Toxic cyanobacterial monitoring in the future:

Genetic testing of harmful algal blooms (cyano-HABs)

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Current monitoring: Action criteria (OR DHS)

Cyanobacteria: 100,000 cells/ml
Microcystis or Planktothrix: 40,000/ml
Microcystin: 8 ppb recreational exposure
1 ppb drinking water

Phytoplankton analyses

<table>
<thead>
<tr>
<th>Species</th>
<th>Density #/mL</th>
<th>Density %/mL</th>
<th>Biomass um/mL</th>
<th>Biomass %/mL</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planktothrix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcystin: 8 ppb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ppb drinking water</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Toxin analyses

<table>
<thead>
<tr>
<th>MAP ID</th>
<th>Station</th>
<th>Microcystin</th>
<th>Cell Count - Cell Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>D River</td>
<td>= 0.60</td>
<td>No Scum - Low Density</td>
</tr>
<tr>
<td>B</td>
<td>Campground</td>
<td>= 3.60</td>
<td>Some Scum - Moderate Density</td>
</tr>
<tr>
<td>C</td>
<td>Regatta Grounds</td>
<td>= 2.96</td>
<td>No Scum - Moderate Density</td>
</tr>
<tr>
<td>D</td>
<td>Holmes Road Park</td>
<td>&gt; 10.00</td>
<td>Some Scum - Moderate Density</td>
</tr>
<tr>
<td>E</td>
<td>Sand Point</td>
<td>= 2.48</td>
<td>No Scum - Low Density</td>
</tr>
<tr>
<td>F</td>
<td>East D.L. State Park</td>
<td>= 2.98</td>
<td>Some Scum - Moderate Density</td>
</tr>
<tr>
<td>L</td>
<td>Mid Lake</td>
<td>= 2.86</td>
<td>Moderate Scum - Moderate Density</td>
</tr>
<tr>
<td>I</td>
<td>NE Arm</td>
<td>= 4.26</td>
<td>No Scum - Low Density</td>
</tr>
<tr>
<td>K</td>
<td>NW Arm</td>
<td>= 4.00</td>
<td>No Scum - Low Density</td>
</tr>
<tr>
<td>4</td>
<td>Southern End</td>
<td>= 3.72</td>
<td>No Scum - Low Density</td>
</tr>
<tr>
<td>5</td>
<td>East Thumb</td>
<td>= 3.86</td>
<td>No Scum - Low Density</td>
</tr>
<tr>
<td>6</td>
<td>Deepest Point</td>
<td>= 4.24</td>
<td>Some Scum - Moderate Density</td>
</tr>
</tbody>
</table>

Note: Microcystin is only one of the many toxins produced by cyanobacteria. These test results are only a snapshot in time and are provided for guidance only. Conditions may change quickly.
The problem with morphological ID

Organisms called *Microcystis* should all have the most similar DNA sequences. Sister sequences should be from the same species and genus.

Conclusion: Some of the cyanos whose DNA sequences are in the GenBank database were mis-named using the current morphological approach. This reflects widespread problems with morphological ID.
Microcystis can assume widely different colony morphologies that have confused attempts at species ID.

Otsuka et al. have recommended that species ID of Microcystis be discontinued, and that the species *M. aeruginosa*, *M. ichthyoblabe*, *M. wesenbergii*, *M. viridis*, *M. novacekii*, *M. flos-aquae*, *M. pseudofilamentosa* be merged and referred to as *Microcystis aeruginosa*.

The problem with morphological ID

*Microcystis* colonies

Fresh sample

Sample treated with Lugol’s

Colony disruption during preservation makes ID more difficult
Genetic analysis of cyanobacterial blooms: many research studies but not yet used for making public health decisions

Highly plastic genome of *Microcystis aeruginosa* PCC 7806, a ubiquitous toxic freshwater cyanobacterium

Jöelle Trangetl1, Philippe Guillaumet1, Anne-Marie Casterej, Jean-François Humbert2-4, Hans CP Matthijs3, Diego Cortez2, Andrew Tolomou3-10, Cheng-Cai Zhang5, Simonetta Ghirardi1, Jan-Cristoph Kehr6, Yvonne Jillges6, Nadine Zermer7, Sven Becker8, Emmanuel Talla9, Amel Latifi5, Alain Billault3, Anthony Jepelletier3, Elke Dittmann2, Christiane Bouchier1 and Nicole Tandeau de Marsac2

Detection of Microcystin-Producing Cyanobacteria in Finnish Lakes with Genus-Specific Microcystin Synthetase Gene E (mcyE) PCR and Associations with Environmental Factors

Anne Rantala, Pirjo Rajanen-Wacklin, Christina Lyra, Lisa Lepistö, Jukka Rintala, Joanna Kwiecinska-Boczek, and Kaarina Sivonen

Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake

Gabriel Zwart, Miranda P. Kamat van Agtswijk, Innon van der Wolff, Steven Hemmes, Ferry Hugen, Horst I. Happefeld and Herman J. Gans

Genetic Diversity in *Microcystis* Populations of a French Storage Reservoir Assessed by Sequencing the 16S-23S rRNA Intergenic Spacers

J-F. Humbert1, D. Duris-Latour2, B. Le Berre1, H. Giraudet1 and M-J. Salençon2

Light and the Transcriptional Response of the Microcystin Biosynthesis Gene Cluster

Melanie Kalibernick,1,2 Brett A. Neilan,3,4 Thomas Dörner,2 and Elke Dittmann2

School of Microbiology and Immunology, University of New South Wales, Sydney 2052, Australia,1 and Institute for Biology (Genetics), Humboldt University, Berlin, Germany2.
Future monitoring: Action criteria (OR DHS)

