



HABs in Utah Lake- a molecular insight

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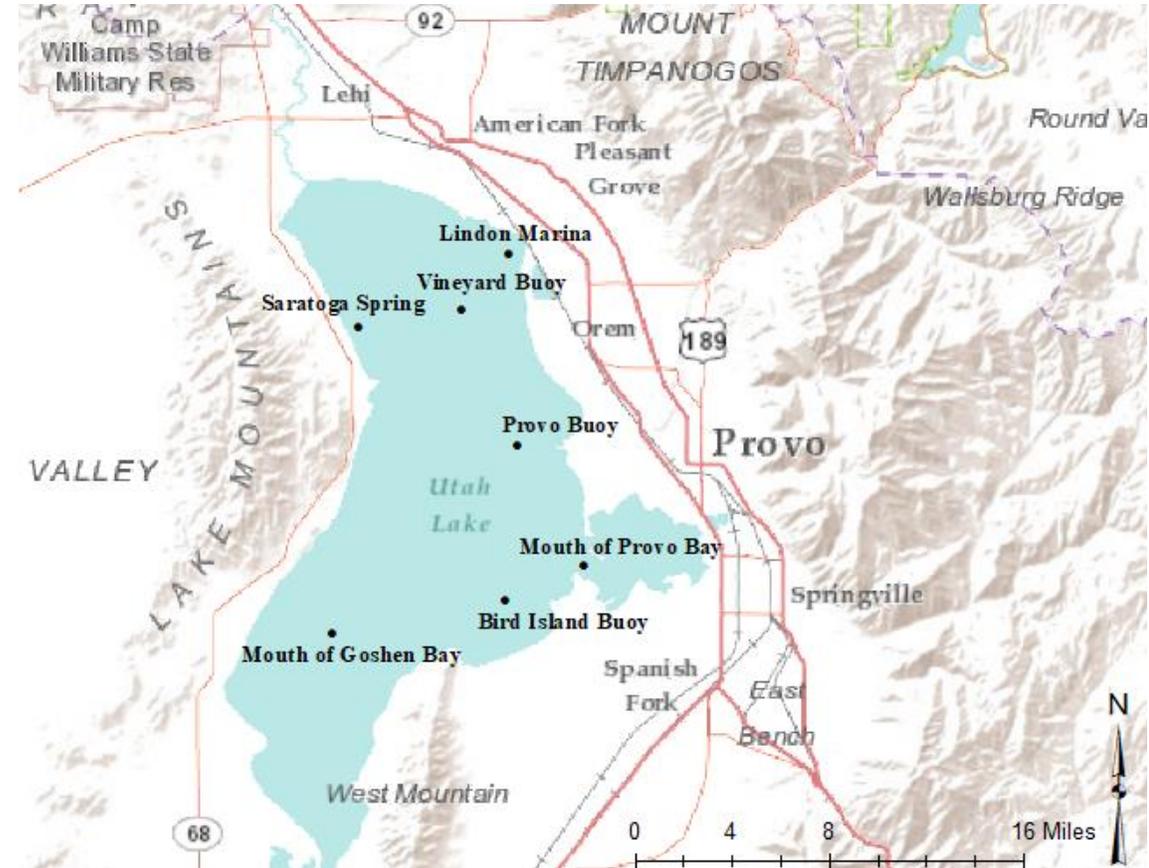
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Funding: EPA Star Grant

The sampling of Utah Lake for Cyano-diversity and ecology

- Sampling conditions:
 - Sampled continuously three years (2016, 2017-2018);
 - Ambient water conditions;
 - Holistic pictures of bacterial community using molecular methods
- Research questions:
 - The overall environmental conditions, cyanobacterial communities and other bacterioplankton
 - How the interactions among them indicate tipping point of algal blooms?



Water quality parameters

- **Physical parameters:**

- Temperature, pH, DO, conductivity, total dissolved solids

- **Nutrient indicators:**

- Ammonia, nitrite, nitrate, TN, TP, chlorophyll a

- **Cyanotoxin measurement:**

- Different variations of microcystins (MC-LR, MC-RR, MC-YR, MC-LA) and nodularin



