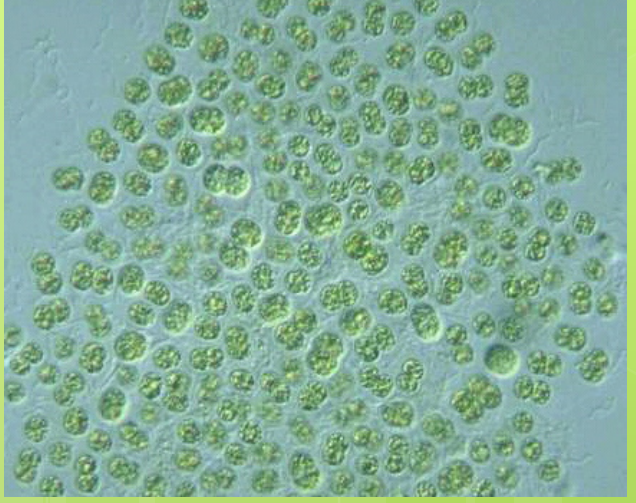
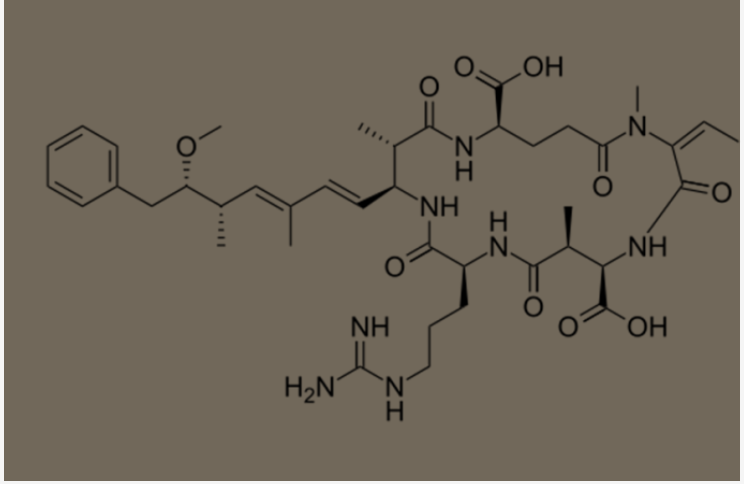




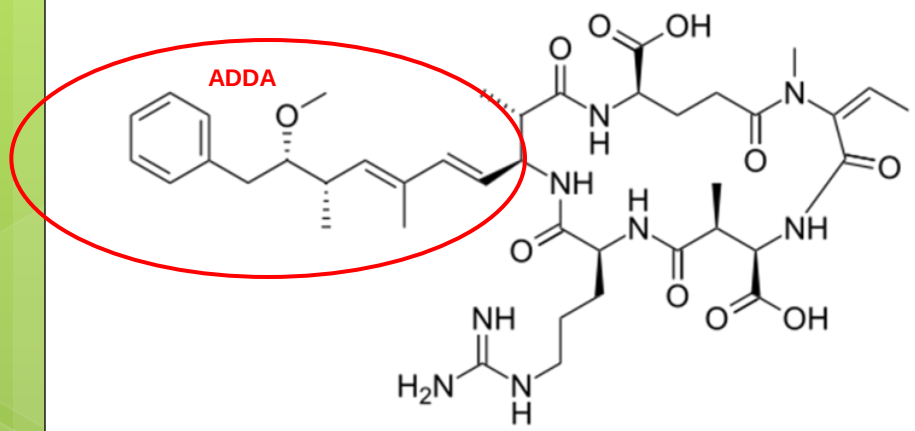
Cyanotoxin Laboratory Analysis

Daniel Orr

CA Department of Fish and Wildlife

Microcystins

- Cyclic heptapeptides (90+)



- Hepatotoxin +
- Tumor promoter
- Stable (weeks-months)
- Most common of the cyanotoxins (occur worldwide); widespread poisonings and <u>most problematic</u>

Exposure Risk and Toxin Concentration (How low do we need to go?)

