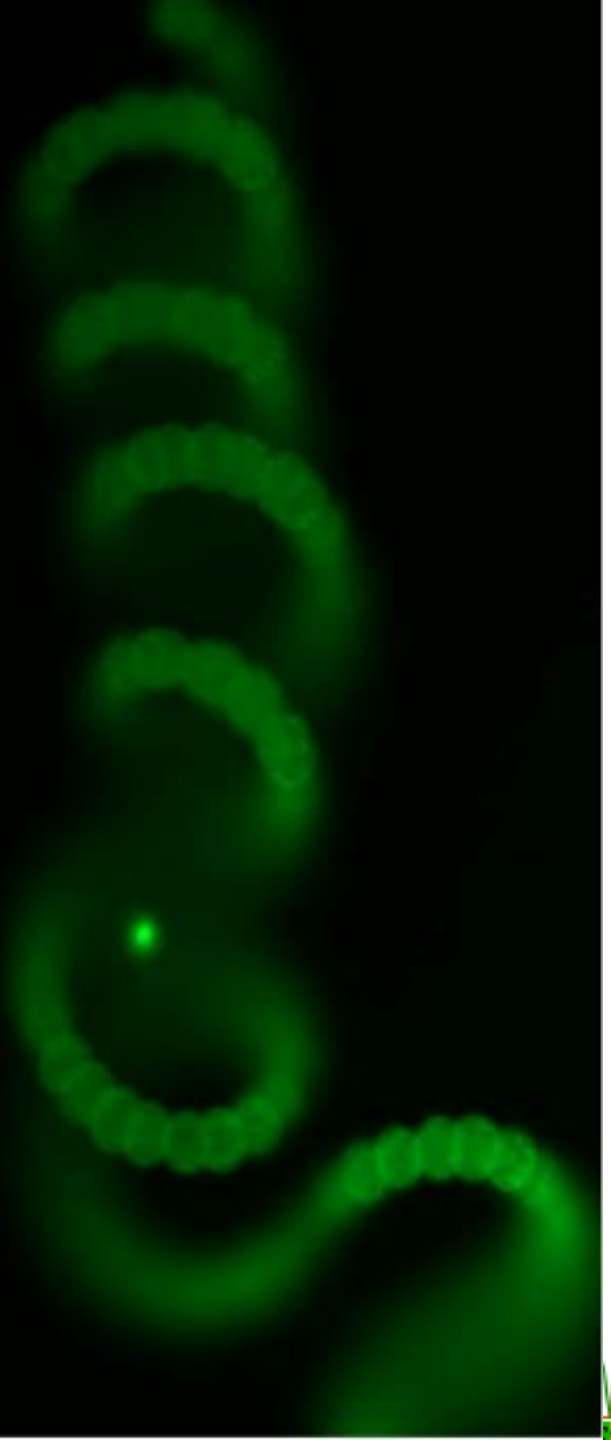
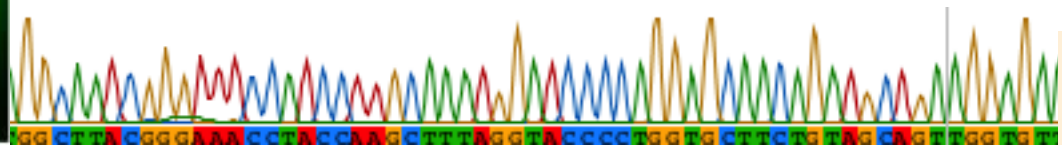


# *Cyanobacterial occurrence in lakes and rivers in the PNW and the role of eutrophication:*

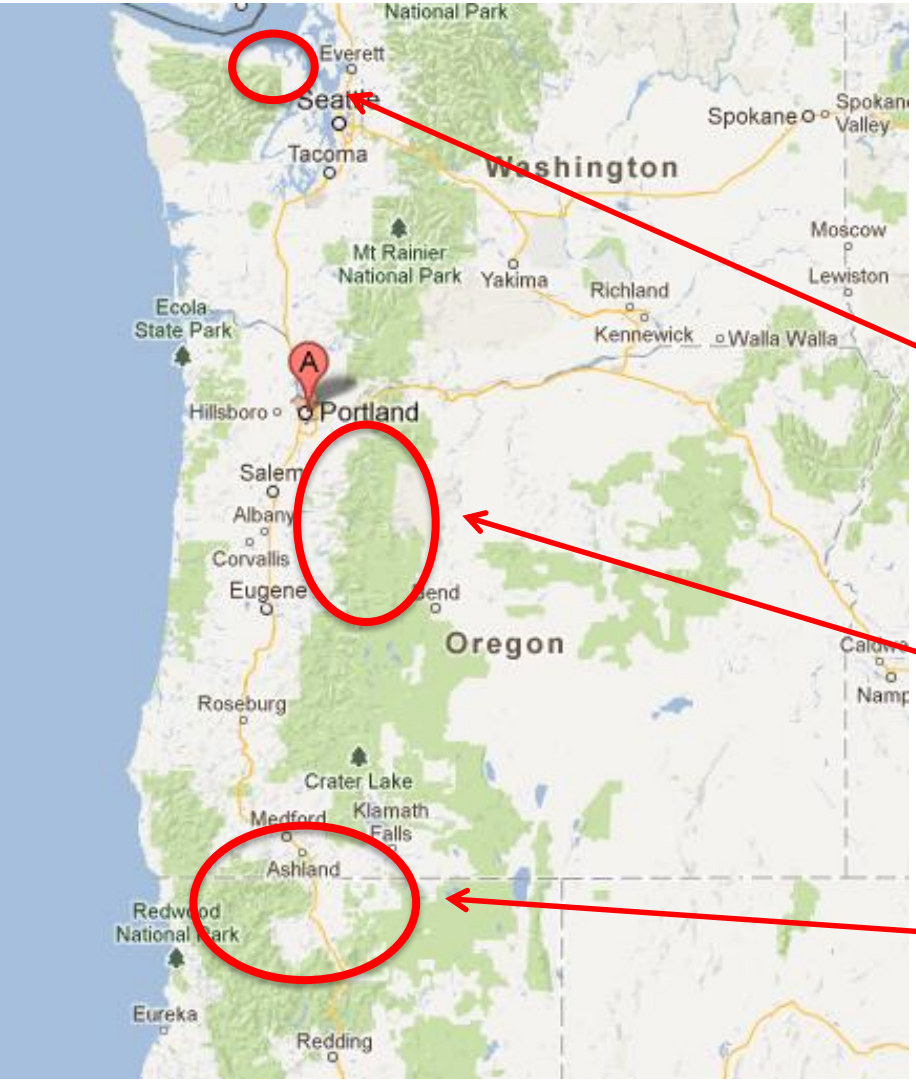
## *Progress through genetic technologies*


