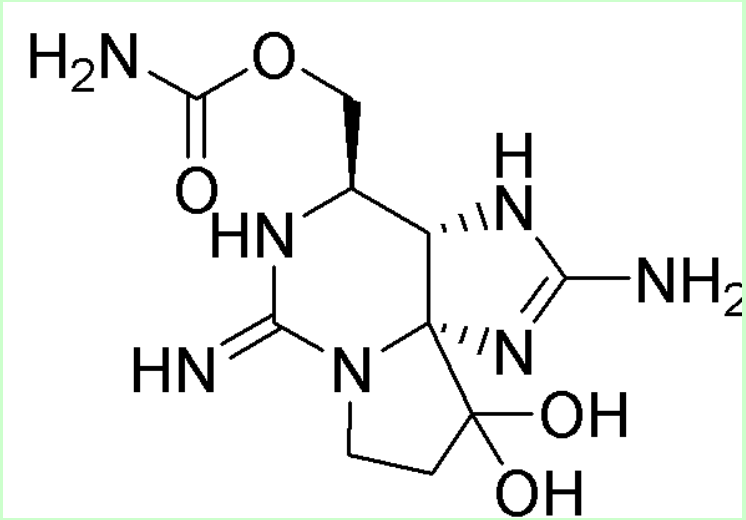
# Summary of Oxidation Treatment Processes Extracellular Toxins

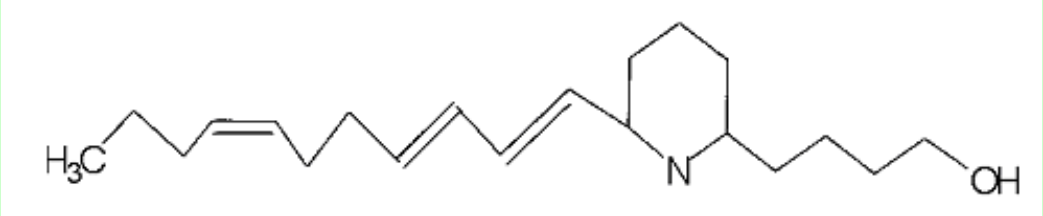
| | Saxitoxin |
|---|---|
| Chlorine | Yes |
| Ozone | No |
| Chloramine | Has not been investigated. |
| Chlorine dioxide | Has not been investigated. |
| Hydroxyl radical | Has not been investigated. |
| Potassium permanganate | No |




Westrick et. al. Anal. Bioanal. Chem. (2010) 397:1705–1714

# 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 $ugl^{-1}$ (Acero et al 2005)

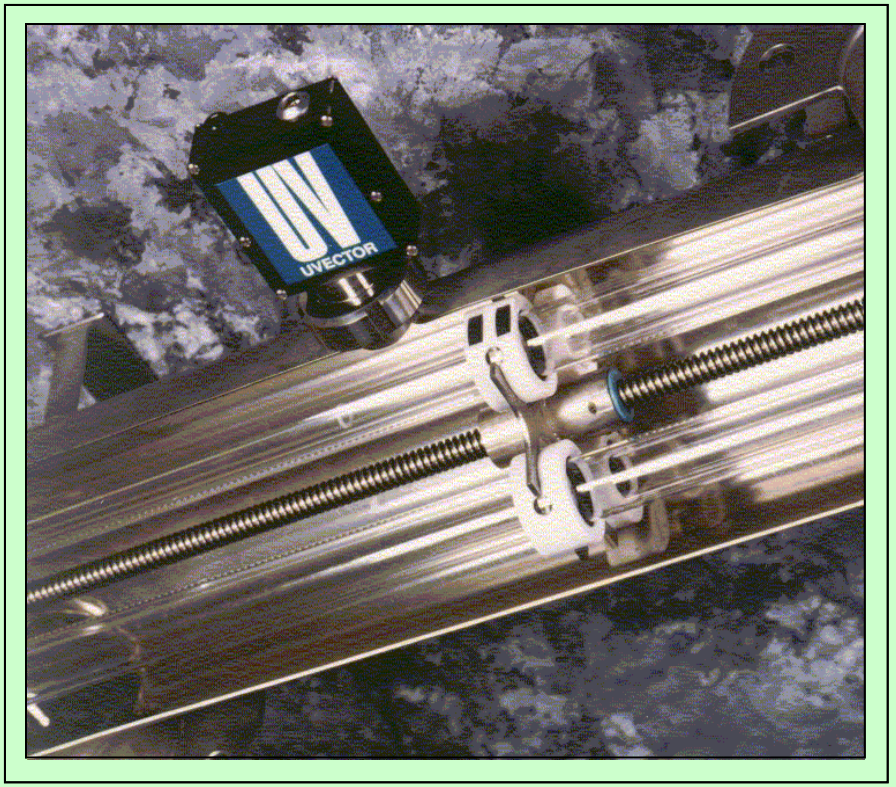
| pH | $[MCLR]_0$ | CT-values, $mgl^{-1}min$ | | | |
|---|---|---|---|---|---|
| | $ugl^{-1}$ | 10°C | 15°C | 20°C | 25°C |
| 6 | 50 | 46.6 | 40.2 | 34.8 | 30.3 |
| | 10 | 27.4 | 23.6 | 20.5 | 17.8 |
| 7 | 50 | 67.7 | 58.4 | 50.6 | 44.0 |
| | 10 | 39.8 | 34.4 | 29.8 | 25.9 |
| 8 | 50 | 187.2 | 161.3 | 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 *Crytosporidium 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/$H_2O_2$
- Fenton Reagent
- Radiolysis
- Ultrasonic degradation
- $TiO_2$ photocatalysis
- Ferrate

