Toxic cyanobacterial monitoring in the future:

genetic testing of harmful algal blooms (cyano-HABs)

Theo Dreher

Department of Microbiology

Center for Genome Research & Biocomputing

Oregon State University

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

Phytoplankton Sample Analysis

Sample: Klamath Basin
Sample Site: TG
Sample Depth: SG
Sample Date: 3-Sep-09

Total Density (#/mL): 1,353
Total Biovolume (um^3/mL): 795,013
Trophic State Index: 48.2

	Species	Density #/mL	Density Percent	Biovolume um^3/mL	Biovolume Percent	Group
1	Epithemia sorex	300	22.2	341,675	43.0	diatom
2	Nitzschia frustulum	171	12.7	20,552	2.6	diatom
3	Diatoma tenue	157	11.6	45,528	5.7	diatom
4	Cocconeis placentula	128	9.5	59,087	7.4	diatom
5	Synedra ulna	86	6.3	170,409	21.4	diatom
6	Rhoicosphenia curvata	57	4.2	6,679	0.8	diatom
7	Nitzschia palea	57	4.2	10,276	1.3	diatom
8	Navicula decussis	43	3.2	8,221	1.0	diatom
9	Rhodomonas minuta	29	2.1	571	0.1	cryptophyte
10	Nitzschia paleacea	29	2.1	2,797	0.4	diatom
11	Diatoma vulgare	29	2.1	55,947	7.0	diatom
12	Gomphonema olivaceum	14	1.1	3,211	0.4	diatom
13	Nitzschia capitellata	14	1.1	5,138	0.6	diatom
14	Selenastrum minutum	14	1.1	285	0.0	green
15	Aphanizomenon flos-aquae	14	1.1	10,790	1.4	bluegreen
16	Cymbella sinuata	14	1.1	1,998	0.3	diatom
17	Gomphonema angustatum	14	1.1	2,569	0.3	diatom
18	Navicula gregaria	14	1.1	2,498	0.3	diatom
19	Navicula capitata	14	1.1	6,851	0.9	diatom
20	Navicula viridula	14	1.1	6,422	0.8	diatom
21	Achnanthes lanceolata	14	1.1	2,569	0.3	diatom
22	Navicula cryptocephala	14	1.1	2,640	0.3	diatom
23	Nitzschia acicularis	14	1.1	3,996	0.5	diatom
24	Pediastrum boryanum	14	1.1	2,854	0.4	green
25	Achnanthes minutissima	14	1.1	714	0.1	diatom
26	Synedra mazamaensis	14	1.1	3,654	0.5	diatom
27	Fragilaria construens	14	1.1	6,394	0.8	diatom
28	Cyclotella meneghiniana	14	1.1	5,423	0.7	diatom
29	Scenedesmus quadricauda	14	1.1	3,711	0.5	green
30	Microcystis aeruginosa	11	0.8	1,553	0.2	bluegreen

Note: 4X count for toxic species.

Aphanizomenon flos-aquae cells/mL = 171

Microcystis aeruginosa cells/mL = 194

Aquatic Analysts | Sample ID: MA90

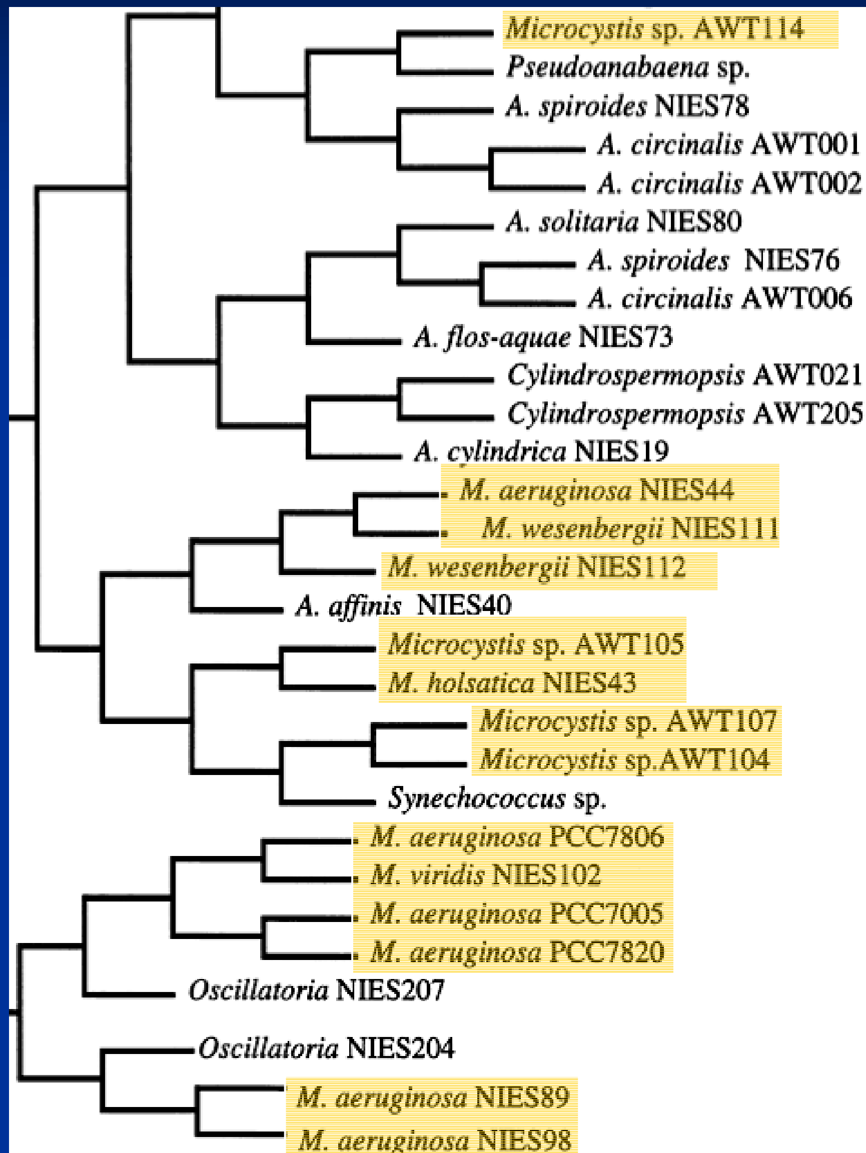
Toxin analyses

MAP ID	Station	Microcystin	Cell Count - Cell Density
A	D River	= 0.60	No Scum - Low Density
B	Campground	= 3.60	Some Scum - Moderate Density
C	Regatta Grounds	= 2.96	No Scum - Moderate Density
D	Holmes Road Park	> 10.00	Some Scum - Moderate Density
E	Sand Point	= 2.48	No Scum - Low Density
F	East D.L. State Park	= 2.98	Some Scum - Moderate Density
1	Mid Lake	= 2.86	Moderate Scum - Moderate Density
2	NE Arm	= 4.26	No Scum - Low Density
3	NW Arm	= 4.00	No Scum - Low Density
4	Southern End	= 3.72	No Scum - Low Density
5	East Thumb	= 3.86	No Scum - Low Density
6	Deepest Point	= 4.24	Some Scum - Moderate Density

Note: Microcystin is only one of the many toxins produced by cyanobacteria. These tests results are only a snap-shot in time and are provided for guidance only. Conditions may change quickly.

The problem with morphological ID

Microcystis sp.

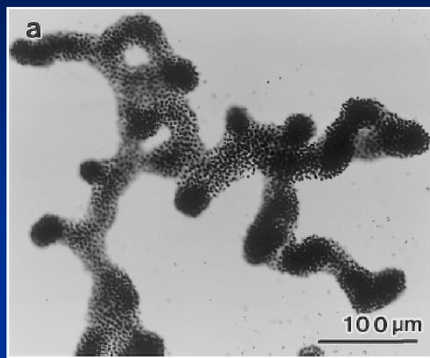


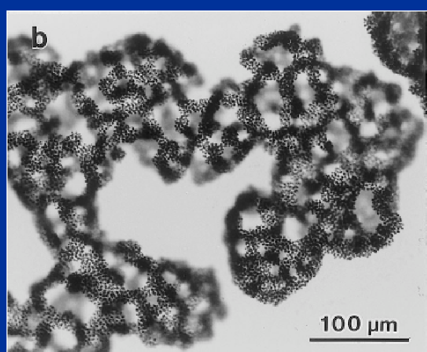
Organisms called *Microcystis* should all have the most similar DNA sequences

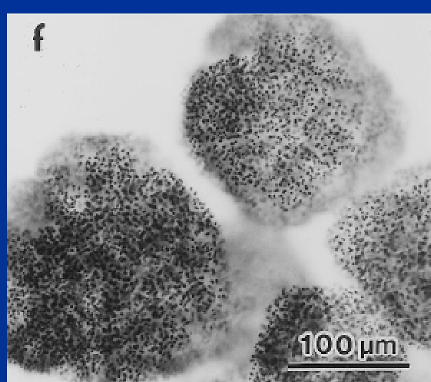
Sister sequences should be from the same species and genus

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.

The problem with morphological ID

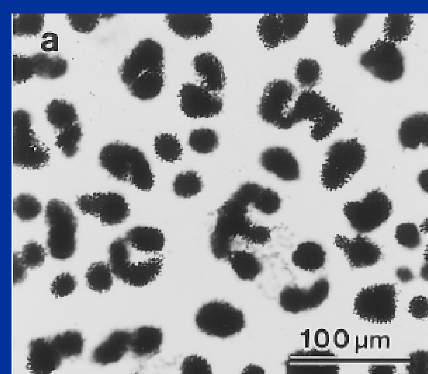


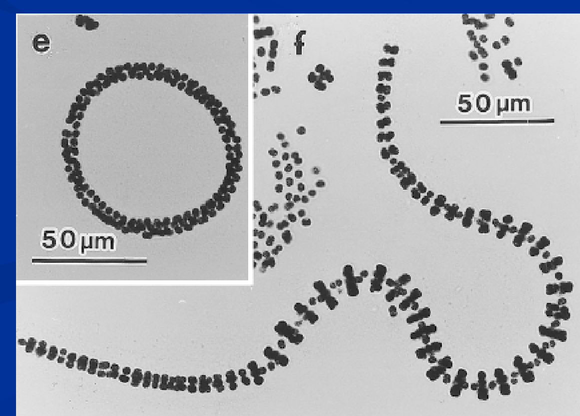




Microcystis **can assume widely different colony morphologies that have confused attempts at species ID**

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*.





