

## Session C5: Cyanobacteria Detection and Monitoring Methods

Room C123  
3:30 – 5:00 pm

**0223**  
**C5-1**

### **Using Real-Time Monitoring for Assessing Cyanobacteria Algal Blooms and Water Quality Conditions at Two Waterbodies in the Boston Urban Area**

Tom Faber and Liz McCarthy

*US Environmental Protection Agency Region 1, North Chelmsford, Mass., USA*

In response to recent concerns over cyanobacteria blooms, US EPA Region 1 New England has two real-time monitoring stations in the Mystic and Charles River watersheds, which includes the Boston, MA urban area. Cyanobacteria blooms in the Mystic and Charles River watersheds have caused beach closings, posted warnings, and cancelled swimming races. Monitoring results along with challenges encountered in the two years of operation will be presented. Real-time monitoring data includes temperature, specific conductance, dissolved oxygen, pH, turbidity, chlorophyll, and phycocyanin. Field instrument quality assurance/quality control and laboratory results for cell counts and chlorophyll will be discussed. In addition, data collected near the real-time monitoring station (also to assess cyanobacteria concentrations) from partnering organizations including the MA Department of Public Health, Tufts University and a local volunteer monitor for the Mystic and Charles River Watershed Associations will be presented.

**0543**  
**C5-2**

### **New Mass Spectrometry Methods for the Evaluation of Cyanobacterial Blooms**

Claudia S. Maier, Soyoun Ahn, Ievgen Motorykin, Jeff Morre and Theo Dreher *Oregon State Univ., Corvallis, Oreg., USA*

New mass spectrometry methods based on electrospray ionization, gas phase ion mobility separations, and high resolution mass spectrometry were evaluated for profiling cyanobacterial blooms in Pacific Northwest freshwaters. Microcystins and other peptide biotoxins exhibit high structural variability which causes significant analytical challenges for assessing the composition of blooms. Here we report the use of ion mobility separation in combination with high resolution MS/MS approaches (realized in a Waters Synapt G2 High Definition Mass Spectrometry system) for profiling and identification of cyanobacterial peptides. We also discuss the use of a new quadrupole time-of flight instrument, an AB Sciex TripleToF 5600 system, which allows realization of high-resolution “MRM-like” quantification as an alternative method to the well established targeted LC-MS/MS approaches. Spiked samples, cell culture extracts and natural waters were analyzed to show proof-of-performance of these new mass spectrometry platforms.

**0263**  
**C5-3**

### **Suitability of *In Vivo* Fluorometry and Backscatter Data to Assess Short-Term Fluctuations of a Cyanobacteria Bloom, Upper Klamath Lake, Oregon**

Liam Schenk<sup>1</sup>, Tamara Wood<sup>2</sup>, Sara Eldridge<sup>1</sup> and Blake Eldridge<sup>1</sup>

<sup>1</sup>*US Geological Survey, Klamath Falls, Oreg., USA*, <sup>2</sup>*US Geological Survey, Portland, Oreg., USA*

The US Geological Survey has monitored seasonal variability in water quality associated with large blooms of *Aphanizomenon flos aquae* on Upper Klamath Lake, Oregon, since 2002. Monitoring in 2011 included the deployment of field sensors for the measurement of phycocyanin (a pigment in cyanobacteria) using *in vivo* fluorometry at two monitoring sites. The sensors recorded hourly cyanobacteria concentration in cells per milliliter and relative fluorescence units throughout the 2011 field season at a depth of 1 meter from the surface. Due to potential interferences with *in vivo* fluorometry (such as turbidity and temperature) and the variability in cell volume among cyanobacteria, post calibration was performed using cyanobacteria enumeration of samples and compared against sensor data. Preliminary analysis of phycocyanin data from the field sensors shows that the sensors are qualitative tools when attempting to assess concentrations of cyanobacteria blooms *in situ*, and that those data require validation with cyanobacteria enumeration of field samples. Backscatter data from an acoustic Doppler current profiler at one of the sites was used as a surrogate for suspended solids to provide insight into the short-term variability of biomass in the water column. These backscatter data, in conjunction with the phycocyanin sensors, were used to describe the vertical structure of the Cyanobacteria bloom, at 1-hour

intervals throughout the field season. These two types of data also were used to assess short-term (daily to weekly) variability in depth-integrated particulate nutrients. Validation data were provided by water samples collected weekly and during two diurnal sampling events. Use of these techniques will improve the assessment of algal bloom and nutrient dynamics in the water column on short time scales and has applicability to other shallow lake systems.

**0404**  
**C5-4**

#### **Evaluation and Comparison of Sample Splitting and Cell Lysis Techniques for Recovery of Total Microcystins from Cyanobacteria**

Keith Loftin<sup>1</sup>, Barry Rosen<sup>2</sup>, Rachael Lane<sup>3</sup>, Jennifer Graham<sup>4</sup> and Susan Keydel<sup>5</sup>

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Historically, a variety of sample collection and cell lysis techniques have been used to recover total microcystins, a group of toxins produced by some species of cyanobacteria, despite a general lack of validation data. Previous interlaboratory microcystin comparisons using multiple sampling, lysis, and analytical techniques revealed a much greater variability than is required to compare total microcystin between samples or data sets. Five major sources of variability have been identified and include 1) lack of available certified reference standards, 2) split sample irreproducibility, 3) incomplete cell lysis, 4) incomparable analytical methods and data reduction, and 5) matrix effects. Microcystin producing cyanobacterial bloom samples were collected from surface water across the United States. Surface water bloom samples from lakes and rivers were homogenized and split by churn splitter. Aliquots of each churn split sample were also lysed by five techniques: autoclave, boiling, sequential freeze/thaw, sonication, and a commercial reagent called QuikLyse™. All samples were assessed for microcystins by enzyme-linked immunosorbent assay and cell lysis by microscopy. Churn splitter samples also were analyzed for total chlorophyll and morphological cyanobacterial identification and enumeration. Churn splitter replicates showed that percent relative standard deviation (%RSD) of chlorophyll results for individual bloom samples ranged from 8.1 to 150 % with an average of 34 % overall. Percent RSD for replicate measurements of cyanobacterial abundance (cells/L) and total and dissolved microcystin concentrations ranged from 2.7 to 58 % and 10 to 107 % RSD with averages of 16 % and 34% overall. Lower relative microcystin recoveries were observed for the cyanobacteria cell lysis comparison in several cases for QuikLyse™ and sonication compared to autoclave, boiling, and freeze/thaw despite complete cell disruption at some sonication power settings. Autoclave and boiling techniques also released large concentrations of cyanobacterial pigment compared to sequential freeze/thaw which may lead to matrix effects and can effect qualitative and quantitative results produced by various analytical techniques. Generally, filamentous microcystin producers were the most difficult to lyse. These results have implications on the ability to accurately and reproducibly measure chlorophyll, cyanobacterial abundance, and microcystins.