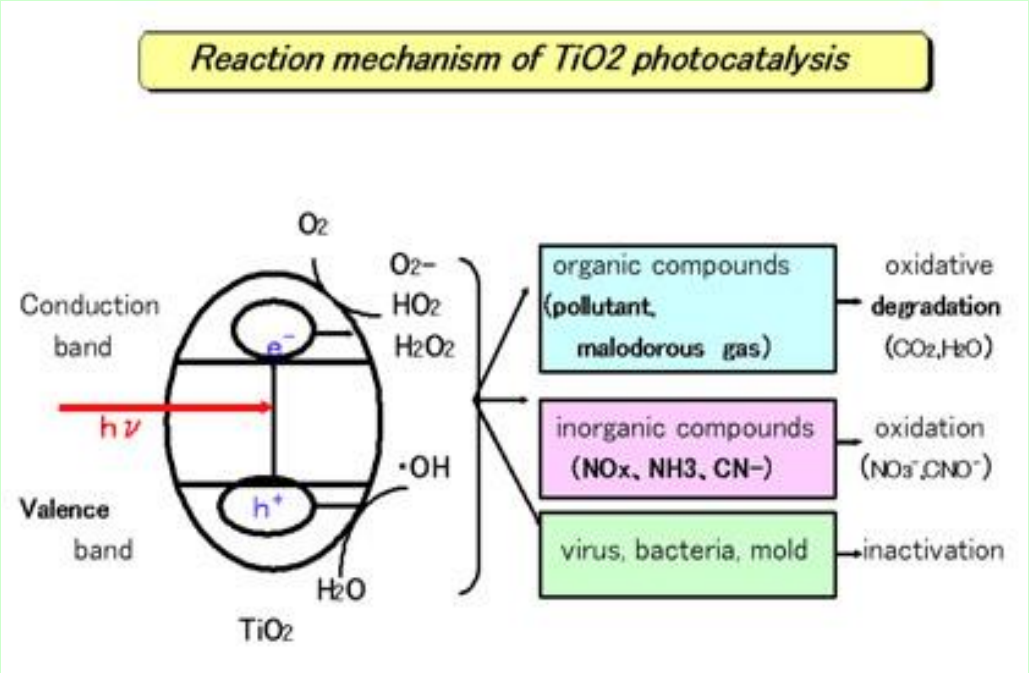
Sharma et. al, Separation and Purification 91 (2012) 3-17