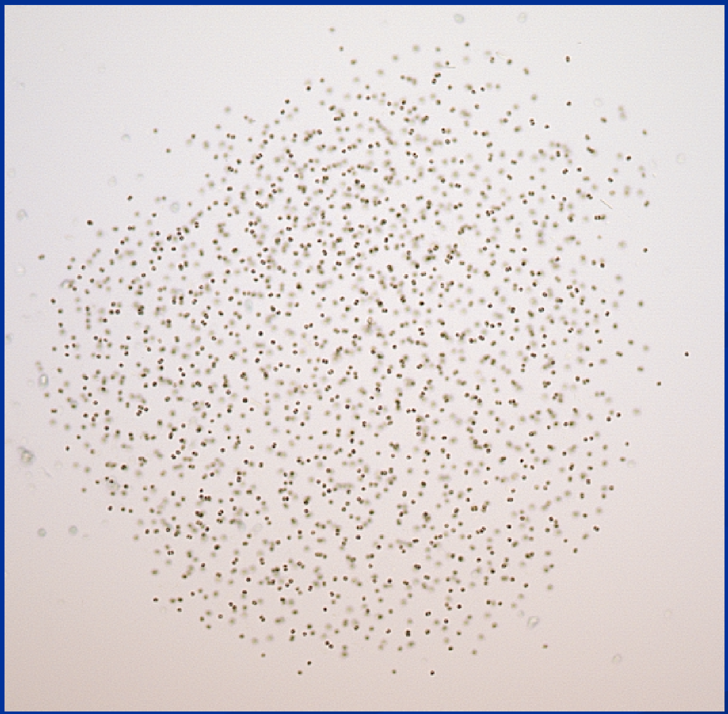
Otsuka et al. J. Gen. Appl. Microbiol. 46 (2000):39-50

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

BMC Genomics BioMed Central

Research article **Open Access**

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

Lionel Frangeul†[1], Philippe Quillardet†[2], Anne-Marie Castets[2], Jean-François Humbert[2,3], Hans CP Matthijs[4], Diego Cortez[5], Andrew Tolonen[2,10], Cheng-Cai Zhang[6], Simonetta Gribaldo[5], Jan-Christoph Kehr[7], Yvonne Zilliges[7], Nadine Ziemert[7], Sven Becker[8], Emmanuel Talla[6], Amel Latifi[6], Alain Billault[9], Anthony Lepelletier[1], Elke Dittmann[7], Christiane Bouchier[1] and Nicole Tandeau de Marsac*[2]

Applied and Environmental Microbiology, Dec. 2003, p. 7289–7297 Vol. 69, No. 12
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.12.7289–7297.2003

Quantitative Real-Time PCR for Determination of Microcystin Synthetase E Copy Numbers for *Microcystis* and *Anabaena* in Lakes

Jaana Vaitomaa,[1] Anne Rantala,[1] Katrianna Halinen,[1] Leo Rouhiainen,[1] Petra Tallberg,[2] Lena Mokelke,[1] and Kaarina Sivonen[1]*

Department of Applied Chemistry and Microbiology[1] and Department of Limnology and Environmental Protection,[2] University of Helsinki, Helsinki, Finland

Applied and Environmental Microbiology, Sept. 2006, p. 6101–6110 Vol. 72, No. 9
0099-2240/06/$08.00+0 doi:10.1128/AEM.01058-06

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

Anne Rantala,[1] Pirjo Rajaniemi-Wacklin,[1] Christina Lyra,[1] Liisa Lepistö,[2] Jukka Rintala,[3] Joanna Mankiewicz-Boczek,[4] and Kaarina Sivonen[1]*

Department of Applied Chemistry and Microbiology, University of Finland, Helsinki, Finland[1]; Finnish Environment Institute,

Microbial Ecology

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

J.F. Humbert[1], D. Duris-Latour[2], B. Le Berre[1], H. Giraudet[2] and M.J. Salençon[3]

(1) INRA, UMR CARRTEL, BP 511, 74203 Thonon Cedex, France
(2) Université J. Monnet, Lab. de Biologie Animale et Appliquée, 42023 St Etienne, France
(3) EDFR&D, Laboratoire National d'Hydraulique et Environnement, 6, Quai Watier, 78401 Chatou Cedex, France

Received: 12 January 2004 / Accepted: 3 March 2004 / Online publication: 17 June 2005

Environmental Microbiology (2005) 7(3), 365–377 doi:10.1111/j.1462-2920.2004.00715.x

Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake

Gabriel Zwart,* Miranda P. Kamst-van Agterveld, Irene van der Werff-Staverman, Ferry Hagen, Hans L. Hoogveld and Herman J. Gons

Centre for Limnology, NIOO-KNAW Netherlands Institute of Ecology, Rijksstraatweg 6, 3631 AC Nieuwersluis, the Netherlands.

Introduction

The microbial community in the water column of marine and freshwater ecosystems is thought to be less complex than microbial communities in soils and sediments (Torsvik *et al.*, 2002; Weinbauer and Rassoulzadegan, 2004) and indeed, evidence is accumulating that both the marine

Applied and Environmental Microbiology, Aug. 2000, p. 3387–3392 Vol. 66, No. 8
0099-2240/00/$04.00+0

Light and the Transcriptional Response of the Microcystin Biosynthesis Gene Cluster

Melanie Kaebernick,[1,2] Brett A. Neilan,[1]* Thomas Börner,[2] and Elke Dittmann[2]

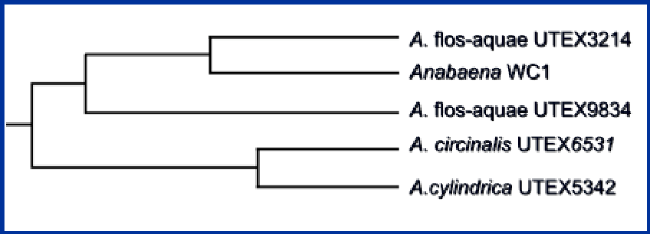
School of Microbiology and Immunology, University of New South Wales, Sydney 2052, Australia,[1] and Institute for Biology (Genetics), Humboldt University, Berlin, Germany[2]

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

```
  1 TTCTCATGTT TGACAGCTTA TCATCGATAA GCTTTAATGC
 61 TTGCTAACGC AGTCAGGCAC CGTGTATGAA ATCTAACAAT
121 CACCGTCACC CTGGATGCTG TAGGCATAGG CTTGGTTATG
181 GCGGGATATC GTCCATTCCG ACAGCATCGC CAGTCACTAT
241 TGCGTTGATG CAATTTCTAT GCGCACCCGT TCTCGGAGCA
301 CCGCCCAGTC CTGCTCGCTT CGCTACTTGG AGCCACTATC
361 CACACCCGTC CTGTGGATCC TCTACGCCGG ACGCATCGTG
421 AGGTGCGGTT GCTGGCGCCT ATATCGCCGA CATCACCGAT
```



+/–

Phytoplankton analyses

Phytoplankton Sample Analysis

Sample: Klamath Basin
Sample Site: TG
Sample Depth: SG
Sample Date: 3-Sep-09

Total Density (#/mL): 1,353
Total Biovolume (um³/mL): 795,013
Trophic State Index: 48.2

	Species	Density #/mL	Density Percent	Biovolume um³/mL	Biovolume Percent	Group
1	Epithemia sorex	300	22.2	341,675	43.0	diatom
2	Nitzschia frustulum	171	12.7	20,552	2.6	diatom
3	Diatoma tenue	157	11.6	45,528	5.7	diatom
4	Cocconeis placentula	128	9.5	59,087	7.4	diatom
5	Synedra ulna	86	6.3	170,409	21.4	diatom
6	Rhoicosphenia curvata	57	4.2	6,679	0.8	diatom
7	Nitzschia palea	57	4.2	10,276	1.3	diatom
8	Navicula decussis	43	3.2	8,221	1.0	diatom
9	Rhodomonas minuta	29	2.1	571	0.1	cryptophyte
10	Nitzschia paleacea	29	2.1	2,797	0.4	diatom
11	Diatoma vulgare	29	2.1	55,947	7.0	diatom
12	Gomphonema olivaceum	14	1.1	3,211	0.4	diatom
13	Nitzschia capitellata	14	1.1	5,138	0.6	diatom
14	Selenastrum minutum	14	1.1	285	0.0	green
15	Aphanizomenon flos-aquae	14	1.1	10,790	1.4	bluegreen
16	Cymbella sinuata	14	1.1	1,998	0.3	diatom
17	Gomphonema angustatum	14	1.1	2,569	0.3	diatom
18	Navicula gregaria	14	1.1	2,498	0.3	diatom
19	Navicula capitata	14	1.1	6,851	0.9	diatom
20	Navicula viridula	14	1.1	6,422	0.8	diatom
21	Achnanthes lanceolata	14	1.1	2,569	0.3	diatom
22	Navicula cryptocephala	14	1.1	2,640	0.3	diatom
23	Nitzschia acicularis	14	1.1	3,996	0.5	diatom
24	Pediastrum boryanum	14	1.1	2,854	0.4	green
25	Achnanthes minutissima	14	1.1	714	0.1	diatom
26	Synedra mazamaensis	14	1.1	3,654	0.5	diatom
27	Fragilaria construens	14	1.1	6,394	0.8	diatom
28	Cyclotella meneghiniana	14	1.1	5,423	0.7	diatom
29	Scenedesmus quadricauda	14	1.1	3,711	0.5	green
30	Microcystis aeruginosa	11	0.8	1,553	0.2	bluegreen

Note: 4X count for toxic species.

Aphanizomenon flos-aquae cells/mL = 171

Microcystis aeruginosa cells/mL = 194

Aquatic Analysts — Sample ID: MA90

Toxin analyses

MAP ID	Station	Microcystin	Cell Count - Cell Density
A	D River	= 0.60	No Scum - Low Density
B	Campground	= 3.60	Some Scum - Moderate Density
C	Regatta Grounds	= 2.96	No Scum - Moderate Density
D	Holmes Road Park	> 10.00	Some Scum - Moderate Density
E	Sand Point	= 2.48	No Scum - Low Density
F	East D.L. State Park	= 2.98	Some Scum - Moderate Density
1	Mid Lake	= 2.86	Moderate Scum - Moderate Density
2	NE Arm	= 4.26	No Scum - Low Density
3	NW Arm	= 4.00	No Scum - Low Density
4	Southern End	= 3.72	No Scum - Low Density
5	East Thumb	= 3.86	No Scum - Low Density
6	Deepest Point	= 4.24	Some Scum - Moderate Density

Note: Microcystin is only one of the many toxins produced by cyanobacteria. These tests results are only a snap-shot in time and are provided for guidance only. Conditions may change quickly.

Future cyanobacterial database

Willow Creek Reservoir, Heppner

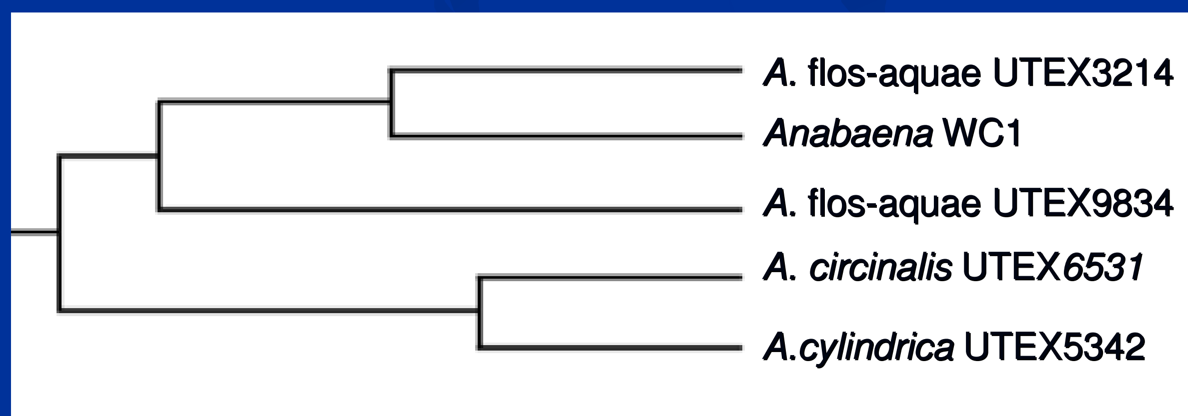
Collection: June 10, 2008

Morphological ID: *Anabaena flos-aquae*

Genotypic ID: *Anabaena* WC1

16S rDNA
rDNA ITS
Phycocyanin *cpcBA*

```
  1 TTCTCATGTT TGACAGCTTA TCATCGATAA GCTTTAATGC
 61 TTGCTAACGC AGTCAGGCAC CGTGTATGAA ATCTAACAAT
121 CACCGTCACC CTGGATGCTG TAGGCATAGG CTTGGTTATG
181 GCGGGATATC GTCCATTCCG ACAGCATCGC CAGTCACTAT
241 TGCGTTGATG CAATTTCTAT GCGCACCCGT TCTCGGAGCA
301 CCGCCCAGTC CTGCTCGCTT CGCTACTTGG AGCCACTATC
361 CACACCCGTC CTGTGGATCC TCTACGCCGG ACGCATCGTG
421 AGGTGCGGTT GCTGGCGCCT ATATCGCCGA CATCACCGAT
```



