Cyanobacterial occurrence in lakes and rivers in the PNW and the role of eutrophication: Progress through genetic technologies

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Cyanobacterial blooms in the PNW and Northern California

Anderson Lake, Washington
Unusually high levels of anatoxin-a
Toxin producer identified

Willamette Valley reservoirs, Oregon
Potential toxicity in drinking water sources
Toxicity and annual strain successions

Klamath Valley, Oregon/California
Toxic Microcystis in reservoirs and river
Genotype trends over 9 years
Long-distance river transport
Anabaena/Aphanizomenon/Dolichospermum with varied toxigenicities: null, \textit{ana}+, \textit{cyr}+, \textit{mcy}+

Anderson Lake, Washington
Anatoxin-a up to 1090 µg/L
\textit{Anabaena} sp. WA102 \textit{Ana}+ culture

Cascade Range reservoirs & lakes, Oregon
Potential toxicity in drinking water sources
\textbf{Dexter Res: Non-toxic}
\textbf{Detroit Res: 7-epi-Cylindrospermopsin @ 195 µg/L}
\textbf{Odell Lake: Microcystin @ 675 µg/L}

Upper Klamath Lake, Oregon
\textit{Aphanizomenon flos-aquae} (AFA)
Harvested for “nutritional supplements”
\textit{AFA} UKL13 culture
Microcystis and microcystin in the PNW

**Washington State**
*Microcystis* and microcystin common in Washington State lakes

**Oregon**
Toxic *Microcystis* at low altitude W. Oregon, E. Oregon, and Willamette River in downtown Portland

**Klamath Valley, Oregon/California**
*Microcystis* in Upper Klamath Lake, Klamath River reservoirs and 300 km of Klamath River
# Eutrophication: Trophic State Index and PNW cyanobacterial blooms

<table>
<thead>
<tr>
<th>Trophic Class</th>
<th>Trophic State Index</th>
<th>Chl-a (µg/L)</th>
<th>TP (µg/L)</th>
<th>Secchi Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligotrophic</td>
<td>&lt;40</td>
<td>&lt;2.6</td>
<td>&lt;12</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Mesotrophic</td>
<td>40 - 50</td>
<td>2.6 - 20</td>
<td>12 - 24</td>
<td>2 - 4</td>
</tr>
<tr>
<td>Eutrophic</td>
<td>50 - 70</td>
<td>20 - 56</td>
<td>24 - 96</td>
<td>0.5 - 2</td>
</tr>
<tr>
<td>Hypertrophic</td>
<td>&gt;70</td>
<td>&gt;56</td>
<td>&gt;96</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lake</th>
<th>Chl-a (µg/L)</th>
<th>TP (µg/L)</th>
<th>TN (µg/L)</th>
<th>Trophic Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson, WA</td>
<td>40 - 80 (150)</td>
<td>1000 - 1300</td>
<td></td>
<td>Eutrophic</td>
</tr>
<tr>
<td>Dexter, OR</td>
<td>20 - 30</td>
<td>50 - 80</td>
<td>500 - 750</td>
<td>Eutrophic</td>
</tr>
<tr>
<td>Copco, CA</td>
<td>40 - 200</td>
<td>200</td>
<td>800 - 1500</td>
<td>Hypertrophic</td>
</tr>
<tr>
<td>Taihu, China</td>
<td>40 - 80</td>
<td>100 - 250</td>
<td>2000 - 4000</td>
<td>Hypertrophic</td>
</tr>
</tbody>
</table>

**Sources:** Jefferson Co. Report 2013 (And); Dreher unpub (Dex); Asarian et al. 2009 (Copco); Xu et al. 2015 (Taihu)
CyanoHAB challenges in the PNW

Abatement through reduced nutrient inputs
• But we face elevated P levels from volcanic geology

Abatement by other means,
e.g., flow control, temperature regulation, lower fish stocking

Assessment & monitoring of threats to public & ecological health:
• Drinking water: toxins, taste & odor compounds
• Recreational exposure, incl. pets (esp. dogs)
  • Food contamination through irrigation
• Toxin transfer to remote sites downriver & bioaccumulation in shellfish
  • Benthic sources of toxins
• Food-web and ecological disruptions; e.g., role as threat to endangered sucker fish in Upper Klamath Lake (OR)
  • Impacts of climate change, esp. increasingly dry summers

Genetic-inspired research is essential in crafting the best management & public health strategies to these challenges
Genome sequencing of CyanoHABs

Genome sequencing (human and gut microbes) has revolutionized medicine.
Genome sequencing of freshwater cyanobacteria has similar potential.

- Clarify evolutionary and taxonomic relationships between isolates.
- Reveal pathways relevant to ecological fitness & differences between isolates.

