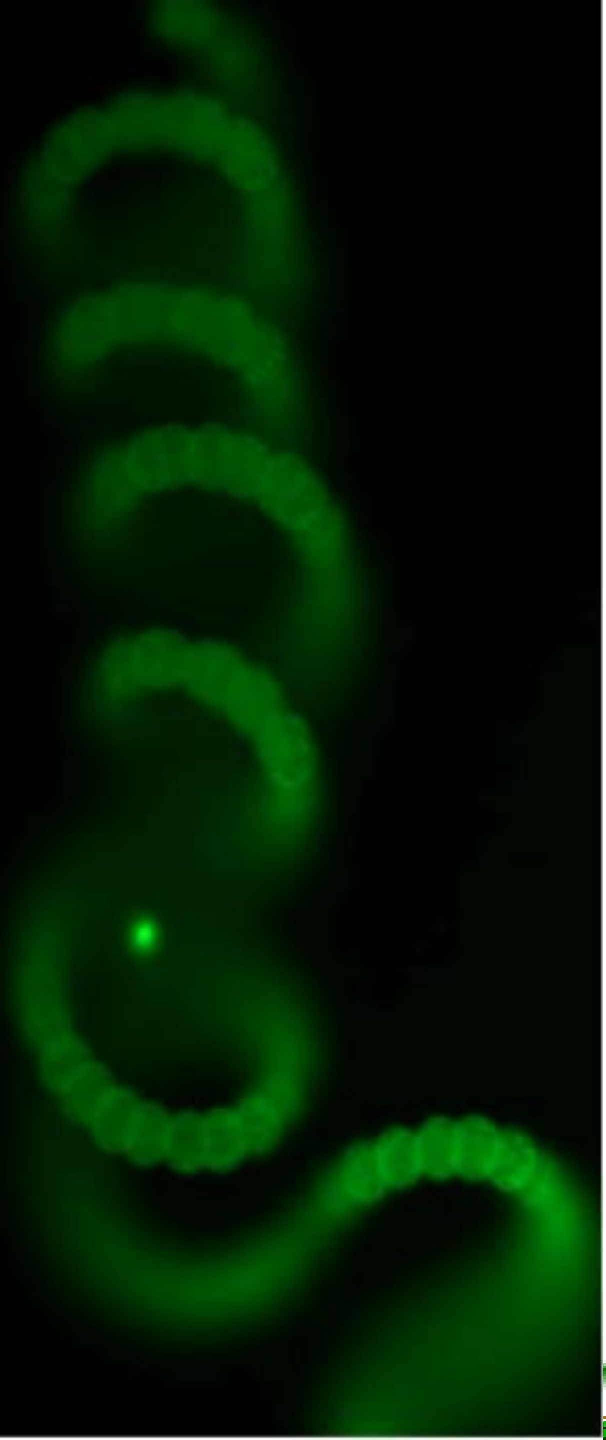
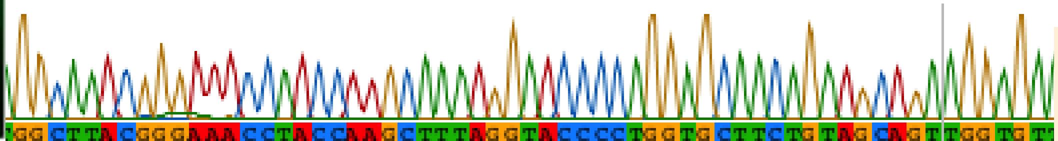


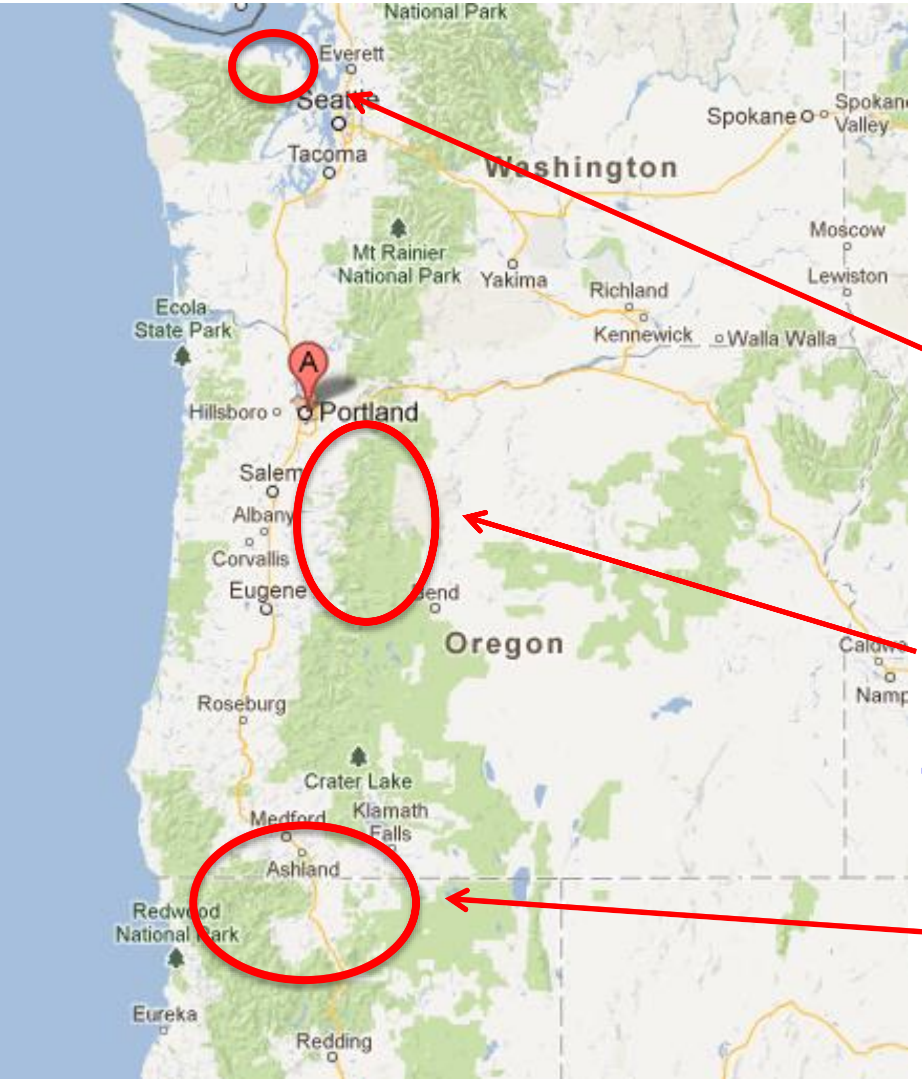
Cyanobacterial occurrence in lakes and rivers in the PNW and the role of eutrophication: Progress through genetic technologies