**Theo W. Dreher**

**Department of Microbiology**
**Center for Genome Research & Biocomputing**
**Oregon State University, Corvallis, OR**

# 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, *ana+, cyr+, mcy+*

**Anderson Lake, Washington**

Anatoxin-a up to 1090 µg/L

***Anabaena* sp. WA102 Ana+ culture**

*Anabaena flos-aquae/ lemmermannii* morphology

**Cascade Range reservoirs & lakes, Oregon**

Potential toxicity in drinking water sources

**Dexter Res: Non-toxic**

**Detroit Res: 7-epi-Cylindrospermopsin @ 195 µg/L**

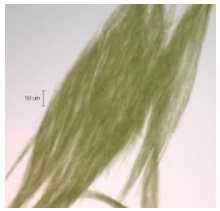
**Odell Lake: Microcystin @ 675 µg/L**

**Upper Klamath Lake, Oregon**

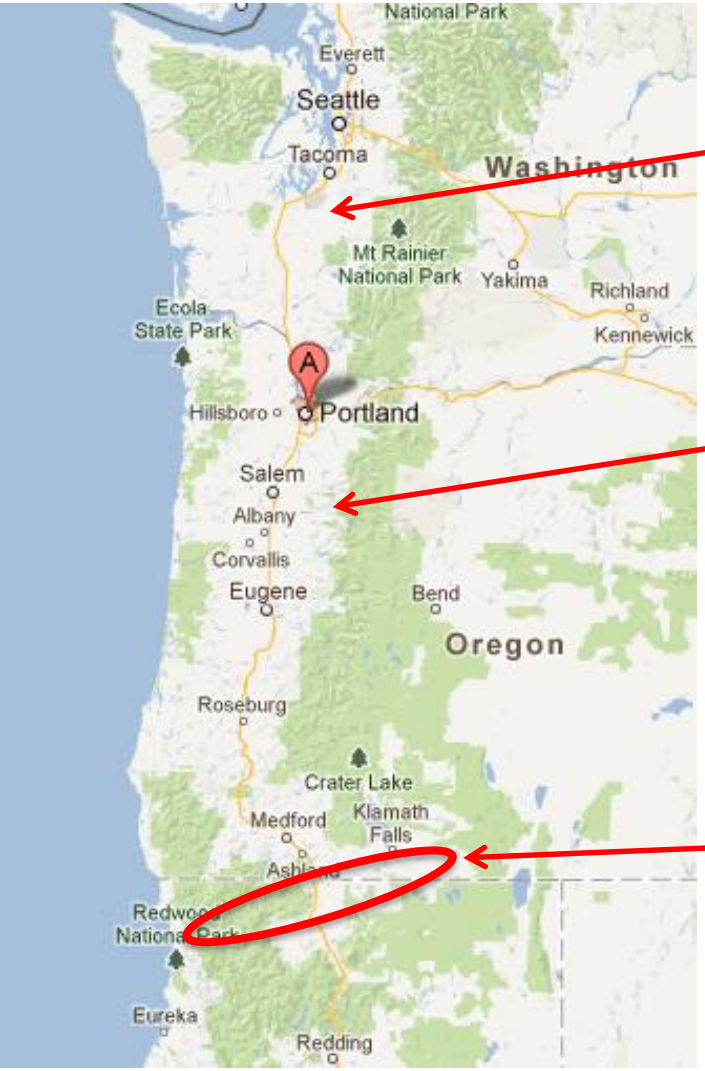
*Aphanizomenon flos-aquae* (AFA)

Harvested for "nutritional supplements"

**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

| Trophic Class | Trophic State Index | Chl-a (µg/L) | TP (µg/L) | Secchi Depth (m) |
|---|---|---|---|---|
| **Oligotrophic** | <40 | <2.6 | <12 | >4 |
| **Mesotrophic** | 40 - 50 | 2.6 - 20 | 12 - 24 | 2 - 4 |
| **Eutrophic** | 50 - 70 | 20 - 56 | 24 - 96 | 0.5 - 2 |
| **Hypertrophic** | >70 | >56 | >96 | <0.5 |

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

**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:

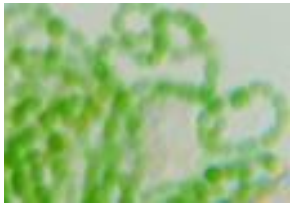
- Drinking water: toxins, taste & odor compounds
- Recreational exposure, incl. pets (esp. dogs)
- Food contamination through irrigation
- Toxin transfer to remote sites downriver & bioaccumulation in shellfish
- Benthic sources of toxins
- Food-web and ecological disruptions; e.g., role as threat to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

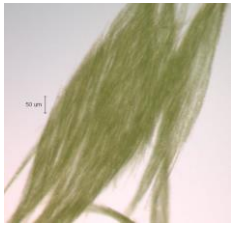
**Genetic-inspired research is essential in crafting the best management & public health strategies to these challenges**

# Genome sequencing of CyanoHABs

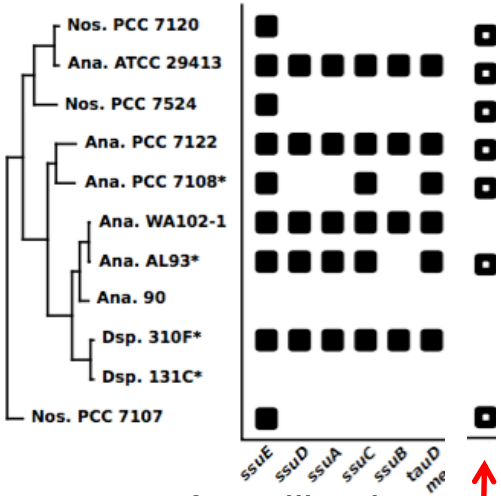
Genome sequencing (human and gut microbes) has revolutionized medicine
**Genome sequencing of freshwater cyanobacteria has similar potential**

- **Clarify evolutionary and taxonomic relationships between isolates**





- **Reveal pathways relevant to ecological fitness & differences between isolates**