* Genes for utilization of reduced forms of S
  Phycoerythrin synthesis
 Genome sequencing of CyanoHABs

Genome sequencing (human and gut microbes) has revolutionized medicine
Genome sequencing of freshwater cyanobacteria has similar potential

- Allow optimal design of primers for PCR-based high-throughput monitoring

<table>
<thead>
<tr>
<th>Forward primer (NB78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphanizomenon sp. WA102</td>
</tr>
<tr>
<td>GCTTAATGGTTTGCGCGAA</td>
</tr>
<tr>
<td>A T T Mismatches</td>
</tr>
</tbody>
</table>

- Provide reference genomes for possible future proteomic strain identification, as widely used in clinical bacterial ID
DNA and RNA analysis for determining the biological influences of population dynamics

Differential niche competition between cyanobacteria
Availability of symbiotic “phycosphere” bacteria
Top-down predators
Cyanophages
Bacterial predators
Zooplankton
Chytrid fungi
Amoebae
Physiological stress

Sample

1.2 μm GF/C Filter

0.2 μm Filter

Concentrate w/ Microcon

0.02 μm Filter

Retain Flow-through

Extract DNA

Cyanos (DNA, RNA)
Eukaryotes
Bacteria
Archaea
Viruses
Free DNA (lysed cells)
Anderson Lake, WA: Anatoxin-a up to 1090 µg/L

*Anabaena* sp. WA102 culture; *ana*+ Major anatoxin producer in Anderson Lake

Shotgun metagenome analysis to study all DNA present in a sample

*Anabaena* sp. WA102 genome cluster

Contigs with anatoxin biosynthetic genes

Relative read depth Culture WA102-1

9 July 2012 187 µg/L anatoxin-a

Relative read depth Anderson Lake (GF/C), 7 July 2012

Nathan Brown
Anderson Lake, WA: Anatoxin-a up to 1090 µg/L

Shotgun metagenome analysis to study all DNA present in a sample

Contigs with anatoxin biosynthetic genes

Anabaena sp. WA102 genome cluster

Aphanizomenon flos-aquae WA102 genome cluster

20 May 2013
12.5 µg/L anatoxin-a

20 May 2013
Anderson Lake (GF/C), 20 May 2013

Nathan Brown
Anabaena sp. WA102

5.7 Mbp
Single chromosome

Completely assembled genome PacBio
2 SMRT cells
72x av. Coverage
Av Phred score = 82

Illumina: ~700 contigs

Gaps in Illumina coverage

Repetitive elements:
transposases & rRNA (5)

Nathan Brown
Extreme gene order randomization between *Anabaena sp. WA102* and *sp. 90*
Av. nucleotide identity = 92%

*Anabaena sp. 90*
Isolated 1986
Baltic Sea
5.1 Mbp
Two chromosomes
(4.3 + 0.8)
*mcy+*
Wang et al., 2012

*Anabaena sp. WA102*
Isolated 2013
5.7 Mbp
Single chromosome
*ana+*

Nathan Brown
Cyanobacterial successions in Dexter Reservoir

** All nontoxic **

Biovolume (µm³/mL)

- Anab. flos-aquae
- Anab. crassa
- Apha. flos-aquae
- Gloeotrichia
Shotgun metagenonomic analysis
Dexter Reservoir 2014

Relative read depth
0.2 - 1.2 µm fraction

Relative read depth
>1.2 µm (GF/C) fraction

Illumina HiSeq metagenomes assembled using IDBA, prokaryotic contigs taxonomically sorted with PhylopythiaS+, and clustered with mmgenome

Tim Otten
Upper Klamath Lake *Aphanizomenon flos-aquae* non-axenic culture community genome sequencing

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Genome size (Mbp)</th>
<th>Genome completion %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aphanizomenon flos-aquae</em></td>
<td>4.25</td>
<td>97</td>
</tr>
<tr>
<td><em>Alphaproteobacterium</em></td>
<td>3.5</td>
<td>100</td>
</tr>
<tr>
<td><em>Betaproteobacterium</em></td>
<td>3.4</td>
<td>100</td>
</tr>
<tr>
<td><em>Bacteroidetes</em></td>
<td>3.2</td>
<td>100</td>
</tr>
</tbody>
</table>

PacBio SMRT cell sequencing

- The **3 novel bacterial genomes** indicate heterotrophs dependent on *Aphanizomenon* for N (probably provided as NH$_3$), C and maybe even reduced forms of S.
- *Bacteroidetes* seems to encode a secreted peroxidase, which AFA lacks; may provide protection against extracellular reactive oxygen species (ROS)

Connor Driscoll
Microcystis aeruginosa blooms and microcystin levels exceed WHO guidelines annually in Copco and Iron Gate Reservoirs .......... and frequently in 300 km of river downstream
Copco Reservoir *Microcystis* strain dynamics 2007-2015

Phycocyanin gene *cpcBA* Illumina amplicon analysis

CPC-A encompasses the major **toxic strain**
CPC-B, C, D are correlated with low toxicity

Tim Otten
SNP DNA fingerprinting connects *Microcystis* populations in Iron Gate Reservoir and 300 km of downstream Klamath River (2012)

SNP, single nucleotide polymorphism

454 pyrosequencing ($n = 98,029$ cpcBA amplicon reads)
Genetic technologies enable ....

- Identification of genetically distinct cyanobacteria present in a bloom; development of strain-specific PCR-based primers for high-throughput monitoring
- Inventory of toxin and T&O genes present; in which cyanos?
- Deduction of physiological optima for different strains from genome content; prediction and experimental study of strain competition and succession scenarios
  - Development of strain-specific predictive models for competitiveness under nutrient reduction or climate change scenarios
- Use of gene tracking to study spread and emergence of CyanoHABs
Acknowledgments

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