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)

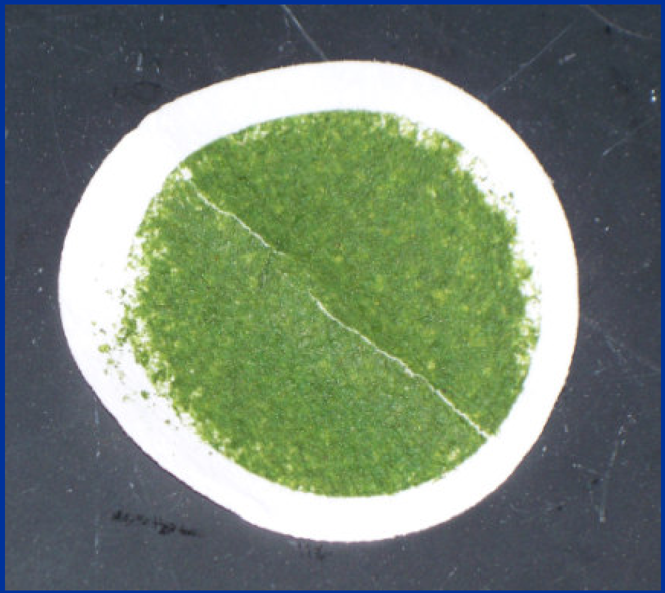
Sample collection

Storage & shipping on ice, not preserved

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

Sample preparation

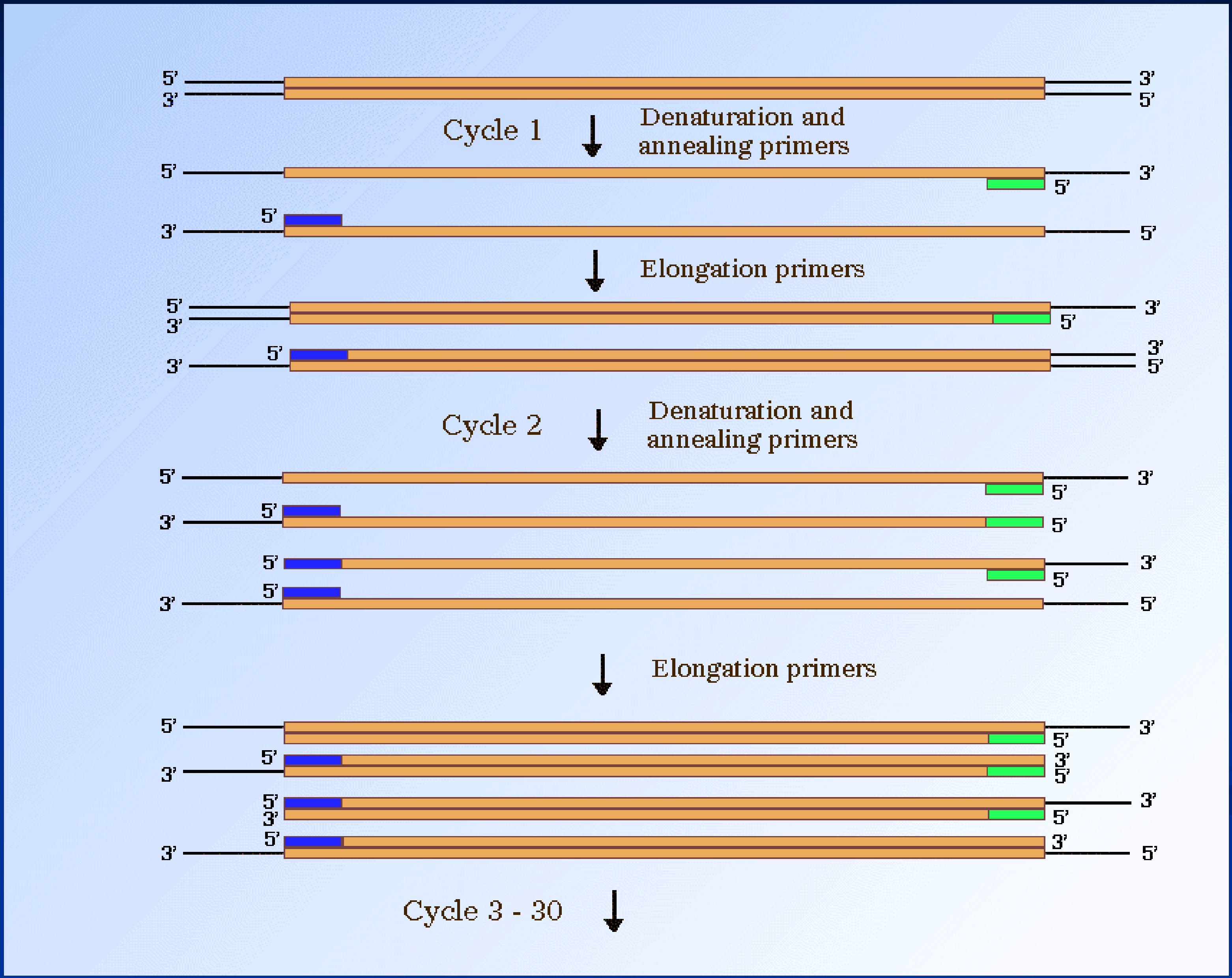
Samples are filtered for immediate use or storage in freezer for subsequent DNA extraction



0.45 micron glass fiber filter

0.22 micron Millipore filter

Polymerase Chain Reaction: PCR



Cyano-HAB PCR targets

There are multiple options that can be considered

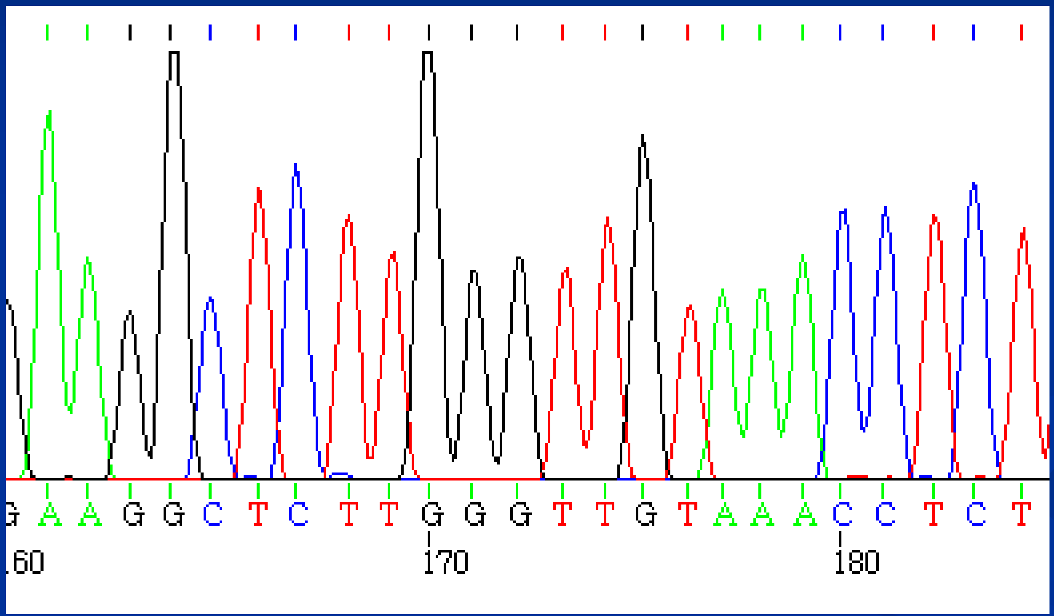
- PCR designed to detect all HAB-forming cyanobacteria
- PCR directed at particular genera or species
- PCR follwed by DNA sequencing
- Quantitative PCR to measure gene numbers

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)



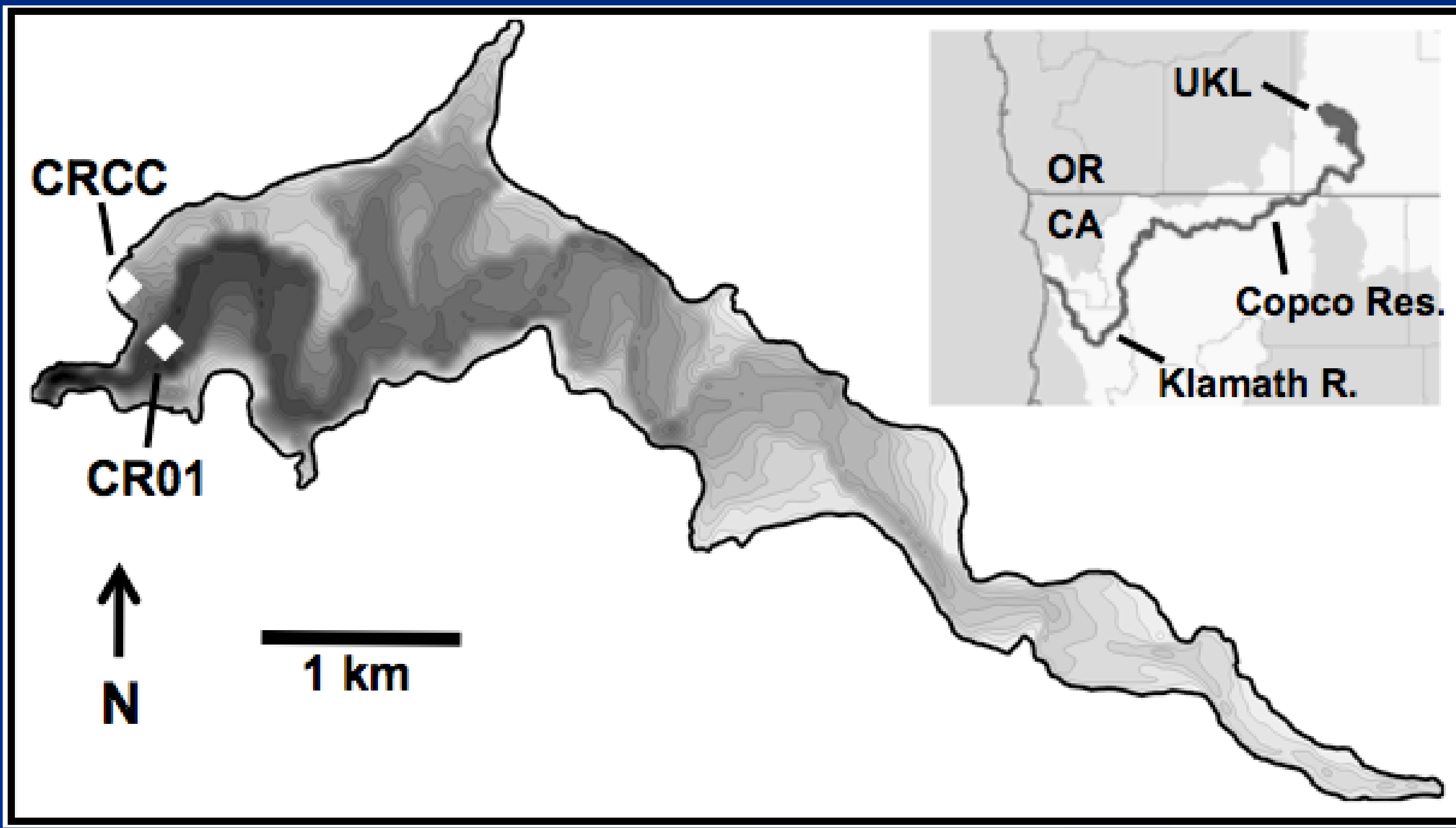
TGCTCCCGGCCGCCATGGCGGCCGCGGGAATTCGATTCCTACGGGAGGCA
GCAGTGGGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGCAATACCGC
GTGAGGGAAGAAGGCTCTTGGGTTGTAAACCTCTTTTCTCAGGGAAGAAA
AAAATGACGGTACCTGAGGAATAAGCATCGGCTAACTCCGTGCCAGCAGC
CGCGGTAATACGGAGGATGCAAGCGTTATCCGGAATGATTGGGCGTAAAG
GGTCCGCAGGTGGCATTGTAAGTCTGCTGTTAAAGAGTCTGGCTCAACCA
GATAAAAGCAGTGGAAACTACAAAGCTAGAGTATGGTCGGGGCAGAGGGA
ATTCCTGGTGTAGCGGTGAAACGCGTAGATATCAGGAAGAACACCGGTGG
CGAAGGCGCTCTGCTAGGCCAAAACTGACACTGAGGGACGAAAGCTAGGG

