The distribution, ecology and genetics of *Microcystis* blooms throughout the SF Bay Delta

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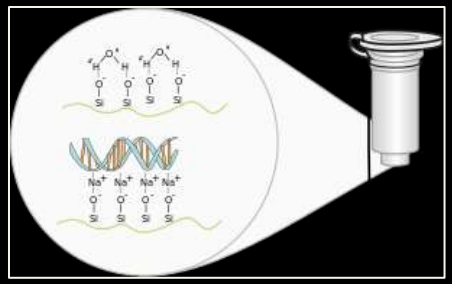
Presentation Overview

- Sample processing and genetic tools used to answer the following questions:

1. What is the *Microcystis* distribution throughout SFE and are these populations connected?
2. Which bioactive metabolites can it produce?
3. How do blooms influence microbial community structure?
4. What are the food web implications of these blooms relative to the Pelagic Organism Decline?

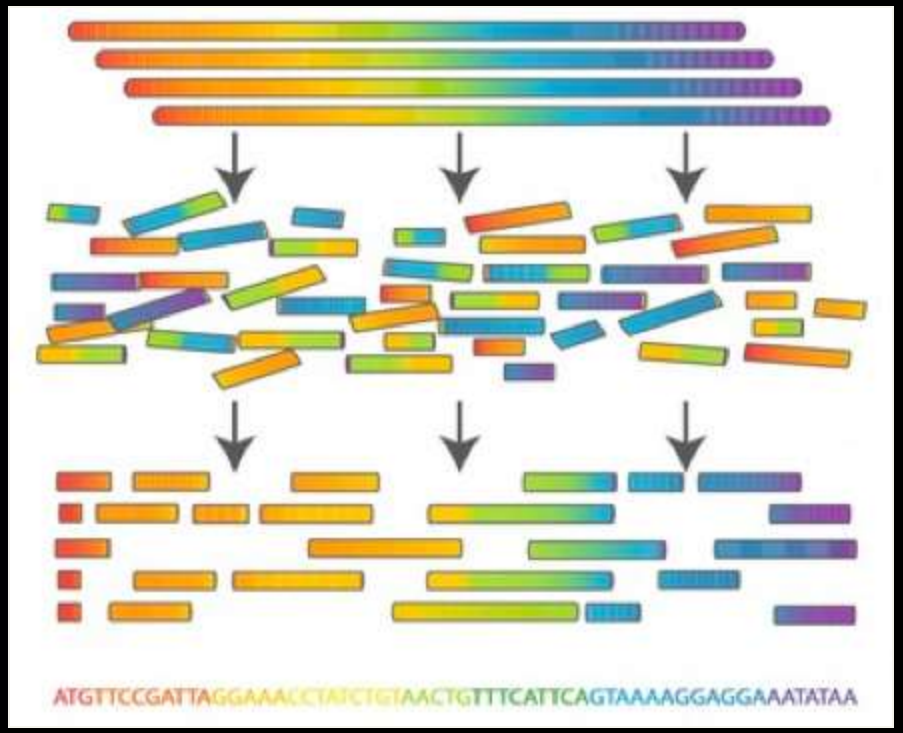
Sample Processing

1. Concentrate sample



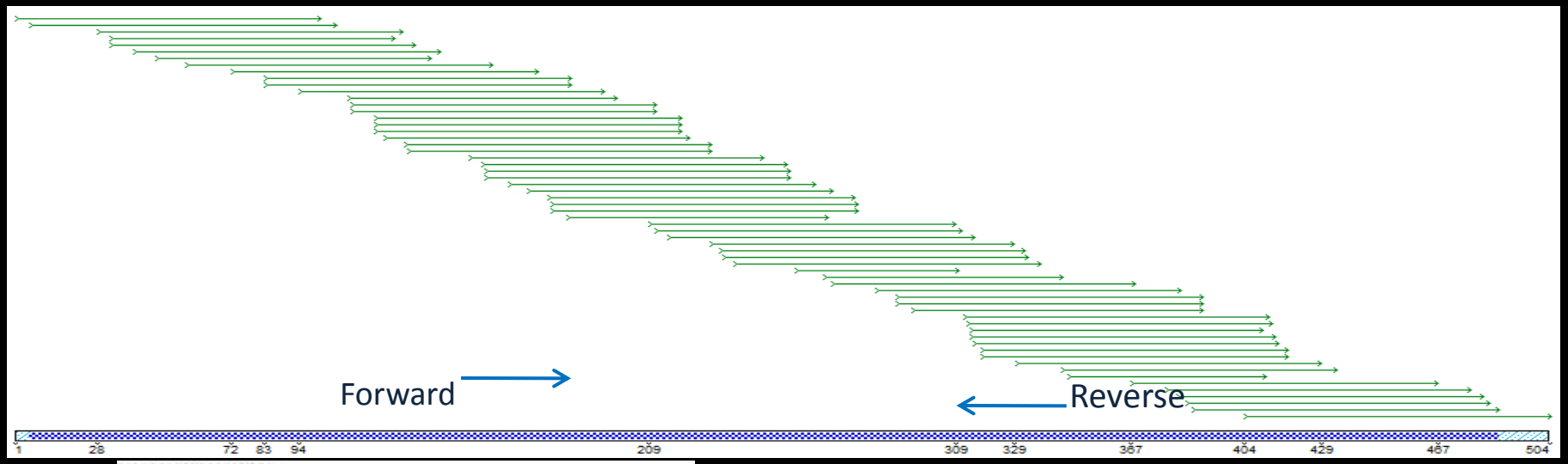
2. Extract DNA

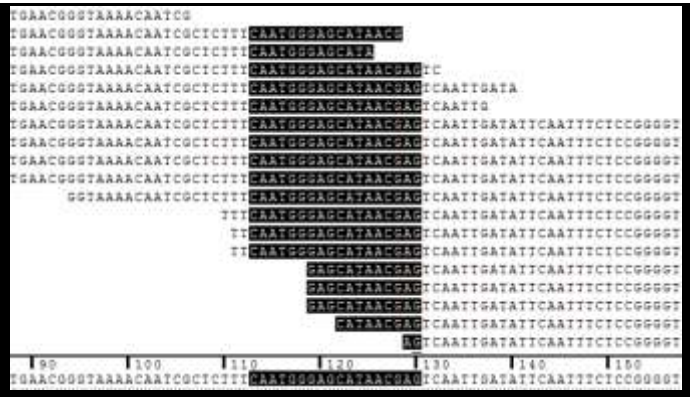
3. DNA is now ready for downstream applications such as PCR/QPCR or sequencing