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

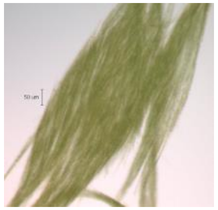
Odell Lake: Microcystin @ 675 µg/L

Upper Klamath Lake, Oregon

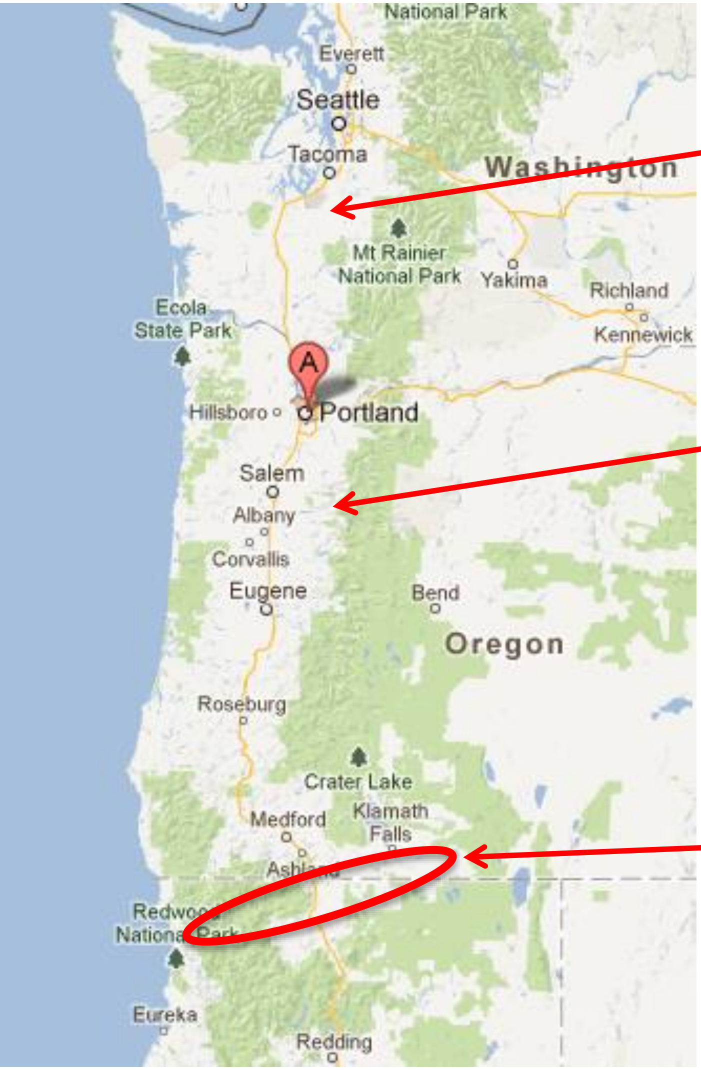
Aphanizomenon flos-aquae (AFA)

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:

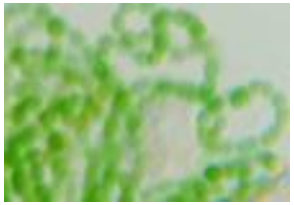
- Drinking water: toxins, taste & odor compounds
- Recreational exposure, incl. pets (esp. dogs)
- Food contamination through irrigation
- Toxin transfer to remote sites downriver & bioaccumulation in shellfish
- Benthic sources of toxins
- Food-web and ecological disruptions; e.g., role as threat to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

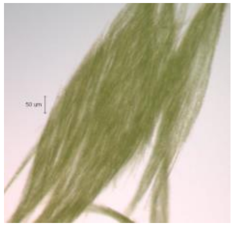
Genetic-inspired research is essential in crafting the best management & public health strategies to these challenges

Genome sequencing of CyanoHABs

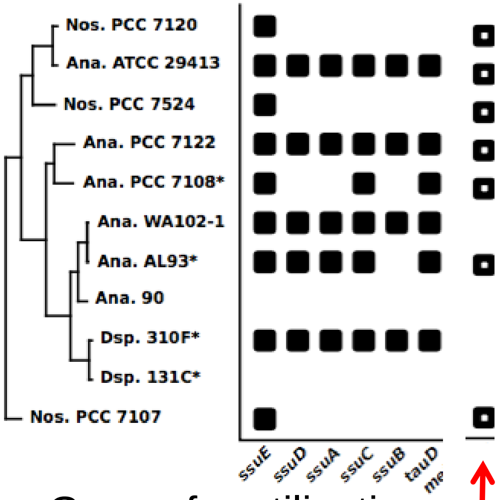
Genome sequencing (human and gut microbes) has revolutionized medicine
Genome sequencing of freshwater cyanobacteria has similar potential