Genes for utilization of reduced forms of S

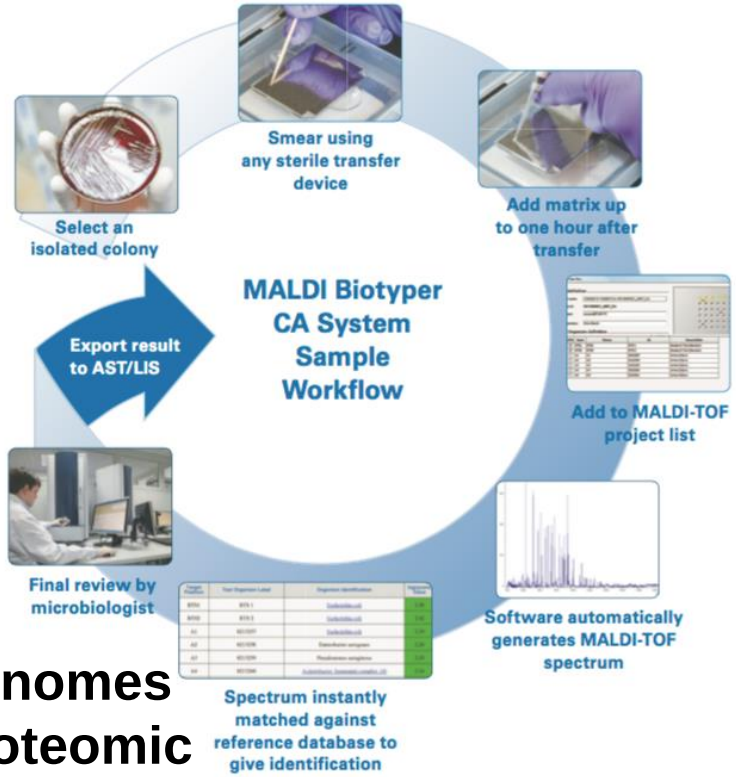
Phycoerythrin synthesis

# Genome sequencing of CyanoHABs

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

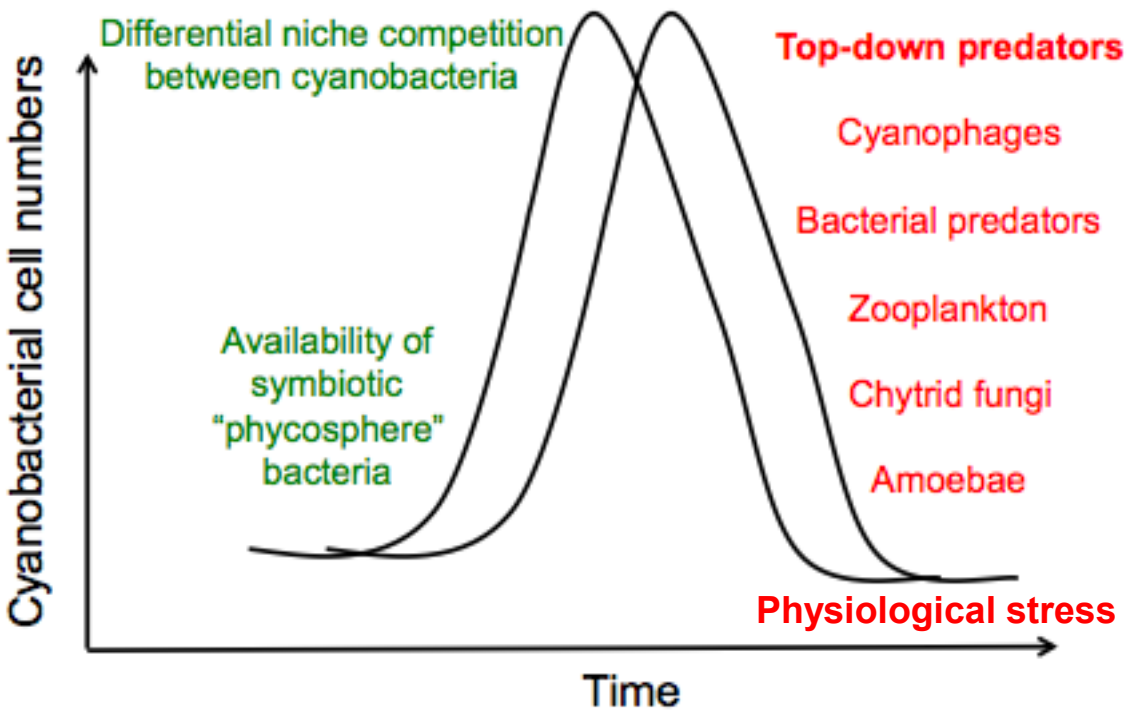
- **Allow optimal design of primers for PCR-based high-throughput monitoring**

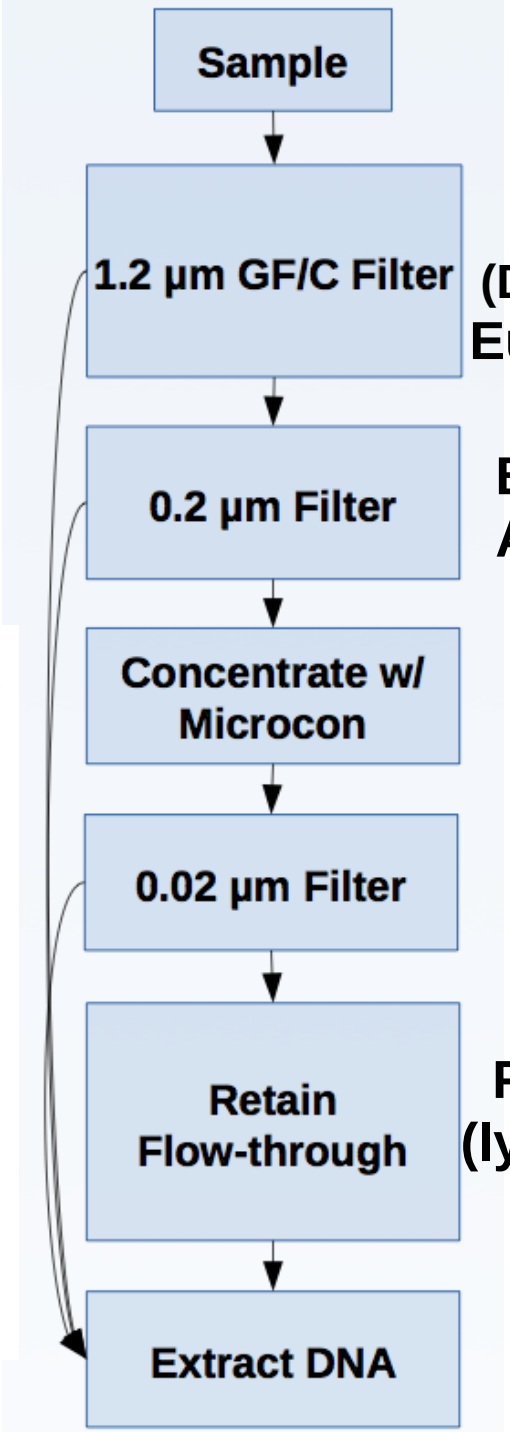
| | Forward primer (NB78) |
|---|---|
| *Aphanizomenon* sp. WA102 | GCTTAAATGGTTTGCGCGAA |
| Mismatches | A T T |
| Primers | GCTTGAACGGCTTGCGCGAA |
| Mismatches | A T |
| *Anabaena* sp. WA102 | GCTTAAATGGCTTGCGCGAA |




- **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




Cyanos (DNA, RNA) Eukaryotes

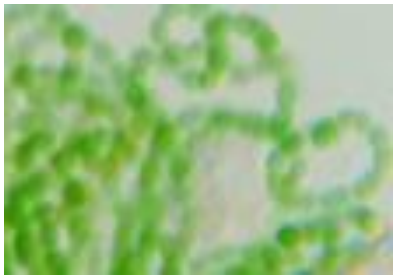
Bacteria Archaea

Viruses

Free DNA (lysed cells)