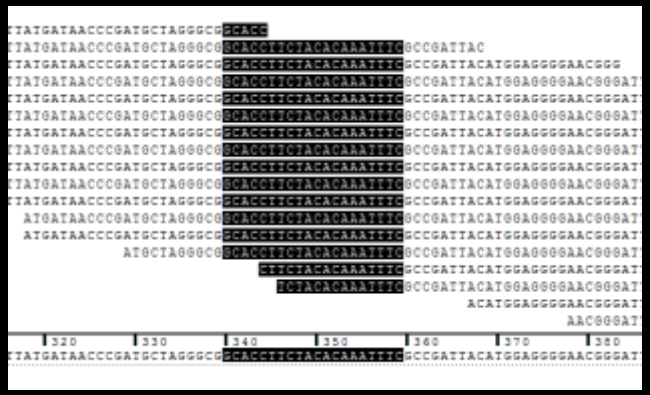


How metagenomics advances our understanding of an ecosystem

- Unbiased view of community structure and genetic capability



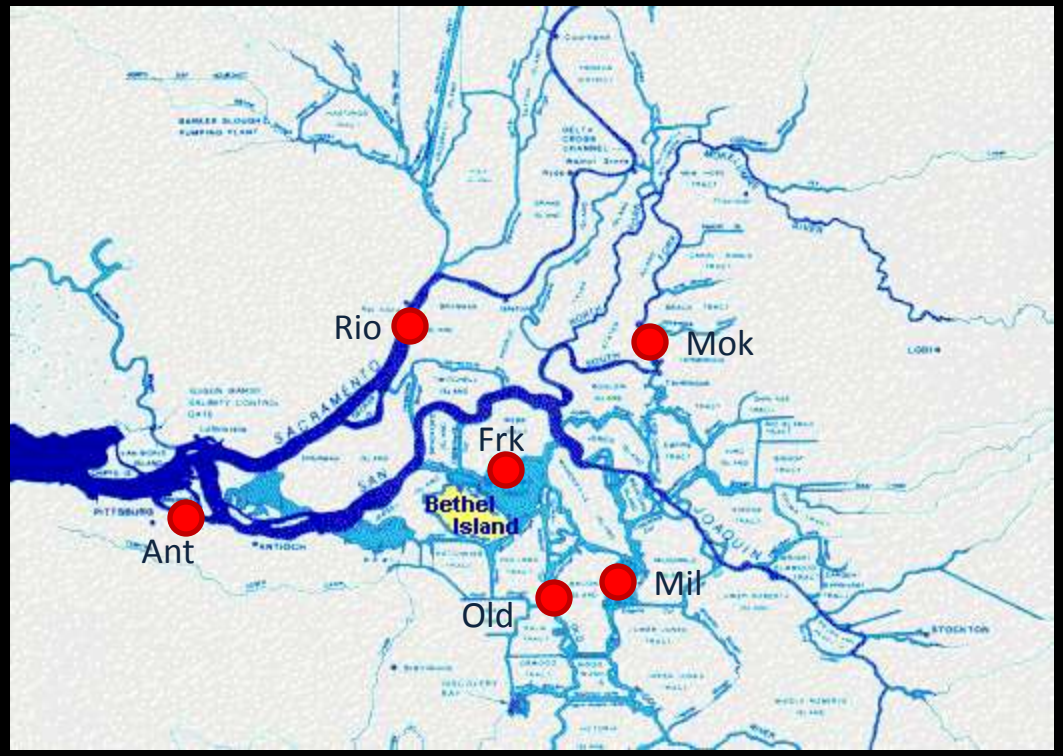




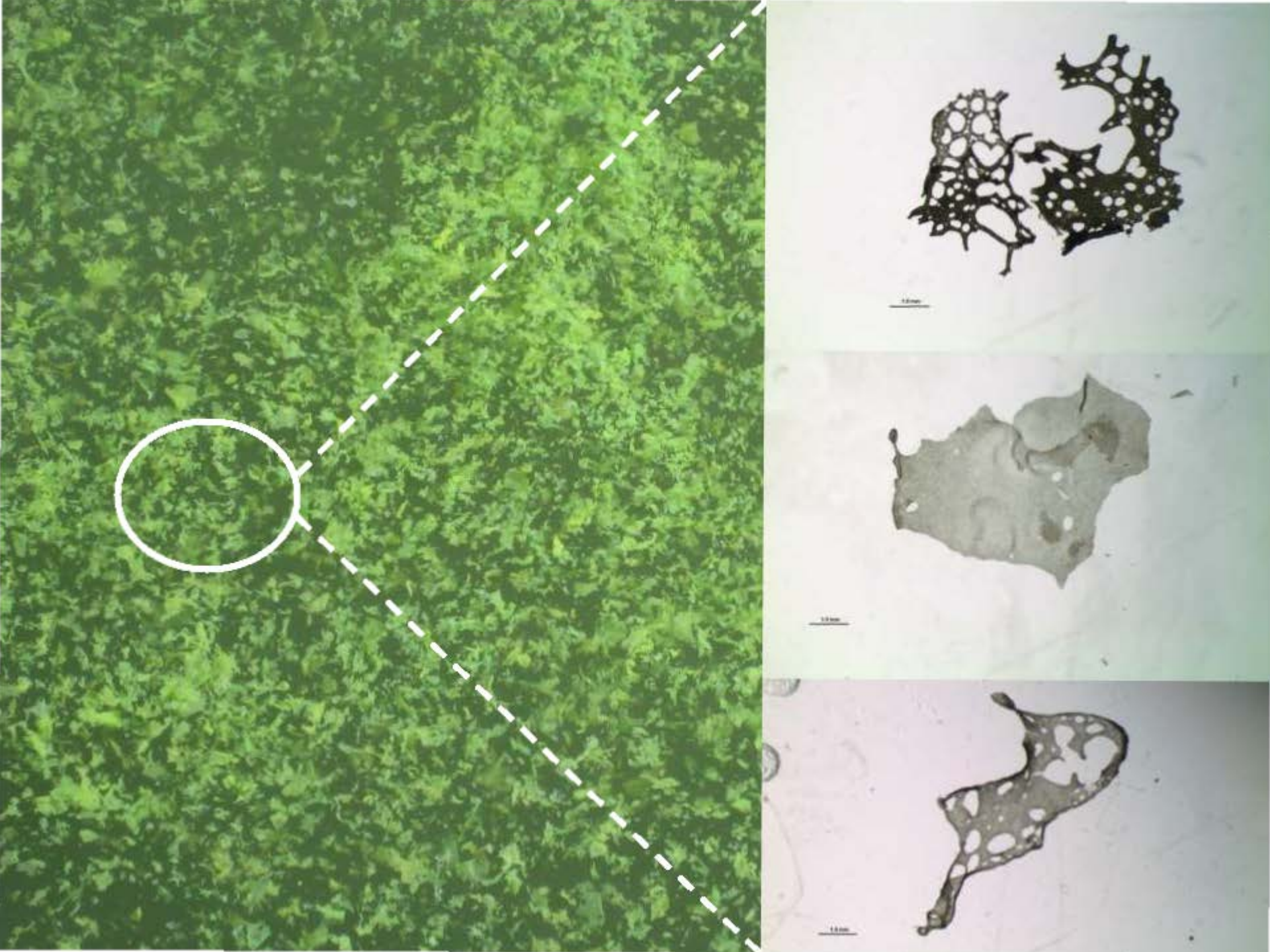
Spatiotemporal Patterns

1. Are different *Microcystis* subtypes endemic to certain regions of the SFE?

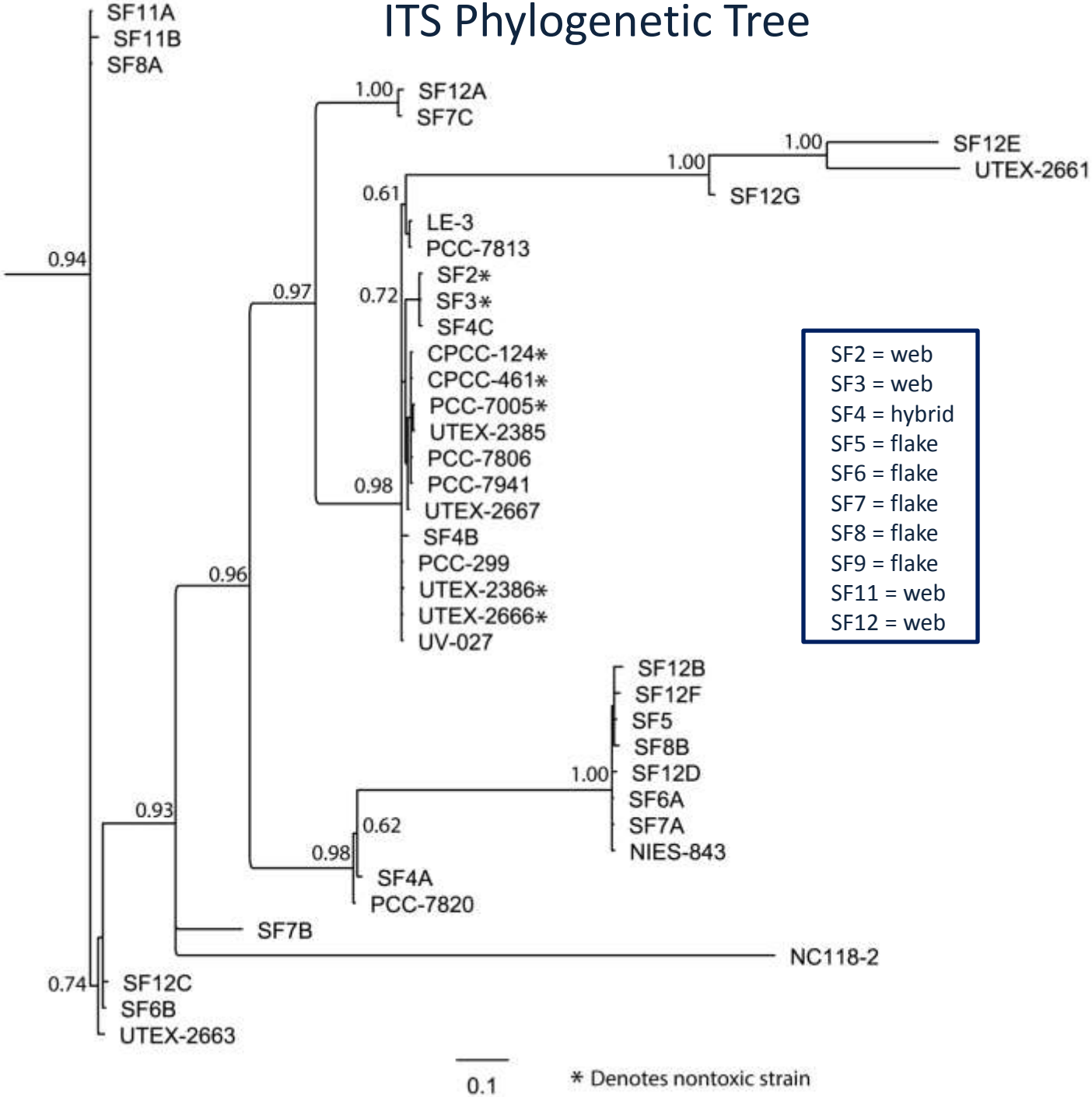
2. Are samples from the confluence of Antioch representative of all *Microcystis* diversity?