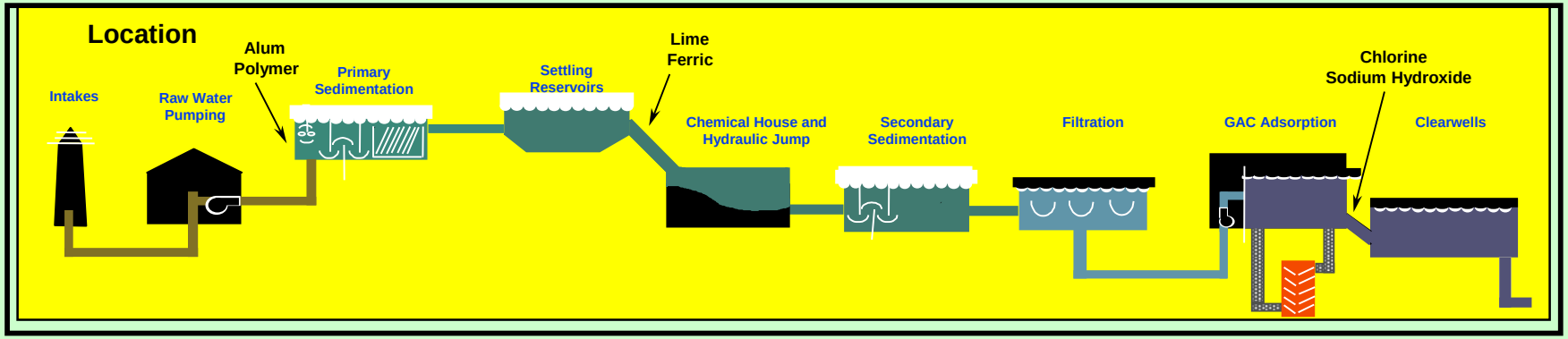
# Ultrasonic Degradation

- 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

# $TiO_2$ 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

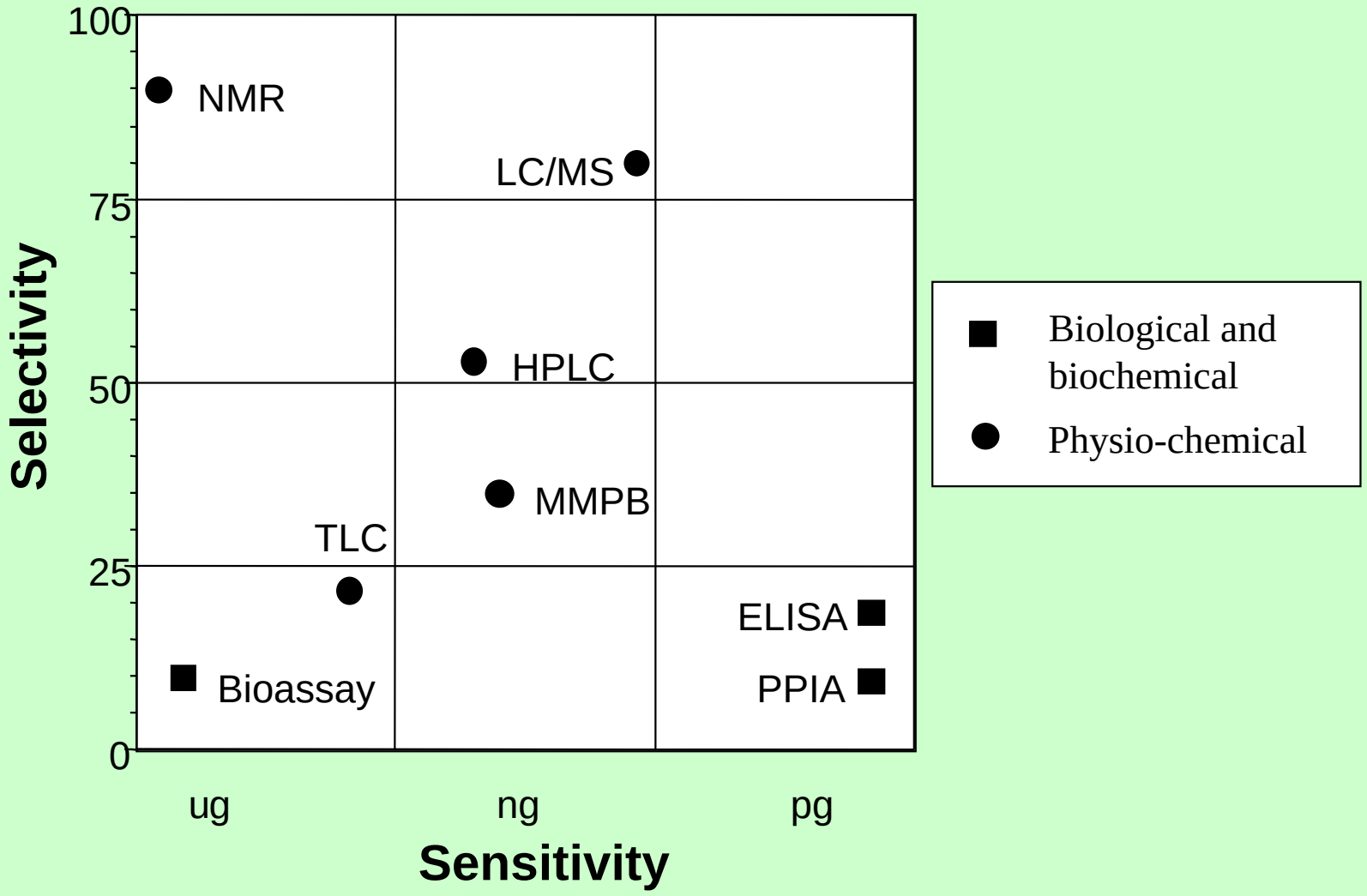
# Pilot and Plant Studies

- 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

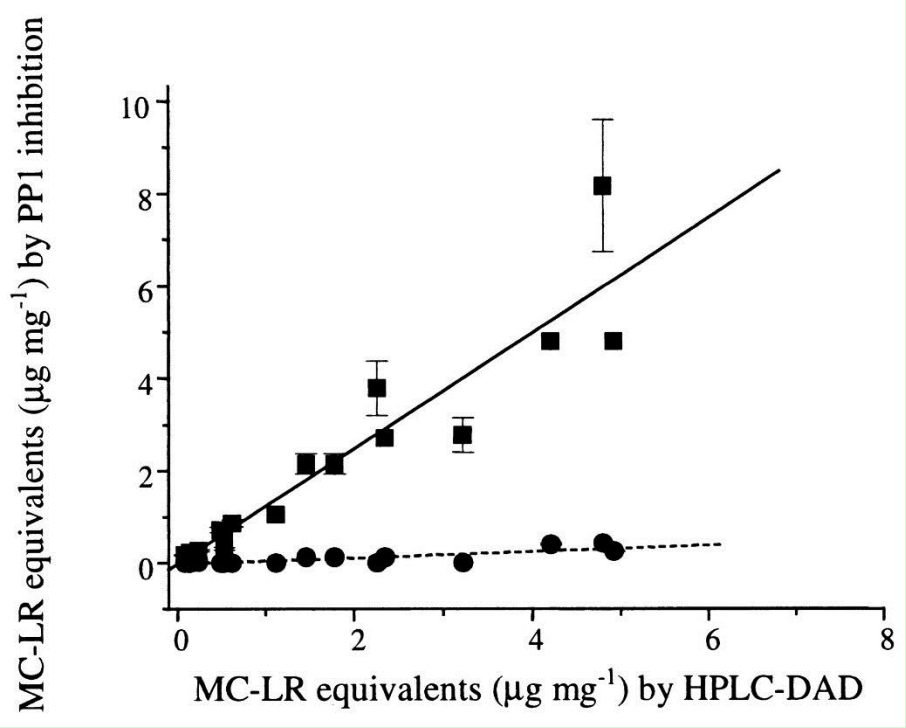
# Selectivity and Sensitivity Relationships between Analytical Methods for Microcystins



# Bioassay Based Detection

- Protein Phosphatase Inhibition Assay (PPIA)
  - No commercial kit available
  - End Point Kinetics

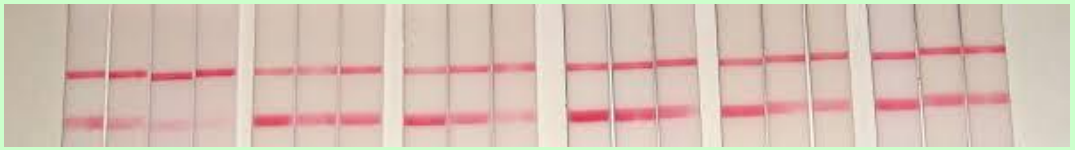
**Comparison of microcystin-LR (MC-LR) equivalents determined by HPLC with DAD and PP1 inhibition**