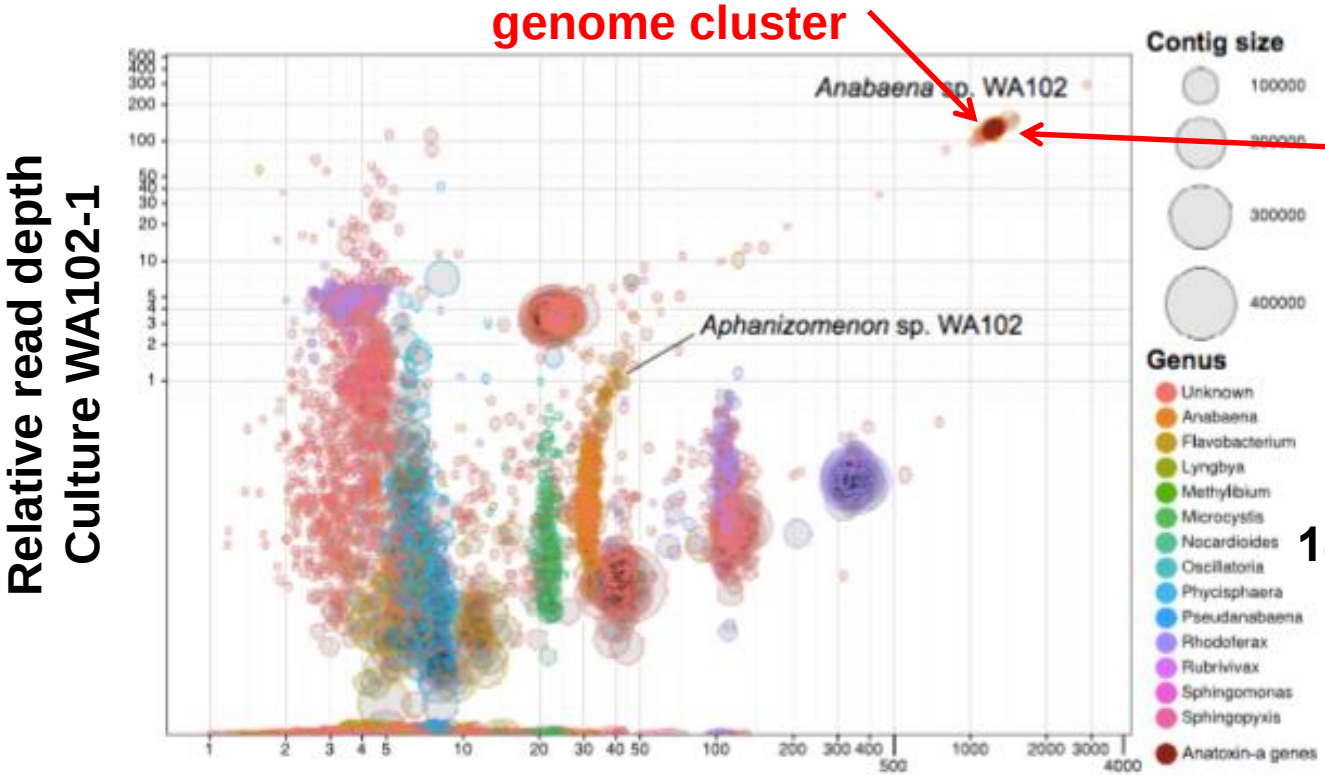
# Anderson Lake, WA: Anatoxin-a up to 1090 µg/L