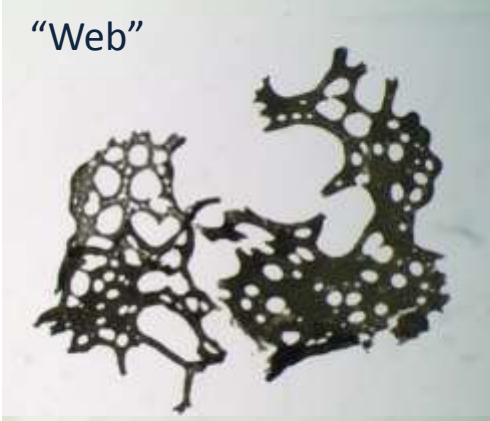
Large River – Antioch and Rio Vista
Small River – Old River and Mokelumne River
Flooded Islands – Mildred Island and Franks Tract

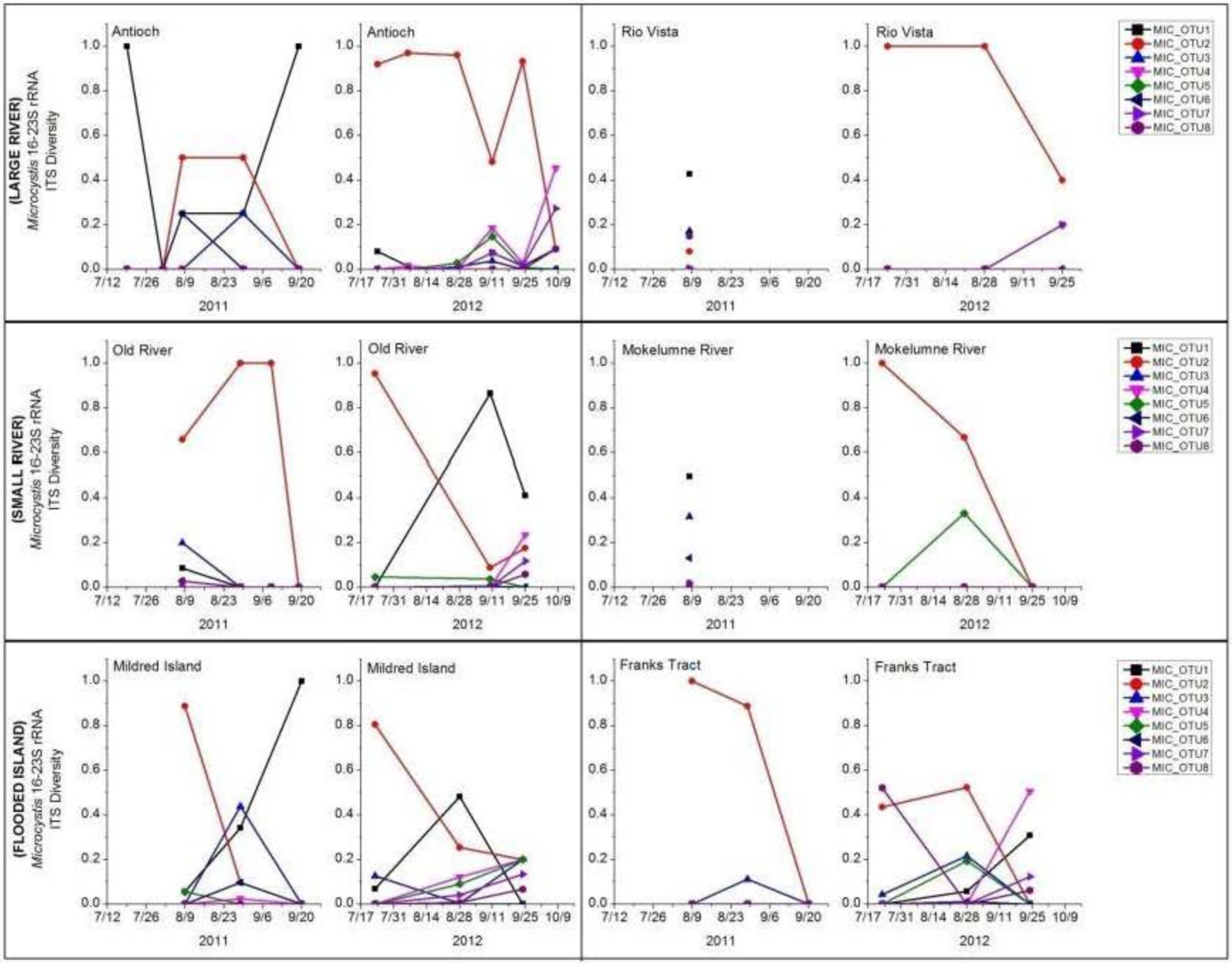


ITS Phylogenetic Tree

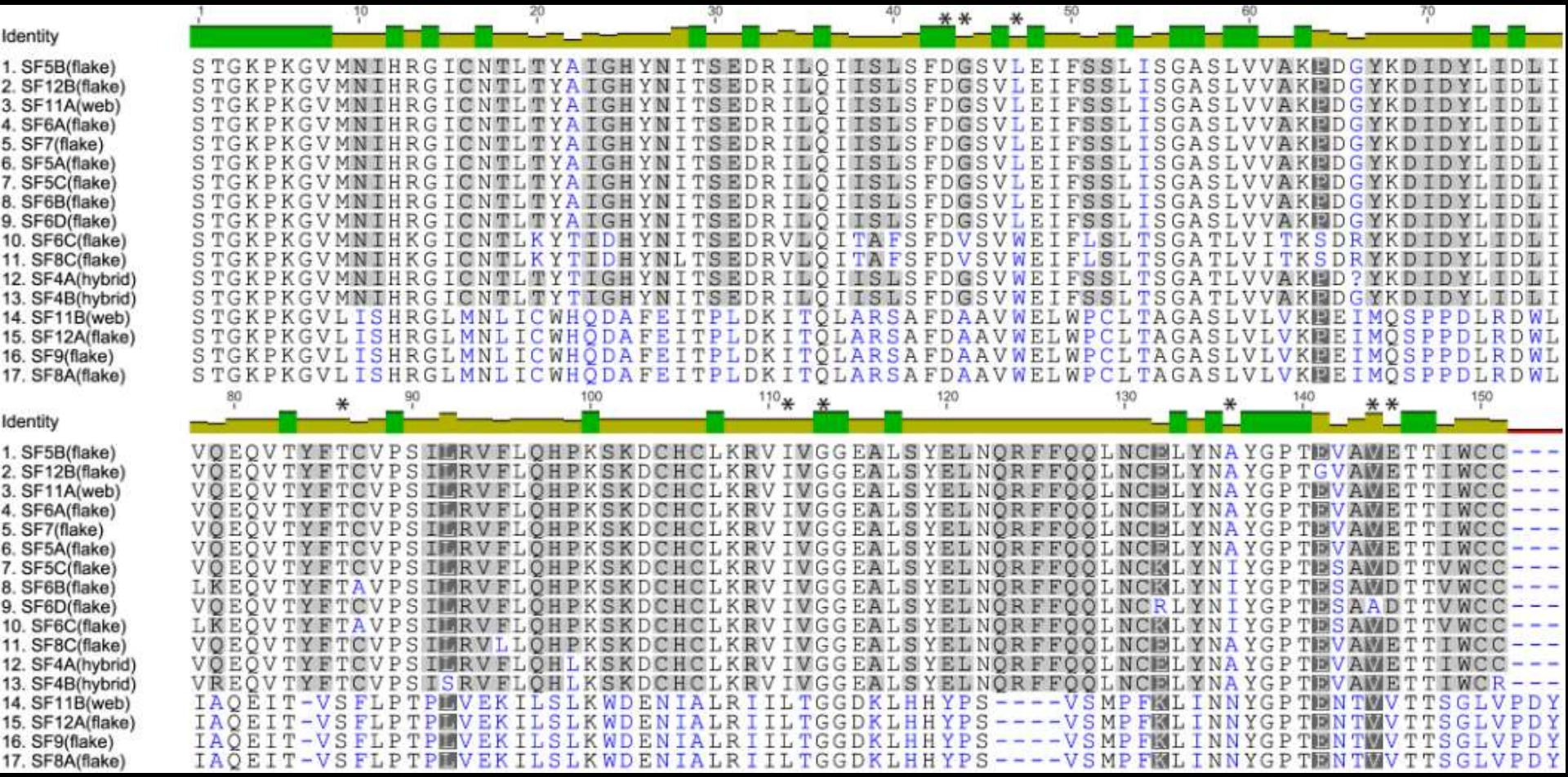


SF2 = web
SF3 = web
SF4 = hybrid
SF5 = flake
SF6 = flake
SF7 = flake
SF8 = flake
SF9 = flake
SF11 = web
SF12 = web



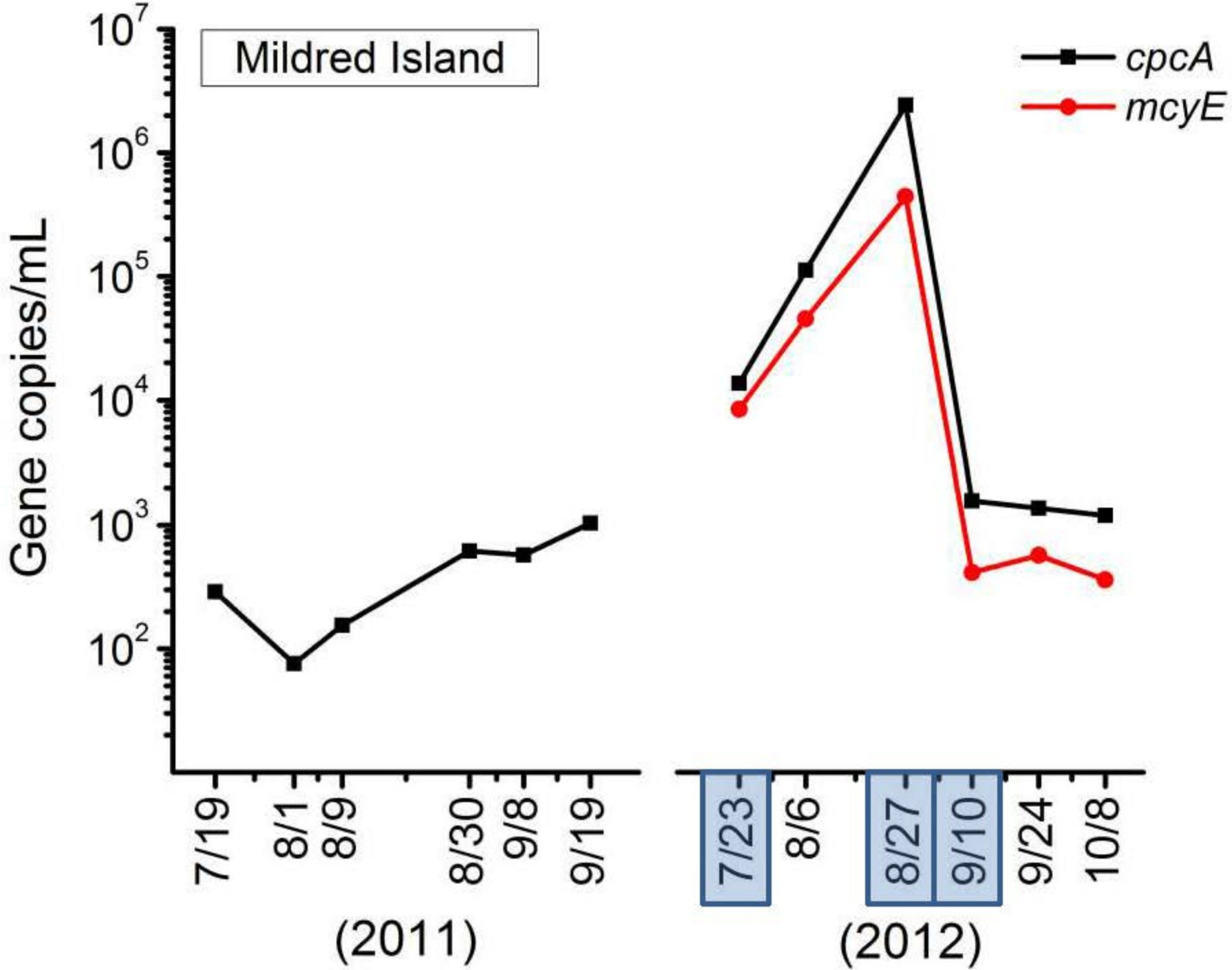

(LARGE RIVER)
Microcystis 16-23S rRNA ITS Diversity
Antioch
Antioch
Rio Vista
Rio Vista
(SMALL RIVER)
Microcystis 16-23S rRNA ITS Diversity
Old River
Old River
Mokelumne River
Mokelumne River
(FLOODED ISLAND)
Microcystis 16-23S rRNA ITS Diversity
Mildred Island
Mildred Island
Franks Tract
Franks Tract
MIC_OTU1
MIC_OTU2
MIC_OTU3
MIC_OTU4
MIC_OTU5
MIC_OTU6
MIC_OTU7
MIC_OTU8
7/12 7/26 8/9 8/23 9/6 9/20
2011
7/17 7/31 8/14 8/28 9/11 9/25 10/9
2012