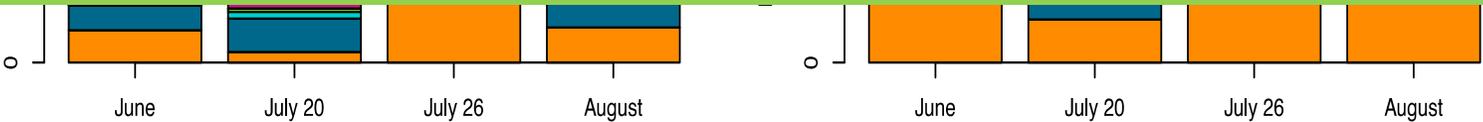
Phylum level- Total bacteria 2016

Pelican Point

Lincoln Beach

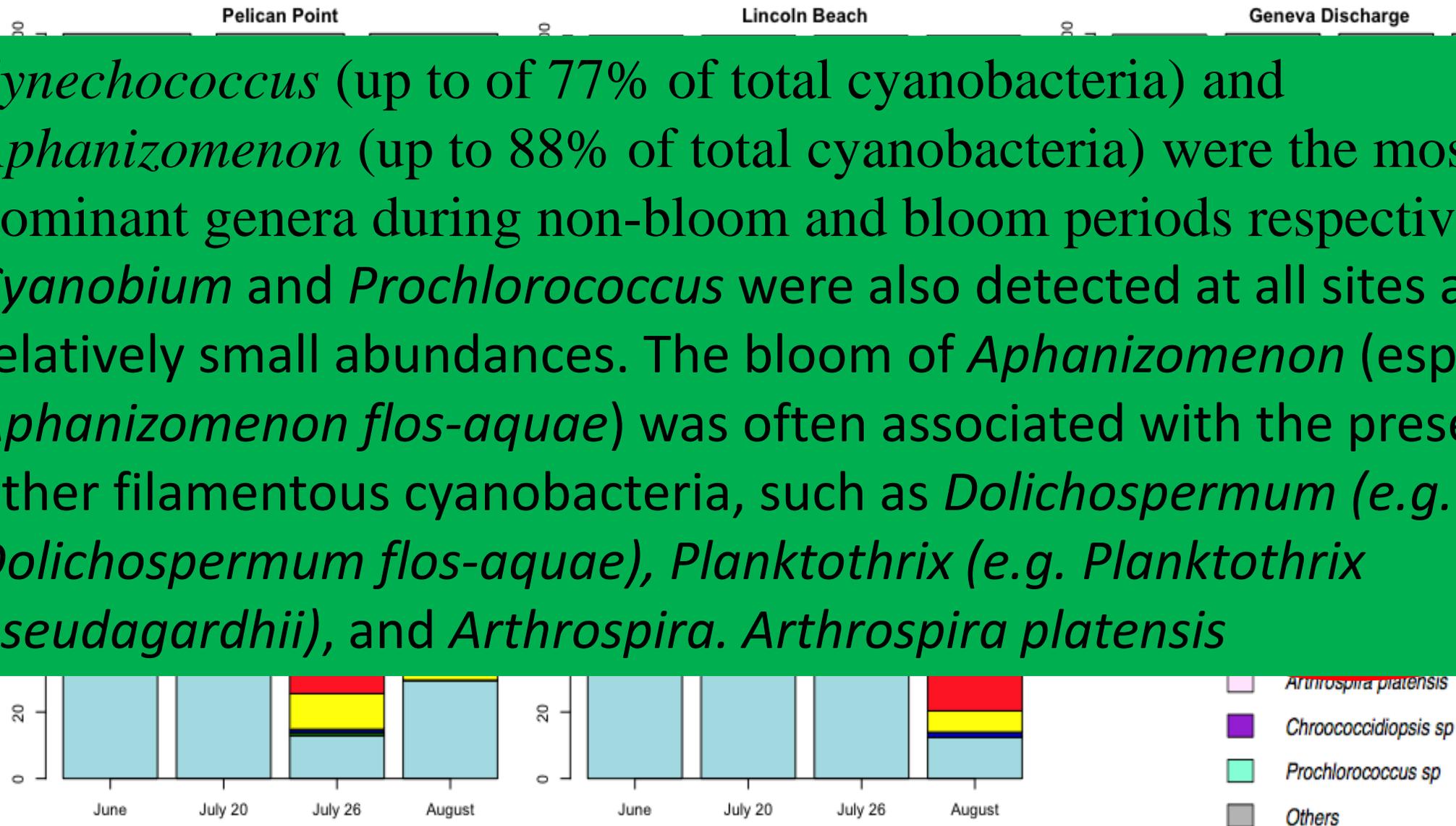
Geneva Discharge

For phylum level, total of 20 prokaryotic phyla were observed each year. *Cyanobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Verrucomicrobia* were the dominant bacteria phylum in both years. *Proteobacteria* accounted for around 50% of the total bacteria communities in 2016. *Bacteroidetes* was the second largest phylum, which occupied a larger relative abundance during the bloom. *Cyanobacteria* was not the dominant bacteria community during the harmful algal bloom. In fact, the relative abundance of *cyanobacteria* decreased from 10-30% before bloom to less than 1% during the bloom in 2016. The relative abundance was recovered to 7-33% after the bloom.

