Development of a Molecular Toolbox for Analyses of Bloom-Forming and Toxin-Producing Cyanobacteria in Upper Klamath Lake, Oregon 2013-2014

Sara Eldridge

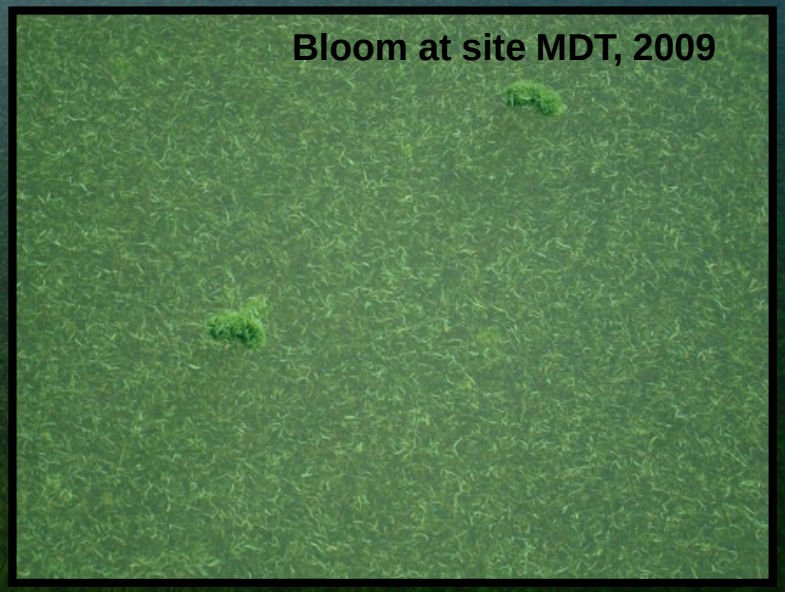
Oregon Water Science Center,

Klamath Falls Field Station

National Monitoring Conference

Cincinnati, OH 2014

U.S. Department of the Interior
U.S. Geological Survey



Acknowledgements

- USGS, Office of Water Quality and NAWQA
- Erin Stelzer, Rebecca Bushon, and personnel USGS Ohio Water Microbiology Lab
- Heather Johnson, Natasha Isaacs, and personnel, USGS Michigan Bacteriological Research Lab
- Theo Dreher, Department of Microbiology, Oregon State University
- Tamara Wood, Oregon Water Science Center

USGS

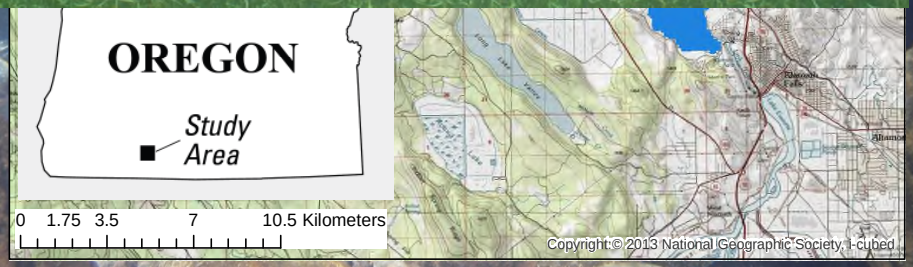
Upper Klamath Lake, Oregon

- Large (305 km^2), shallow (2.6 m, average)
- Hypereutrophic
- Seasonal blooms of *Aphanizomenon flos-aquae* (AFA; > 90 %)
- Microcystins, *M. aeruginosa*
- Endangered Lost River and shortnose suckers