WHO risk definitions (*Chorus and Bartram, 1999*):

- Low risk: less than 10 micrograms per liter (µg/L)
- Moderate risk: 10–20 µg/L
- High risk: 20–2,000 µg/L
- Very high risk: greater than 2,000 µg/L

WHO provisional guideline for drinking water

- 1 µg/L for microcystin-LR

Analytical reporting limit needed - (1 µg/L÷10)

- 0.1 µg/L (ppb)

OEHHA recommended recreation action level

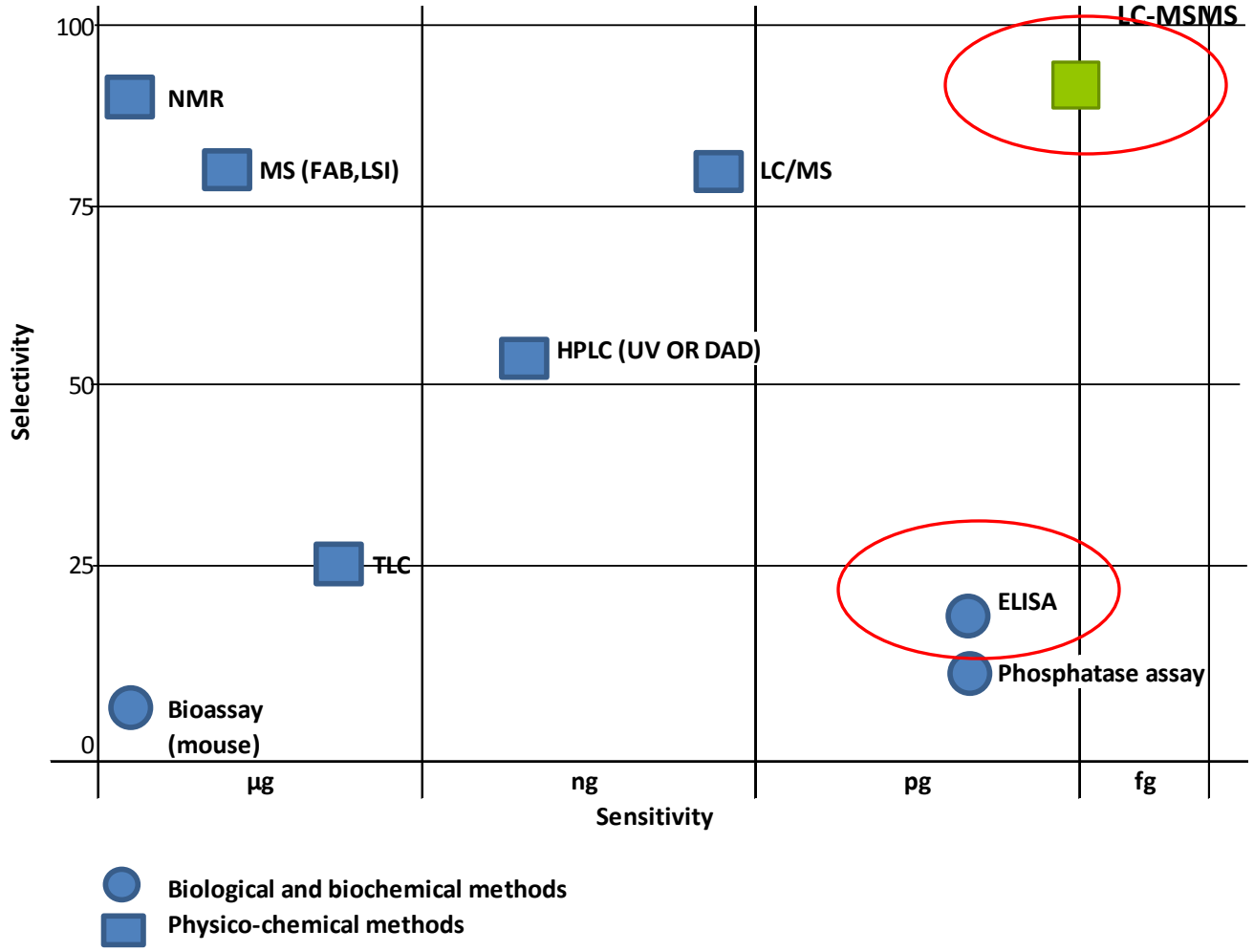
- 0.8 µg/L

Analytical Challenges

- An area of active research: over 90 microcystin variants known to exist[1]
- Few standardized analysis methods exist
- Need selective and sensitive methods
- Need low cost screening method(s) for large numbers of samples
- Analytical standards exist for only a few microcystin variants
- Toxin-producing genera generally produce more than one cyanotoxin[2]