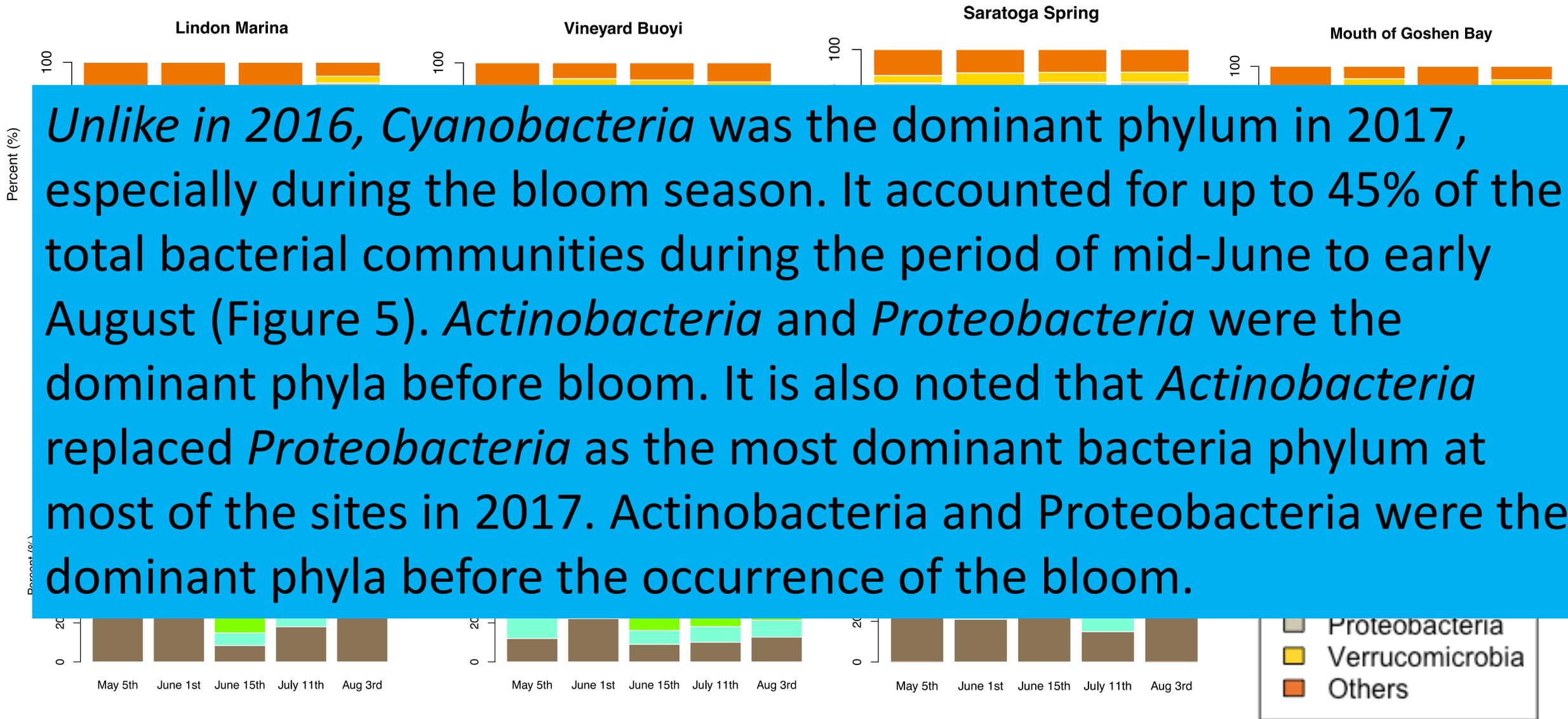


Summer 2016 Cyanobacteria- 16S rDNA based amplicon sequencing

Synechococcus (up to of 77% of total cyanobacteria) and *Aphanizomenon* (up to 88% of total cyanobacteria) were the most dominant genera during non-bloom and bloom periods respectively. *Cyanobium* and *Prochlorococcus* were also detected at all sites albeit in relatively small abundances. The bloom of *Aphanizomenon* (especially *Aphanizomenon flos-aquae*) was often associated with the presence of other filamentous cyanobacteria, such as *Dolichospermum* (e.g. *Dolichospermum flos-aquae*), *Planktothrix* (e.g. *Planktothrix pseudagardhii*), and *Arthrospira*. *Arthrospira platensis*



Phylum level- Total bacteria 2017



Cyanobacteria Speciation- 2017

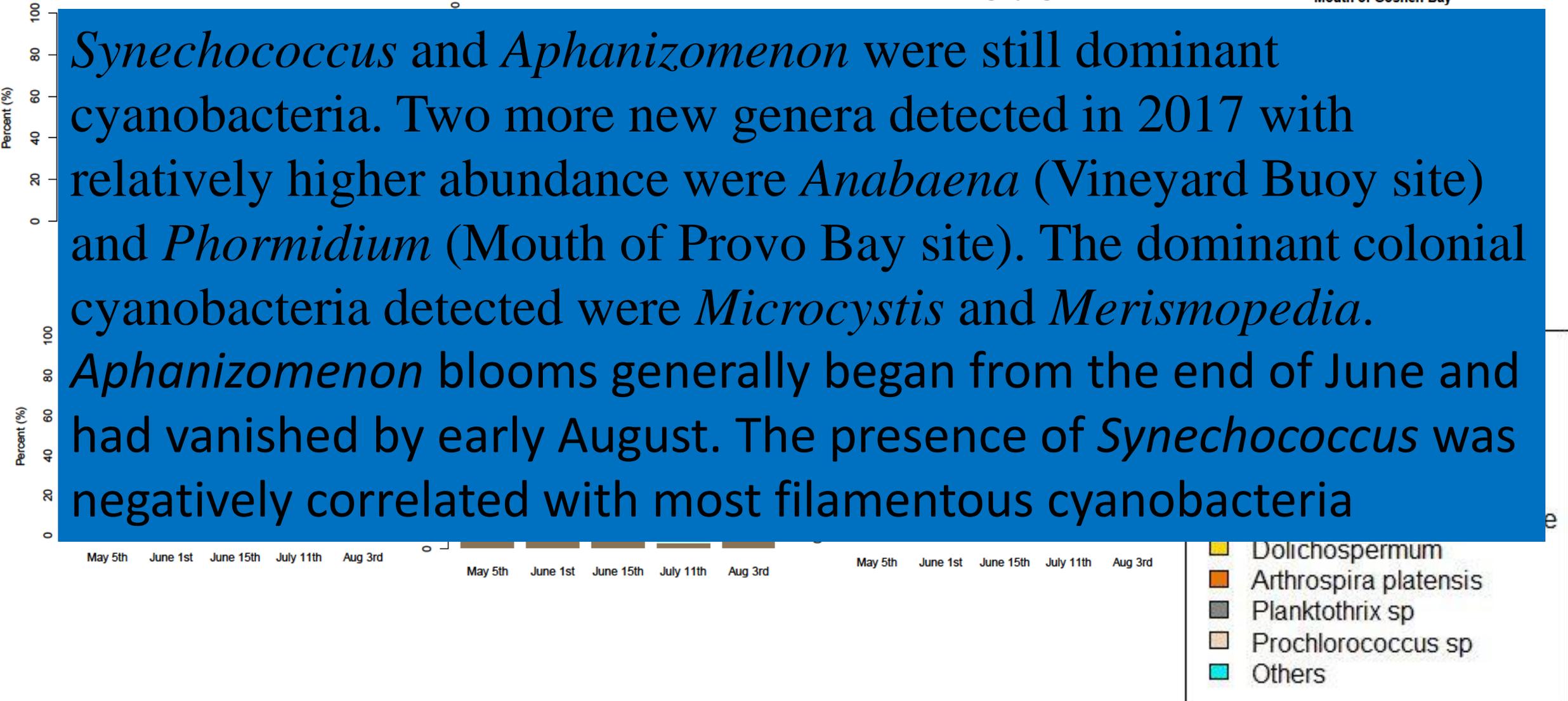
Lindon Marina

Vineyard Buoy

Saratoga Spring

Mouth of Goshen Bay

Synechococcus and *Aphanizomenon* were still dominant cyanobacteria. Two more new genera detected in 2017 with relatively higher abundance were *Anabaena* (Vineyard Buoy site) and *Phormidium* (Mouth of Provo Bay site). The dominant colonial cyanobacteria detected were *Microcystis* and *Merismopedia*. *Aphanizomenon* blooms generally began from the end of June and had vanished by early August. The presence of *Synechococcus* was negatively correlated with most filamentous cyanobacteria