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

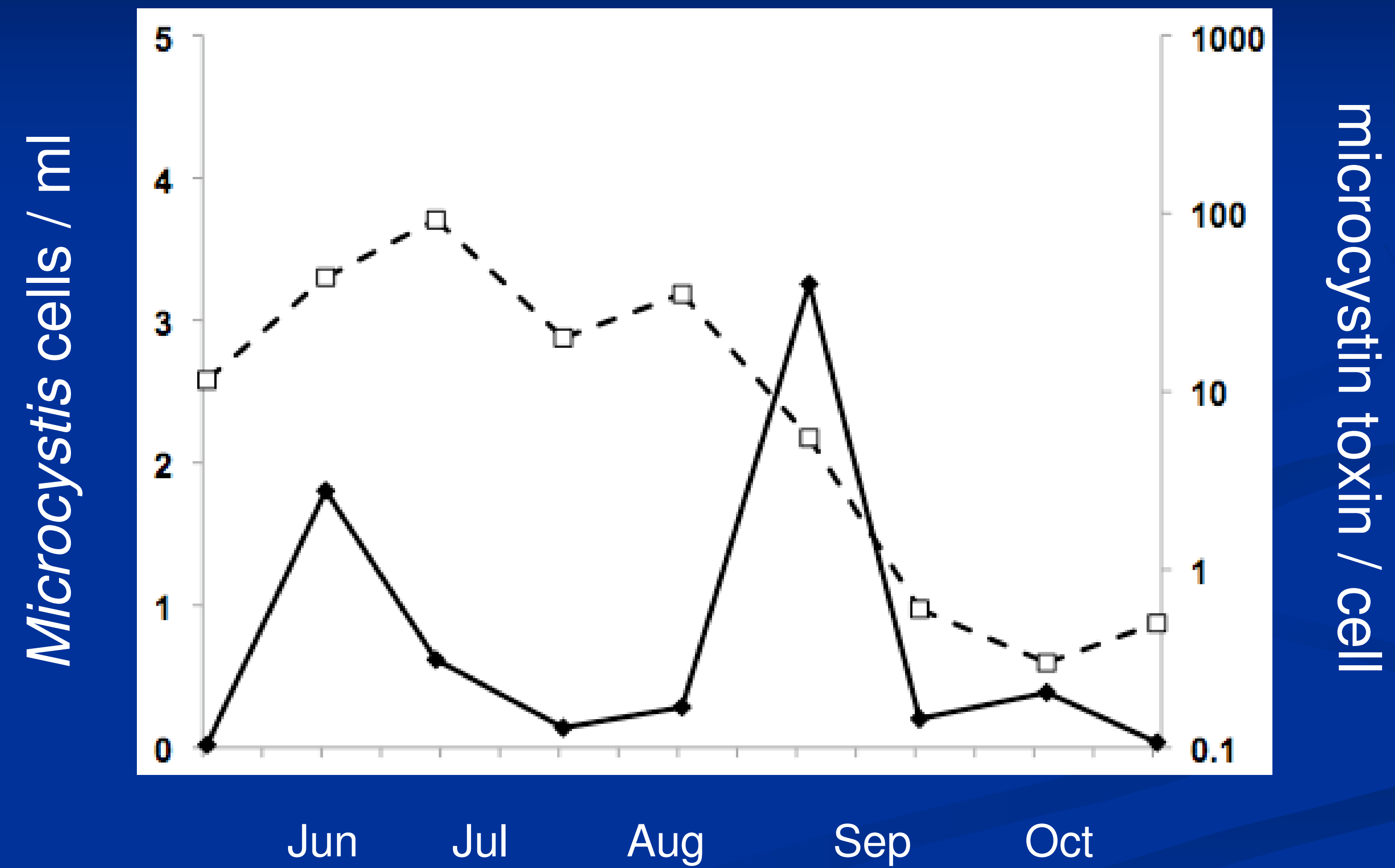
- initial focus on toxic *Microcystis* in Copco Res.
- apply info and techniques to develop assays useful for management decisions

Copco Reservoir sampling sites



Copco *Microcystis* bloom

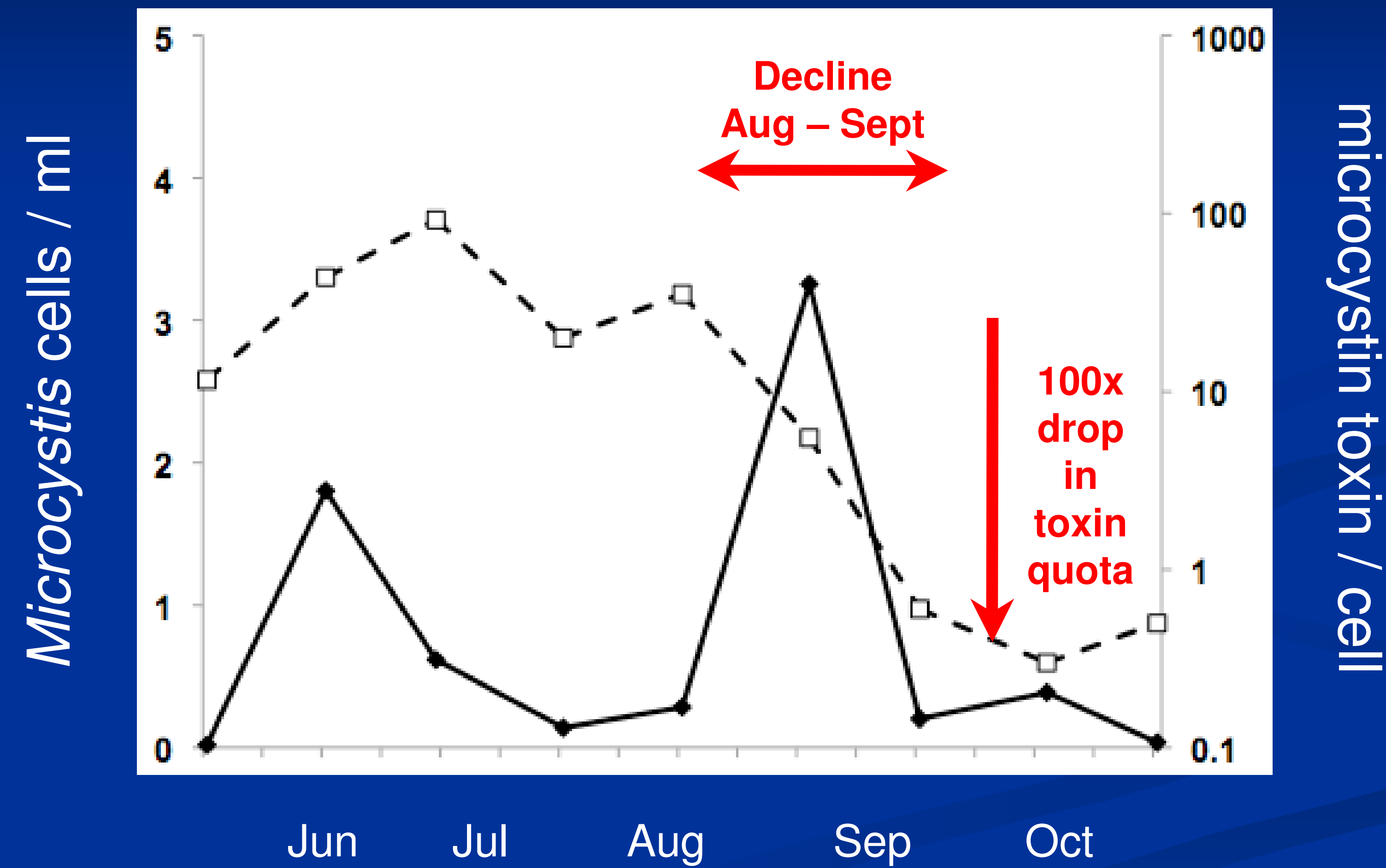
Conventional cell counts and toxin analysis