[1] Walker and Von Dohren, 2006, FEMS Microbiology Reviews, v.30, p. 530-563
[2]Keith Loftin, USGS

Relationship Between Sensitivity & Selectivity of Analytical Methods for Microcystins*



*Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management, Ch 13, WHO 1999

Currently Offered at WPCL

- ELISA (ADDA) total Microcystins(freshwater or seawater with prior arrangement)
- LC-MSMS microcystins quantitative (waters and tissue [fillet, liver, heart])
  - 9 congeners, nodularin, okadaic acid, and domoic acid
- Anatoxin A (RBA) fresh or saltwater
- Anatoxin A by LC-MSMS (freshwater only)

ELISA Screening

- ADDA kit for waters reported as total microcystins
- Selective for the common structural element of microcystins
- Provides a semi-quantitative total that is not dependent on available standards
- Relatively low cost
- Follow up with LC-MSMS for positive samples especially where congener specific information is needed

Comparison of ADDA vs DM ELISA

- No ELISA is MC-LR Specific
- Most cross-reactivites are unknown (CRMs)

ADDA

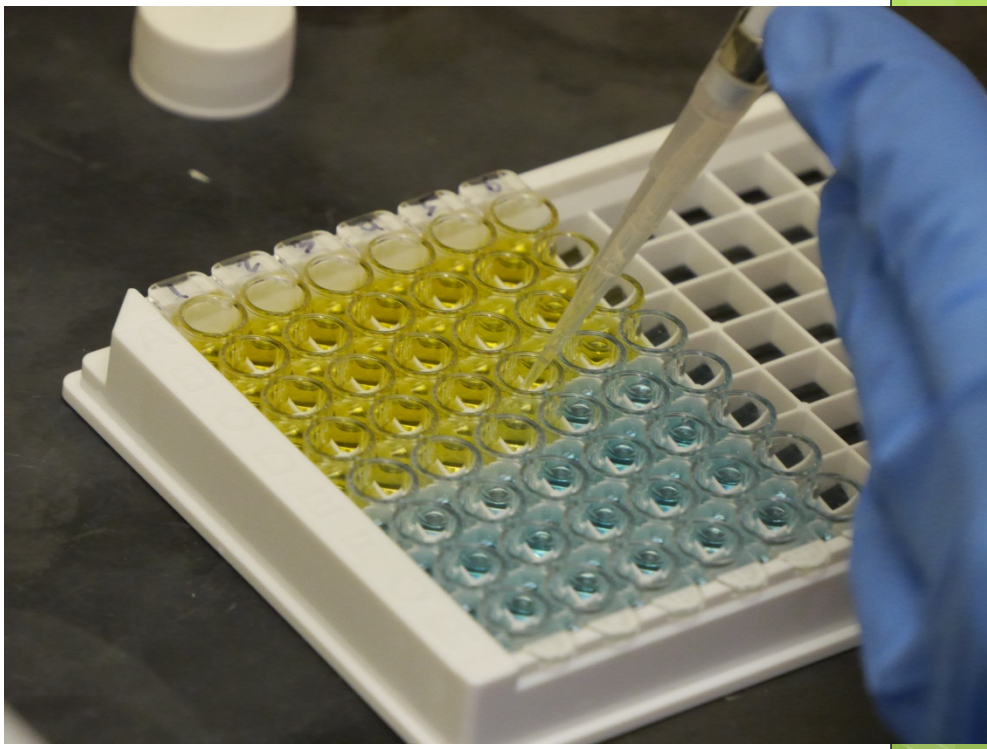
- Broad cross reactivity
- High bias some congeners
- Reports of false positives in tissue
- Semi-quantitative

Direct Monoclonal (DM)