Snapshot of Cyano-diversity in 2016 and 2017

- 2016
 - Total **20** species of cyanobacteria detected through high throughput sequencing
 - Dominant species:
Synechococcus- non-bloom
Aphanizomenon- bloom forming
 - Toxin-producers
Mainly *Microcystis*
– microcystin producers

- 2017
 - Total **36** species of cyanobacteria were detected
 - Dominant species
Synechococcus
Aphanizomenon – July
 - More new species thrive (mostly toxic)

Merismopedia sp: lipopolysaccharides- skin irritation
Phormidium sp (Provo bay): anatoxin
Anabaena sp: anatoxin-a
Dolichospermum sp: microcystin, anatoxins
Microcystis sp: Microcystin
Arthrospira platensis: non-toxic
Prochlorococcus sp: picoplankton

A total of 13 shared phyla and 17 unique cyano operational taxonomic units between 2016 and 2017 with more diversity in 2017.

Different cyanobacteria

Synechococcus, *Cyanobium* and *Prochlorococcus* all belong to picoplankton which has cell size ranges from 0.2 to 2.0 μm (Sieburth et al., 1978). Some of the studies have confirmed their capacity of producing hepatotoxin, neurotoxins, dermatotoxins

Aphanizomenon can produce cyanotoxins of anatoxin-a, cylindrospermopsins, and shellfish toxins. The environmental factors affecting the production of anatoxin-a include 10-30 $^{\circ}\text{C}$, light irradiance in the range of 2-128 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$. *Aphanizomenon* contain toxigenic genes such as *mcyE* that could lead to the production of MC under the right environmental conditions (Lyon-Colbert et al., 2018).

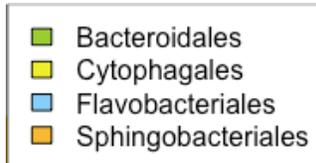
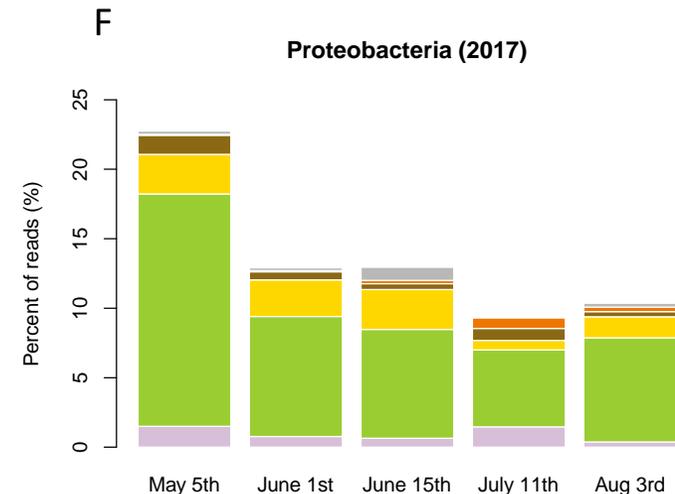
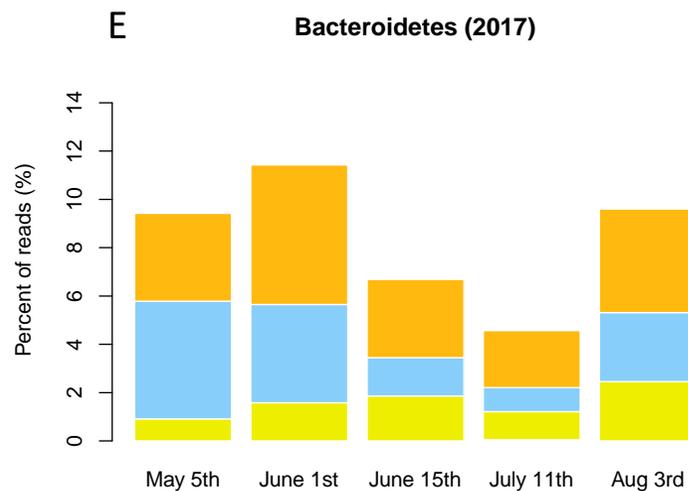
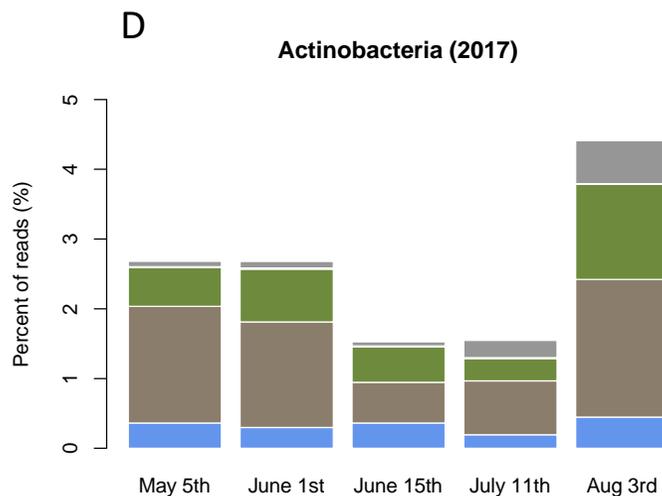
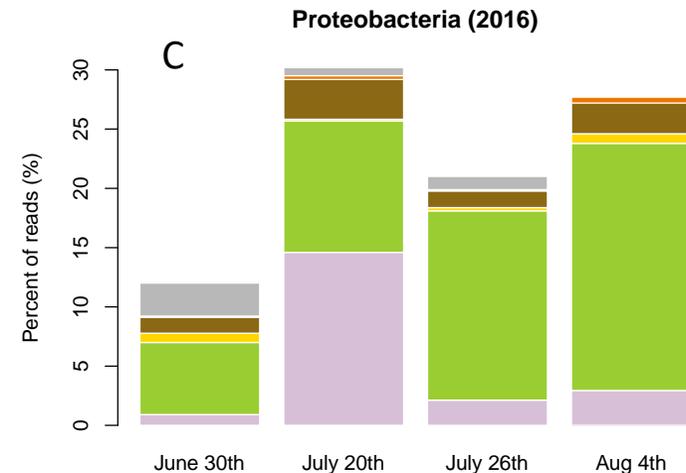
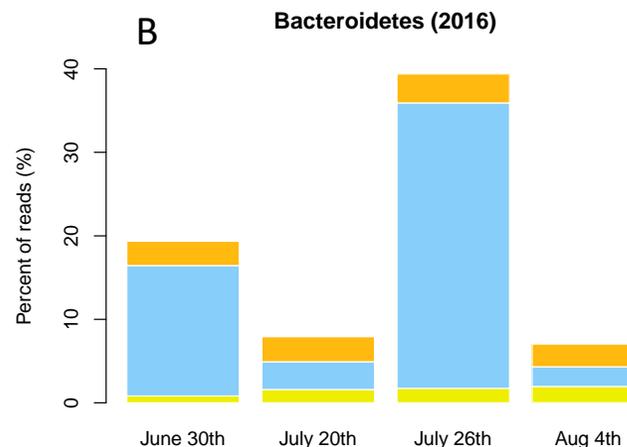
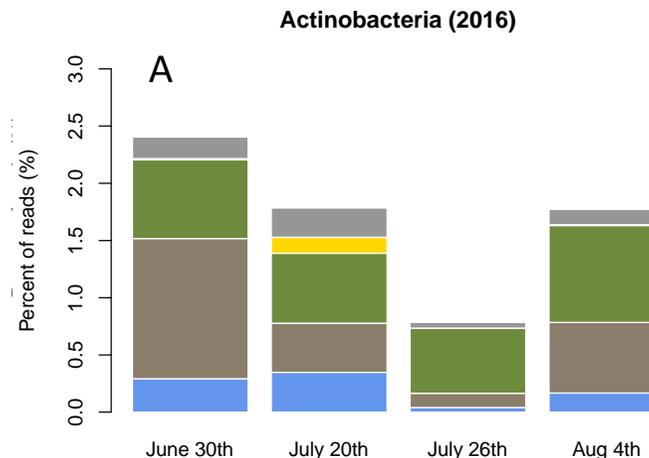
Dolichospermum is also one of the most ubiquitous bloom-forming cyanobacterial genera. *Dolichospermum* is also capable of fixing nitrogen from atmosphere. Despite of the N-fixation advantages, studies have found the *Dolichospermum* blooms mostly form under eutrophication conditions and various nitrogen: phosphorus conditions.