- **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

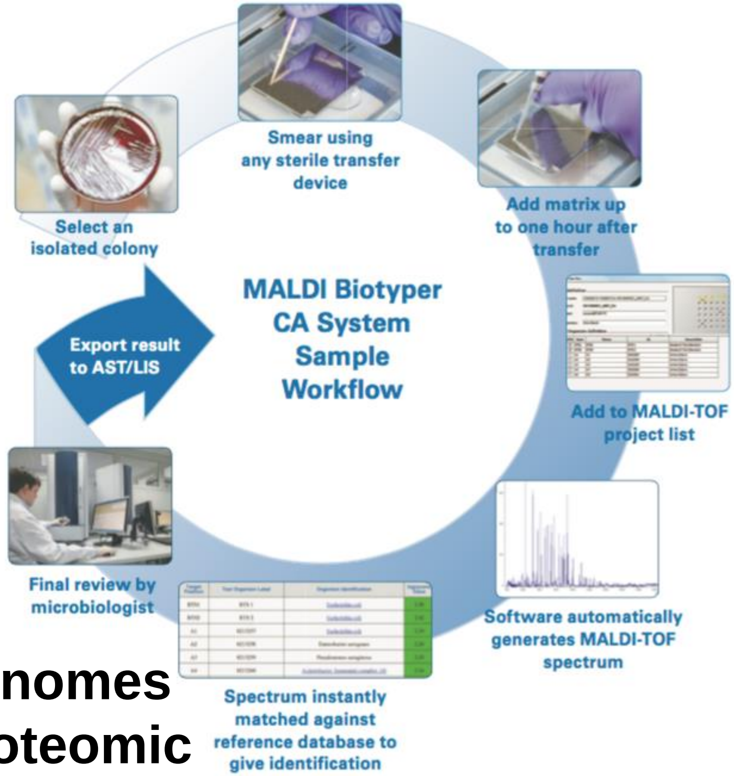
Genome sequencing of CyanoHABs

Genome sequencing (human and gut microbes) has revolutionized medicine

Genome sequencing of freshwater cyanobacteria has similar potential

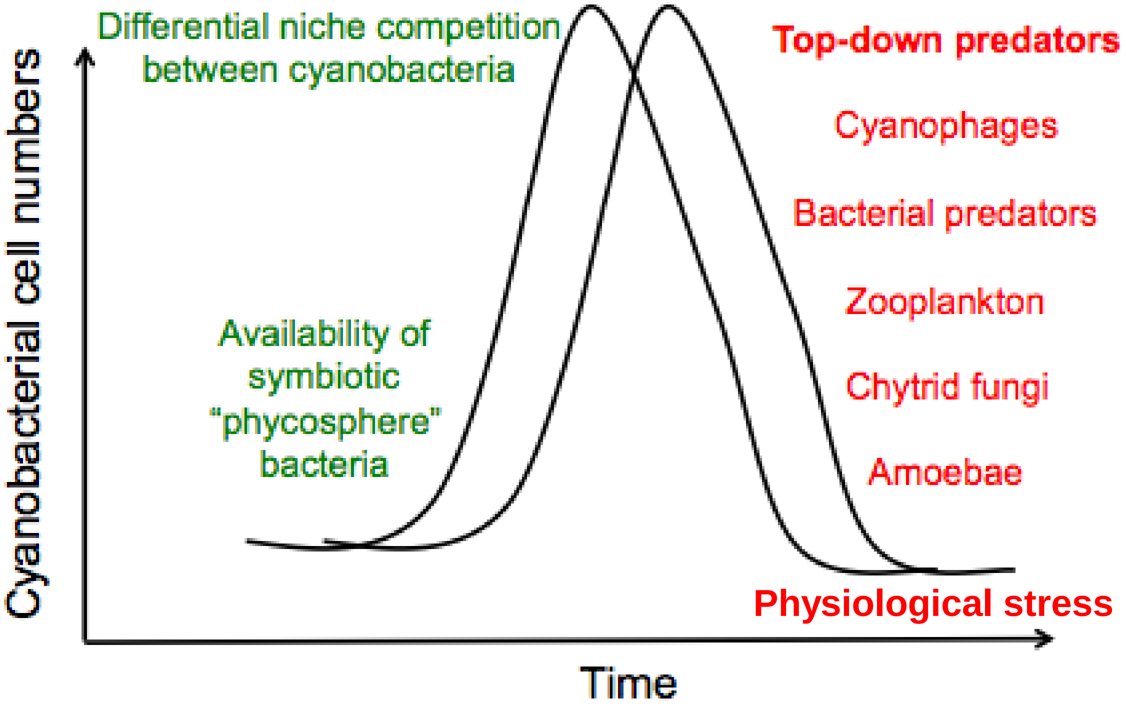
- **Allow optimal design of primers for PCR-based high-throughput monitoring**

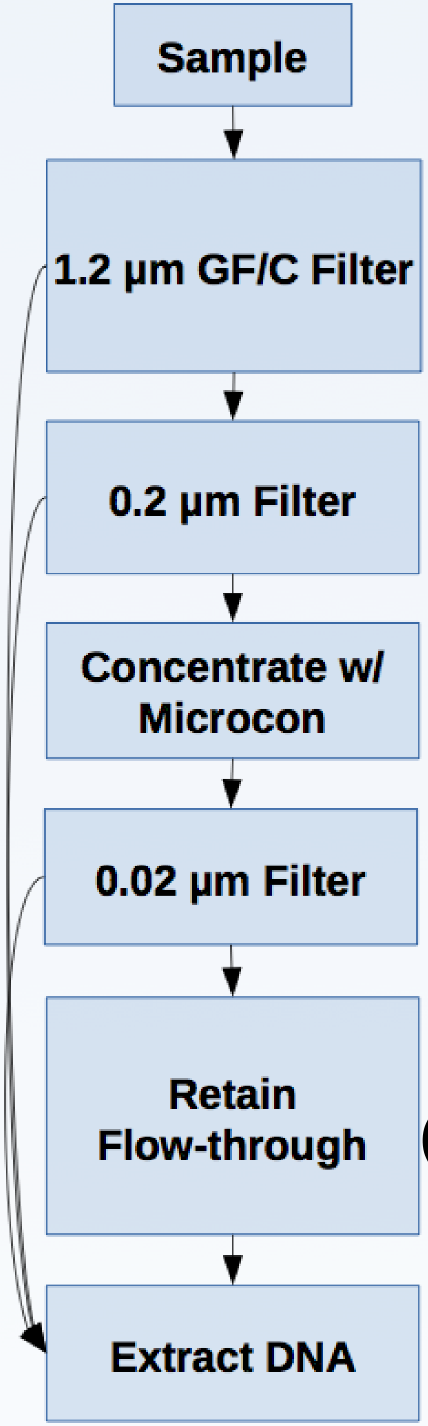
```
                          Forward primer (NB78)
Aphanizomenon sp. WA102   GCTTAAATGGTTTGCGCGAA   🟥
Mismatches                    A  T  T
Primers                   GCTTGAACGGCTTGCGCGAA
Mismatches                    A  T
Anabaena sp. WA102        GCTTAAATGGCTTGCGCGAA   ✓
```



- **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

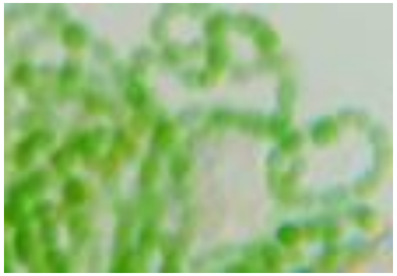
Bacteria
Archaea

Viruses

Free DNA (lysed cells)

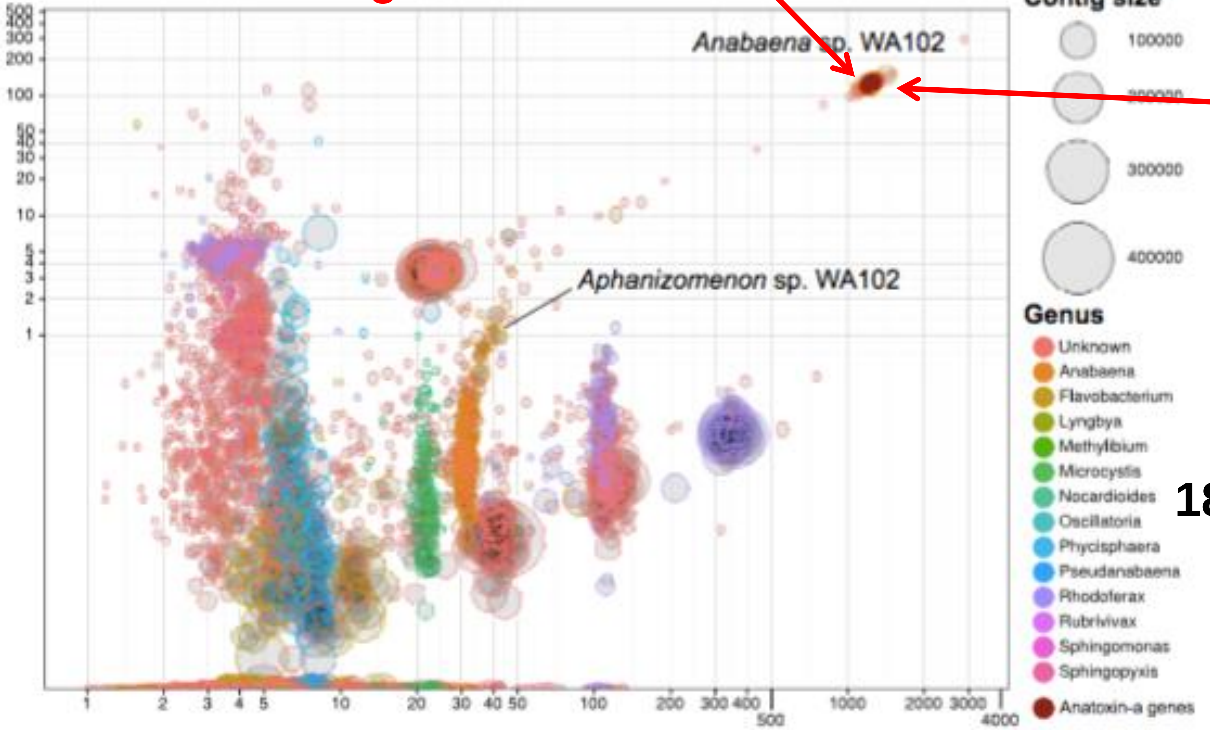
Anderson Lake, WA: Anatoxin-a up to 1090 µg/L