- Selective cross reactivity
- Low bias some congeners
- Cost advantage
- Semi-quantitative

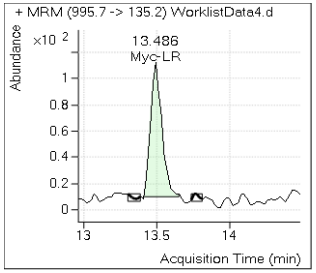
Wells, Stats, and Prices

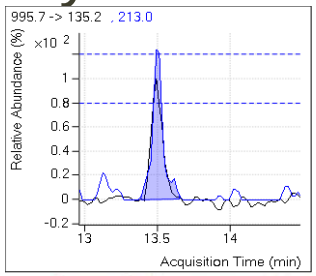
- Recommend/request triplicate wells
- Moderately temperature sensitive
- Plate size, turn around time, and quality control drive cost
- Analyst - good technique is important for precision
- Matrix effects
- Kit manufactures may advertise coefficients of variation (CVs) based on absorbance not concentration
- Confirm the calculation that provides the best curve

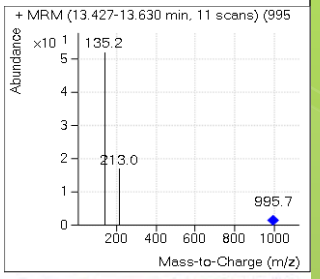


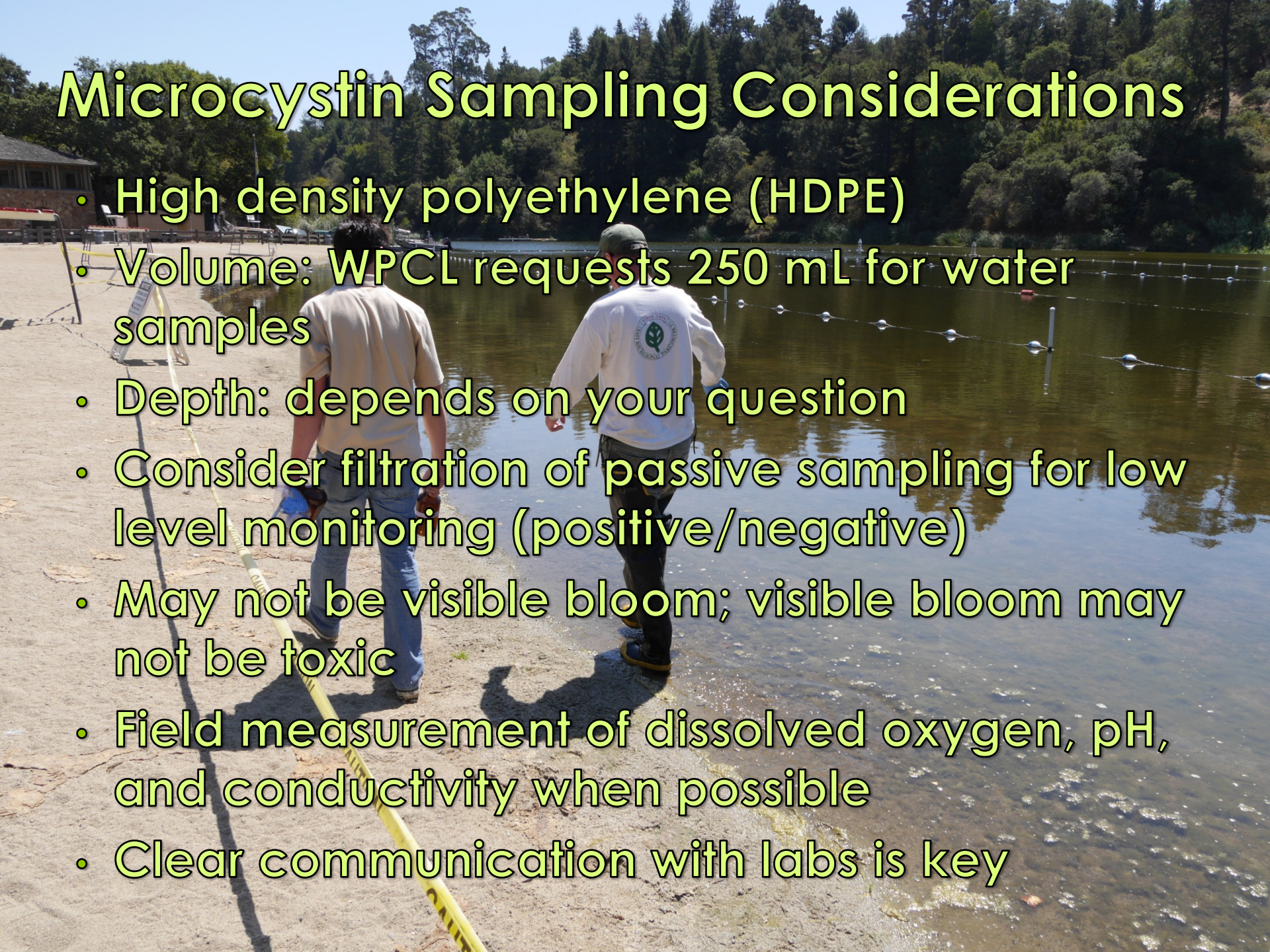
LC-MSMS of Microcystins

- Reporting limit 0.02 ppb for individual toxin
- Analyte list based on availability of standards
  - Microcystin-LR (MC-LR)
  - Desmethyl MC-LR
  - MC-RR
  - Desmthyl MC-RR
  - MC-LA
  - MC-LF
  - MC-LW
  - MC-LY
  - MC-YR
  - Nodularin, Domoic Acid, Okadaic Acid
- Higher funding and time requirements









Microcystin Sampling Considerations

- High density polyethylene (HDPE)
- Volume: WPCL requests 250 mL for water samples
- Depth: depends on your question
- Consider filtration of passive sampling for low level monitoring (positive/negative)
- May not be visible bloom; visible bloom may not be toxic
- Field measurement of dissolved oxygen, pH, and conductivity when possible
- Clear communication with labs is key

Sampling: Risks and Control

Risk: Wet Biomass

- Oral
- Cutaneous and mucous membrane
- Inhalation

Control

- Avoid accidental immersion