Planktothrix is a dominant cyanobacterial genus forming toxic blooms in temperate freshwater ecosystems. It's a filamentous cyanobacteria but not commonly believed to have heterocyst. A recent study reported the benthic *Planktothrix* strains showed unexpected characteristics such as buoyancy, nitrogen fixation capacity and unique natural product features

Phormidium is a toxic benthic mat-forming cyanobacteria that has being increasingly reported in freshwater systems

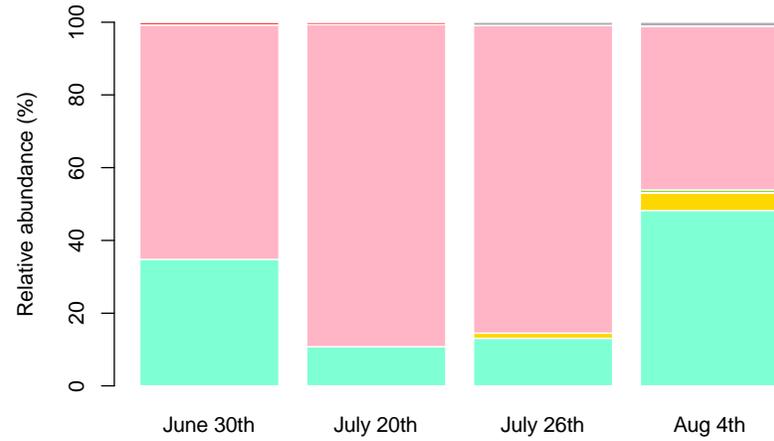
The genus *Arthrospira* comprises filamentous, non-heterocystous cyanobacteria that are generally found in tropical and subtropical regions in warm bodies of water with high carbonate/bicarbonate content, elevated pH, and salinity

Dominant heterotrophic bacterioplankton community (note the differences in y axis)

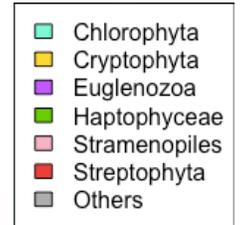
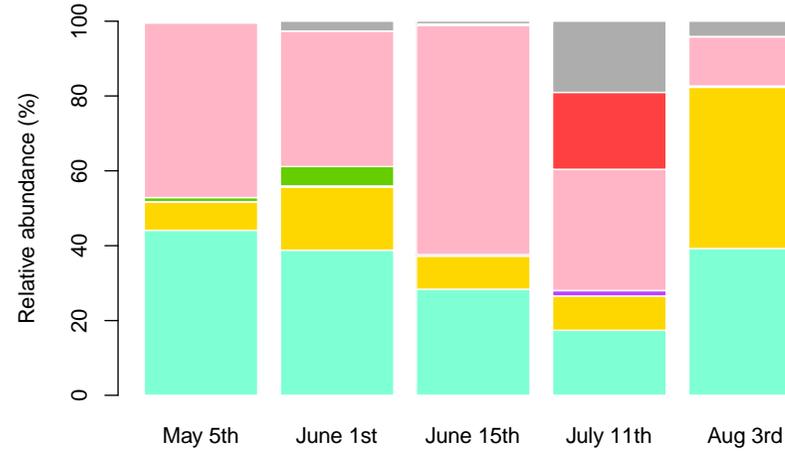