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**



Relative read depth
Culture WA102-1

Contigs with anatoxin biosynthetic genes

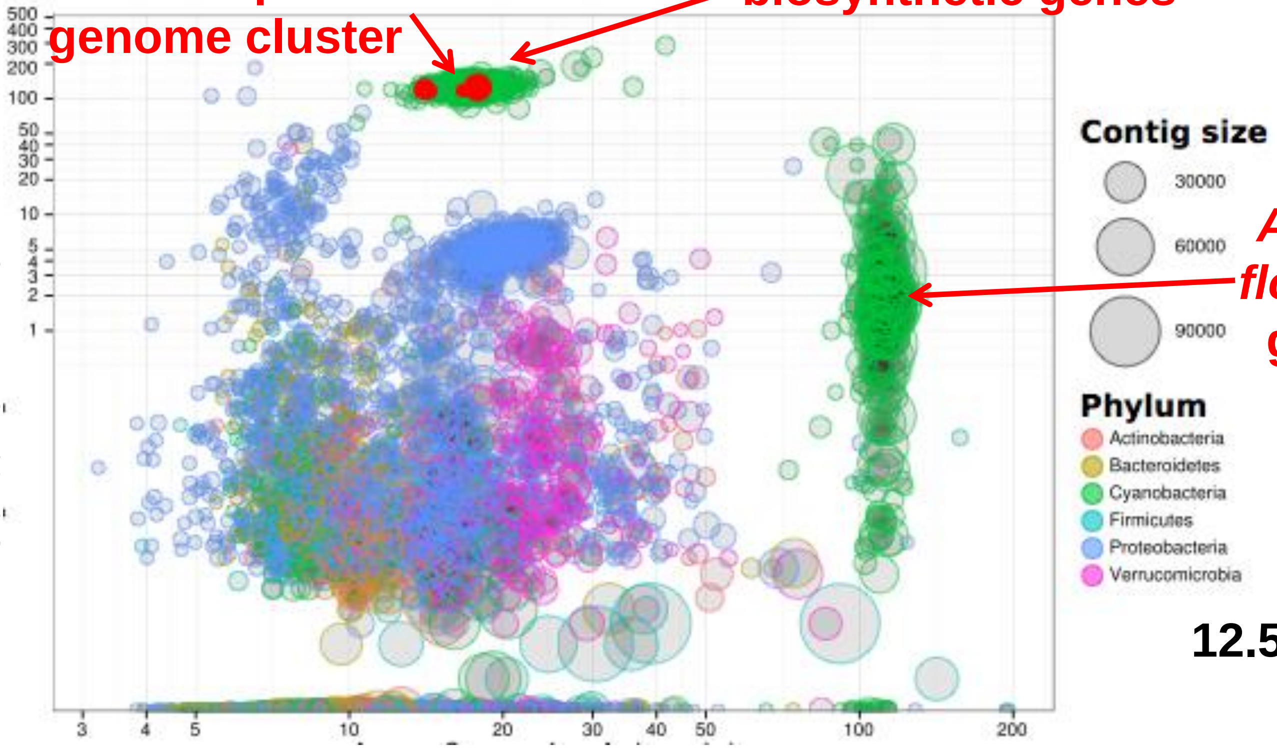
9 July 2012
187 µg/L anatoxin-a

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



Anabaena sp. WA102 genome cluster

Contigs with anatoxin biosynthetic genes

Aphanizomenon flos-aquae WA102 genome cluster

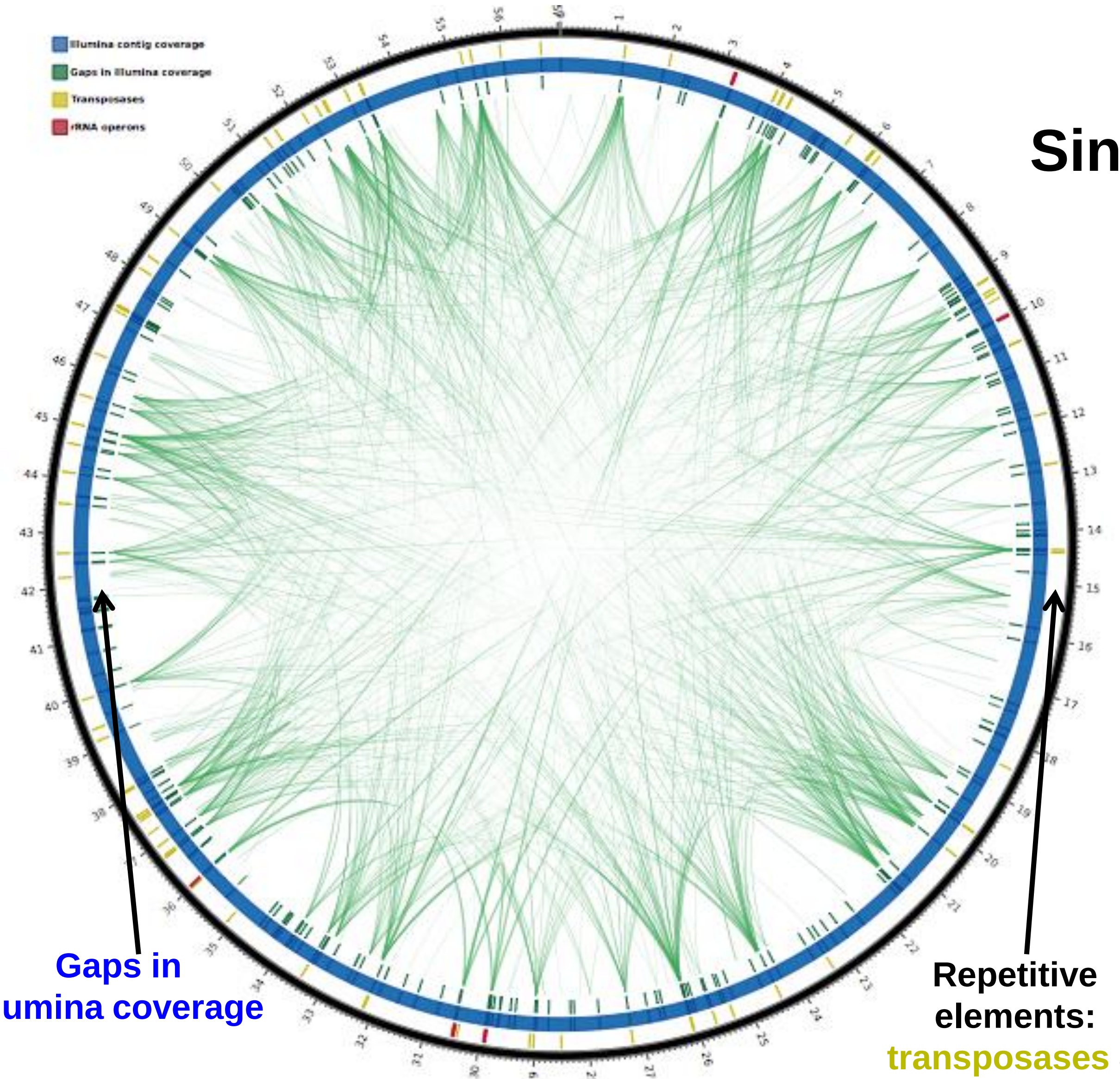
Relative read depth
Culture WA102-1

Relative read depth
Anderson Lake (GF/C), 20 May 2013

20 May 2013
12.5 µg/L anatoxin-a

Nathan Brown

Anabaena sp. WA102



Gaps in Illumina coverage

Repetitive elements:
transposases & rRNA (5)