Variable microcystin congeners

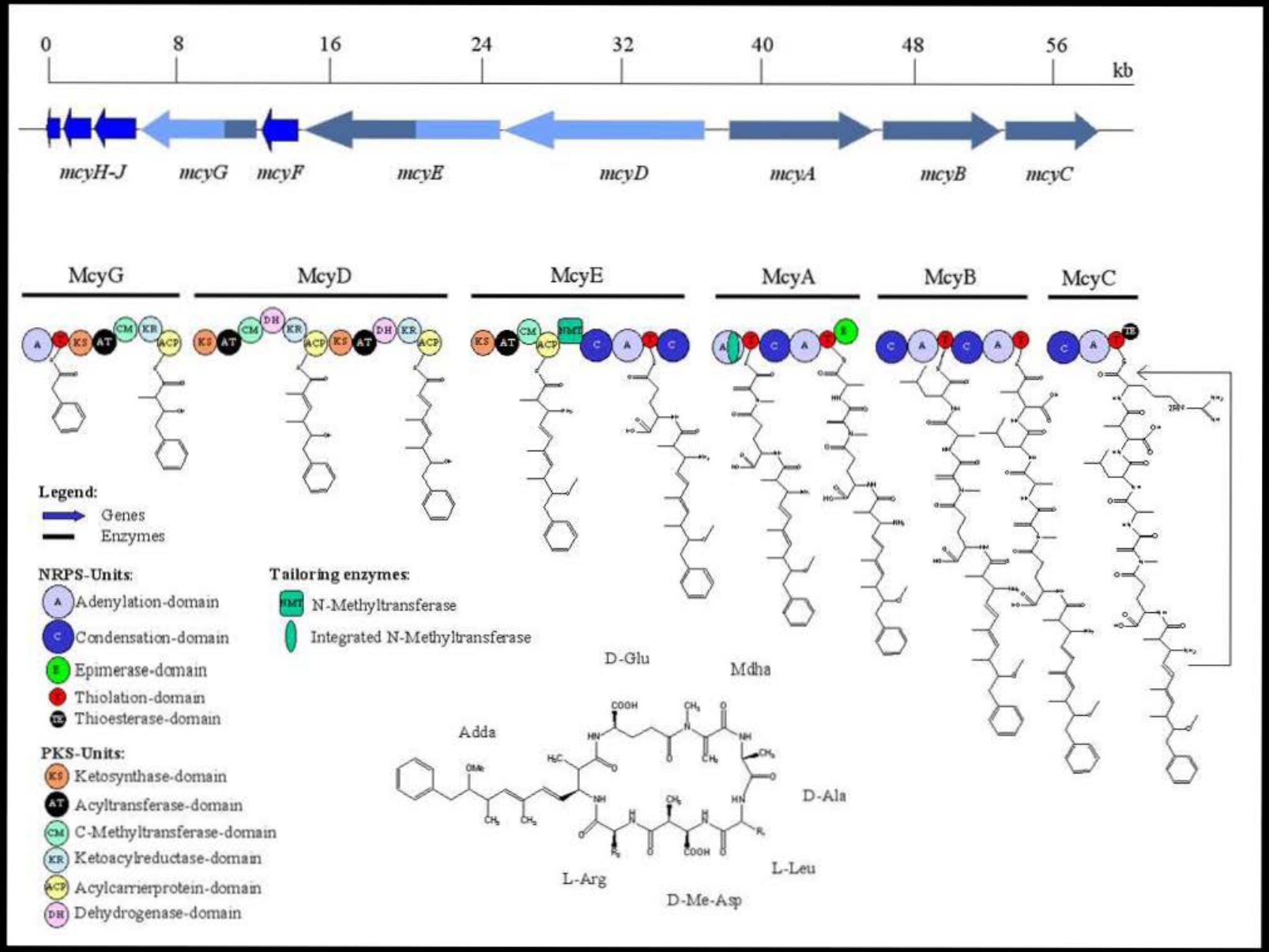


Analysis of A-domain binding pockets suggests

- **Top group produces MC-LR, RR, YR**
- **Middle group produces MC-RR**
- **Bottom group produces MC-LR**

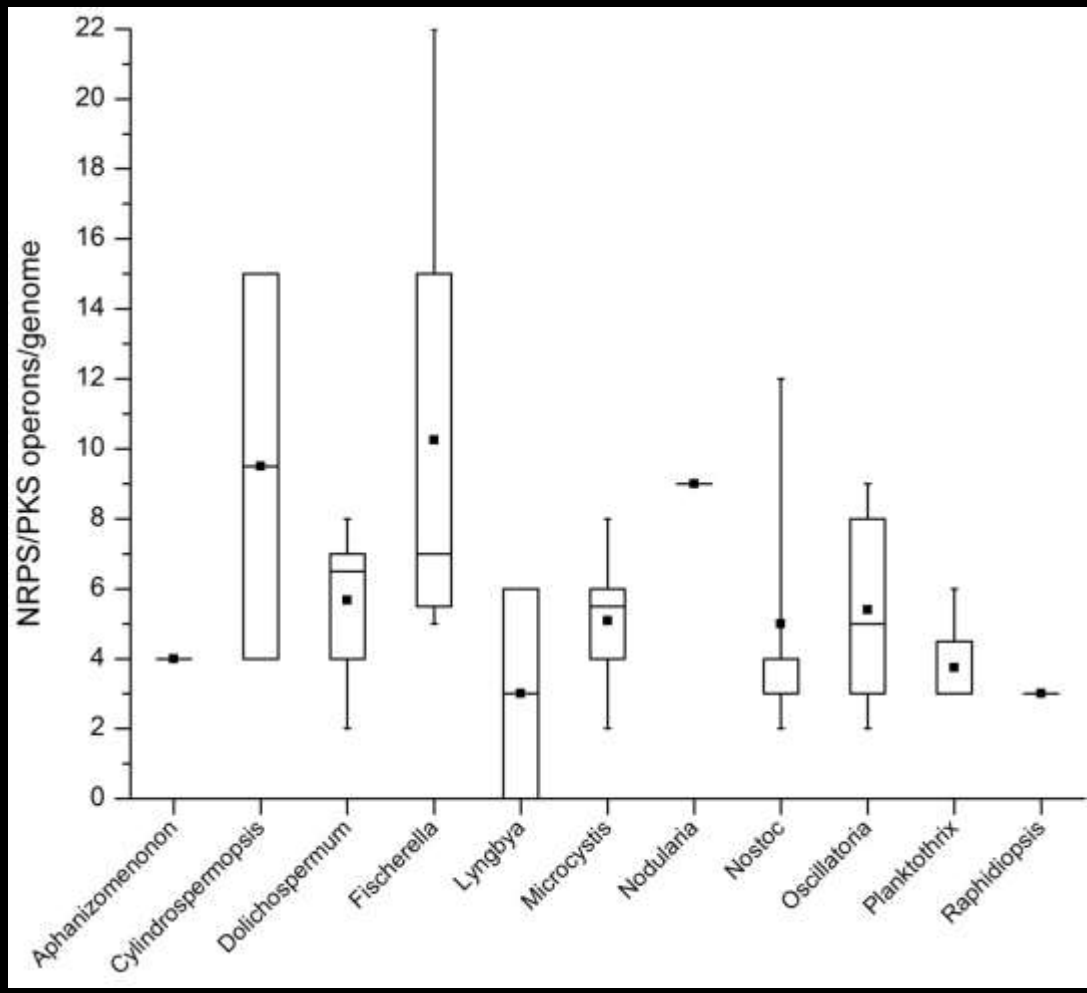

Mildred Island
cpcA
mcyE
Gene copies/mL
10^7
10^6
10^5
10^4
10^3
10^2
7/19
8/1
8/9
8/30
9/8
9/19
7/23
8/6
8/27
9/10
9/24
10/8
(2011)
(2012)

Microcystin biosynthesis (NRPS/PKS)