Eukaryotic phytoplankton

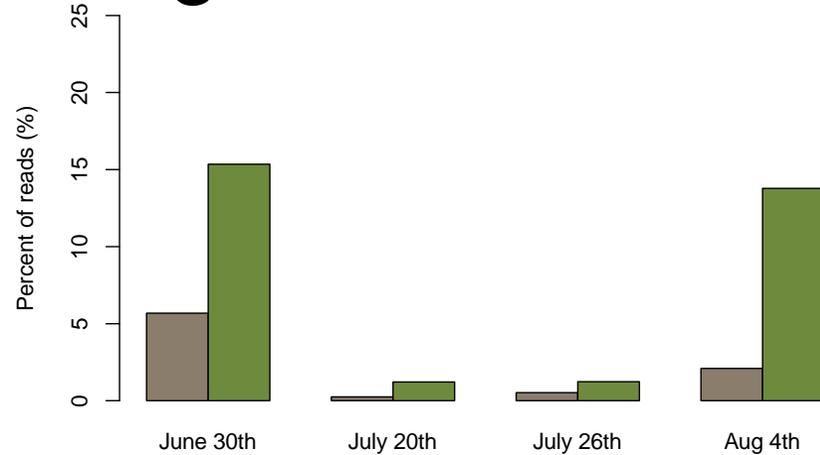
A Chloroplast community (2016)



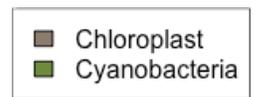
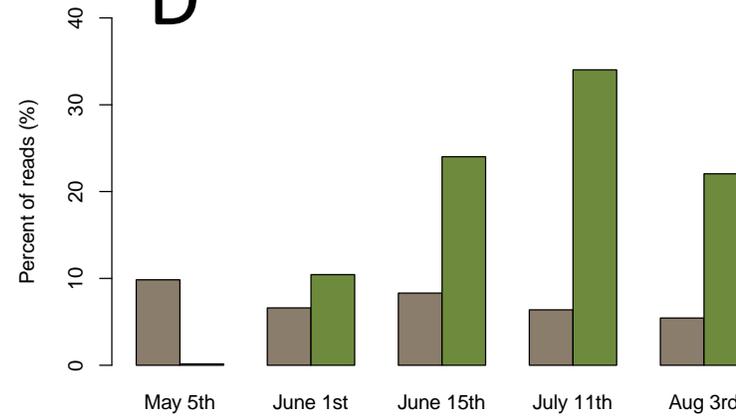
B Chloroplast community (2017)



C Chloroplast vs Cyanobacteria (2016)



D Chloroplast vs Cyanobacteria (2017)



Correlations for Environmental Factors and Species

A

PCA plot -2016

B

PCA plot -2017

Cyanobacteria were found to be negatively linked with heterotrophs and nitrate in both years. Environmental factors such as Chl a, pH, temperature and cBOD are positively correlated with each other. Occurrence of chloroplast communities were observed to have the same trend as cyanobacteria. A clear pattern was observed when individuals are grouped based on months rather than geographical locations. In 2016, heterotrophs dominated the algal bloom in July. Cyanobacteria and chloroplast community accounted for higher relative abundance before and after the bloom. By the contrast, the dominance of cyanobacteria in July was mostly related to temperature in 2017

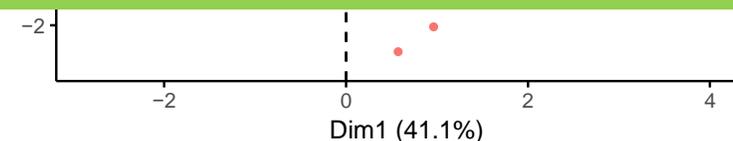
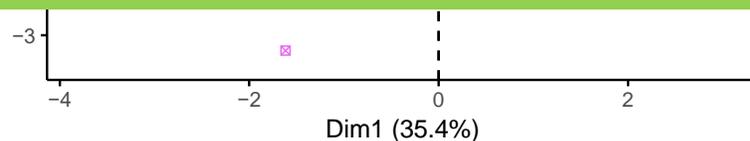
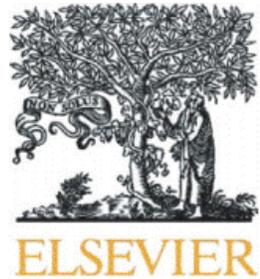


Figure : Two-dimensional Principle components analysis (PCA) biplots linking dominant bacterial communities with environmental factors. (A) Biplot for 2016. (B) Biplot for 2017. Temperature (Temp), Chlorophyll a (Chl.a), total dissolved solids (TDS), carbonaceous biochemical oxygen demand (cBOD), Cyanobacteria (Cyano), Chloroplast (Chloro) and Heterotrophs (Bacteria).