**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

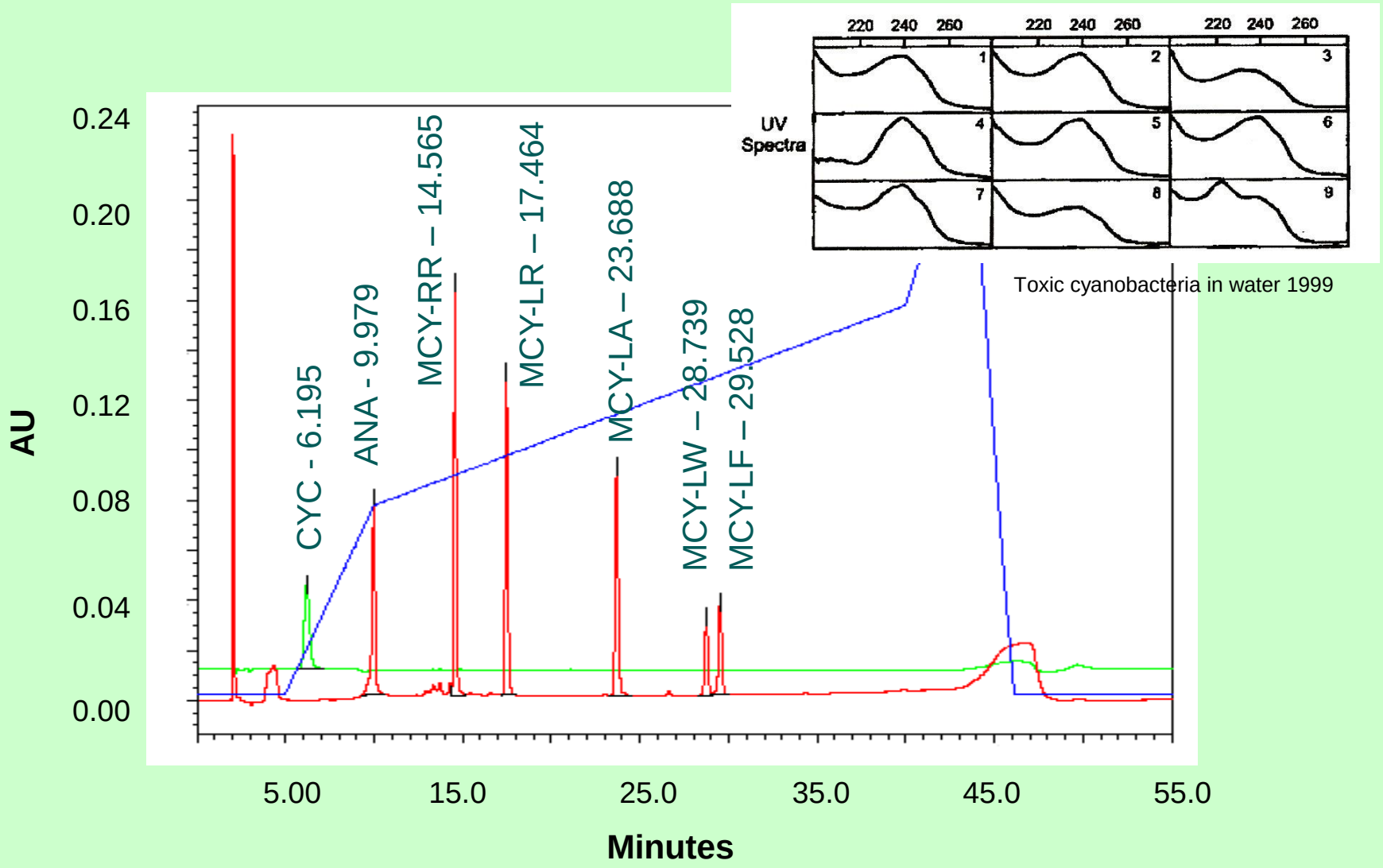
# Liquid Chromatography

- 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

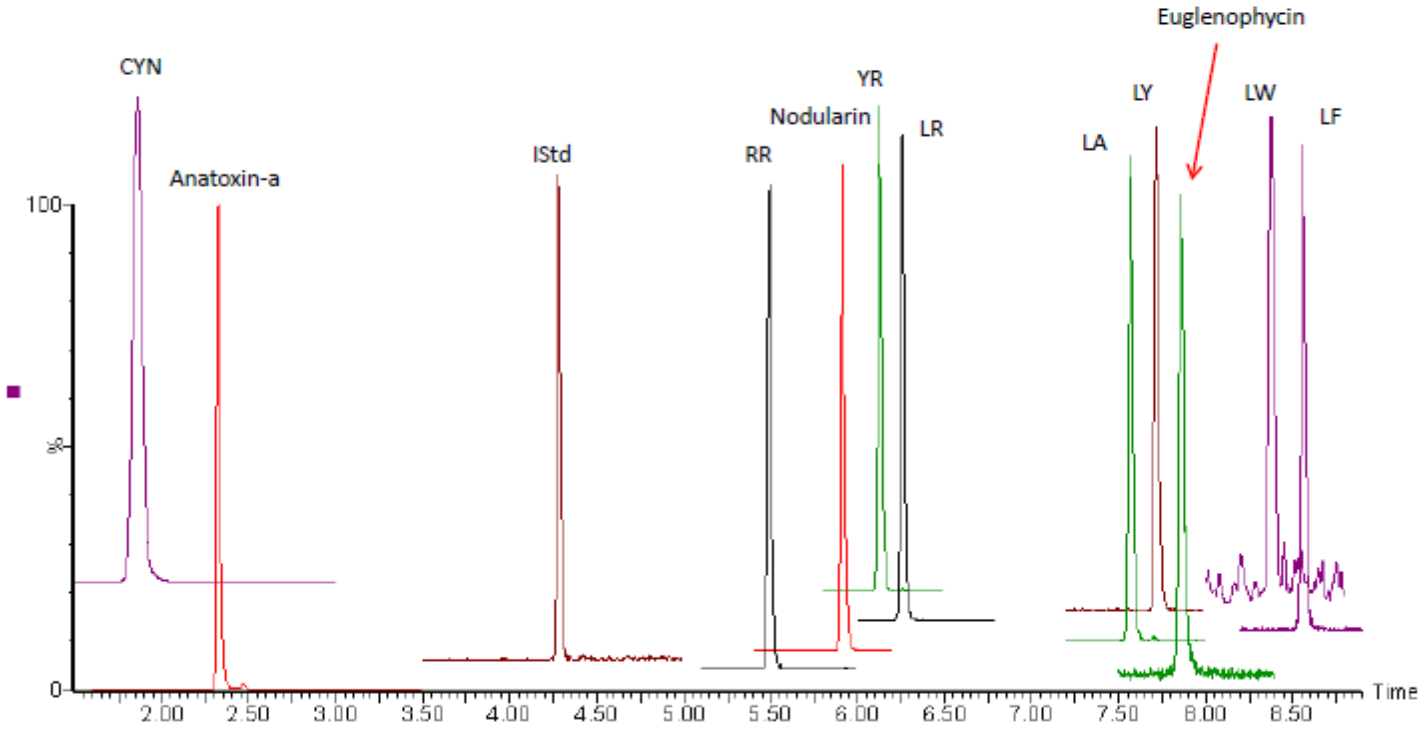
# Separation of the Cyanotoxins by HPLC-PDA