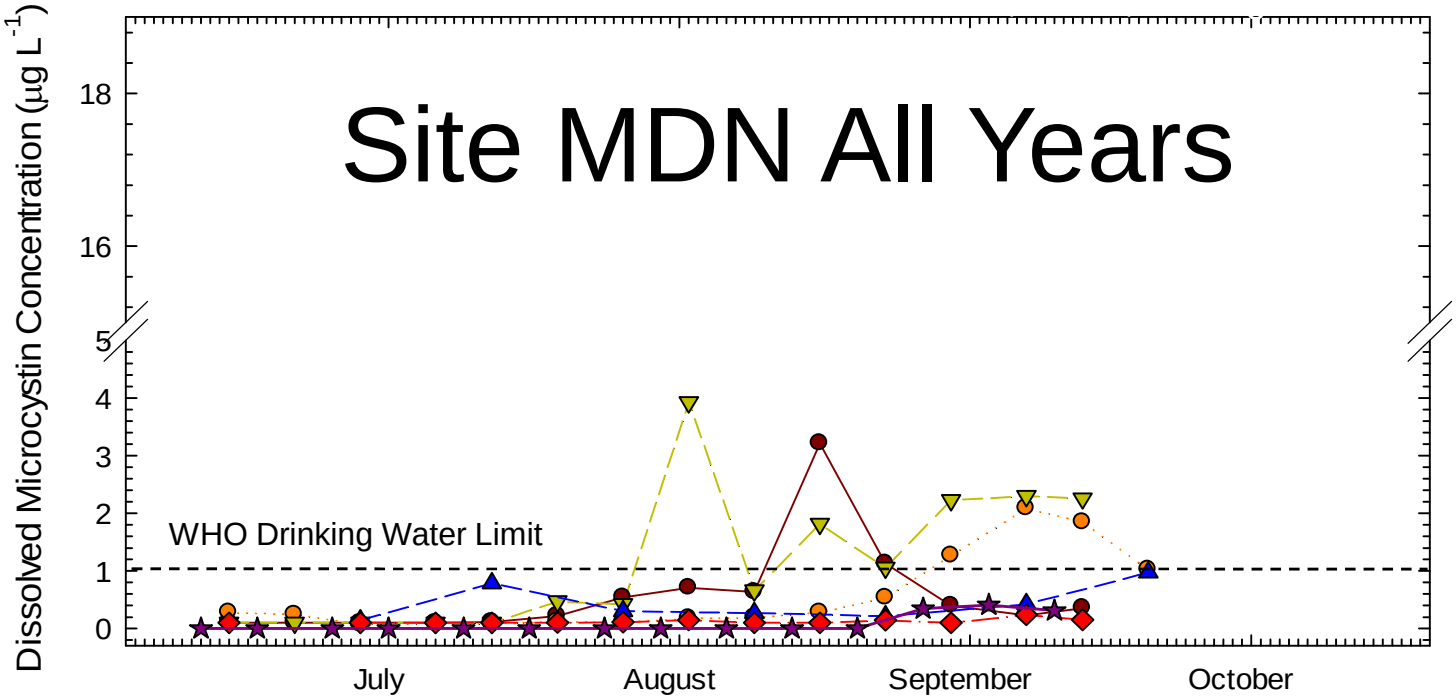
http://or.water.usgs.gov/klamath/

USGS

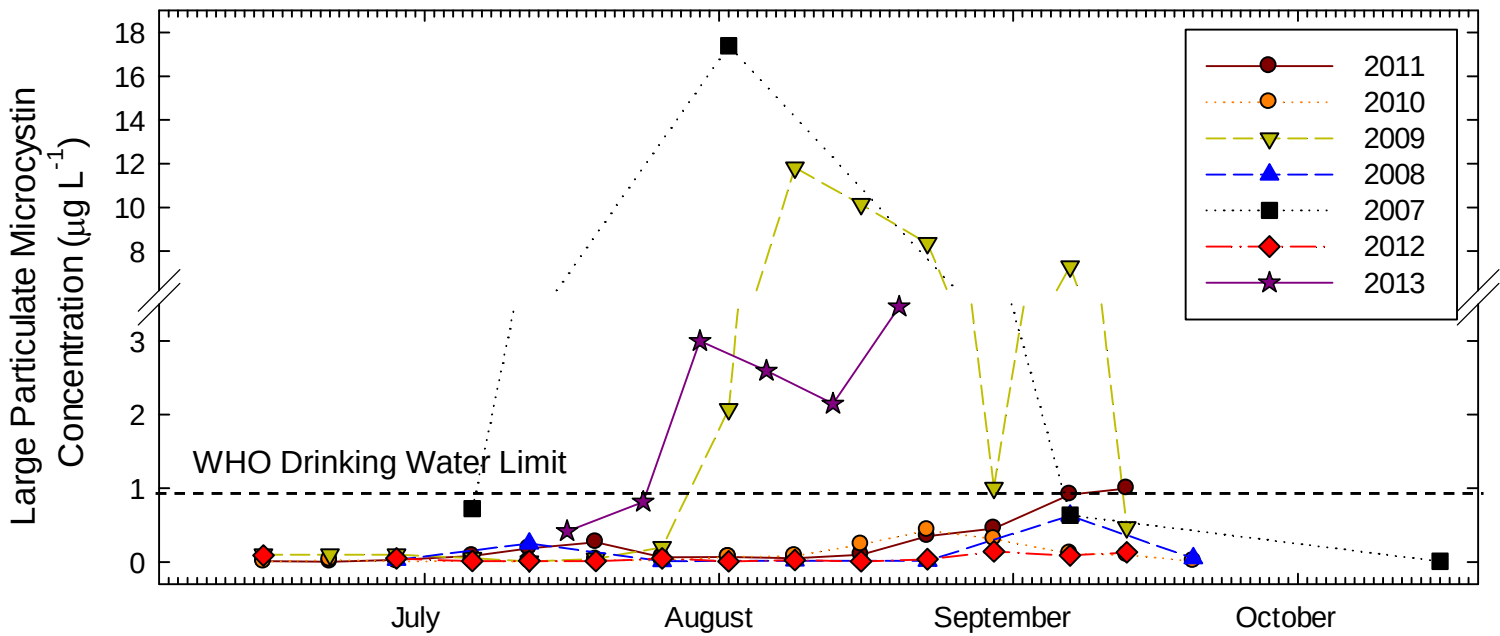
- Bloom Growth
  - High pH (> 9)
  - High [NH_3]
  - O_2 super-saturation
- Bloom decline
  - High dissolved N, P concentrations
  - Severe anoxia