Summary of findings from 2016 and 2017 sampling

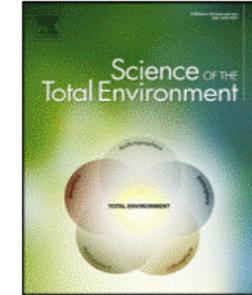
- Bacterial community level:
 - **Filamentous (bloom) and pico-cyanobacteria (non-bloom) are the dominant groups**
 - Potential toxin-producers presence but not the dominant strains
 - Proteobacteria, Bacteroidetes and Actinobacteria are the main bacterioplankton community
 - Ecosystem level: significant community variations with time changes
- The interactions between bacterial community and environmental factors:
 - Pico-cyanobacteria and filamentous are negatively correlated
 - Cyanobacteria and bacterioplankton are negatively correlated
 - **Filamentous cyanobacteria negatively responded to nitrate in the lake**



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High-throughput DNA sequencing reveals the dominance of pico- and other filamentous cyanobacteria in an urban freshwater Lake



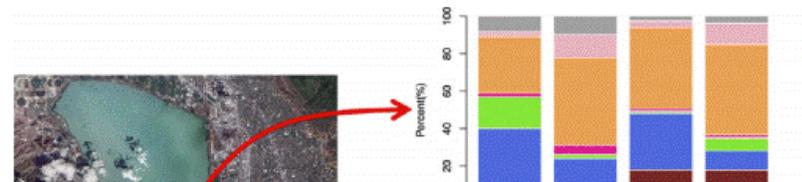
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HIGHLIGHTS

- Occurrence of harmful algal blooms (HABs) is a worldwide problem.
- A holistic approach using deep rDNA sequencing was employed to understand HABs.

GRAPHICAL ABSTRACT



Questions raised on current cyanoHABs

- Why would filamentous cyanobacteria dominated the cyanoHABs?
- What's the nutrient limitation conditions of the lake?
- Does the presence of them correlate with other bacterial communities (e.g., potential cyanotoxin-producers)?
- **Hypotheses:**
 - The lake is not only P limited but significantly N limited under certain conditions
 - Filamentous cyanobacteria overcame nutrient limits by activating N-fixation and P affinity systems
 - The fixed nitrogen in the lake was used to support the growth of potential MC-producers