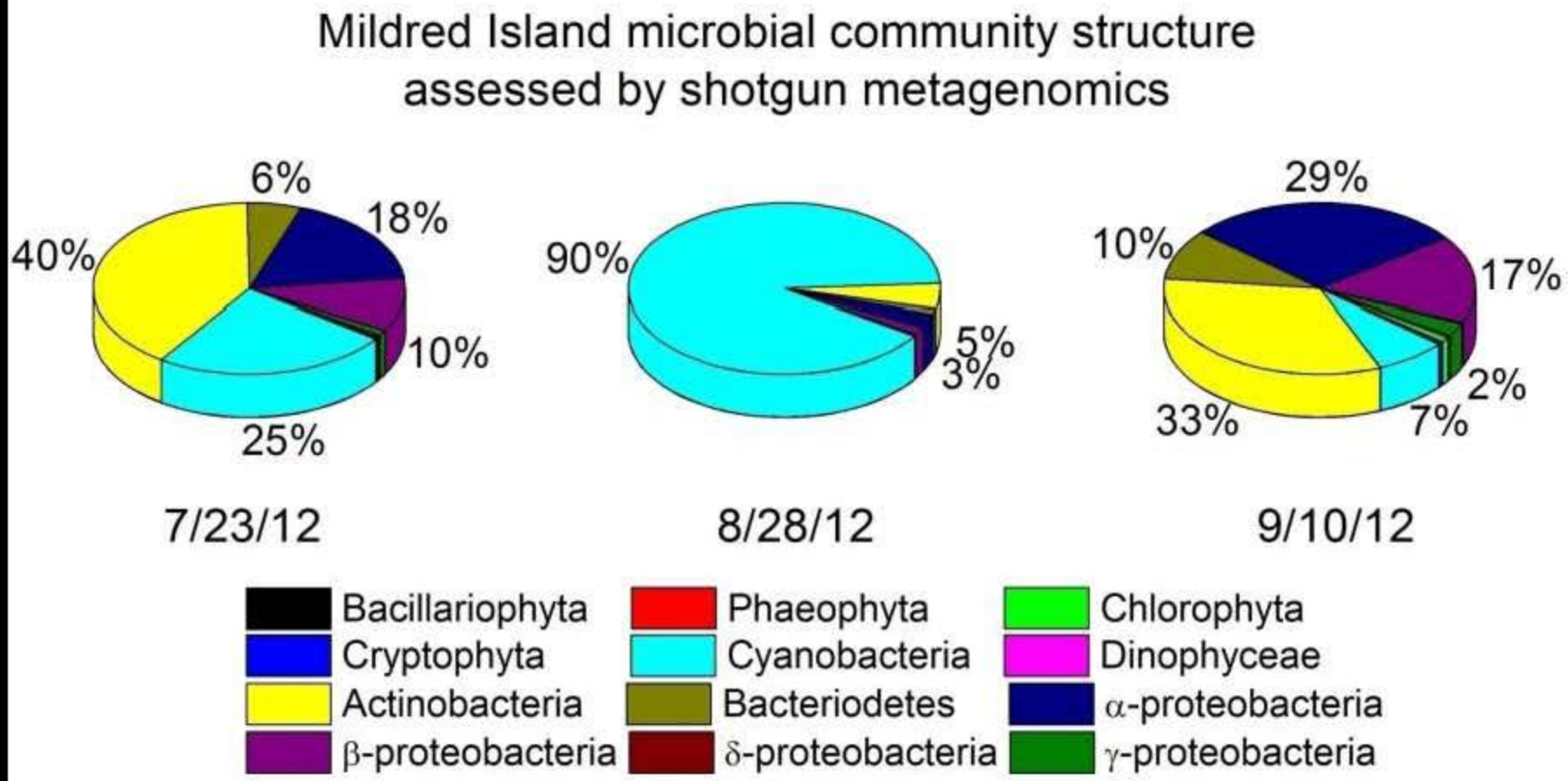


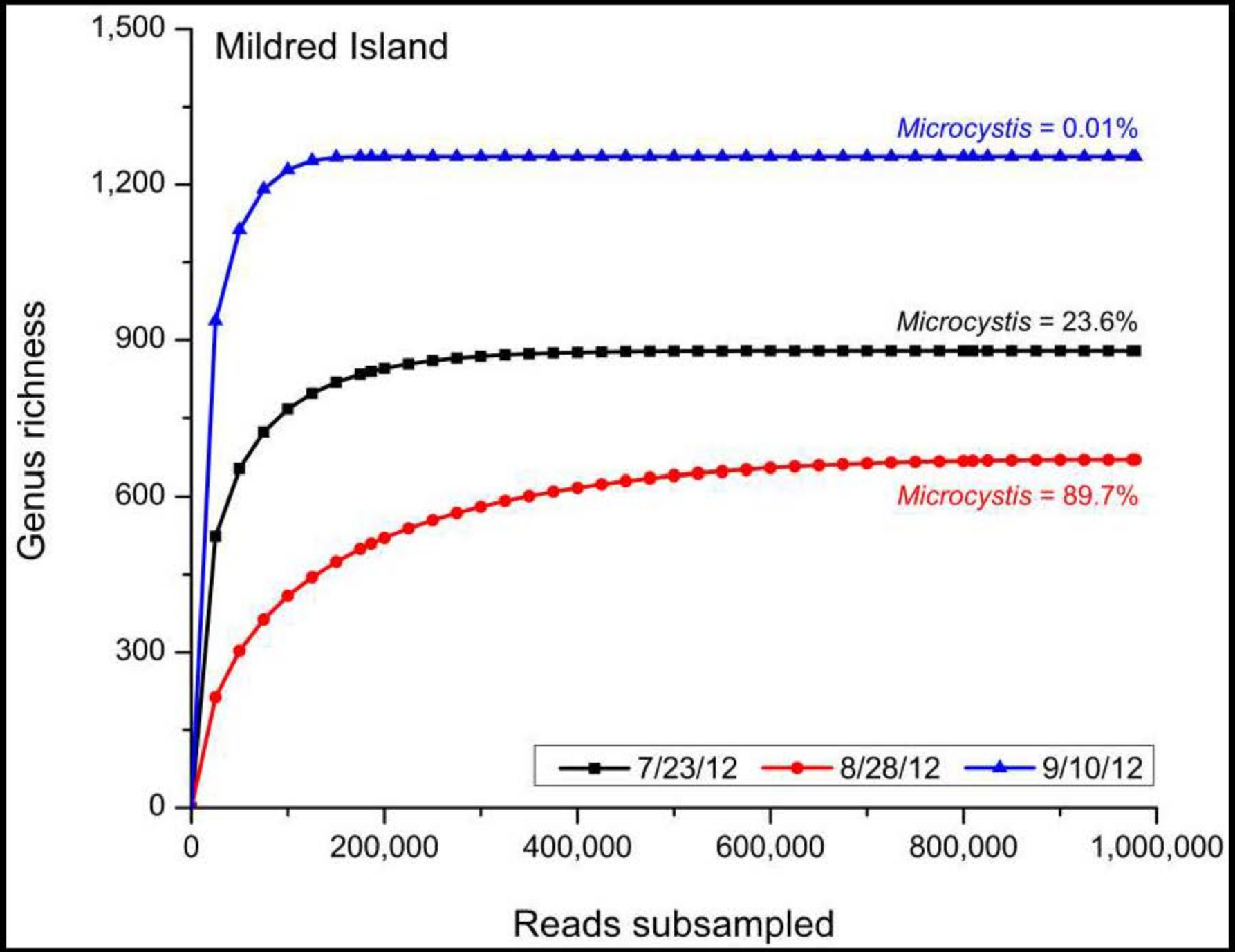
SF Delta *Microcystis* 2º metabolite biosynthesis

1. Aeruginosin (27.7 Kb)
2. Anabaenopeptin (27.8 Kb)
3. Cyanopeptolin
4. Microcystin (55.2 Kb)
5. Microginin (22.3 Kb)
6. Microviridin (9 Kb)
7. Piricyclamide (29.2 Kb)
8. + Several other uncharacterized NRPS and PKS modules