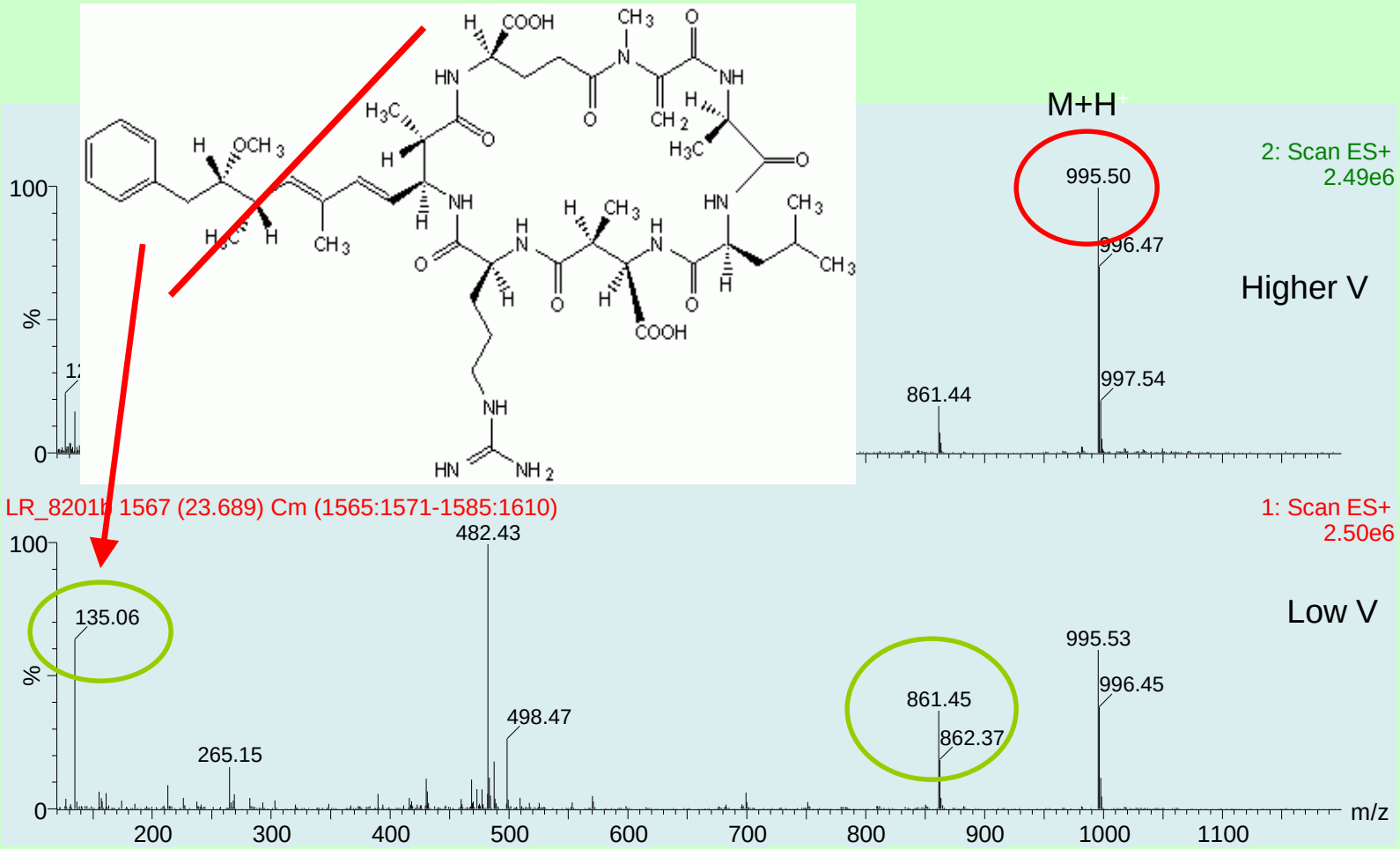
# LC/MS(MS)

- Analysis can be preformed 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



