Development of Strategies and Methods for Monitoring for Algal Blooms and Occurrence of Toxic Cyanobacteria Using Next Generation qPCR and Phylochip Microarrays

Laura Webb, EPA Region 7
Project Team

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- Eric Villegas, Jingrang Lu – ORD Exposure Methods and Measurements, National Exposure Research Laboratory, Microbial Exposure Branch
- Heath Mash – ORD National Risk Management Research Laboratory, Water Systems Division, Drinking Water Systems Branch
Regional Applied research effort (RARE)

- Collaborative effort between and ORD office or lab and a regional office or lab
- Any high priority research need that the region has and that ORD has the expertise to address
- Each region nominates projects and selects, based on merit and funding, which ones move forward
- 2 year project in 5 regional lakes
Goals

• Purpose is to develop a good screening tool to determine if and what genus and or species of cyanobacteria are present and to determine if they are capable of producing toxins
• qPCR (R7) and the qPCR/RT-qPCR (ORD)
  – Using a commercial kit (CyanoDTec) will yield Total Cyanobacteria, Toxin Producing Genes (Microcystin/Nodularin, Saxitoxin, Cylindrospermopsin)
    • Pro: It’s a commercially available kit that has gone through NIST equivalence testing and certification *product of Australia
    • Con: Not sure if the gene targets used are the same as those that reside here in the US and can cost $60/sample to analyze
  – ORD using RT-qPCR
    • Pro: They have many studied methods, gene targets and research to verify the validity of the CyanoDTec
    • Con: They don’t have a standardized protocol/method
  – Correlate molecular and biological results with chemical results
Phylochip and High Throughput Sequencing

- The high throughput sequencing will be used to build a whole genome map of the microbial community.
- Alongside the Phylochip, we will look for any connections in the community composition and bloom formation/toxicity.
- With PhyloChip we are also trying to evaluate the types of cyanobacteria from Phylum down to species.
  - Phylochip has over 800 cyanobacteria listed.
- With the Phylochip we are also trying to develop the Microbial source tracking capabilities.
- Work is still being done on this and not all data has been received and or interpreted.
Sites

- Five lakes chosen within easy driving distance to STC
- Two lakes chosen to represent “reference” conditions, or less impacted area (Bethany, MO and 9 Eagles State Park, IA)
- Three lakes chosen to represent known HAB locations (Smithville Lake, MO, Milford and Lake Perry, KS)
## Watershed Characteristics

<table>
<thead>
<tr>
<th>Land Cover Type</th>
<th>Open Water</th>
<th>Forest</th>
<th>Shrub/Scrub</th>
<th>Herbaceous</th>
<th>Wetlands</th>
<th>Dev, Open Space</th>
<th>Dev, Low Intensity</th>
<th>Dev, Med Intensity</th>
<th>Dev, High Intensity</th>
<th>Pasture/Hay</th>
<th>Crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nine Eagles Lake</td>
<td>6.79</td>
<td>78.37</td>
<td>0.08</td>
<td>2.08</td>
<td>0.43</td>
<td>4.54</td>
<td>0.56</td>
<td>0.00</td>
<td>0.00</td>
<td>5.92</td>
<td>1.24</td>
</tr>
<tr>
<td>Bethany Reservoir</td>
<td>11.11</td>
<td>20.88</td>
<td>2.07</td>
<td>1.92</td>
<td>5.12</td>
<td>1.89</td>
<td>0.00</td>
<td>0.00</td>
<td>50.79</td>
<td>6.22</td>
<td></td>
</tr>
<tr>
<td>Smithville Lake</td>
<td>6.12</td>
<td>9.70</td>
<td>0.33</td>
<td>0.82</td>
<td>1.97</td>
<td>4.98</td>
<td>1.52</td>
<td>0.19</td>
<td>0.04</td>
<td>38.64</td>
<td>35.57</td>
</tr>
<tr>
<td>Lake Perry</td>
<td>2.51</td>
<td>12.35</td>
<td>0.21</td>
<td>8.79</td>
<td>1.00</td>
<td>3.99</td>
<td>0.84</td>
<td>0.13</td>
<td>0.02</td>
<td>45.60</td>
<td>24.49</td>
</tr>
<tr>
<td>Milford</td>
<td>0.41</td>
<td>0.67</td>
<td>0.04</td>
<td>42.38</td>
<td>0.71</td>
<td>3.11</td>
<td>0.31</td>
<td>0.05</td>
<td>0.01</td>
<td>0.65</td>
<td>51.50</td>
</tr>
</tbody>
</table>
Sampling Plan

- Sampled each lake weekly on Tuesday for 26 weeks
- Surface water samples at 3 locations at each beach
- Composited grab samples for chemical
- Sterile grab samples for molecular, composited post-sampling
- Phytoplankton net for taxonomy
- *In situ* using two YSI sondes
Analytes

- **Chemical**: Metals, PAH, pesticides, herbicides, pharmaceuticals, nutrients, anions, personal care products, hormones, endocrine disrupters, chlorophyll a
- **Cyanotoxins**: ELISA (MC and CYL), LCMSMS MC congeners (ORD)
- **Biological**: cyanobacteria, e coli
- **Molecular**: cyanobacteria DNA, community DNA, p-PCR
- **In situ**: DO, pH, turbidity, conductivity, temperature, chlorophyll a, phycocyanin
Molecular samples