5.7 Mbp
Single chromosome

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

Illumina: ~700 contigs

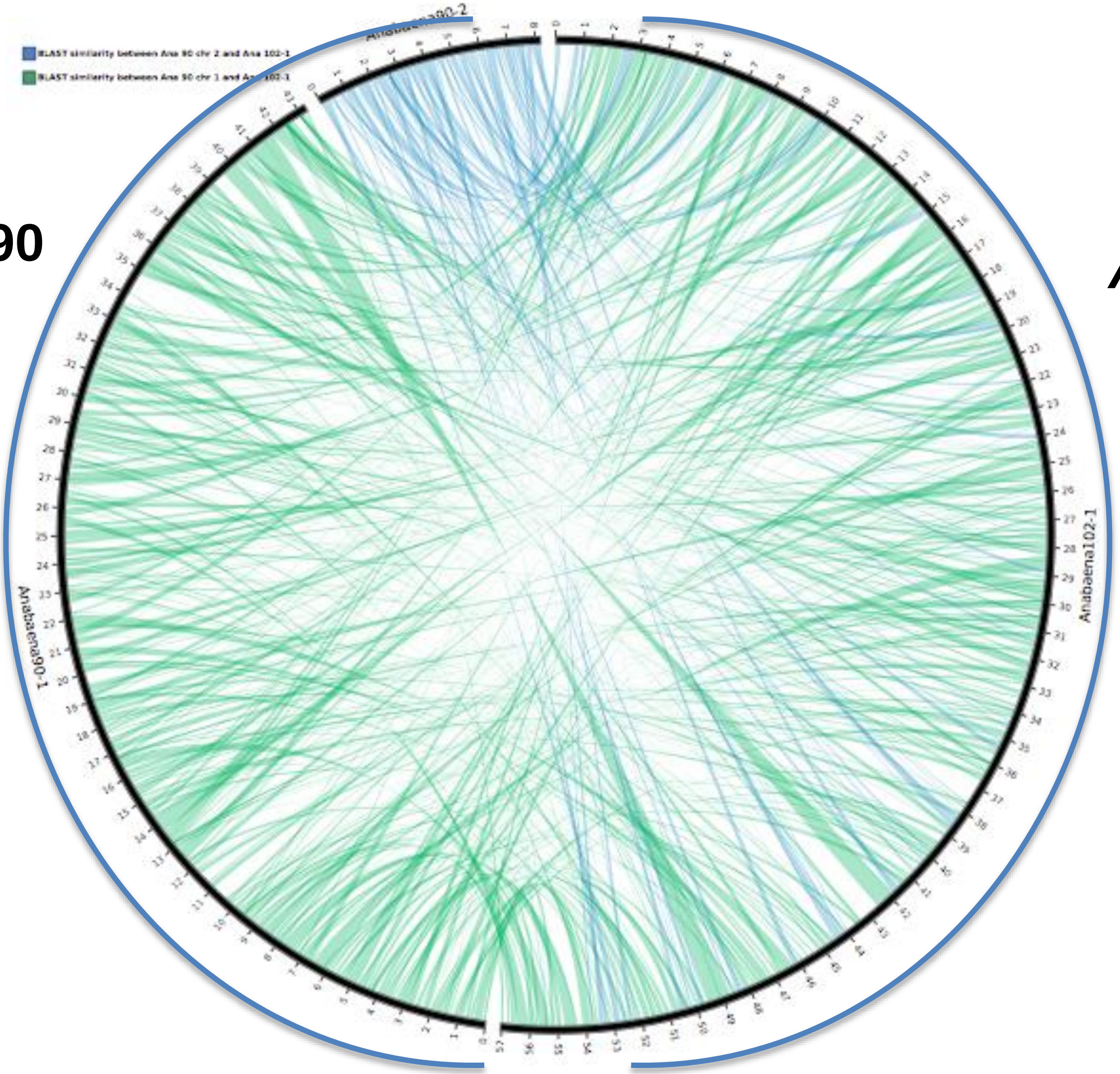
Nathan Brown

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

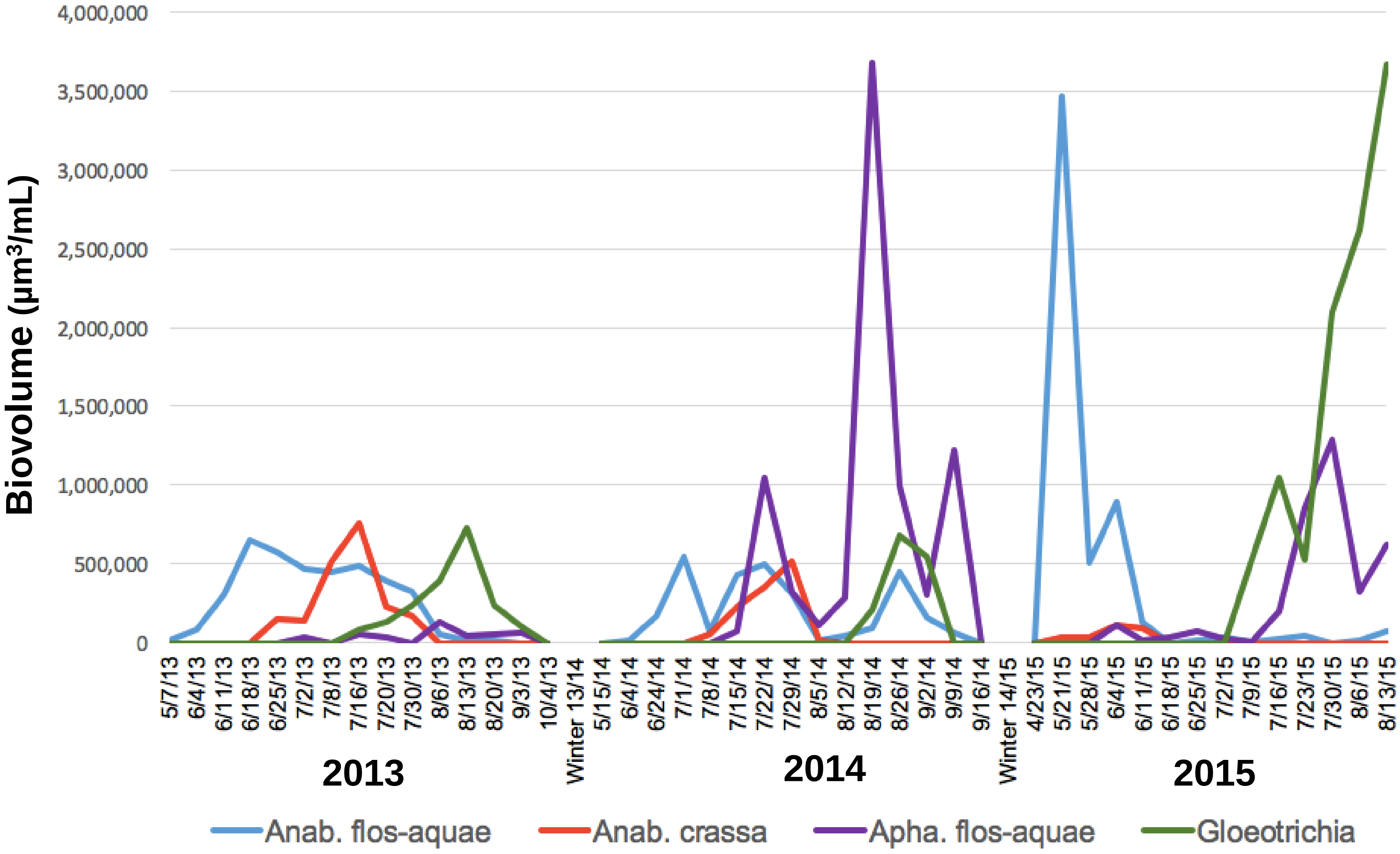


***Anabaena* sp.**
WA102
Isolated 2013

5.7 Mbp
Single