Dissolved, 2008-2013

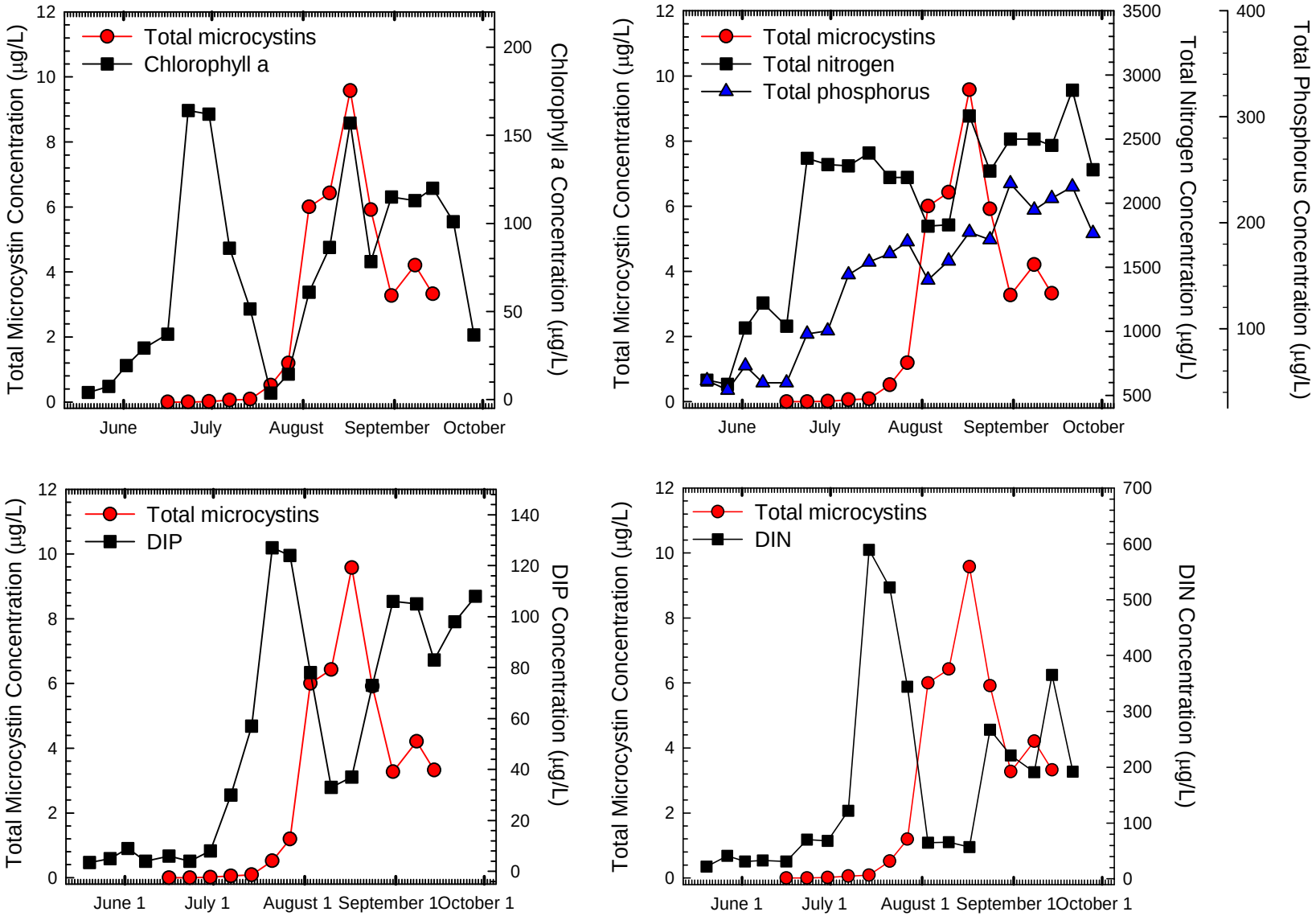


Large Particulate Fraction, 2007-2013



2013 data are provisional

Total Microcystins vs. Chlorophyll *a*, Total and Dissolved Nutrients, 2009

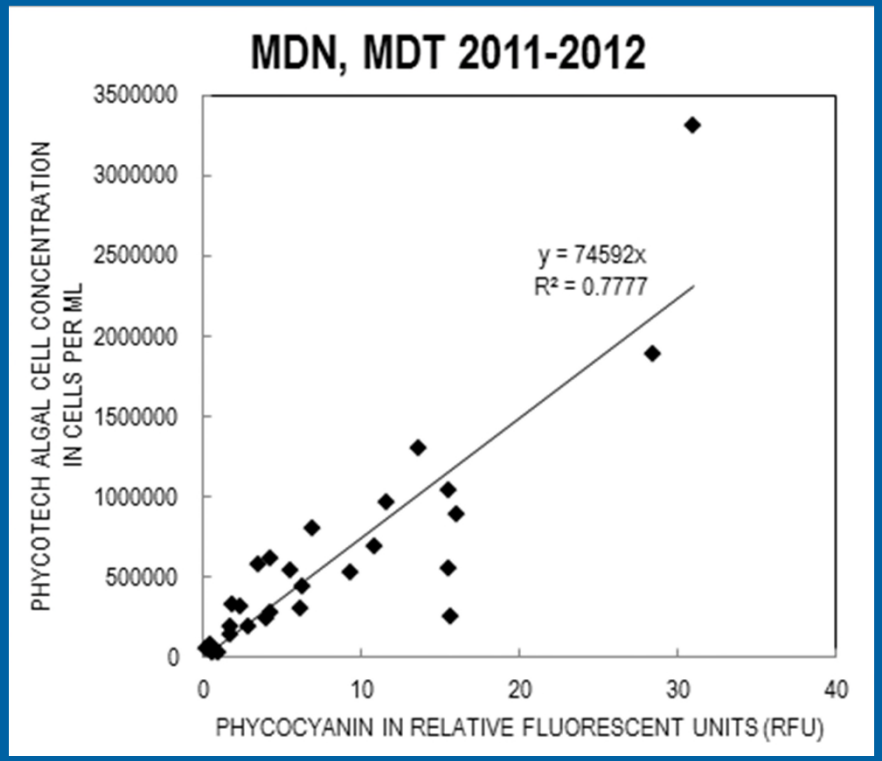


Eldridge and others, 2012: USGS SIR 2012-5069

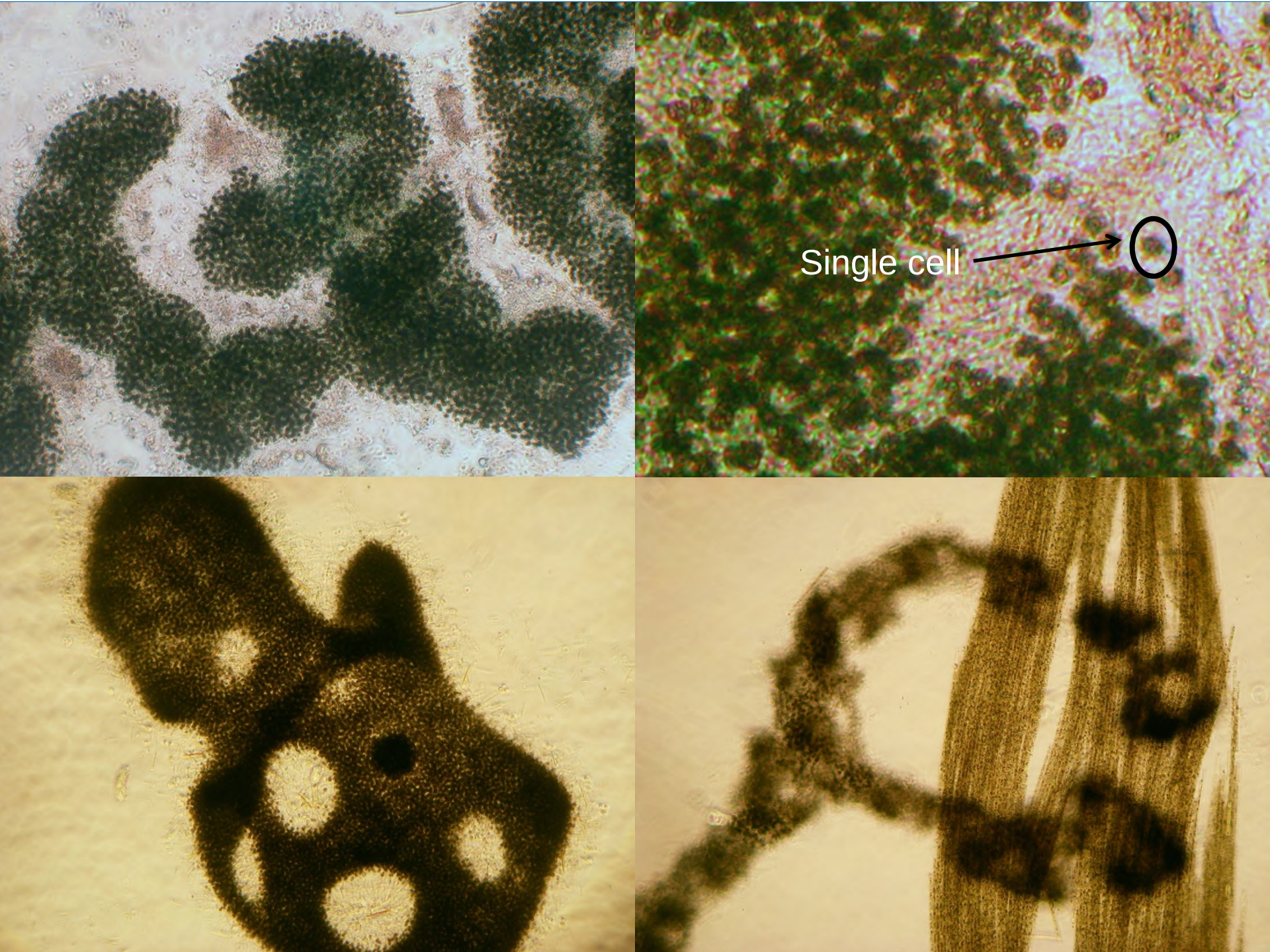
Current Phytoplankton Monitoring

- Microscopic ID, enumeration
- Phycocyanin fluorescence probe, continuous
  - Requires sample enumeration for data correction

Probe RFU vs. sample analysis (cell/mL) to determine ADAPS table 3 correction



USGS


Single cell

Molecular Toolbox Objectives