- Waterproof boots, gloves, waders, avoid splash generation
- Work in calm water condition
- Carry bleach

Risk: Dry Biomass

- Cutaneous and mucous membrane
- Inhalation

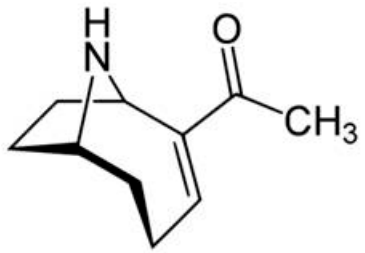
Control

- Gloves, overalls
- Consider wetting before harvest or wear respirator

Stewart Et al. Occupational and environmental hazard assessments for the isolation, purification and toxicity testing of cyanobacterial toxins. Environmental Health. 2009, 8:52

Anatoxin A

- Acute neurotoxin
- Secondary bicyclic amine alkaloid
- Agonist of both neuronal and nicotinic acetocholine receptors
- OEHHA recommended action level 90 µg/L



Anatoxin A

- Receptor-Binding Assay(RBA)
  - Reporting limit 0.2 ppb
  - Range 0.2 – 10 ppb
  - Temperature sensitive
  - Evaporative loss at 37^0C
  - Drift control
  - Recommend triplicate wells
- LCMSMS
  - Reporting limit 0.2 ppb
  - Currently direct injection
  - Freshwater samples only

Sampling Considerations

- Anatoxin A is light sensitive
- Sample pH impacts degradation rate and analytical results

- High density polyethylene (HDPE)
- Volume: WPCL requests 250 mL for water samples
- May not be visible bloom; visible bloom may not be toxic
- Field measurement of dissolved oxygen, pH, and conductivity when possible
- Clear communication with labs is key

Next Steps

- Tissue methods
- Anatoxin a extractions
- Respond to developing regulations
  - Other toxins [cylindrospermopsin, saxitoxin, yessotoxin]
  - Lower quantification needed?
- Identification of species, communities or toxic strains