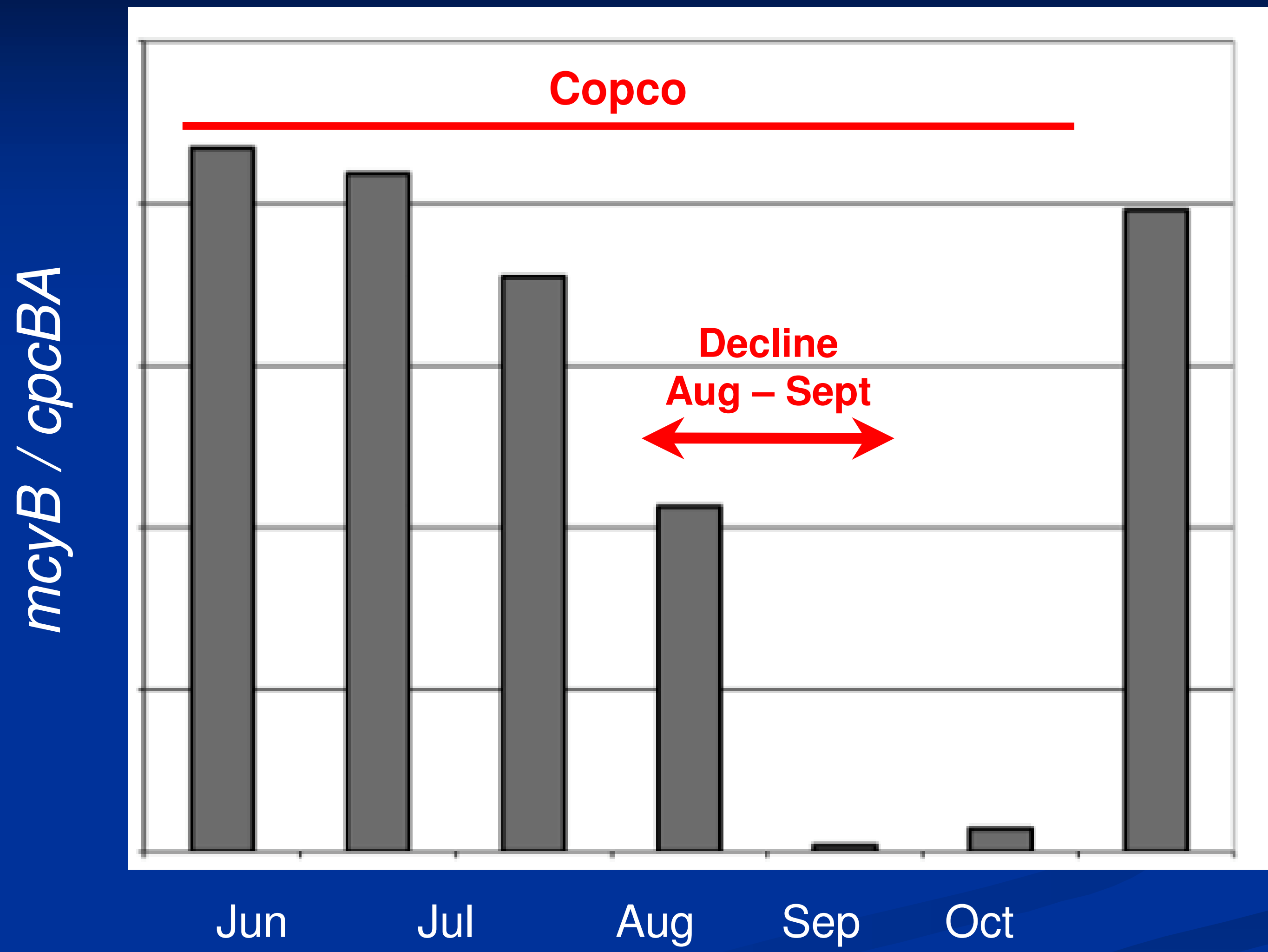
Data from Kann & Corum, 2009

Copco *Microcystis* bloom

Conventional cell counts and toxin analysis



Decline in *mcyB* gene copy number



Quantitative Taqman PCR assay for *Microcystis mcyB* and *cpcBA*

Genetic typing of the *Microcystis* population

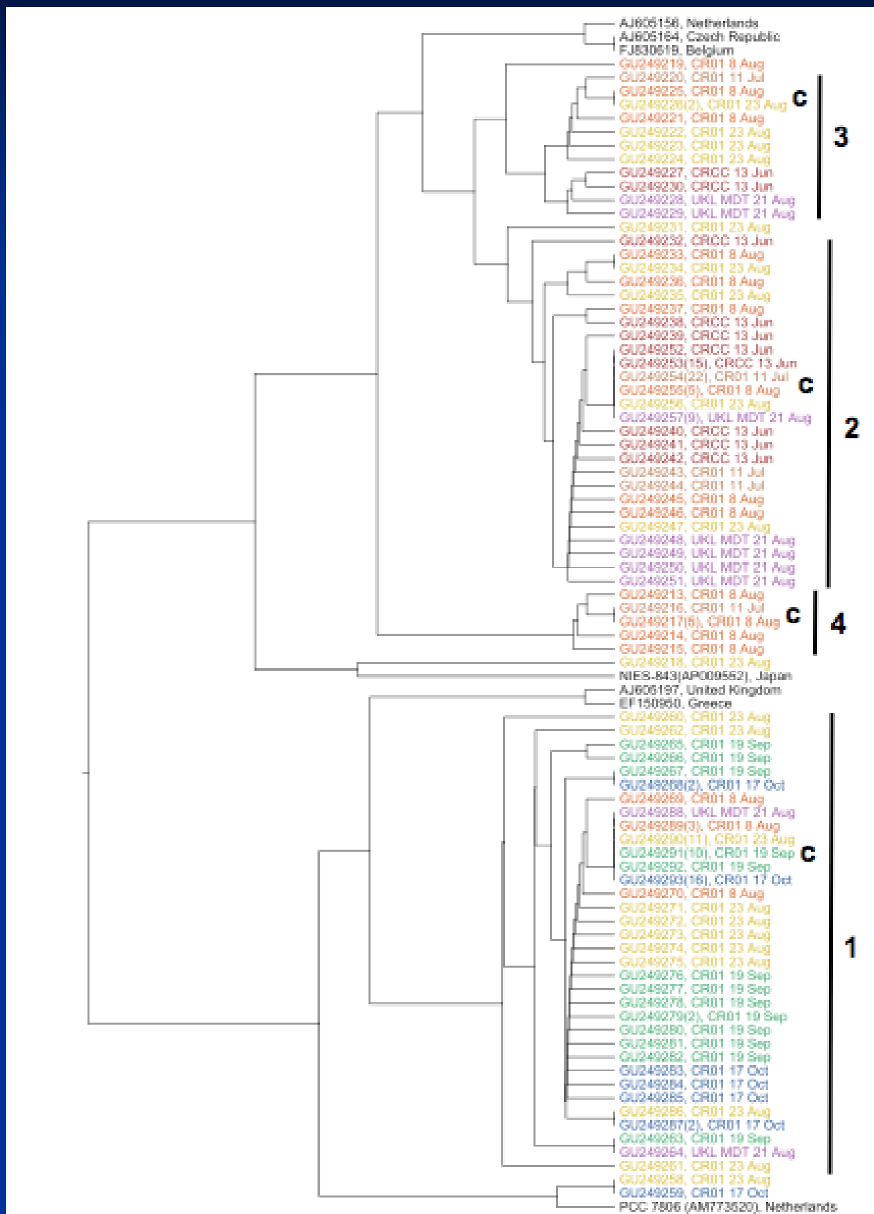
Collect population on a filter by filtering lake water sample

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

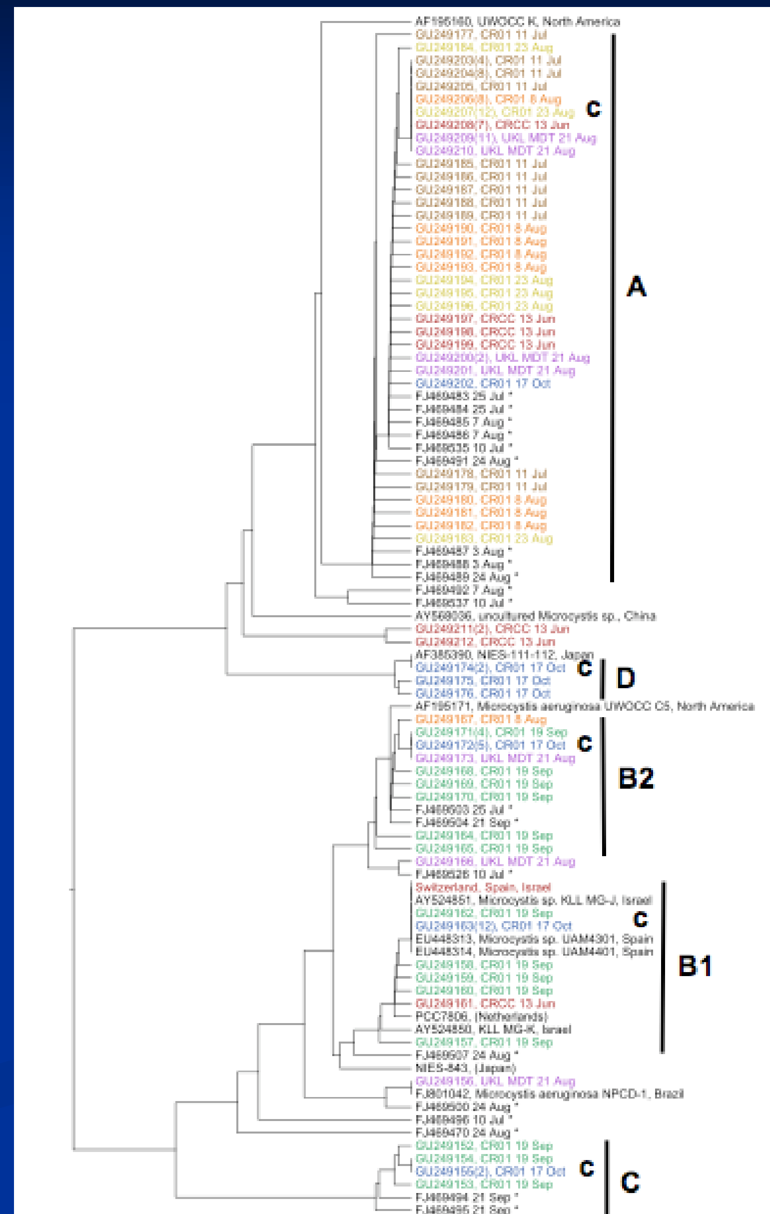
- ITS, ribosomal DNA internal transcribed spacer
- *cpcBA*, phycocyanin intergenic region
- microcystin toxin synthetic gene *mcyA*