chromosome
ana+

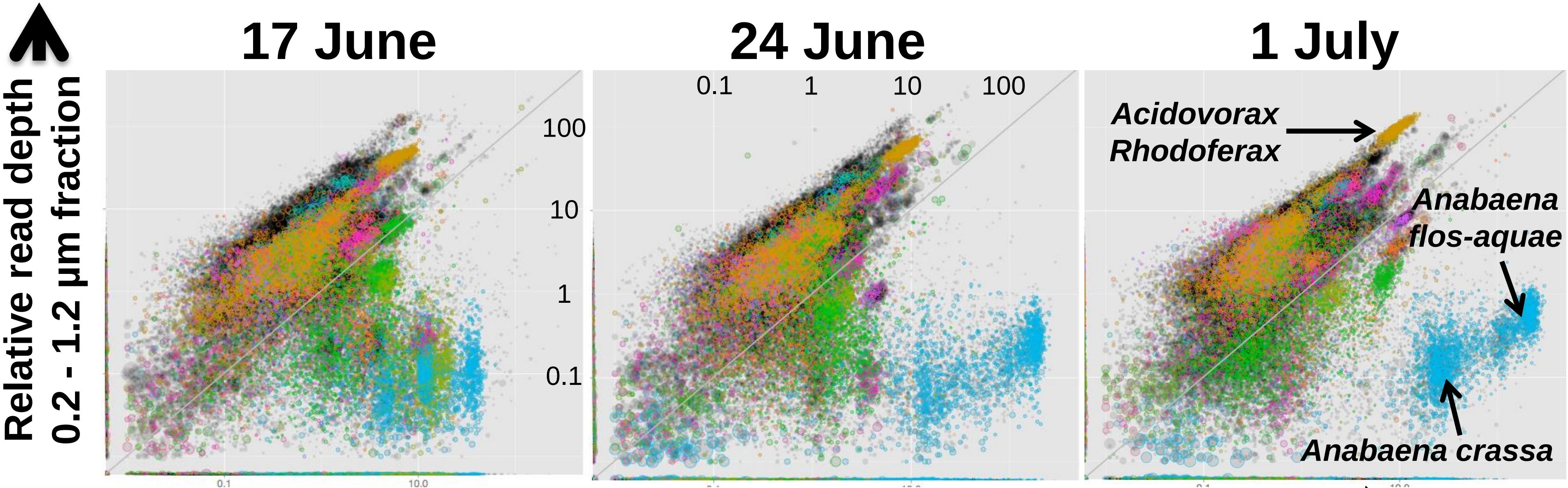
Nathan Brown

Cyanobacterial successions in Dexter Reservoir
** All nontoxic **



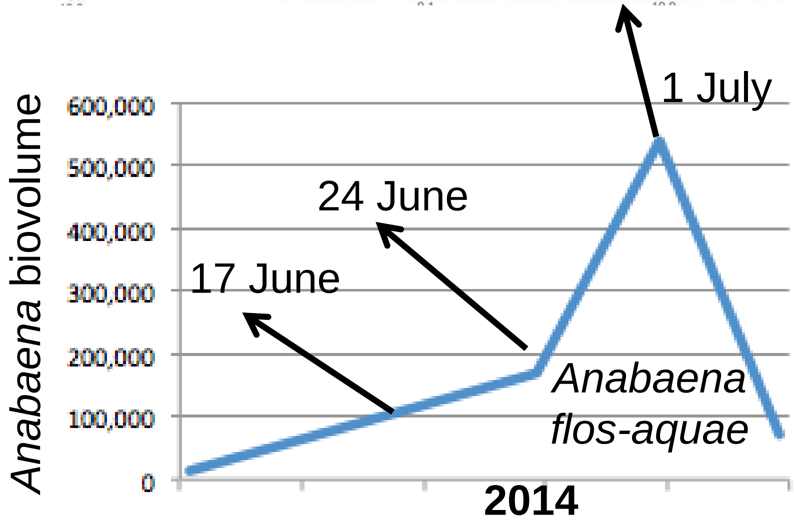
Shotgun metagenomic analysis

Dexter Reservoir 2014



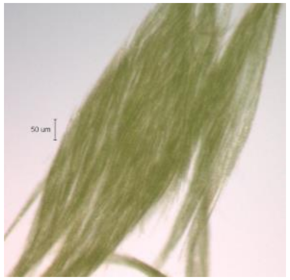
Relative read depth
>1.2 µm (GF/C) fraction