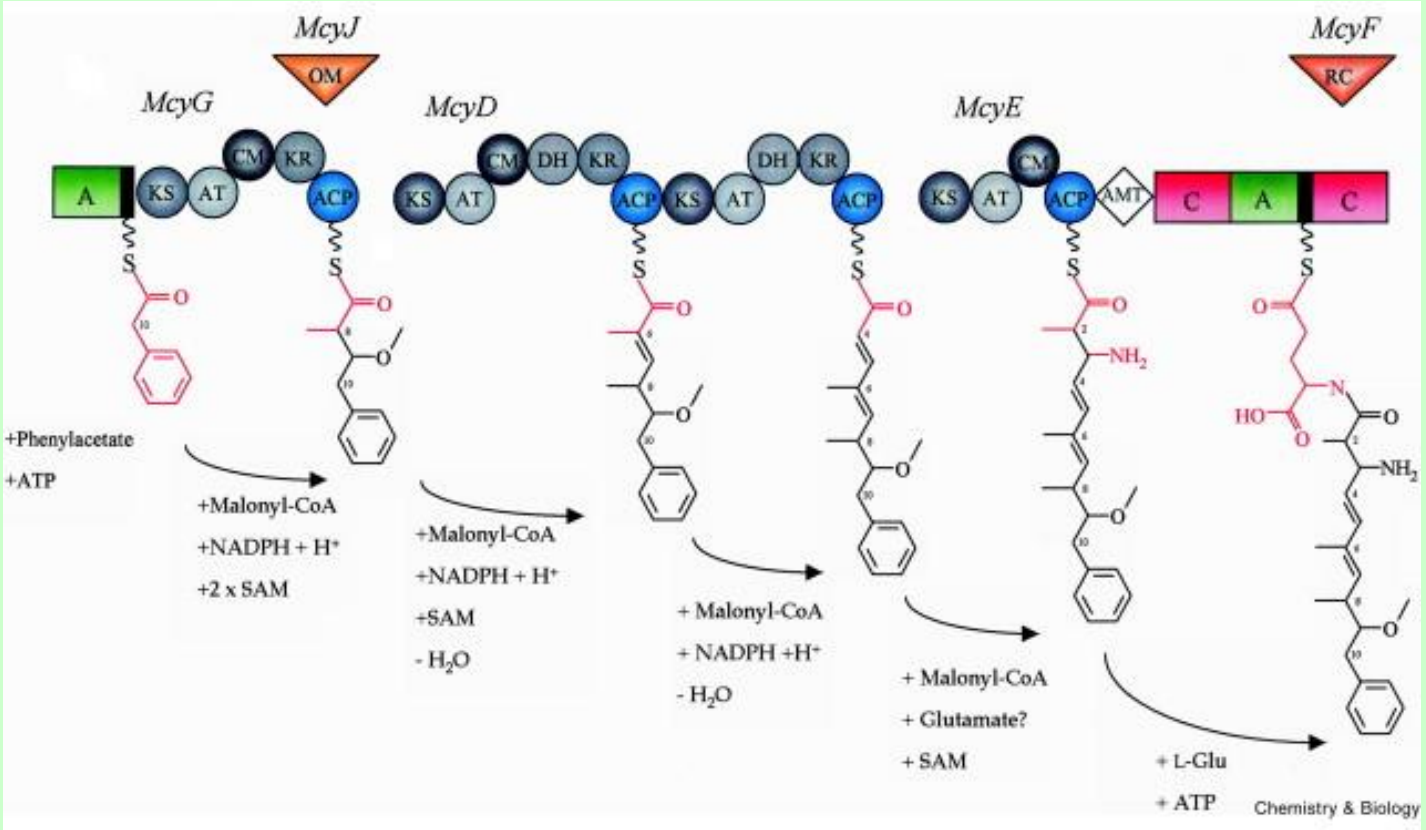
# Microcystin-LR  Mass Spectrum



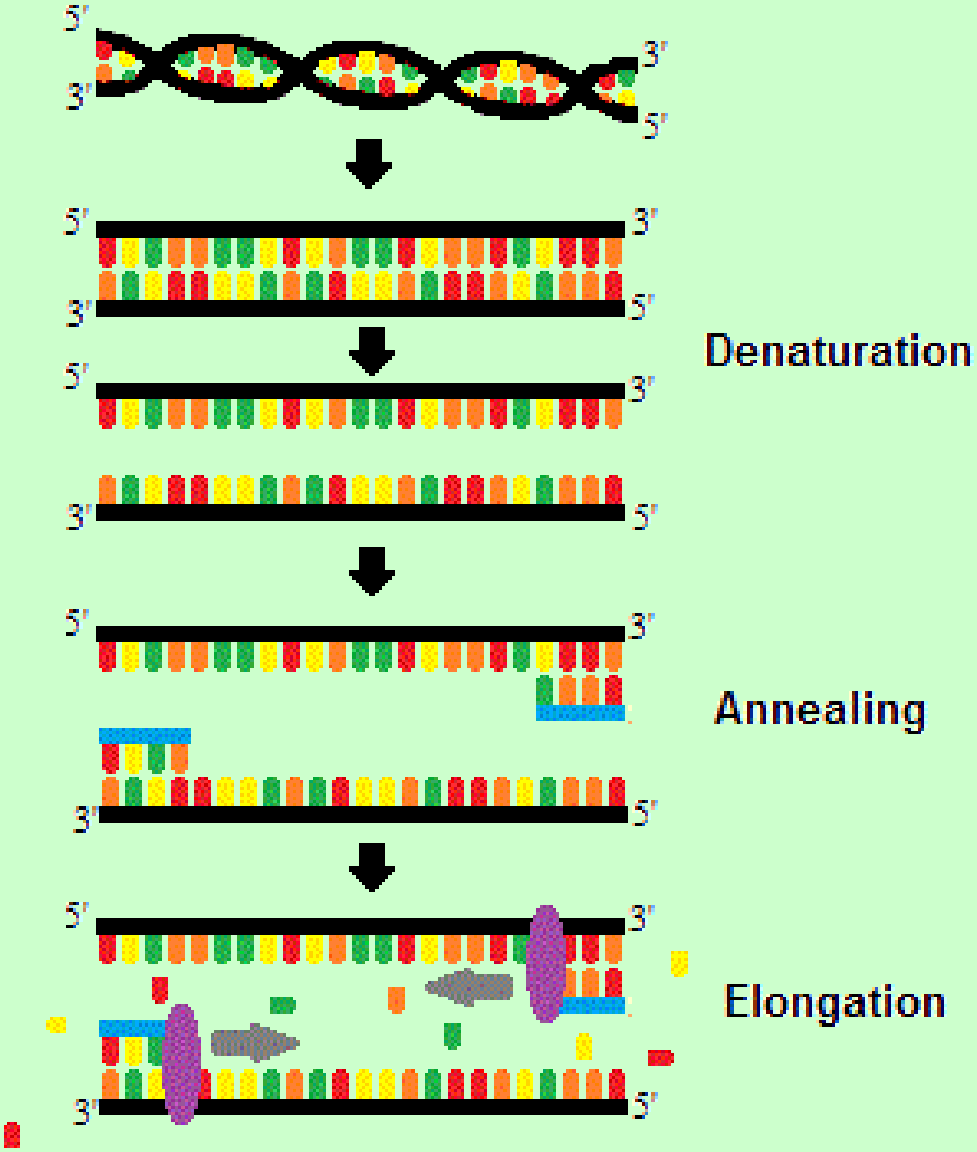
# DNA Based Technologies

- 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

Judy Westrick, Wayne State University