Copco Reservoir *Microcystis* genotypes

ITS sequences



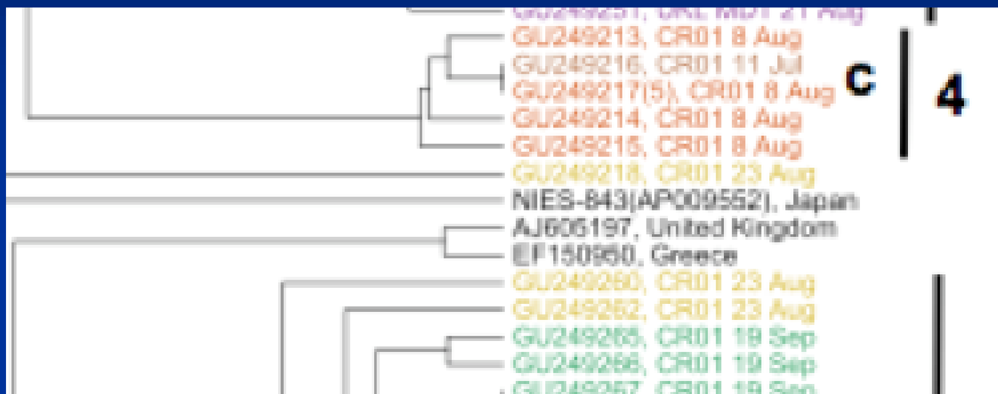
cpcBA sequences



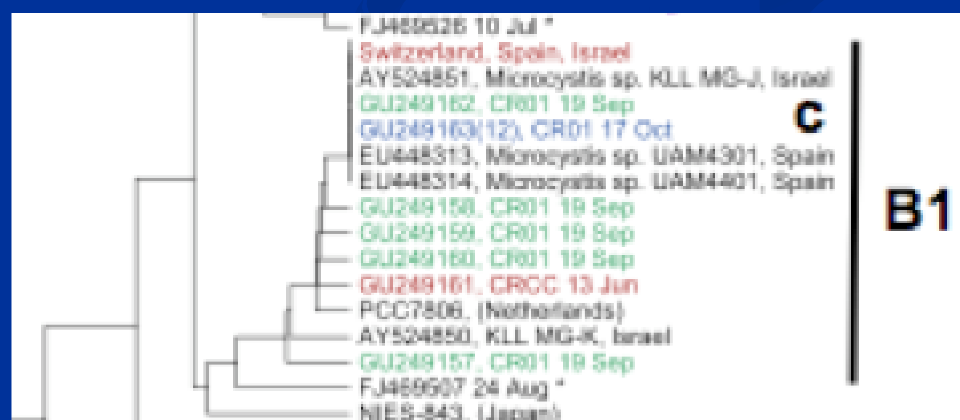
Copco Reservoir *Microcystis* genotypes

Relationship to isolates from other locations

ITS sequences

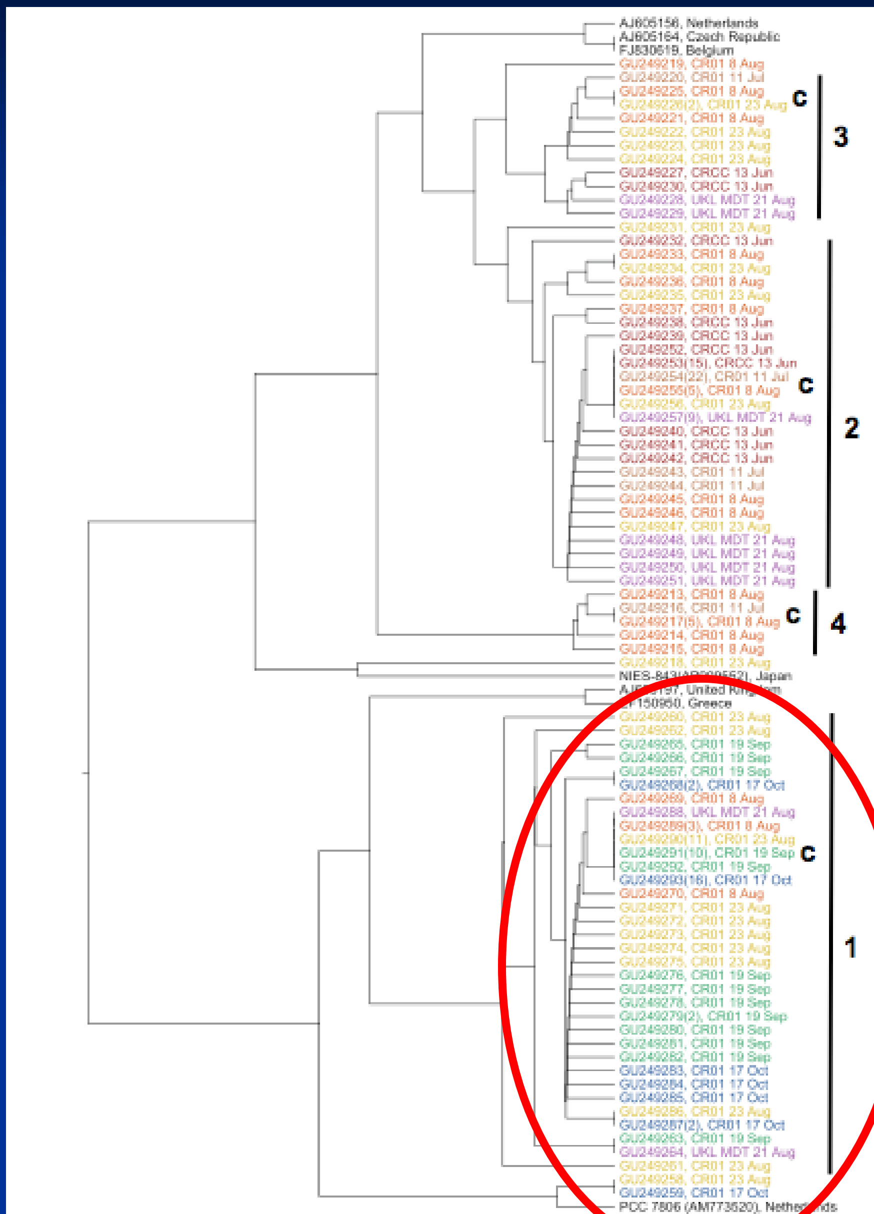


cpcBA sequences

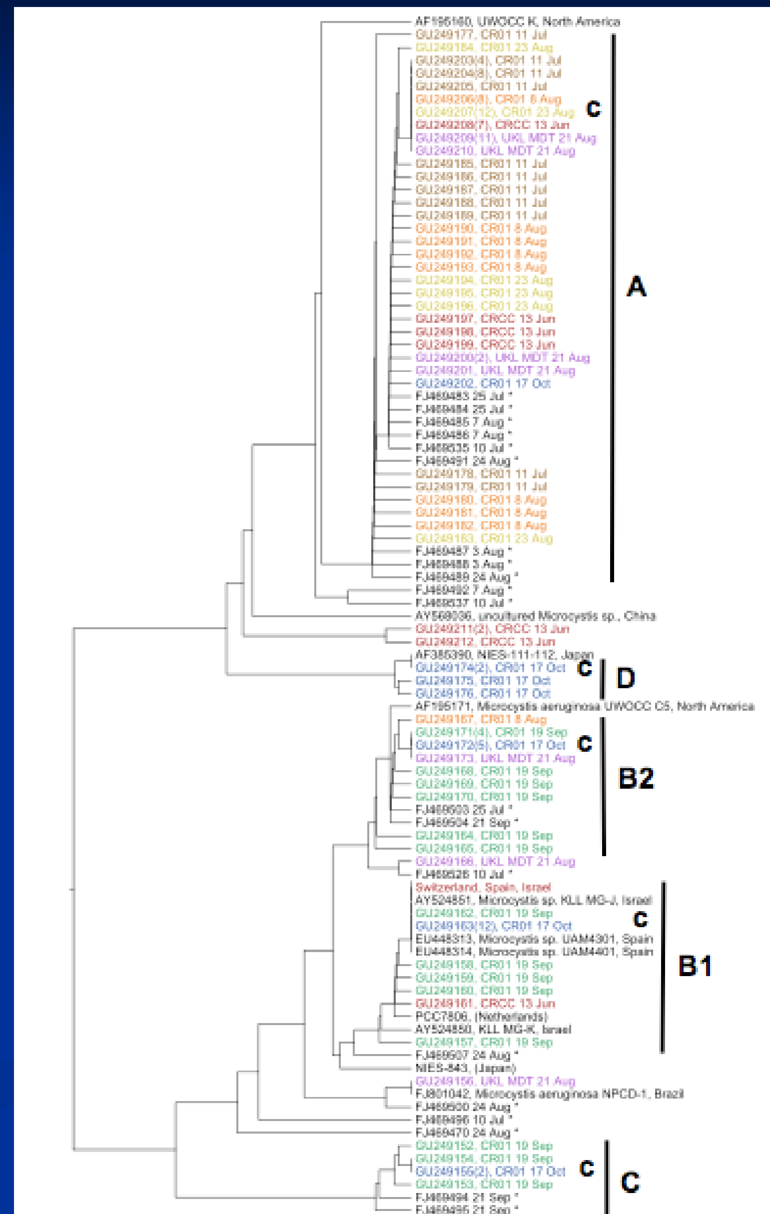


Copco Reservoir *Microcystis* genotypes