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

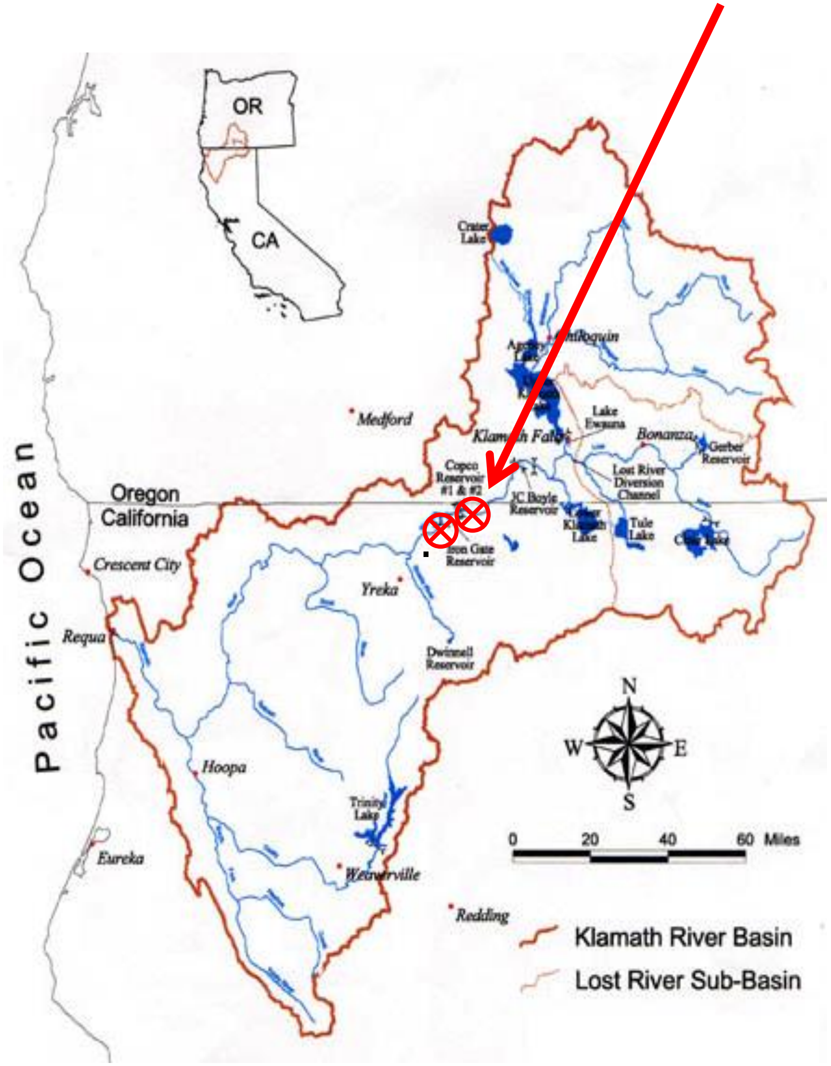
- The **3 novel bacterial genomes** indicate heterotrophs dependent on *Aphanizomenon* for N (probably provided as NH_3), C and maybe even reduced forms of S.
- *Bacteroidetes* seems to encode a secreted peroxidase, which AFA lacks; may provide protection against extracellular reactive oxygen species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