*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**



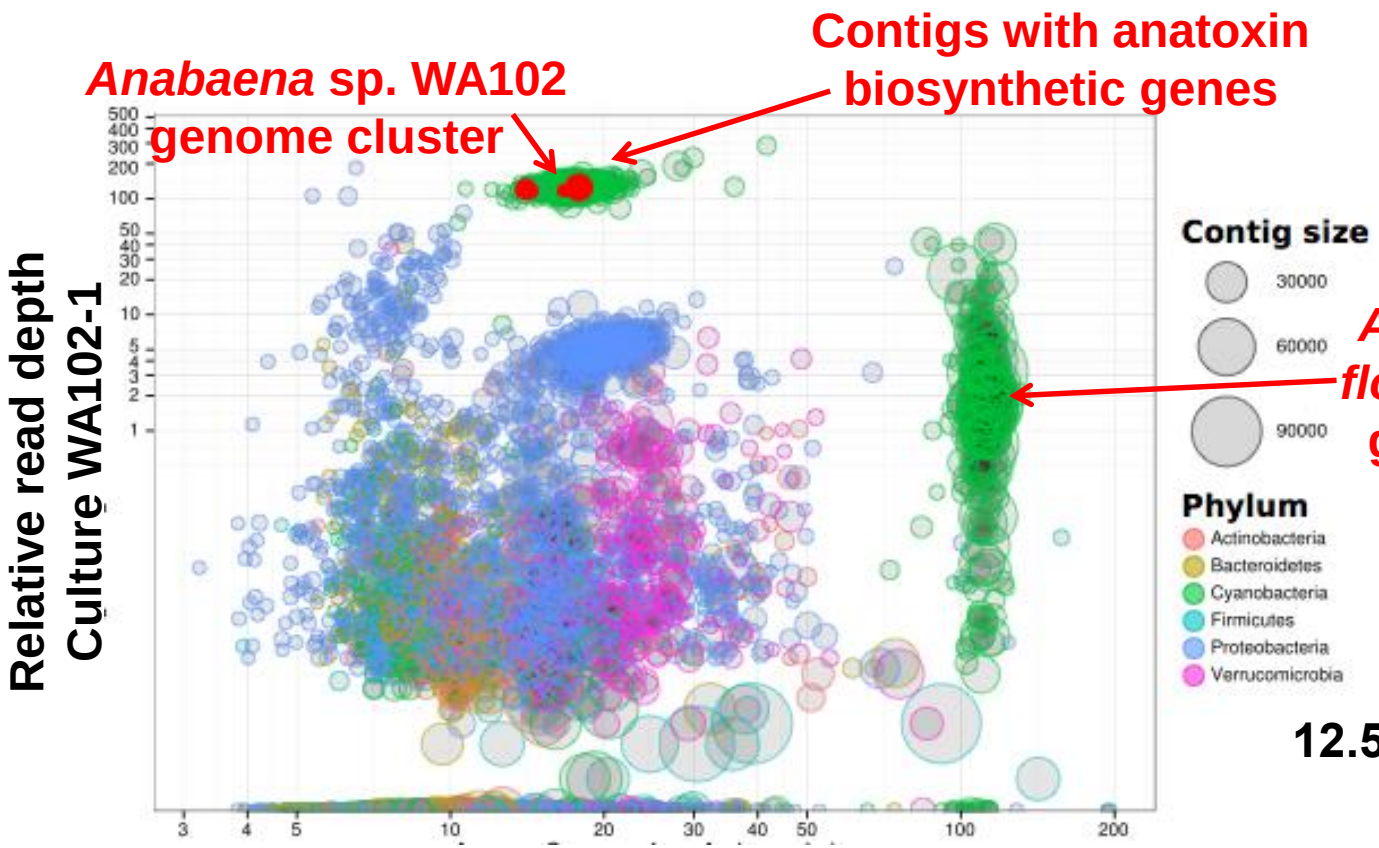

**Contigs with anatoxin biosynthetic genes**

**9 July 2012**
**187 µg/L anatoxin-a**

**Nathan Brown**

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

**Shotgun metagenome analysis to study all DNA present in a sample**

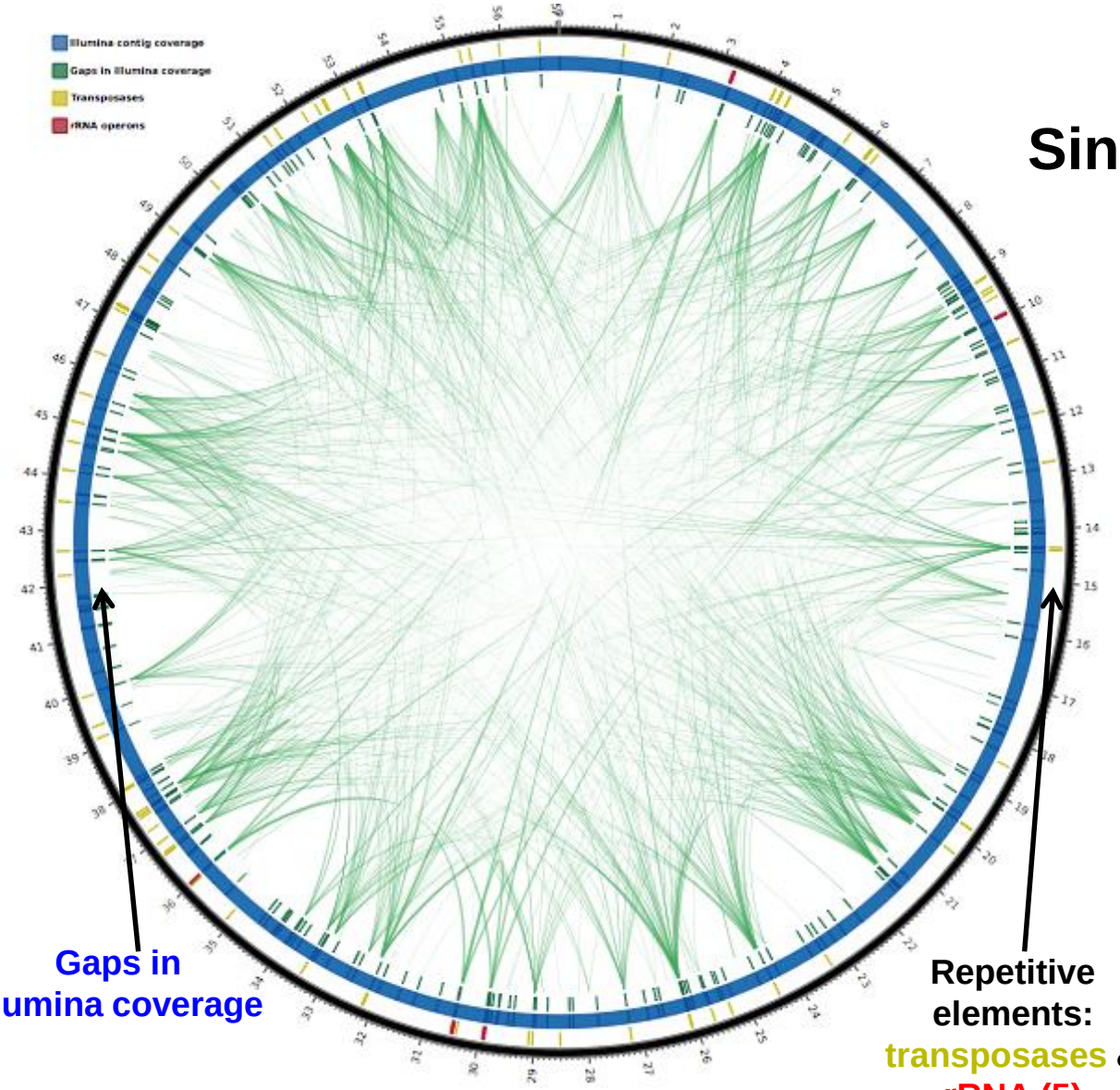



**20 May 2013**
**12.5 µg/L anatoxin-a**

**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**

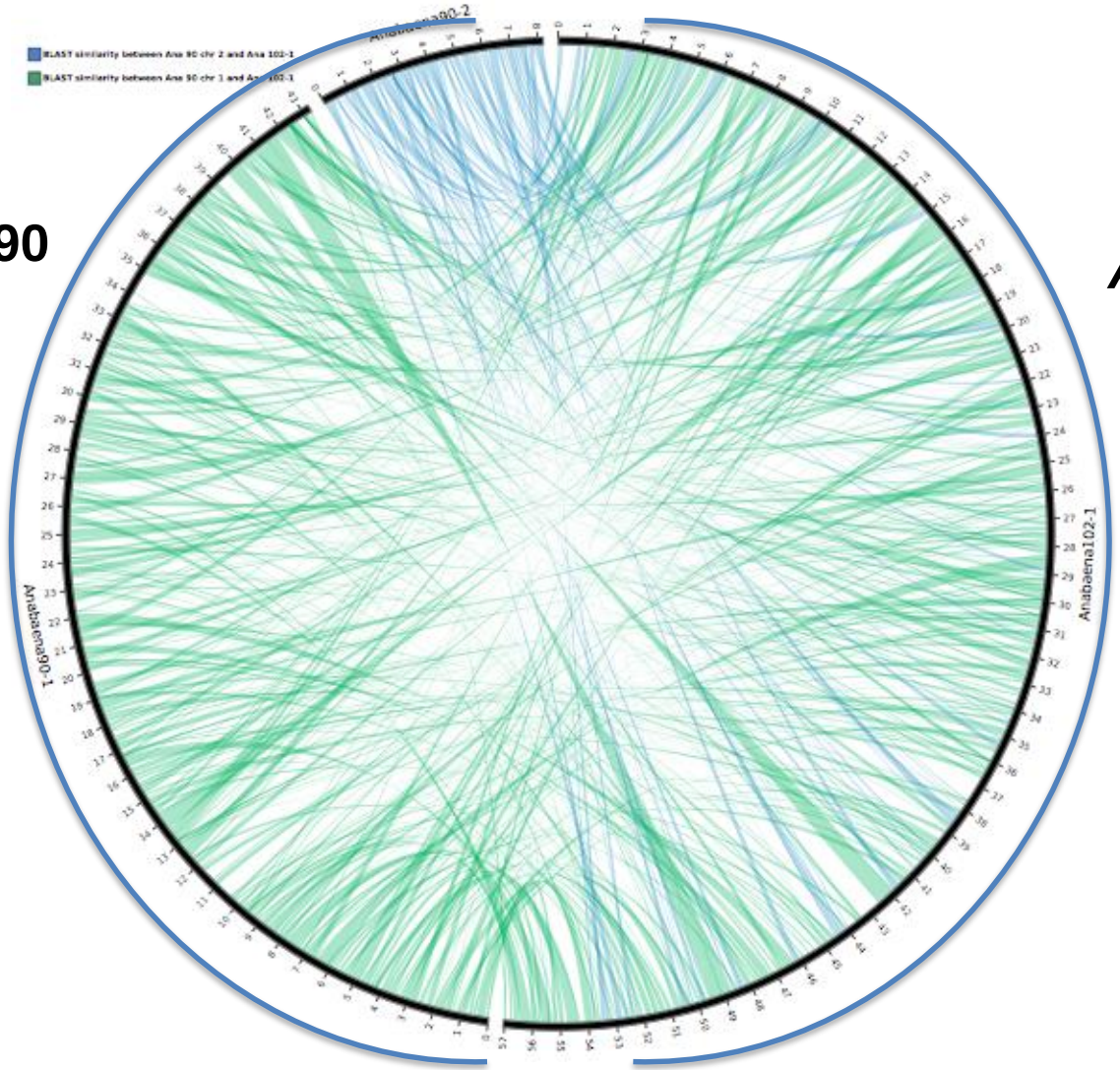
# Extreme gene order randomization between *Anabaena sp.* WA102 and sp. 90