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:

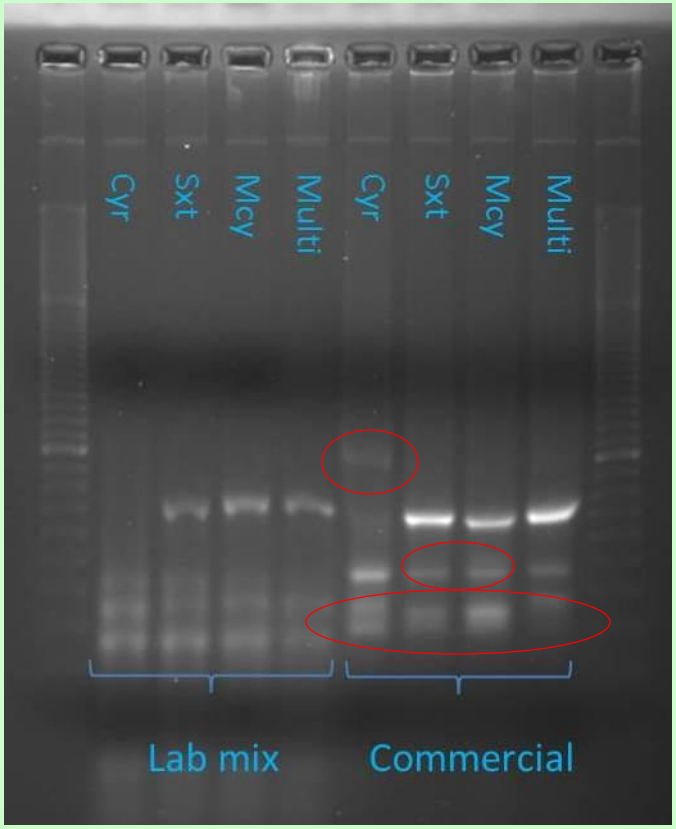
pigments

cell counts

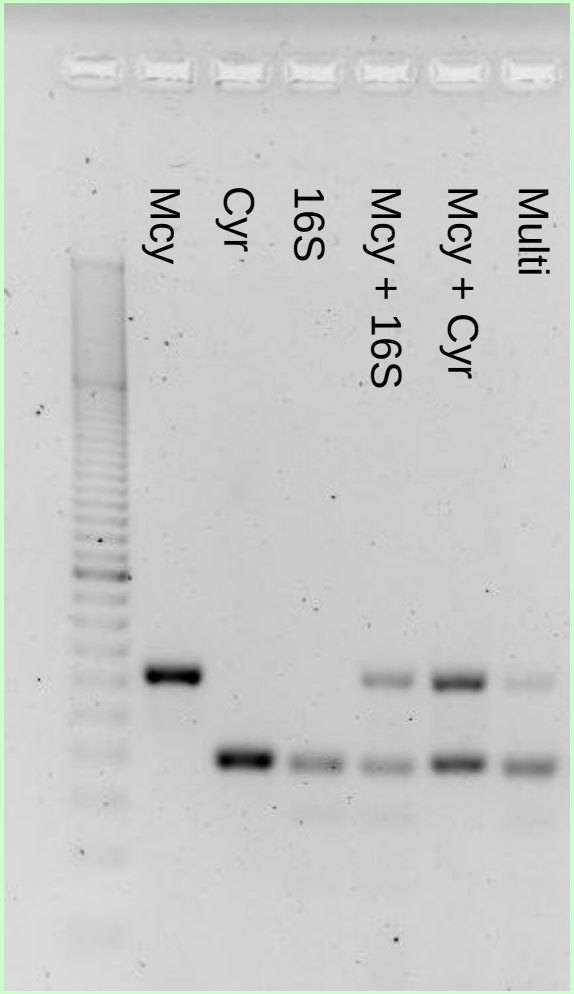
Project Aims:

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 typ
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