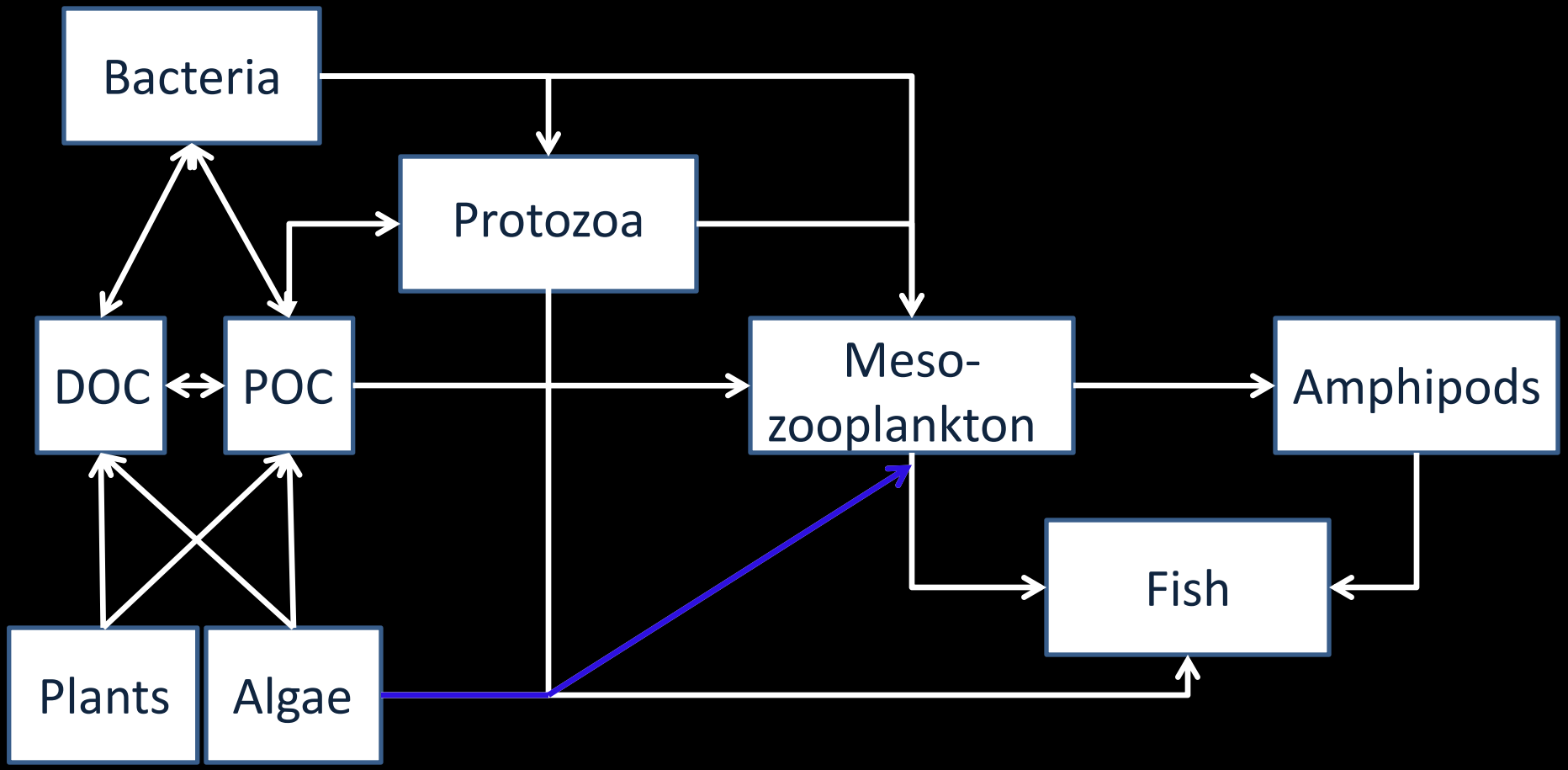


Based on 47 freshwater CyanoHAB genomes

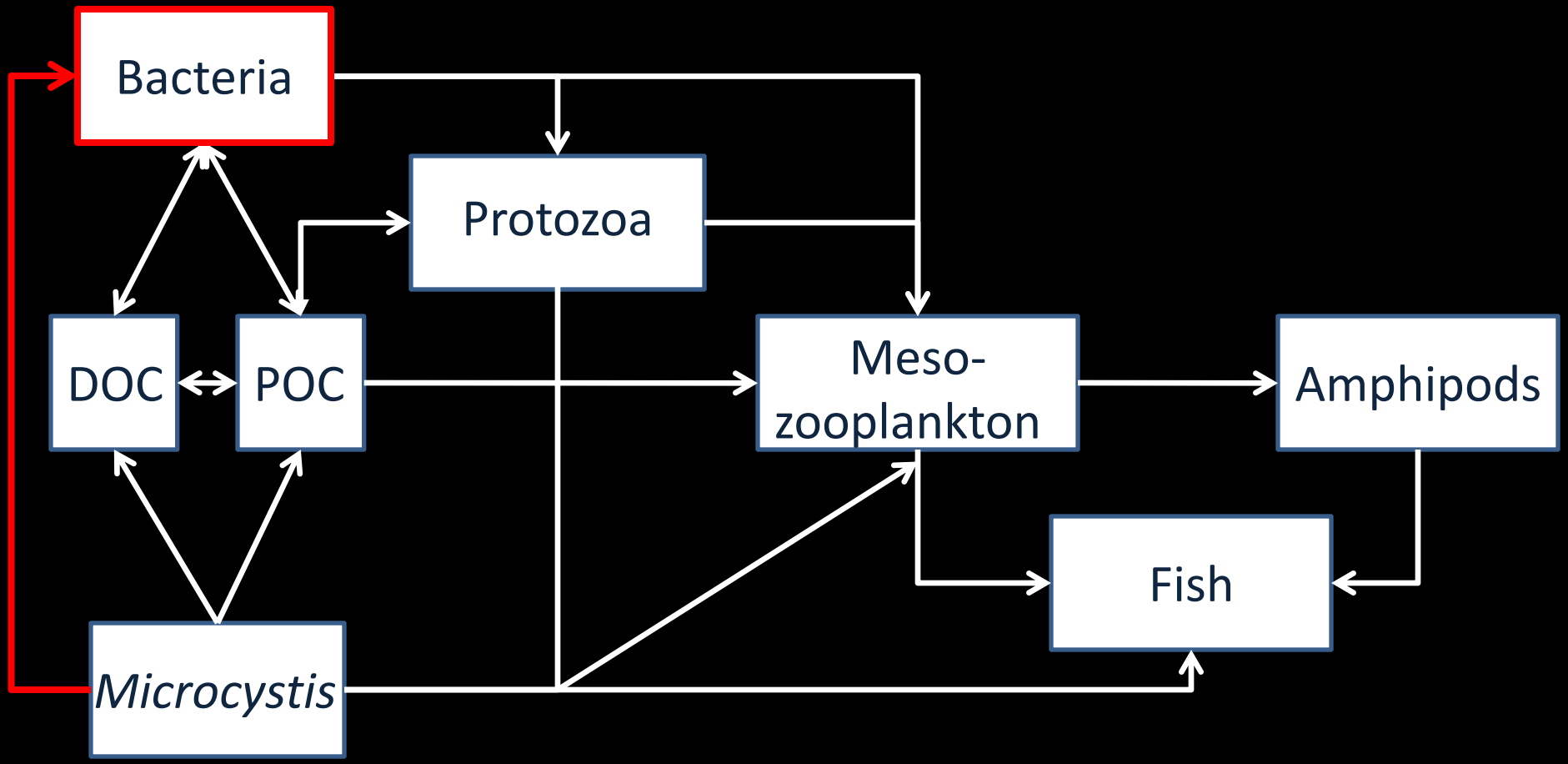

Mildred Island microbial community structure
assessed by shotgun metagenomics
6%
18%
40%
10%
25%
7/23/12
90%
5%
3%
8/28/12
29%
10%
17%
2%
33%
7%
9/10/12
Bacillariophyta
Phaeophyta
Chlorophyta
Cryptophyta
Cyanobacteria
Dinophyceae
Actinobacteria
Bacteriodetes
α-proteobacteria
β-proteobacteria
δ-proteobacteria
γ-proteobacteria


Mildred Island
Microcystis = 0.01%
Microcystis = 23.6%
Microcystis = 89.7%
7/23/12
8/28/12
9/10/12
Genus richness
Reads subsampled
0
300
600
900
1,200
1,500
0
200,000
400,000
600,000
800,000
1,000,000