**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

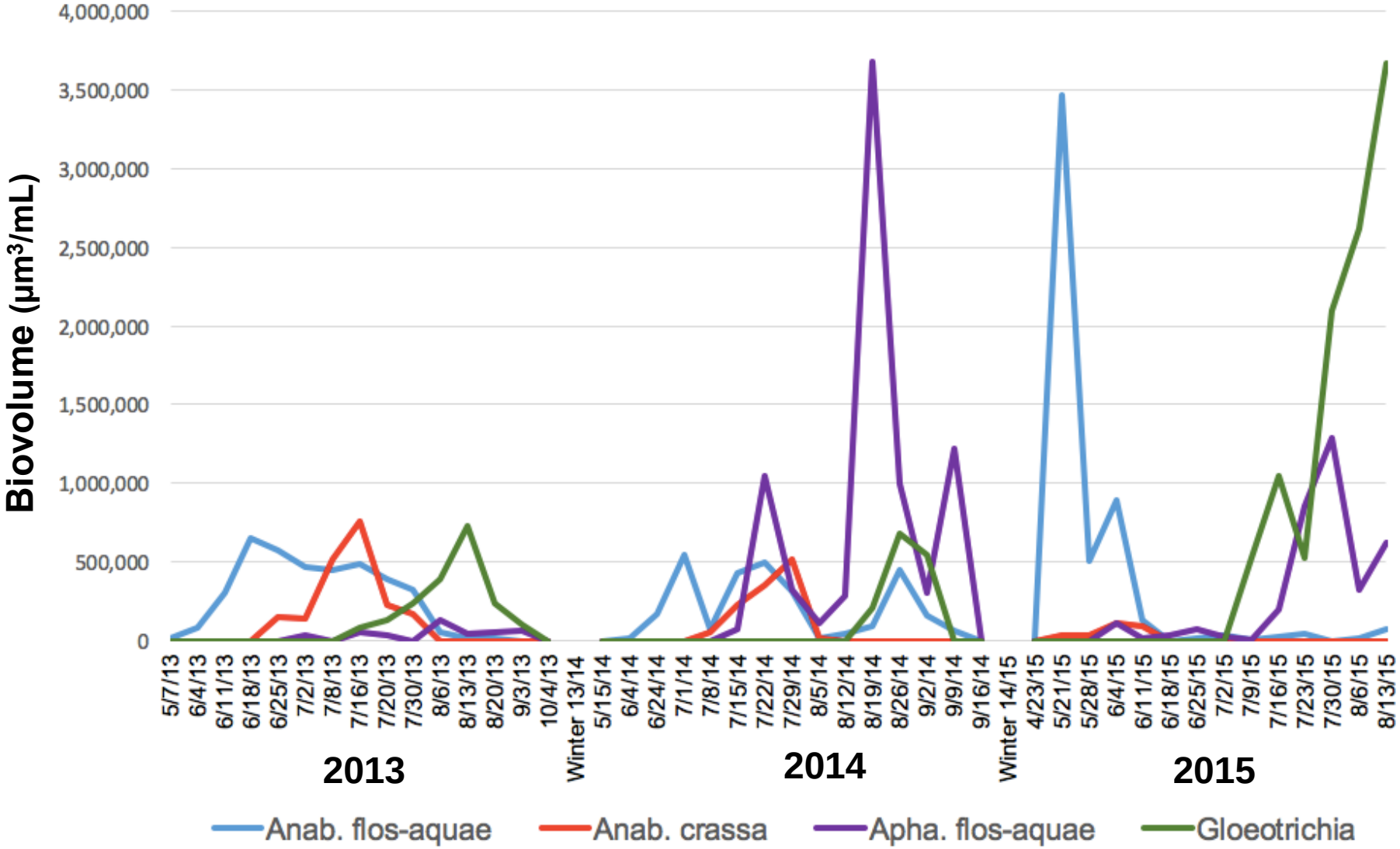



***Anabaena* sp.**
**WA102**
Isolated 2013

5.7 Mbp
Single
chromosome
*ana+*

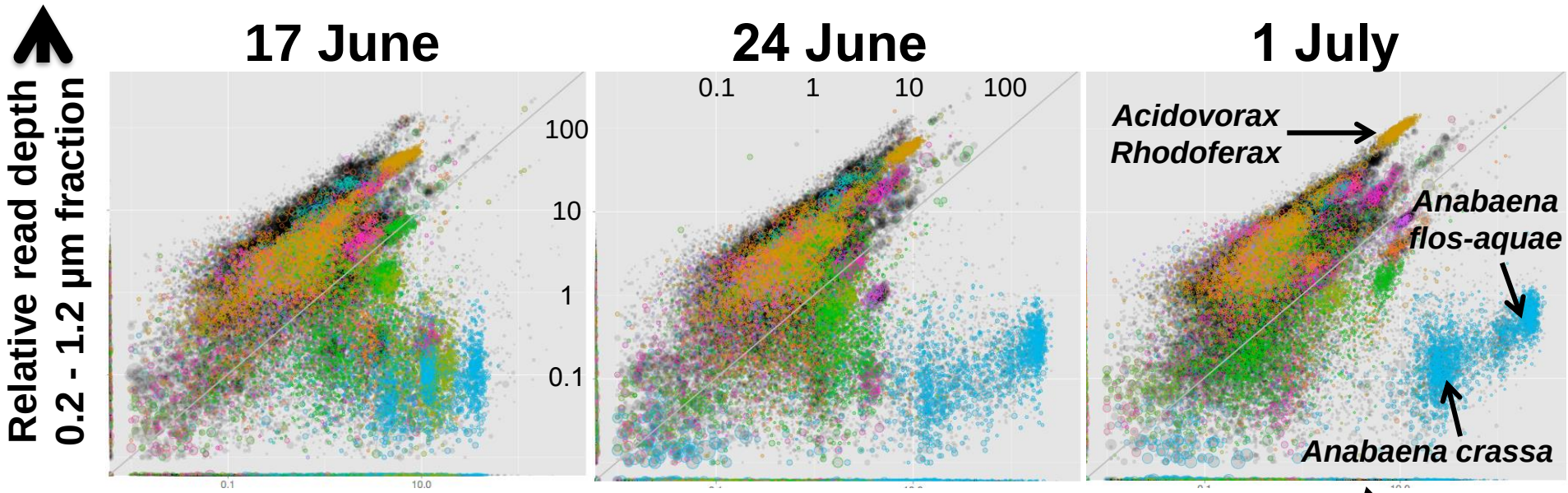
**Nathan Brown**

# Cyanobacterial successions in Dexter Reservoir
# ** All nontoxic **

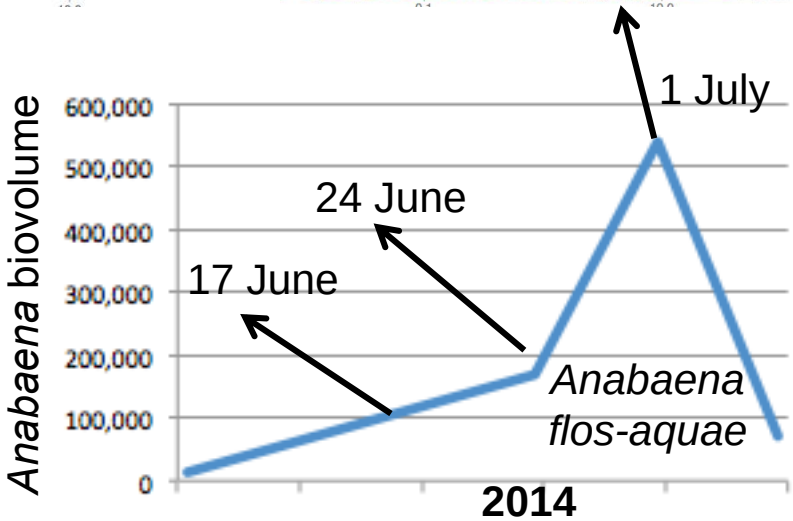


# Shotgun metagenomic analysis

## Dexter Reservoir 2014



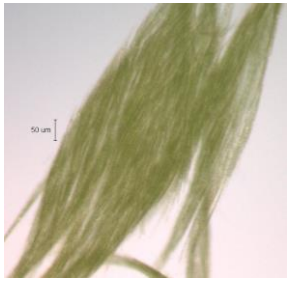

**Relative read depth**
**>1.2 µm (GF/C) fraction**




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



| Bacterium | Genome size (Mbp) | Genome completion % |
|---|---|---|
| *Aphanizomenon flos-aquae* | 4.25 | 97 |
| *Alphaproteobacterium* | 3.5 | 100 |
| *Betaproteobacterium* | 3.4 | 100 |
| *Bacteroidetes* | 3.2 | 100 |