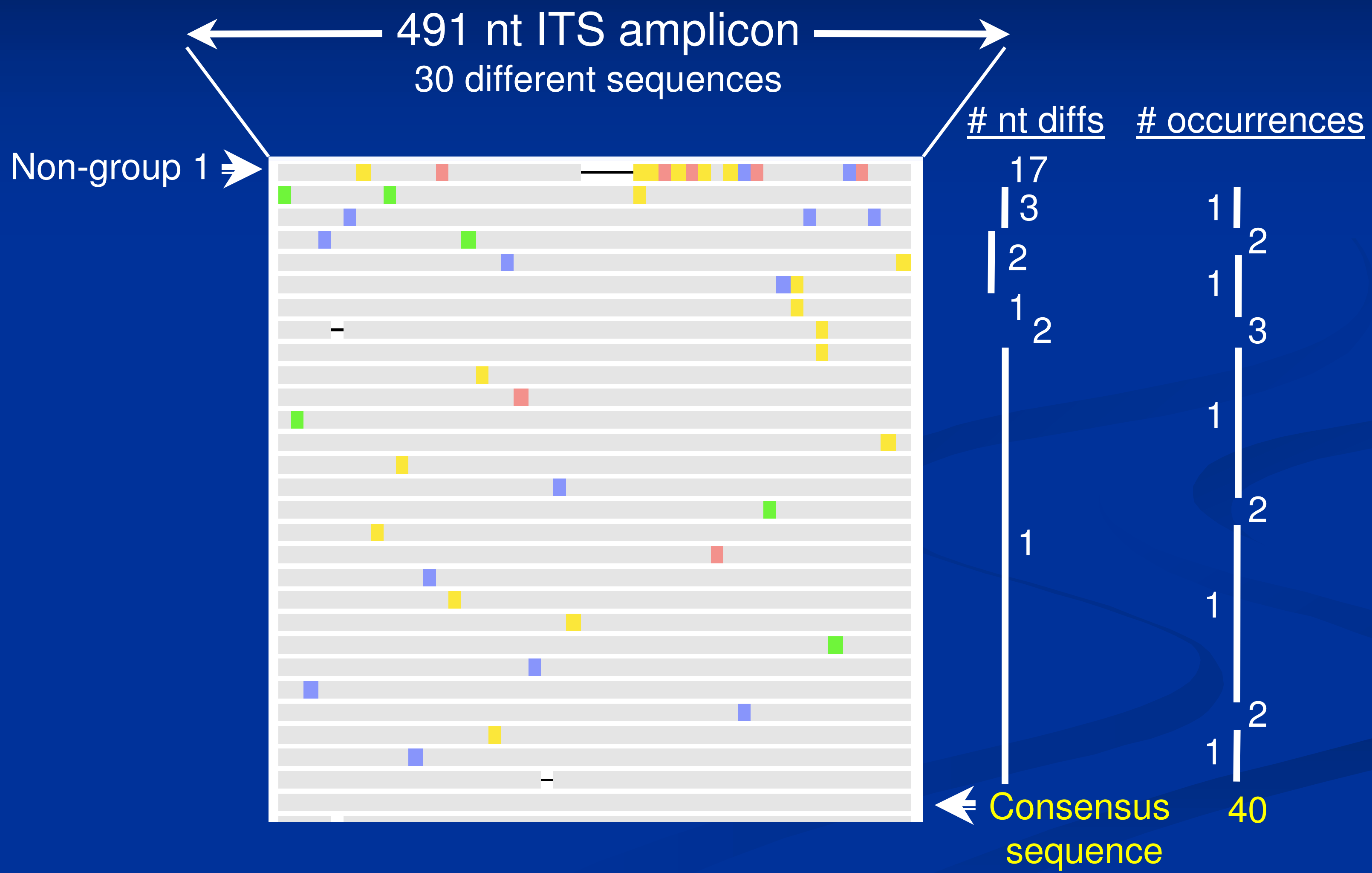
ITS sequences



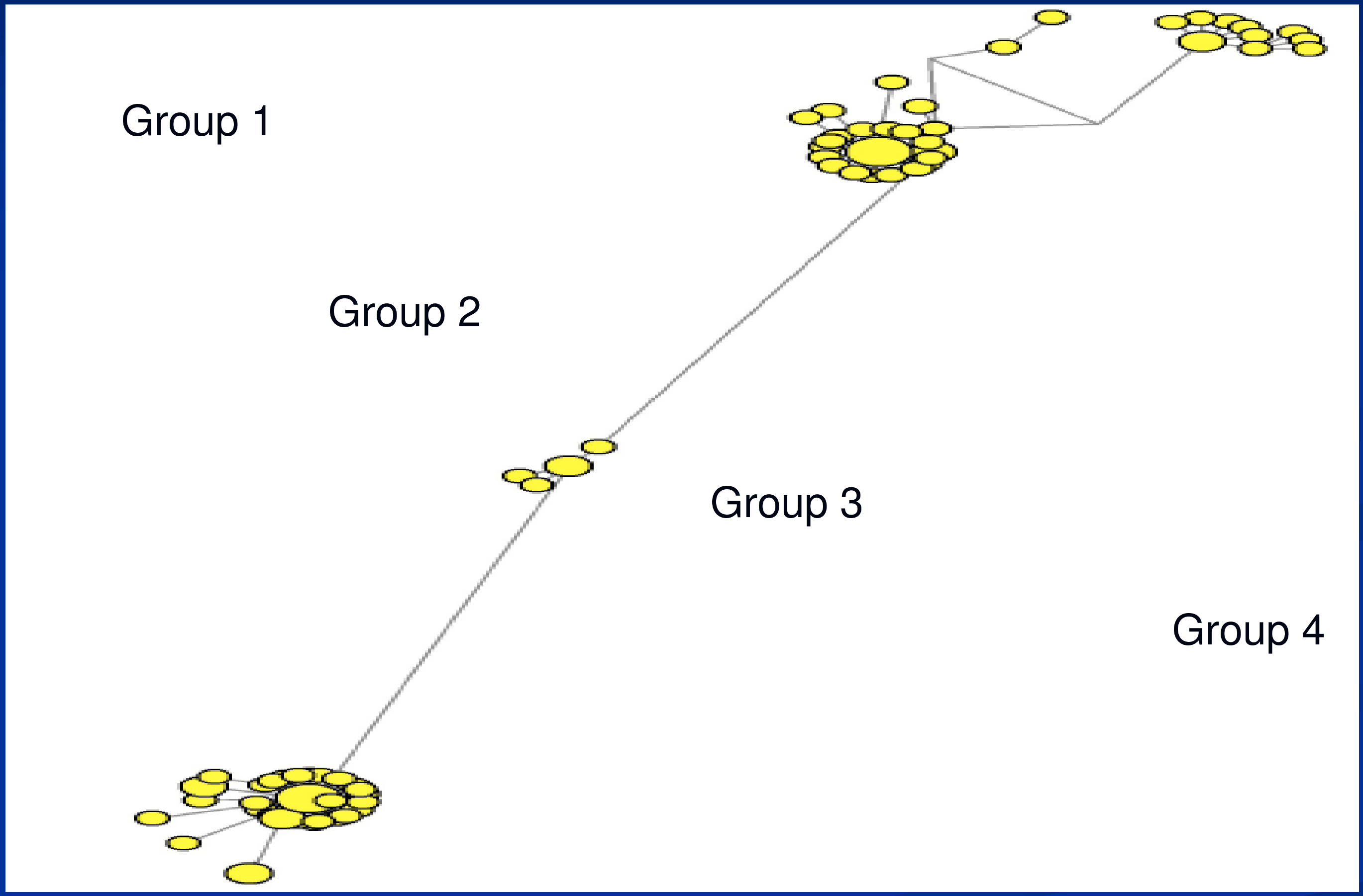
cpcBA sequences



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



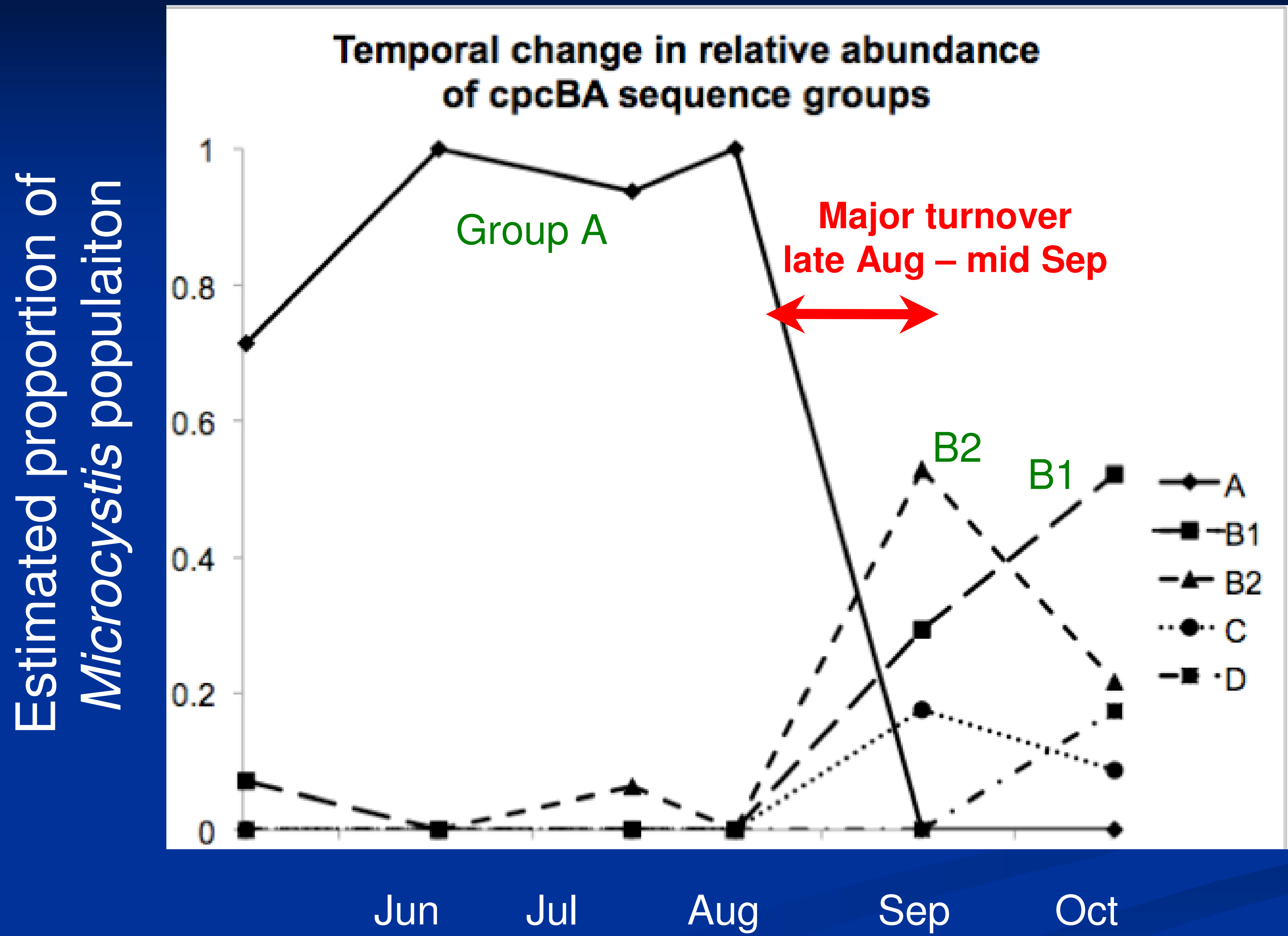
Genetic analysis at the *Microcystis* ITS gene locus (Copco Reservoir)



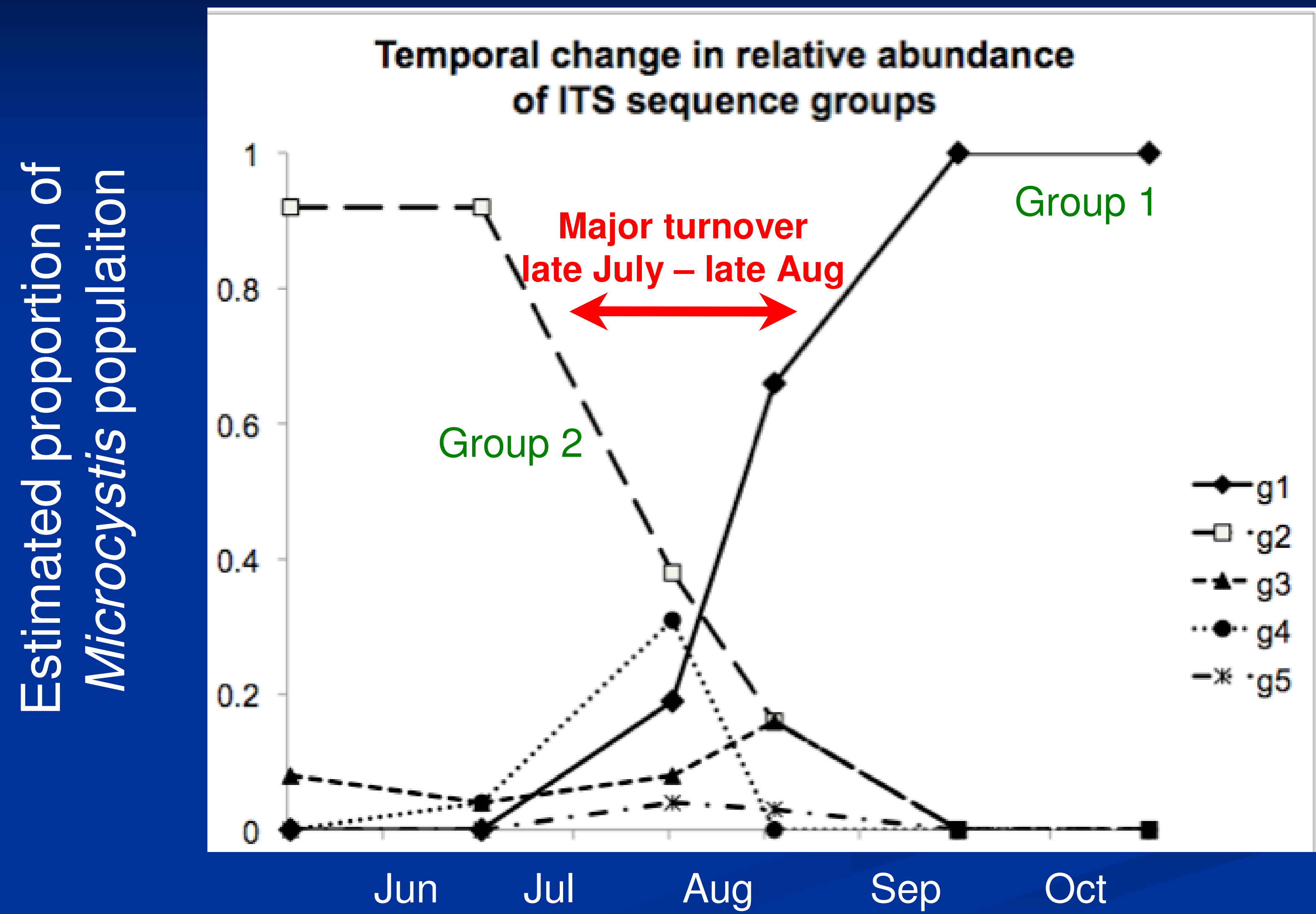
Subgroups are: ◆ well separated ◆ tightly clustered ◆ dominated by single sequences