Cyr
Sxt
Mcy
Multi
Cyr
Sxt
Mcy
Multi
Lab mix
Commercial

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

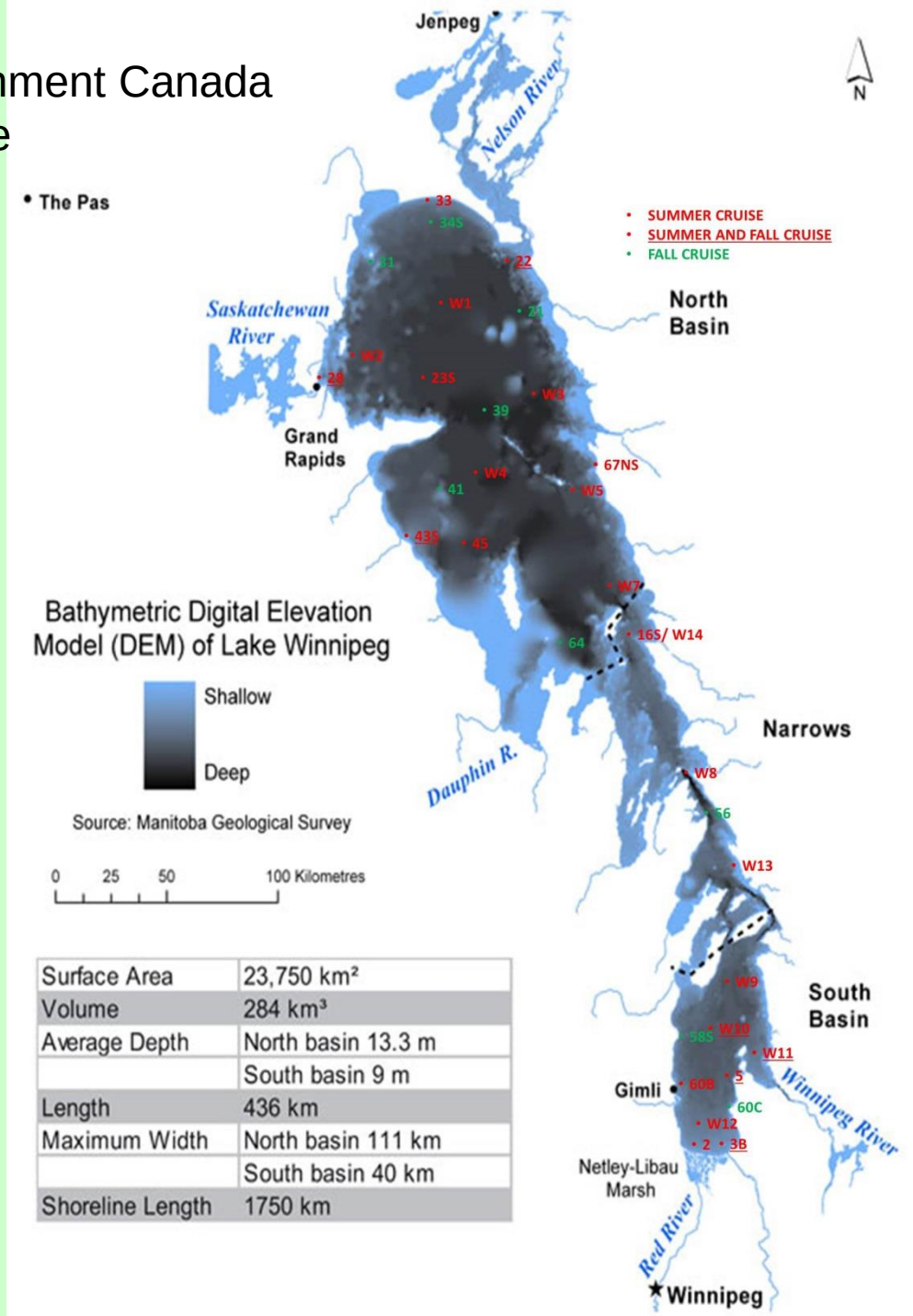
Microcystin: 34 positive by qPCR, 37 positive by LC/MS-MS (borderline qPCR concentrations)

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



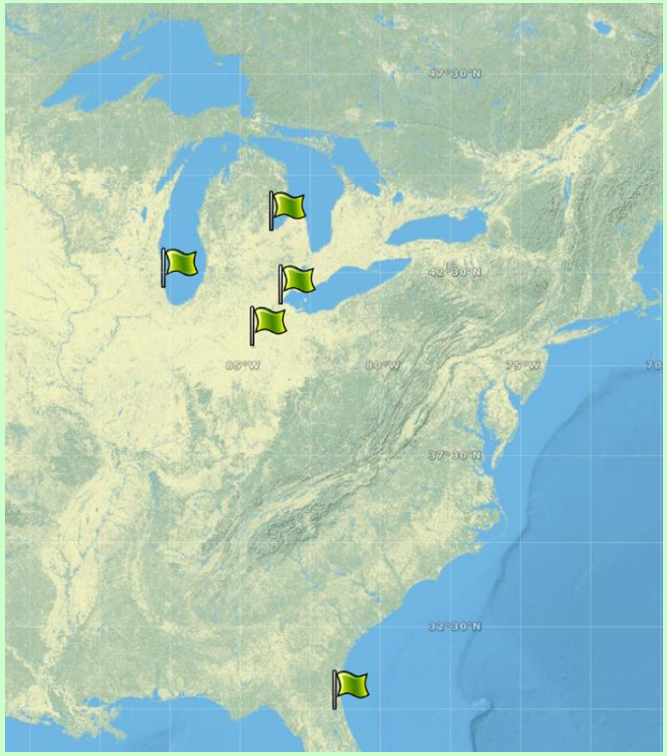

| | |
|---|---|
| Surface Area | 23,750 km² |
| Volume | 284 km³ |
| Average Depth | North basin 13.3 m |
| | South basin 9 m |
| Length | 436 km |
| Maximum Width | North basin 111 km |
| | South basin 40 km |
| Shoreline Length | 1750 km |

# 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 cyano-sites. (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 | *Pseudanabaena* *Anabaena* *Aphanizomenon* *Microcystis* | | | 0.05 (1) | | | | | 0.07 (1) |
| Lake Huron Saginaw Bay Bay City, MI | *Pseudanabaena* *Anabaena* *Aphanizomenon* *Microcystis* | 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 | ***Pseudanabaena*** *Planktothrix* *Anabaena* *Aphanizomenon* *Microcystis* | **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 | *Pseudanabaena* *Anabaena* *Microcystis* | 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 | *Aphanizomenon* | 0.04-0.1 (4) | | 1.33-0.3 (3) | | 0.02 (1) | | | 0.03 (1) |

# Questions?

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