PacBio SMRT cell sequencing

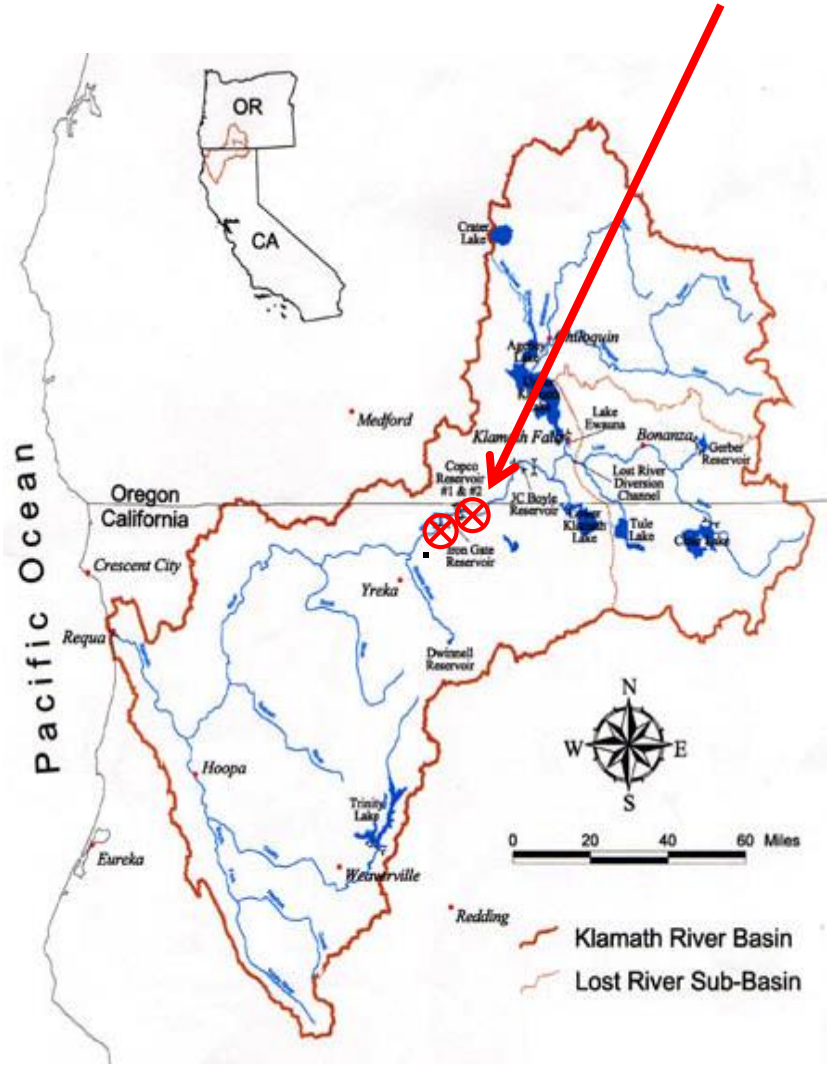
- The **3 novel bacterial genomes** indicate heterotrophs dependent on *Aphanizomenon* for N (probably provided as $NH_3$), C and maybe even reduced forms of S.
- *Bacteroidetes* seems to encode a secreted peroxidase, which AFA lacks; may provide protection against extracellular reactive oxygen species (ROS)