Connor Driscoll

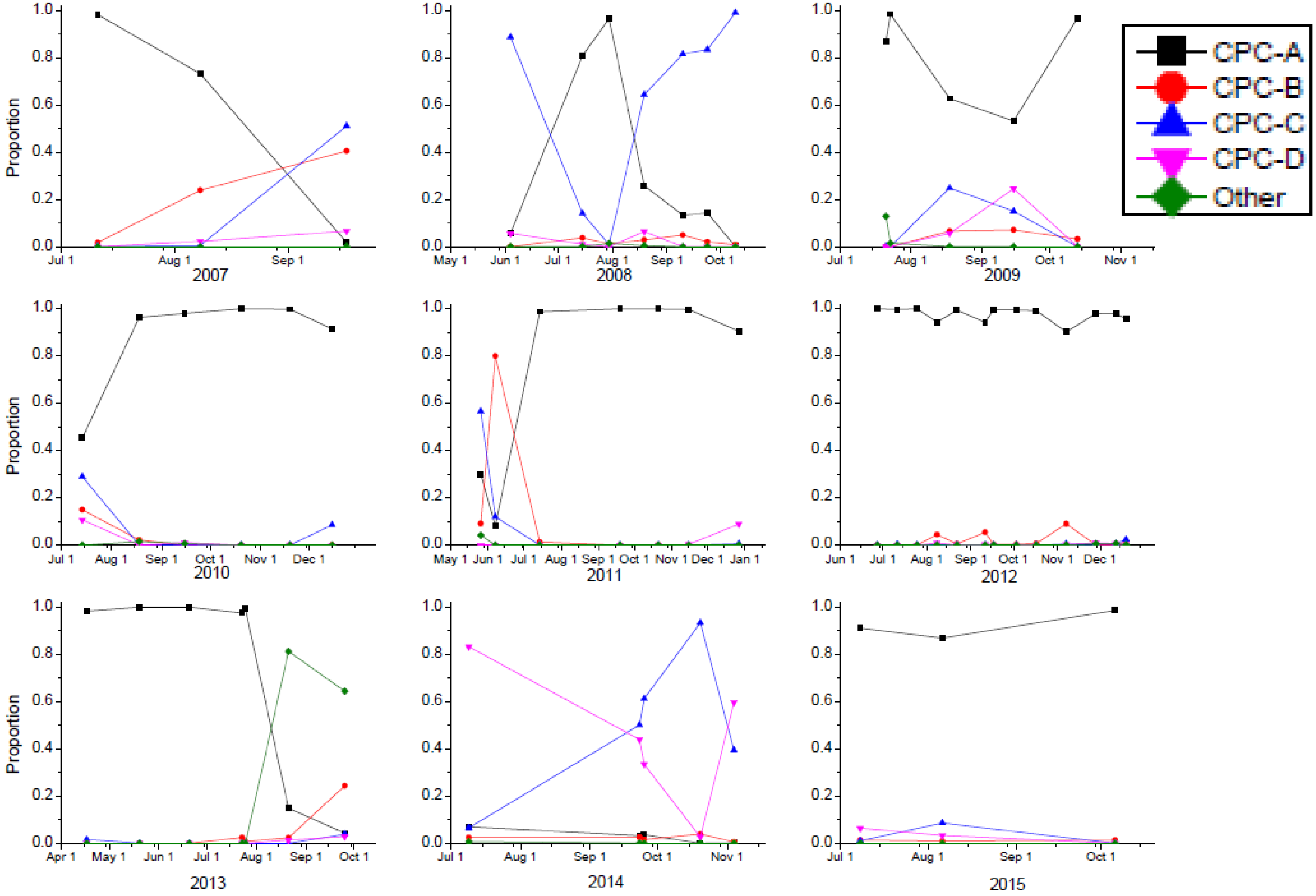
Klamath River Basin

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



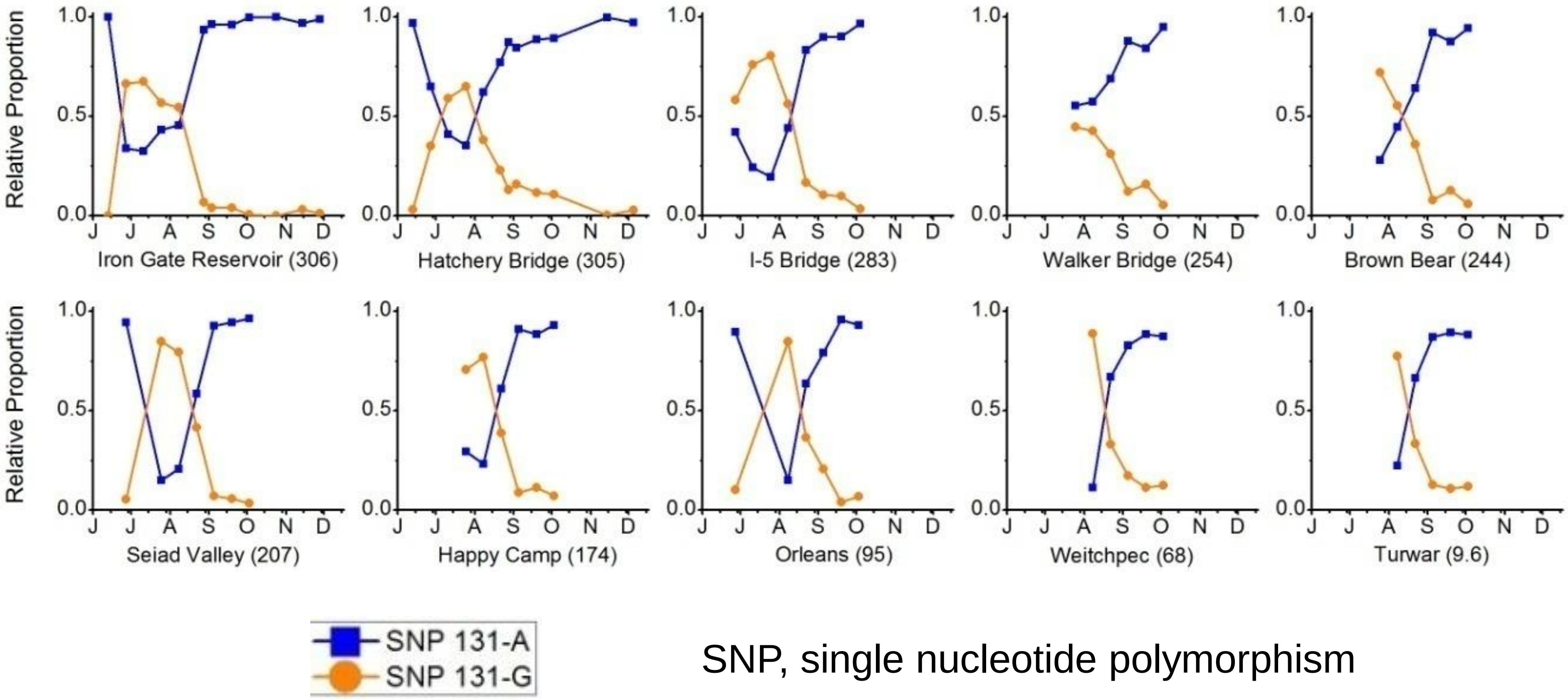
Phycocyanin gene *cpcBA* Illumina amplicon analysis

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)



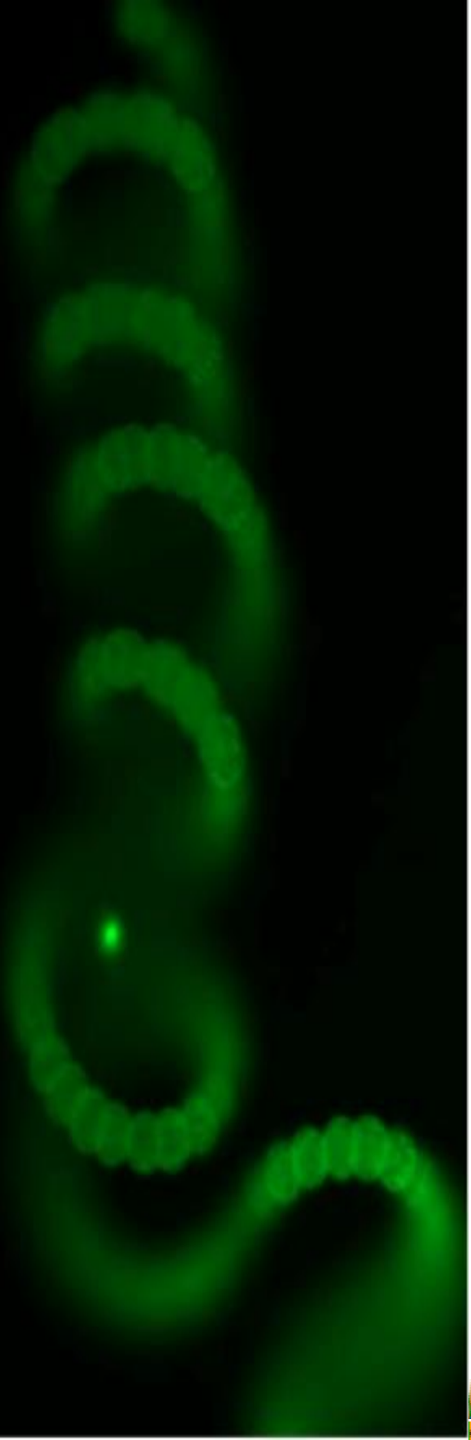
SNP, single nucleotide polymorphism

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



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