GOAL: **Rapid analysis of cyanobacterial community structure and detection and quantification of bloom-forming *Aphanizomenon flos-aquae*, potentially microcystin-producing *Microcystis aeruginosa*, and total cyanobacteria**

OBJECTIVES:

1. **Characterize changes in the cyanobacterial community structure temporally using clone library and DNA sequencing (2013 IP)**
2. **Detect, quantify, and determine changes in the relative abundances of potentially toxigenic and non-toxigenic strains of *M. aeruginosa* using qPCR (2013 completed)**
3. **Quantify cells of *A. flos-aquae**, *M. aeruginosa*, and total cyanobacteria using qPCR and to determine the relative abundance ratios of *A. flos-aquae*, total *M. aeruginosa*, and potentially toxigenic *M. aeruginosa* within the total cyanobacteria community throughout the Upper Klamath Lake sample season (from May to September)**

USGS

Obj 1. Environmental DNA Sequencing

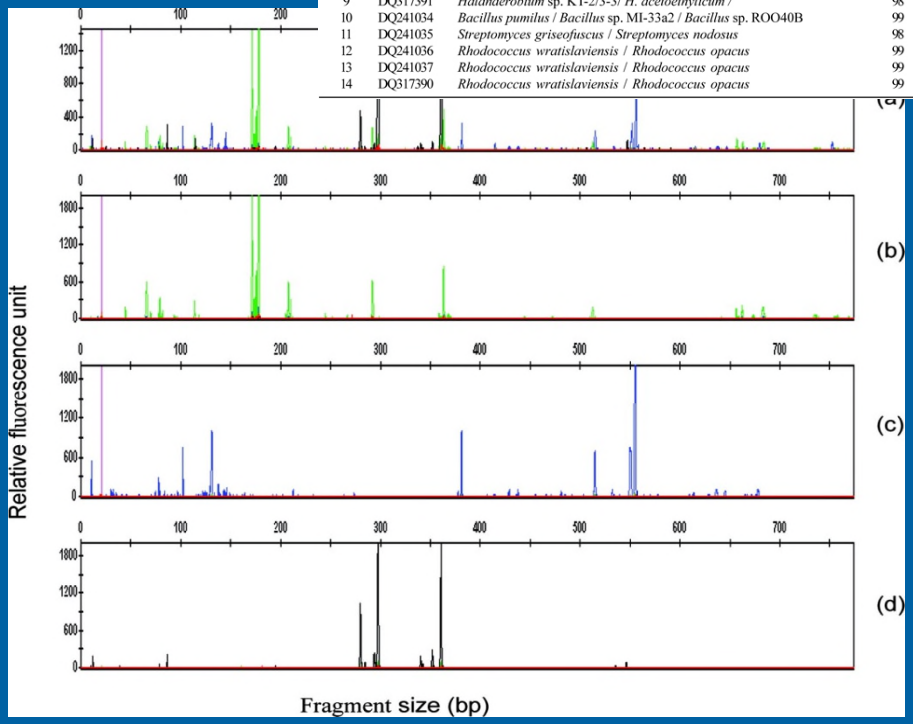
(In progress, USGS MI-BaRL, Lansing, MI)

- Who's there?
- Clone library to isolate genes from community
- Semi-quantitative
- Identify genetic variants
- Reduce cost, time with t-RFLP

EXAMPLE clone library sequence data from Oliveira and others, *Braz. J. Microbiol.* 2008: 39:445-452

Table 1. Sequence identities of formation water and oil clones from petroleum reservoir one.