Such population structure is amenable to subgroup-specfic monitoring

Population turnover during season: *cpcBA*

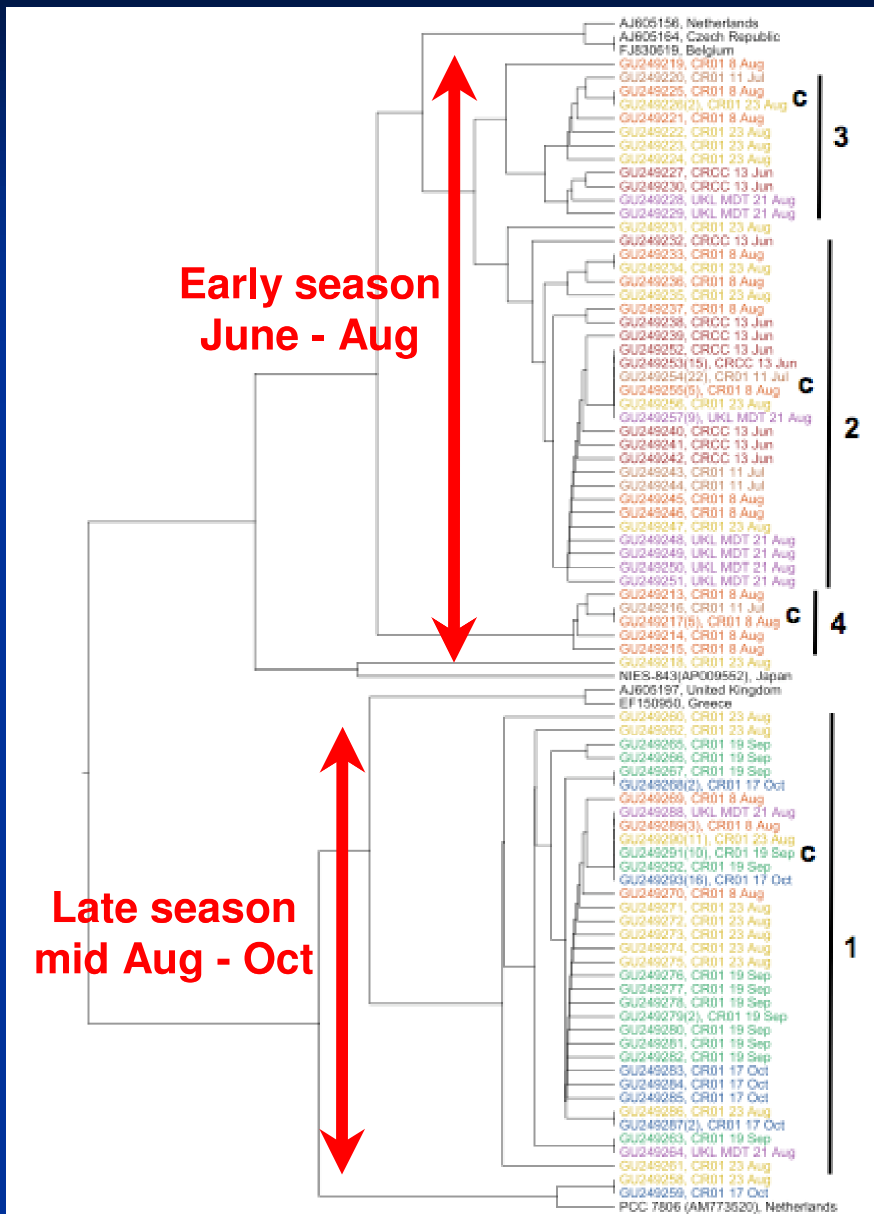


Population turnover during season: ITS

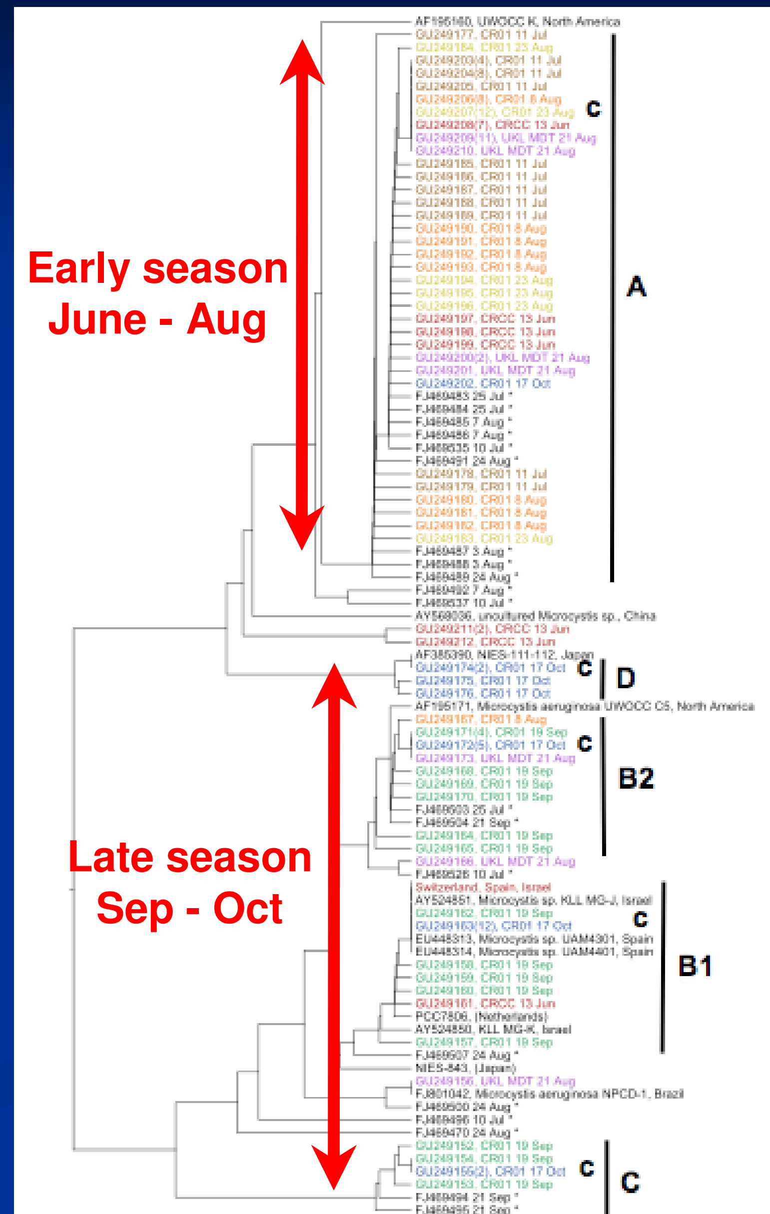


Copco Reservoir *Microcystis* genotypes

ITS sequences

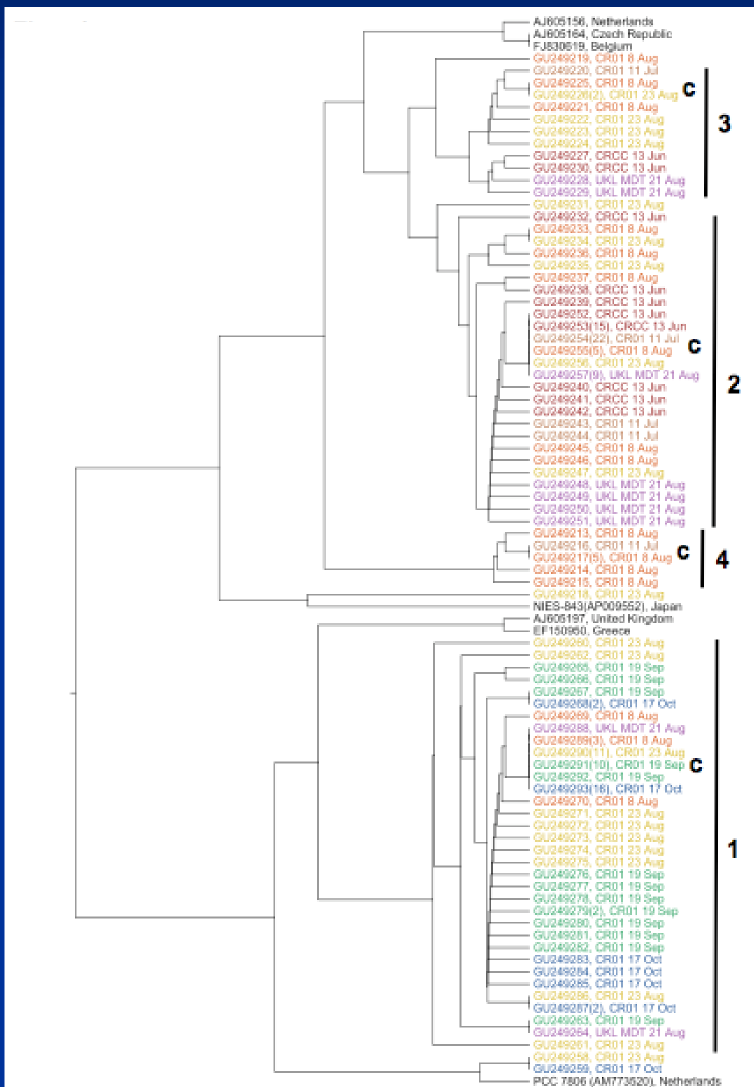


cpcBA sequences



Upper Klamath Lake and Copco Reservoir *Microcystis* genotypes are closely related

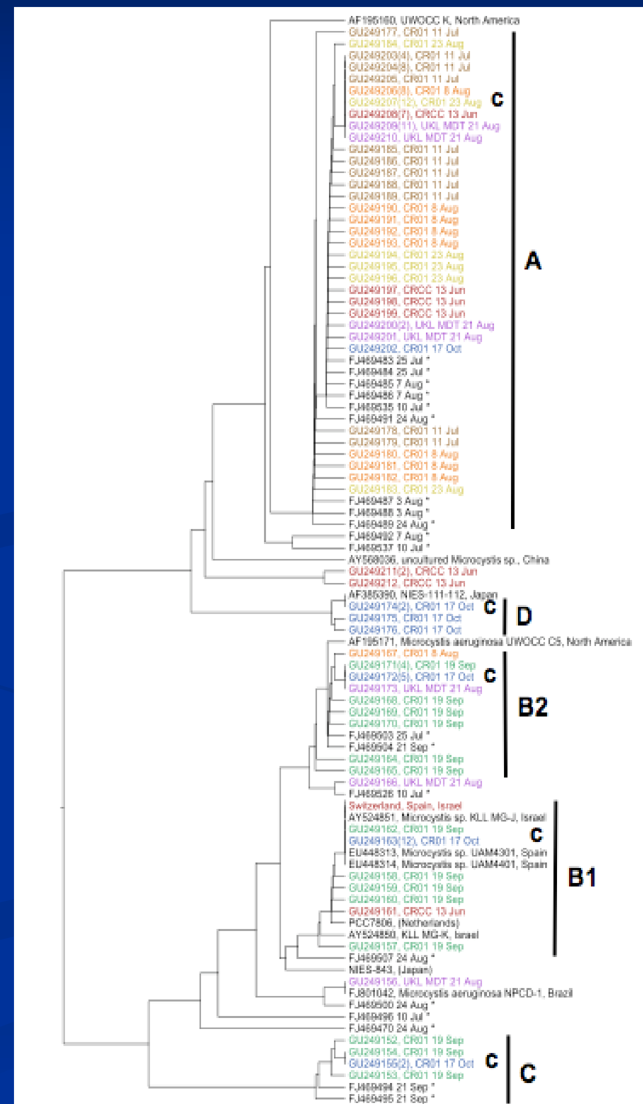
ITS



Upper Klamath Lake sequences

Other colored sequences are from Copco Res.

cpcBA



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