Cyanobacteria: 100,000 genomes/ml

*Microcystis* or *Planktothrix*: 40,000 genomes/ml

Microcystin: 8 ppb recreational exposure

1 ppb drinking water

Genetic analyses: DNA sequence differences among different cyanobacteria strains

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<td>Moderate Scum - Moderate Density</td>
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<td>H</td>
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Note: Microcystin is only one of the many toxins produced by cyanobacteria. These tests results are only a snapshot in time and are provided for guidance only. Conditions may change quickly.
Future cyanobacterial database

Willow Creek Reservoir, Heppner

Collection: June 10, 2008

Morphological ID: *Anabaena flos-aquae*

Genotypic ID: *Anabaena* WC1

- 16S rDNA
- rDNA ITS
- Phycocyanin *cpcBA*

![DNA sequences and phylogenetic tree](image-url)
Why a genetic database and DNA-based monitoring?

What can this do for lake/water managers?

More accurate bloom identification

• species and strain identification + quantitation
• high resolution comparison between lakes (e.g., are the same bloom strains present in adjacent watersheds?)
• detection of toxin genes: early-warning detection
• establish a more accurate understanding of bloom populations: anticipate problems, detect trends

High-throughput detection

• more sampling, quicker, cheaper (more sites, different depths)
Sample collection

Storage & shipping on ice, not preserved

Preservation (Lugol's, glutaraldehyde) may result in decreased mailing costs
Sample preparation

Samples are filtered for immediate use or storage in freezer for subsequent DNA extraction

0.45 micron glass fiber filter

0.22 micron Millipore filter
Cyano-HAB PCR targets

There are multiple options that can be considered
• PCR designed to detect all HAB-forming cyanobacteria
• PCR directed at particular genera or species
• PCR followed by DNA sequencing
• Quantitative PCR to measure gene numbers

There are several common gene targets:
• 16S ribosomal RNA
• ITS, ribosomal RNA internal transcribed spacer
• cpcBA phycocyanin intergenic spacer
• mcy and other toxin biosynthetic genes
• nif nitrogen fixation genes
DNA sequencing

DNA sequencing of cloned DNA (up to 700 run length)
Case study: genetic studies of *Microcystis* population in Klamath River (Dreher lab at OSU)

**Goal:** genetically describe the cyanobacterial population in Klamath waters, esp. in Copco Reservoir, but also in Iron Gate Reservoir and Upper Klamath Lake

- initial focus on toxic *Microcystis* in Copco Res.
- apply info and techniques to develop assays useful for management decisions
Copco *Microcystis* bloom
Conventional cell counts and toxin analysis

Data from Kann & Corum, 2009
Copco *Microcystis* bloom

Conventional cell counts and toxin analysis

![Graph showing Microcystis cells/ml and microcystin toxin/cell from June to October.](image)

- **Microcystis cells/ml**
- **Microcystin toxin/cell**

**June** to **August**

- Decline in Aug – Sept
- 100x drop in toxin quota

**Conventional cell counts and toxin analysis**
Decline in \textit{mcyB} gene copy number

Quantitative Taqman PCR assay for \textit{Microcystis mcyB} and \textit{cpcBA}
Genetic typing of the *Microcystis* population

Collect population on a filter by filtering lake water sample

PCR amplify target gene sequences, produce clone library and determine individual sequences

- **ITS**, ribosomal DNA internal transcribed spacer
- **cpcBA**, phycocyanin intergenic region
- microcystin toxin synthetic gene **mcyA**
Copco Reservoir *Microcystis* genotypes

**ITS sequences**

**cpcBA sequences**
Copco Reservoir *Microcystis* genotypes
Relationship to isolates from other locations

ITS sequences

$cpcBA$ sequences
Copco Reservoir *Microcystis* genotypes

**ITS sequences**

**cpcBA sequences**
Genetic structure of *Microcystis* population: ITS subgroup 1 (Copco Reservoir)

491 nt ITS amplicon
30 different sequences

Non-group 1

Consensus sequence 40

# nt diffs # occurrences
17 1
13 1 2
12 1
2 1 3
1 1 2
1 1 2
1 1 2

1 1 2

1 1
Genetic analysis at the *Microcystis* ITS gene locus (Copco Reservoir)

Subgroups are: • well separated • tightly clustered • dominated by single sequences

Such population structure is amenable to subgroup-specific monitoring
Population turnover during season: *cpcBA*

Estimated proportion of *Microcystis* population

**Temporal change in relative abundance of cpcBA sequence groups**

- Group A
- Major turnover late Aug – mid Sep

- B2
- B1

**X-axis:** Jun, Jul, Aug, Sep, Oct
Population turnover during season: ITS

Estimated proportion of Microcystis population

Major turnover late July – late Aug
Copco Reservoir Microcystis genotypes

ITS sequences

Early season
June - Aug

Late season
mid Aug - Oct

cpcBA sequences

Early season
June - Aug

Late season
Sep - Oct
Upper Klamath Lake and Copco Reservoir * Microcystis* genotypes are closely related.

Other colored sequences are from Copco Res.
It is time ....

• To build a genetic database of cyanobacterial blooms

• To work at implementing genetic (DNA-based) analyses into routine monitoring
Application of cyano-HAB genetic ID

Accurate identification

Differentiate toxic from non-toxic strains and track abundance

Track population dynamics during seasonal bloom development, esp. with respect to toxin production

Explore relationship between blooms in different water bodies

Use in assessing the success of treatment options