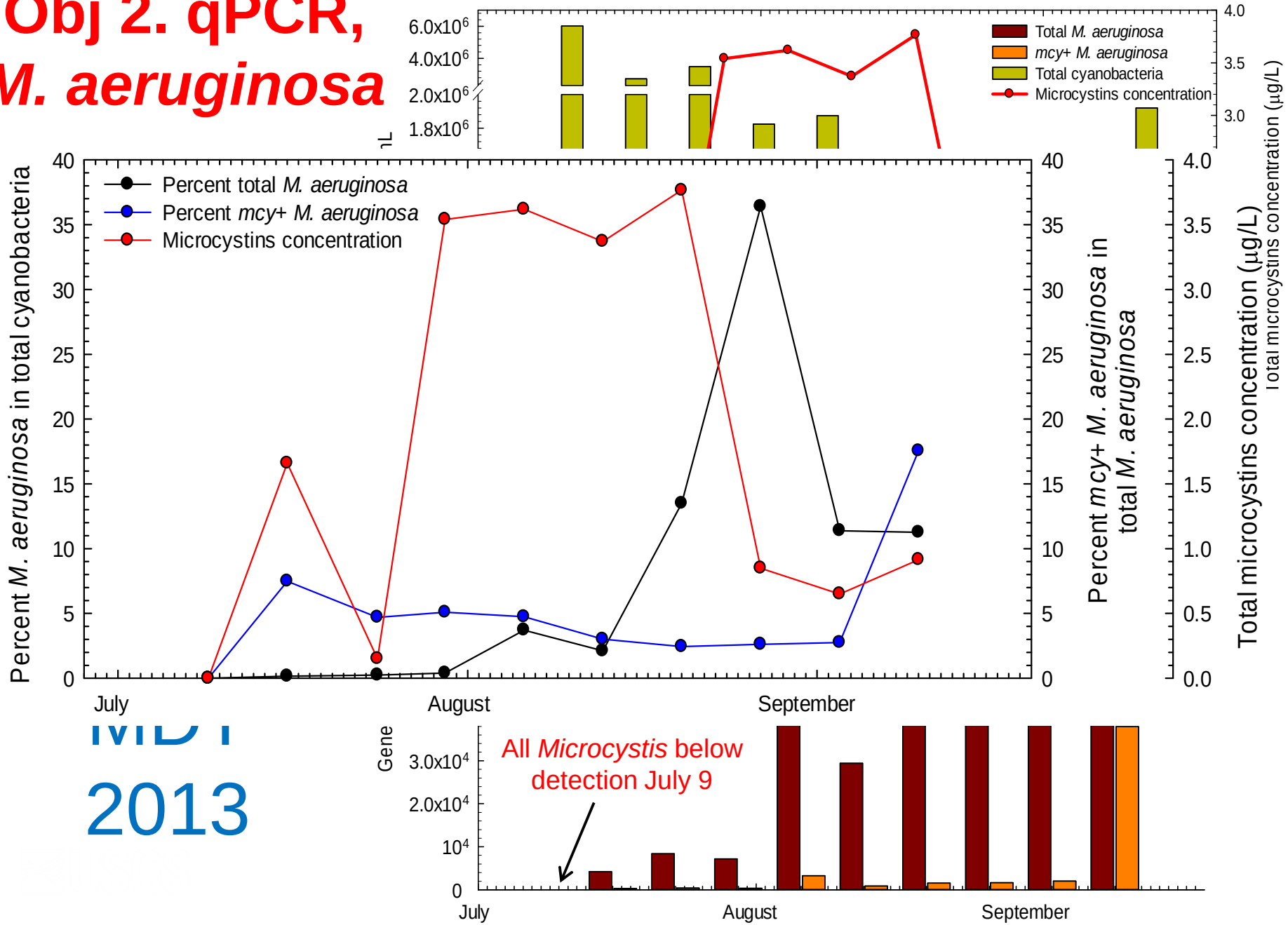
Clone	Accession number	Best match in Blast analysis	Identity scores (% similarity)	Sample
A	DQ241019	*Halanaerobium acetethylicum* / *H. congolense* / *H. saccharolyticum*	98	Water
B	DQ241025	*Halanaerobium saccharolyticum* / *H. acetethylicum* / *H. kushnerii*	98	Water
D	DQ241027	*Bacillus pumilus* / *Bacillus* sp. ROO40B	95	Water
E	DQ241020	*Halanaerobium acetethylicum* / *H. congolense*	98	Water
F	DQ241021	*Halanaerobium acetethylicum*	99	Water
G	DQ241022	*Halanaerobium acetethylicum*	99	Water
H	DQ241023	*Halanaerobium acetethylicum* / *H. congolense* / *H. saccharolyticum*	98	Water
I	DQ241024	*Halanaerobium acetethylicum* / *H. congolense*	98	Water
J	DQ241026	*Halanaerobium saccharolyticum* / *H. acetethylicum* /	98	Water
K	DQ241028	*Halanaerobium acetethylicum* / *H. congolense*	98	Water
1	DQ241029	*Acidithiobacillus ferroxidans*	99	Oil
2	DQ241030	*Halanaerobium* sp. KT-2/3-3 / *H. acetoethylicum* / *H. congolense* / *H. saccharolyticum* subsp. *senegalense*	95	Oil
3	DQ241031	*Halanaerobium* sp. KT-2/3-3/ *H. acetoethylicum*	98	Oil
5	DQ241032	*Alicyclobacillus acidoterrestris* ATCC 49025	97	Oil
8	DQ241033	*Halanaerobium* sp. KT-2/3-3/ *H. acetoethylicum* / *H. congolense* / *H. saccharolyticum* subsp. *senegalense*	97	Oil
9	DQ317391	*Halanaerobium* sp. KT-2/3-3/ *H. acetoethylicum* /	98	Oil