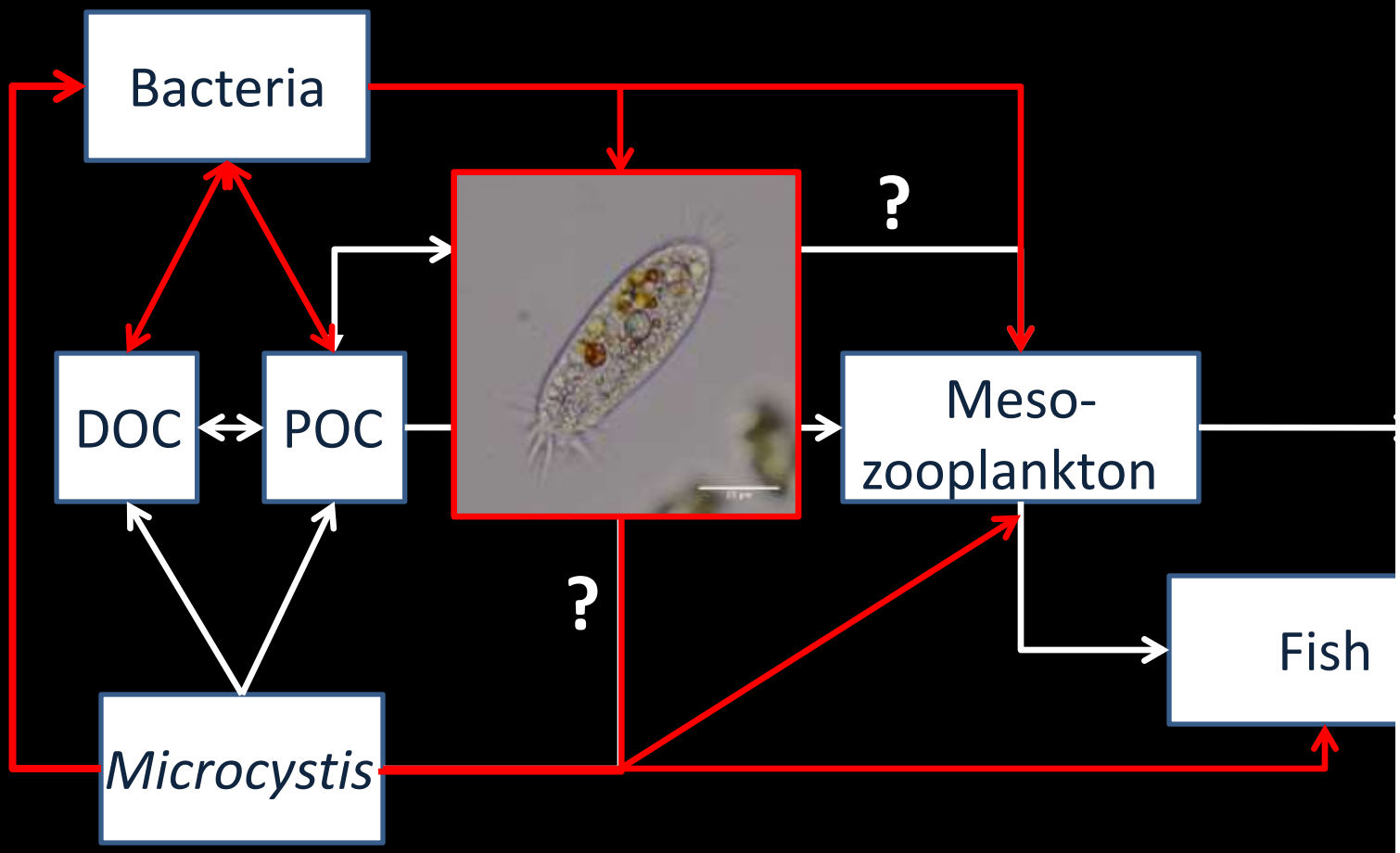
Food web implications – A working model



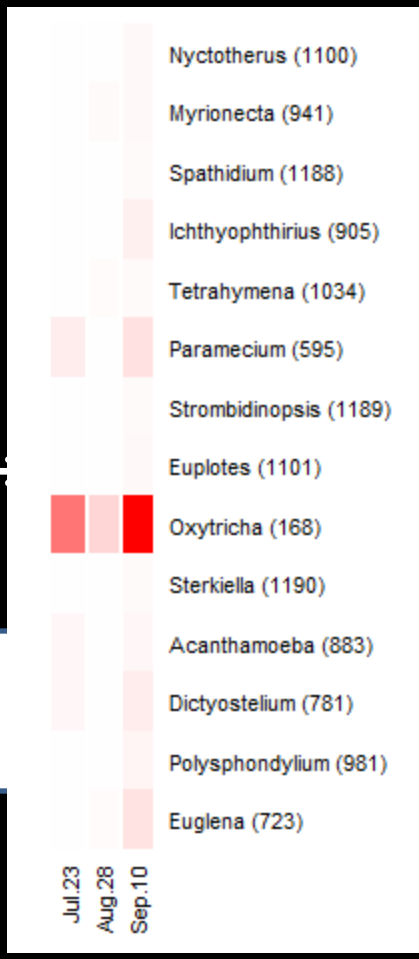
Food web implications – A working model

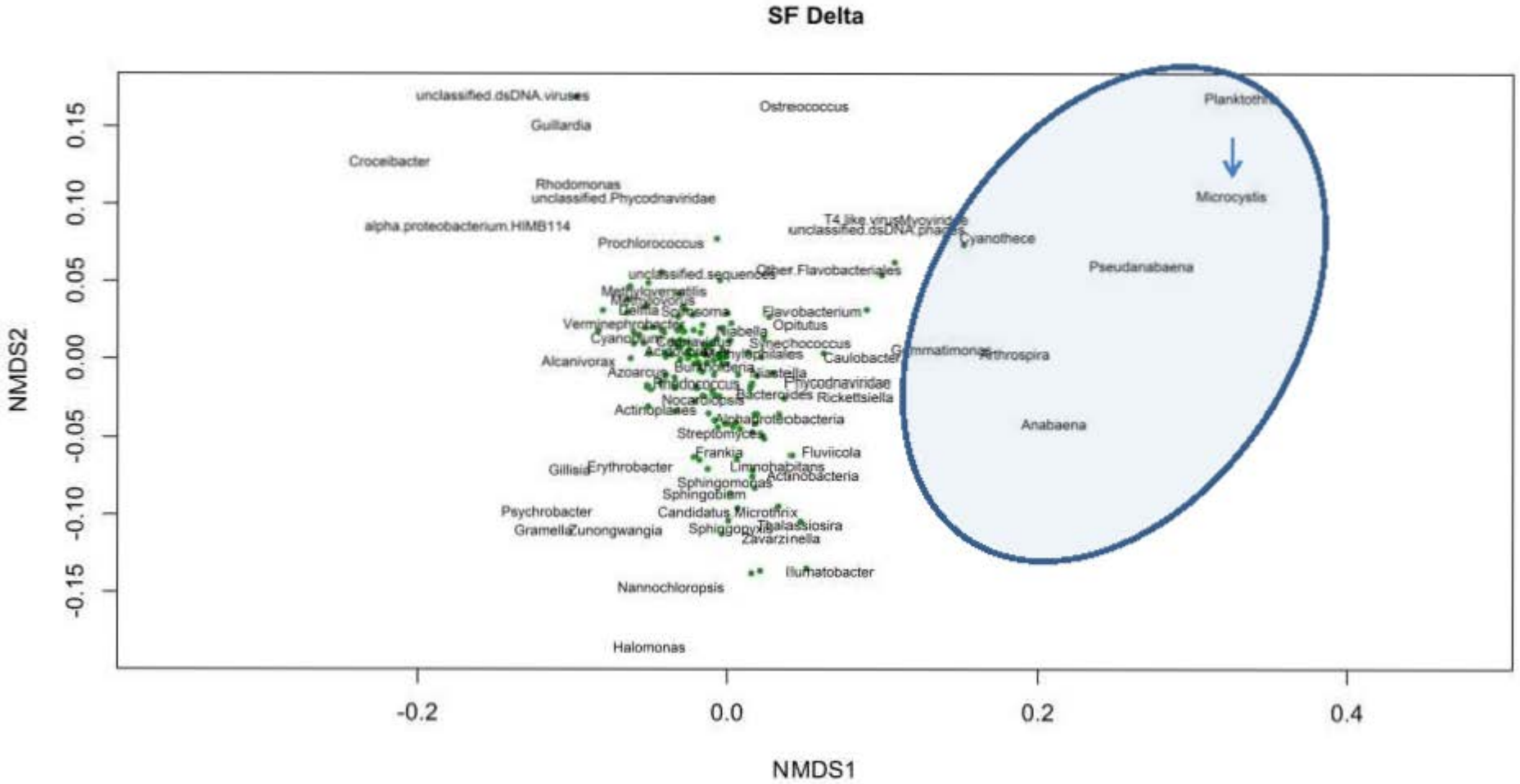


Food web implications – A working model



Protozoa




SF Delta
NMDS2
NMDS1
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.2
0.0
0.2
0.4
unclassified.dsDNA.viruses
Ostreococcus
Guillardia
Crocelbacter
Rhodomonas
unclassified.Phycodnaviridae
alpha.proteobacterium.HIMB114
Prochlorococcus
Microcystis
Cyanothece
Pseudanabaena
Flavobacterium
Opitutus
Synechococcus
Caulobacter
Arthrospira
Anabaena
Phycodnaviridae
Rickettsiella
Alcanivorax
Actinobacteria
Streptomyces
Fluviicola
Erythrobacter
Sphingomonas
Sphingobium
Psychrobacter
Candidatus Microthrix
Thalassiosira
Zavarzinella
Nannochloropsis
Halomonas

Conclusions

- *Microcystis* population composed of numerous strains, many of which are capable of producing several microcystin congeners

- There were no geographically distinct strains, all were ubiquitous

- Morphology was a poor indicator of species/strain or toxicity

- These strains are able to produce a variety of 2° metabolites that may directly or indirectly impact the food web

- During bloom events *Microcystis* cells outnumber all other organisms in the near surface water column and community diversity is halved

- Shotgun metagenomics provides for an unbiased assessment of community structure and function

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- Kimmerer Lab at SFSU
- Kudela Lab at UCSC
- Paerl Lab at UNC

DELTA STEWARDSHIP COUNCIL