• 2017
  – E.coli every 5th week
  – Phylochip every 5th week
  – ORD provided taxonomic sequencing, qPCR, and rt-qPCR
  – R7 qPCR

• 2018
  – Weekly sampling for all molecular parameters
  – R7 qPCR and E.coli
  – ORD qPCR and rt-qPCR
  – Phylochip targeted sampling
**Taxonomic Identification in Bethany**

- **Aphanizomenon**
- **Dolichospermum (Anabaena)**
- **Woronichina**

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### Cyanobacteria & Associated Toxins

<table>
<thead>
<tr>
<th>Toxin Group</th>
<th>Primary Target organ in mammals</th>
<th>Cyanobacterial genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystins</td>
<td>Liver</td>
<td>Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis</td>
</tr>
<tr>
<td>Nodularian</td>
<td>Liver</td>
<td>Nodularia</td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>Nerve Synapse</td>
<td>Anabaena, Planktothrix (Oscillatoria), Aphanizomenon</td>
</tr>
<tr>
<td>Aplysiatoxins</td>
<td>Skin</td>
<td>Lyngbya, Schizothrix, Planktothrix (Oscillatoria)</td>
</tr>
<tr>
<td><strong>Cylindrospermopsins</strong></td>
<td>Liver</td>
<td>Cylindrospermopsis, Aphanizomenon</td>
</tr>
<tr>
<td>Lyngbyatoxin-a</td>
<td>Skin, G.I. Tract</td>
<td>Lyngbya</td>
</tr>
<tr>
<td>Saxitoxins</td>
<td>Nerve Axons</td>
<td>Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>Potential irritant; affects any exposed tissue</td>
<td>ALL</td>
</tr>
</tbody>
</table>
Bethany Temperature and Toxin Relationship

- Cylindrospermopsin found only in Bethany, and always at some level in Bethany
- Found 4 years in a row here
- Highest concentration of toxin found in cooler months
- Never above the EPA Draft water quality criteria/swimming advisory of 8 ug/L
- EPA Drinking Water Health Advisory (10-day)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bottle-fed infants and pre-school children</th>
<th>School-age children and adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylindrospermopsin</td>
<td>0.7 µg/L</td>
<td>3 µg/L</td>
</tr>
</tbody>
</table>

Bethany Reservoir 2017 & 2018

Bethany Reservoir
• Bethany has a significantly different concentration of copper
• Time-line of Bethany shows a drastic spike in copper – perhaps treatment with copper based algaeicide
• Correlation of copper with lower chlorophyll a concentrations – killing off the algae
• Potential for lysing of cyanobacteria cells and releasing the toxin

Phylochip Ave Abund before almost 9000, after was 6500. Had 107 hits before, 57 after
DNA Sequencing

Bethany Reservoir, 2017

- Anabaena
- Cylindrospermopsis raciborskii
- Dolichospermum
- Woronichinia
- Planktothrix
- Pseudanabaena
- Synechococcus

Copper concentration levels:

- Cyanobacteria counts
- DNA Sequencing

Data from EPA United States Environmental Protection Agency.
Smithville Lake

Variable
- Temperature (°C)
- log2(16SDNA)
- log2(16SRNA)

Temperature (°C) vs Log2(copies/L)

04/18/2017 - 10/02/2018

Temperature (°C)

Log2(copies/L)
Relationship between Microcystin and DNA, RNA signals

Smithville Lake

Variable

- Microcystins (ppb) by ELISA
- \(\log_2(\text{mcyE DNA})\)
- \(\log_2(\text{mcyE RNA})\)
Smithville Lake – growing season 2017

Example tile name: L20171482017154.I3m_7D_S3A_CYAN_CI_cyanobacteria_CYAN_CO

If you click on the pixel in QGIS with the information tool, it will give you a number from 0-255, that number can be converted to a quantitative value. To convert the digital number (DN) to cells/mL:

Cl_cyanobacteria concentration or abundance

Cl_cyanobacteria = 10^([DN/100]*0.0001)

Using the 209 value above in the 9/3-9 tile
(DN/100) = (209/100) = 2.09
so... cyanobacteria abundance = 10^(-4)*0.0001 = 0.01293

Cyanobacteria Abundance (cells/mL) = Cl_cyanobacteria * 1.0E+8
0.01293 x 100,000,000 = 1,293,000 cells/mL

Smithville Lake – growing season 2017, continued

8/13-19
8/20-26
8/27-9/2
9/3-9
9/10-16
9/17-23

Don’t know if you want to convert the values to cells per milliliter but I did it for one of the values. You can check my math— not sure I did it right.

Based on these 18 images from last summer, there were some weeks during each month when the eastern arm looked bad, and higher up in the lake it got pretty bad a few times, too.

I didn’t look at any weeks prior to 5/21 nor anything after 5/23 yet.
Phycocyanin begins to increase in early July

CYAN smartphone app shows a signal

Detect Microcystin mid-July through September

Note: toxin concentration never near harmful levels
Lake Perry – growing season 2017

7/2-8
7/9-15
7/16-22
7/23-29
7/30-8/5
8/6-12
8/13-19
8/20-26
8/27-9/2
9/3-9
9/10-16
Kickapoo Tribe reported bloom on Delaware River on 8/7/17
Next:

- Develop the RNA/DNA qPCR methods regionally
- Correlate chemistry, physical, biological and molecular data
- 2019/2020 RARE for Milford Lake

Special thanks to our sampling team!!!