10	DQ241034	*Bacillus pumilus* / *Bacillus* sp. MI-33a2 / *Bacillus* sp. ROO40B	99	Oil
11	DQ241035	*Streptomyces griseofuscus* / *Streptomyces nodosus*	98	Oil
12	DQ241036	*Rhodococcus wratislaviensis* / *Rhodococcus opacus*	99	Oil
13	DQ241037	*Rhodococcus wratislaviensis* / *Rhodococcus opacus*	99	Oil
14	DQ317390	*Rhodococcus wratislaviensis* / *Rhodococcus opacus*	99	Oil



EXAMPLE t-RFLP profiles from Singh, and others, *Appl. Environ. Microbiol*. 2006;72:7278-7285

USGS

Obj 2. qPCR, *M. aeruginosa*



MDT

2013

Obj. 3. qPCR *A. flos-aquae*, *M. aeruginosa*, total cyanobacteria

- New method for *A. flos-aquae* developed at Oregon State University (T. Dreher)

- <u>Results will be used to analyze:</u>
- *A. flos-aquae* vs. *M. aeruginosa* growth dynamics, relation to total cyanobacteria community and other monitored parameters
- Total cyanobacteria measured by qPCR vs. direct counts for adjusting phycocyanin probe data on continuous monitors

USGS

Preliminary Results: Total Cyanobacteria Quantified by qPCR vs. Direct Counts, 2013

- *M. aeruginosa* identified in 90% of qPCR samples, 17% of microscopy samples, MDT
- 14-30% identified only to Family Chroococcaceae in microscopy samples after Aug. 27, MDN and MDT
- Strong correlation between chlorophyll *a* concentration and qPCR or direct counts, MDT

- Next steps: 2014 data collection, compare qPCR results with phycocyanin fluorescence data

USGS