**Connor Driscoll**

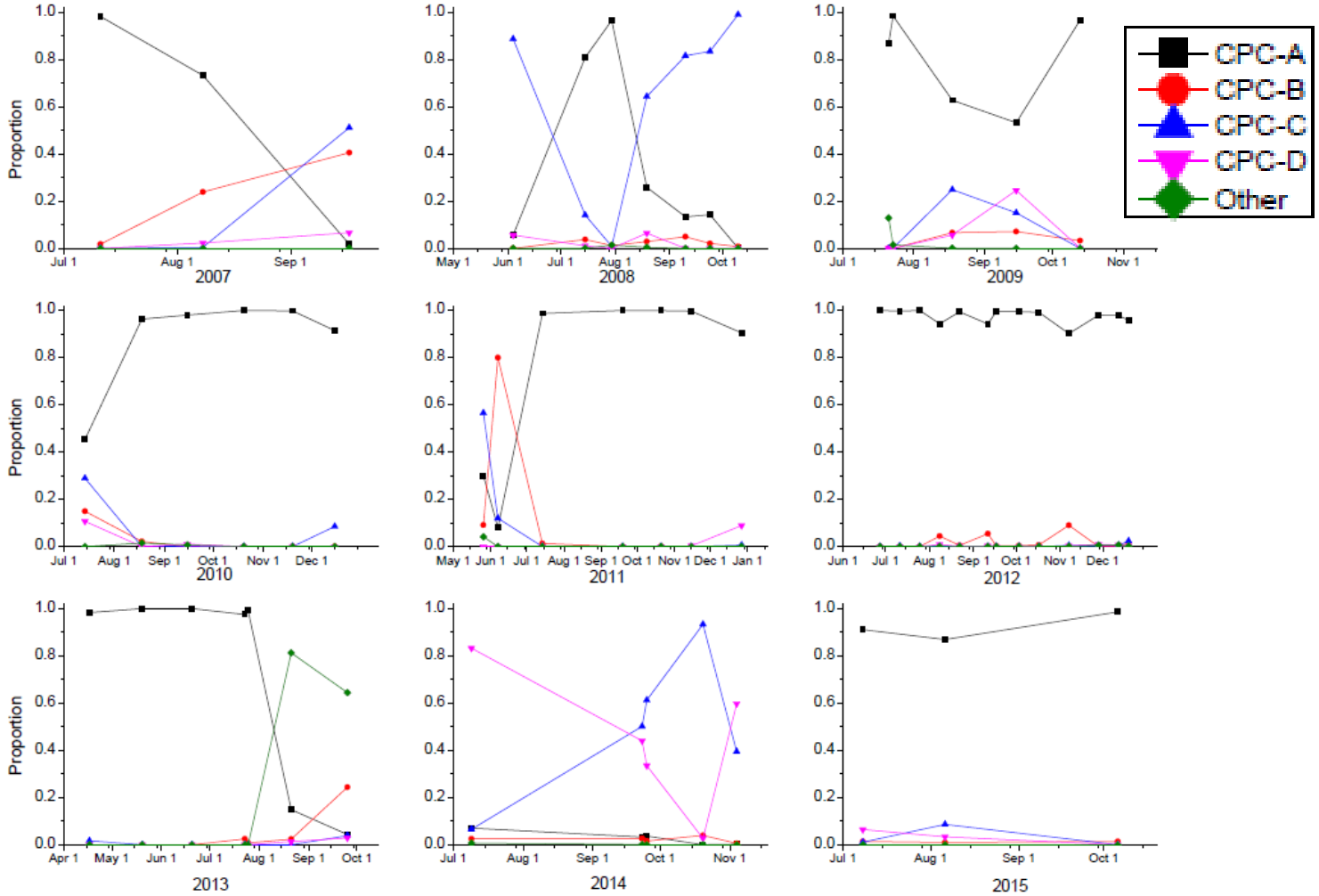
# Klamath River Basin

*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



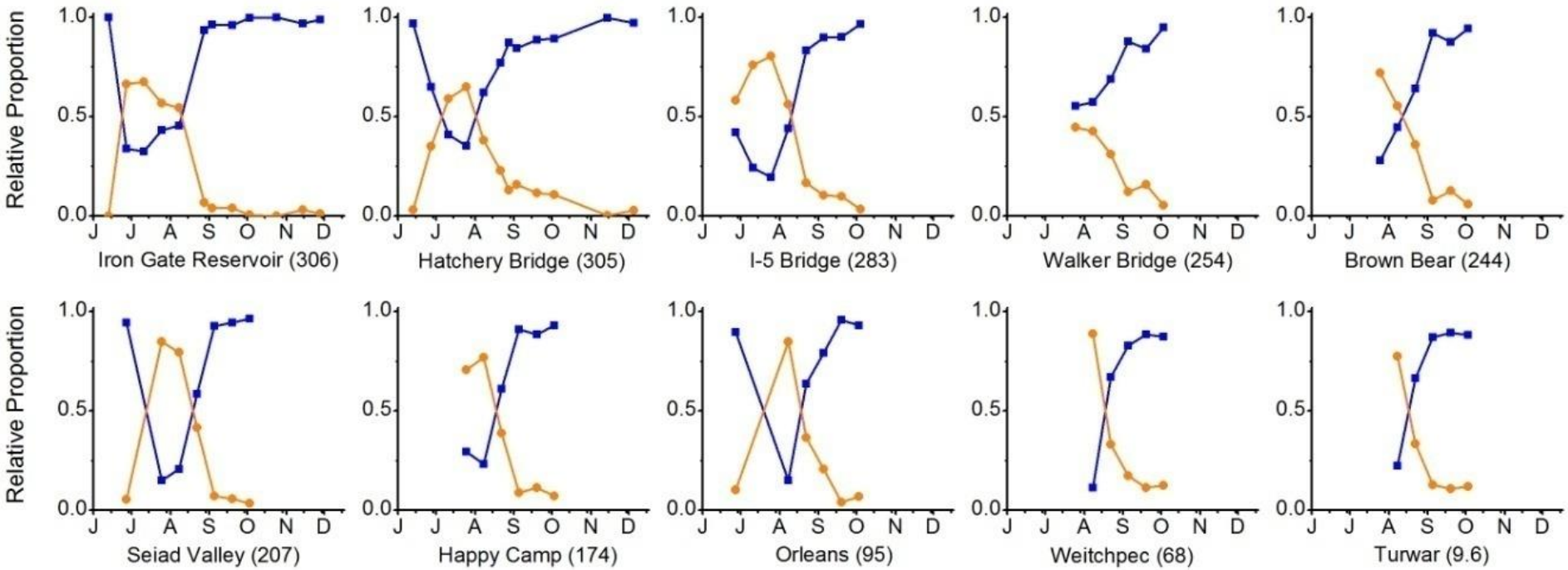

Phycocyanin gene *cpcBA* Illumina amplicon analysis

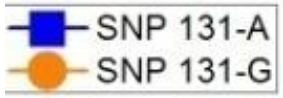
**CPC-A** encompasses the major **toxic strain**

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

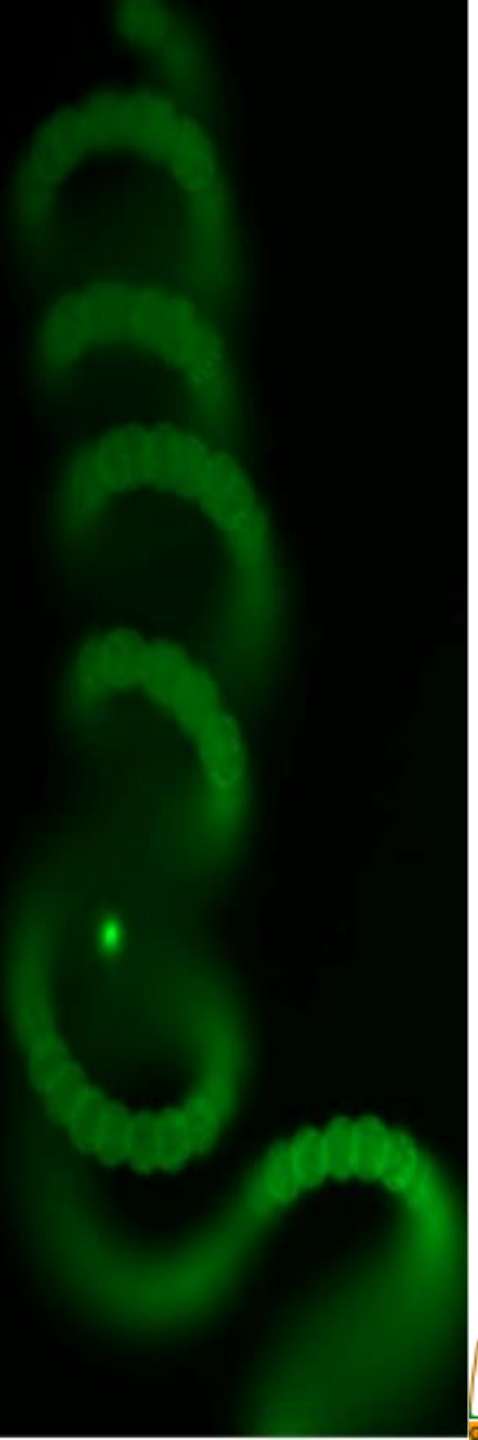
454 pyrosequencing (*n* = 98,029 *cpcBA* amplicon reads)

**Tim Otten**

# 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

Oregon State UNIVERSITY OSU



# Acknowledgments

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**Jennifer Graham**, USGS Kansas Water Science Center

+ many others for providing samples

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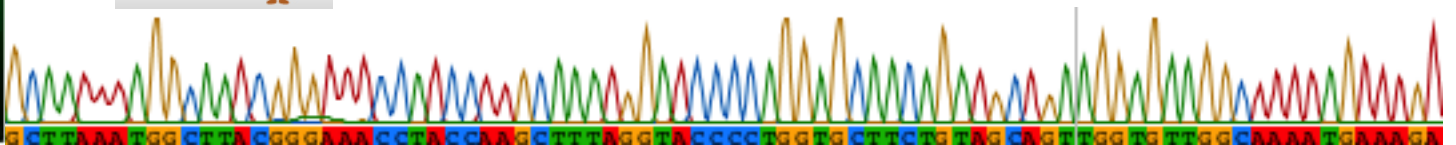
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EHSC new discoveries
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