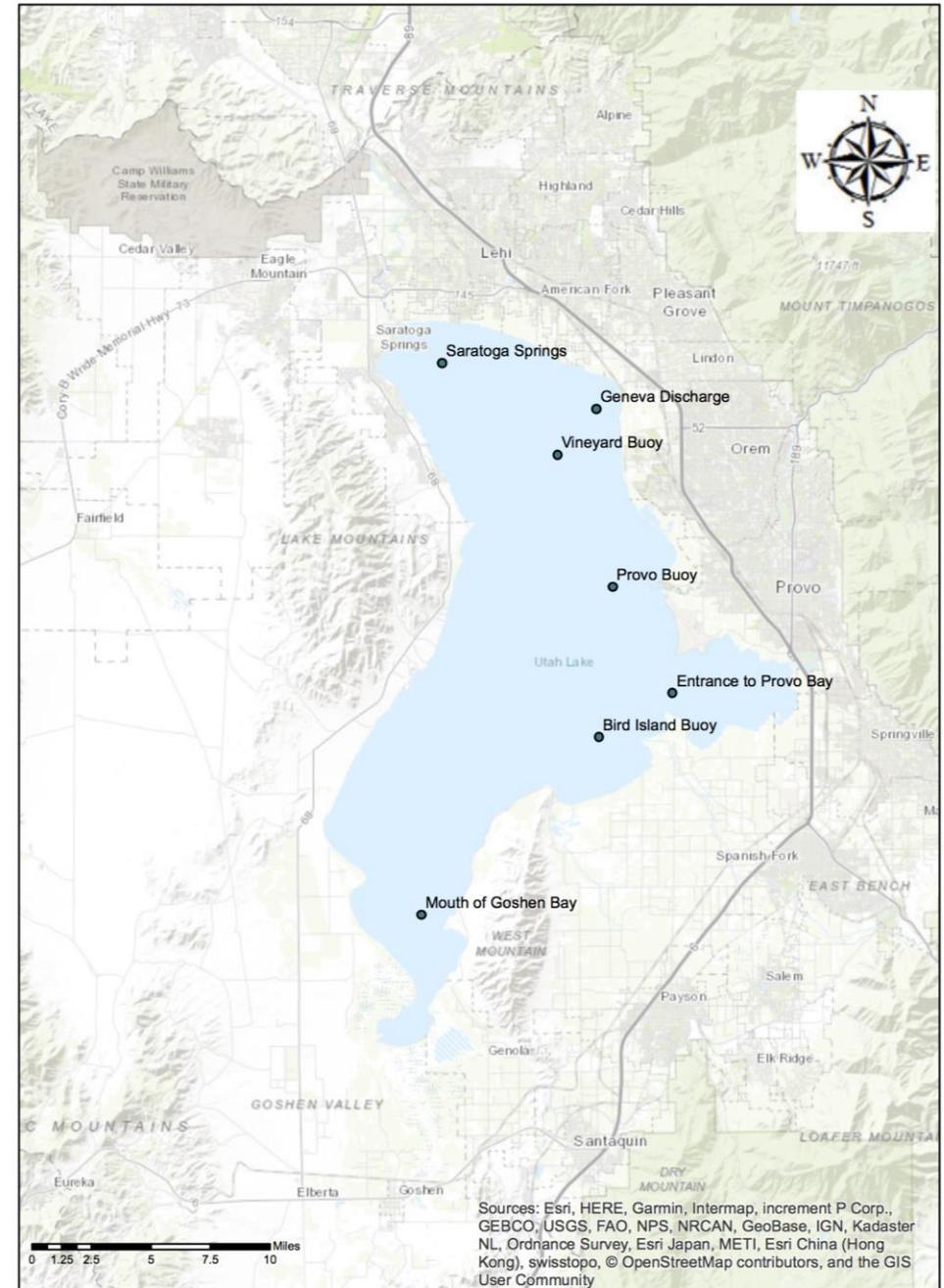
The sampling in 2018

- **The community level:**

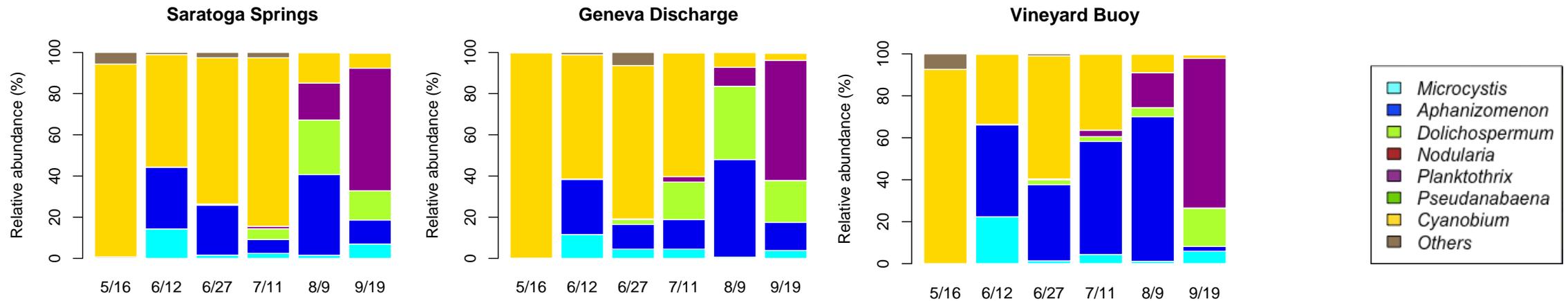
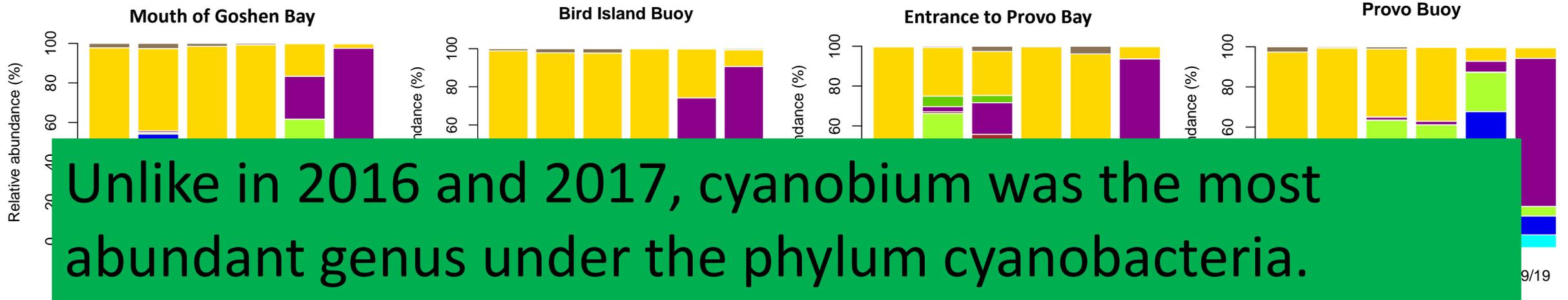
- The presence of cyanobacteria community (filamentous and toxin-producing)
- How bacterial community diversity change vertically?

- **The metabolic level:**

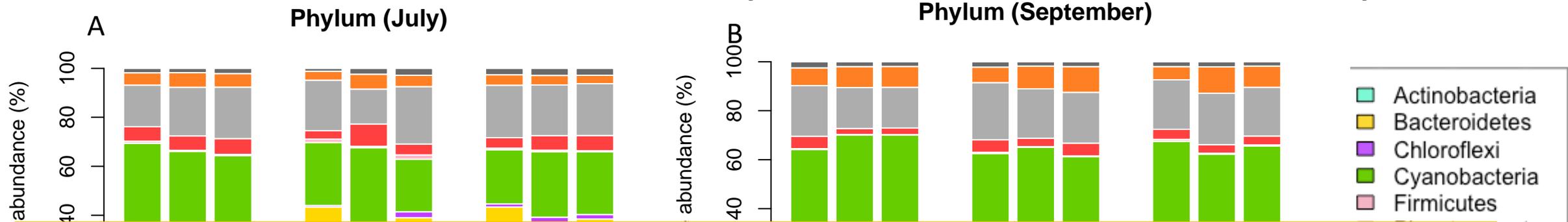
- Whether certain activities (gene and gene expressions) are activated?
- The N-fixation, P affinity, and toxin-producing related



Bacterial composition at the genus level

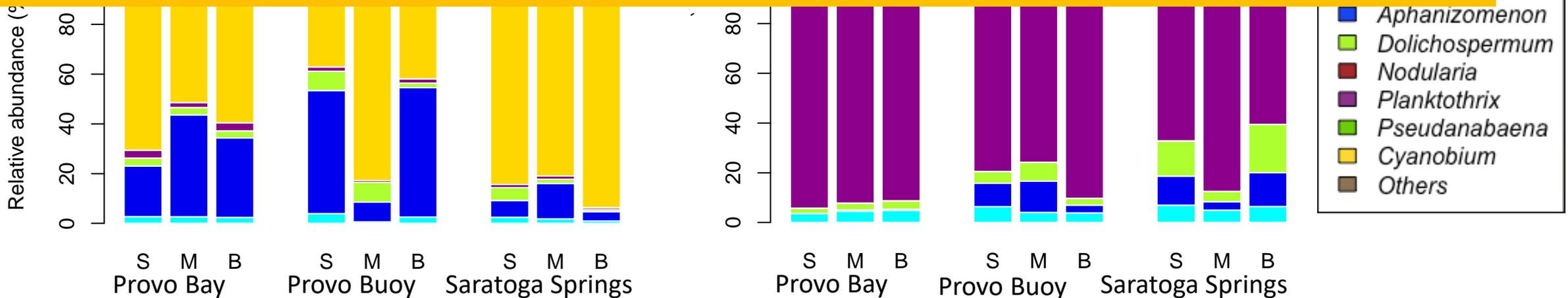


Vertical distributions of cyanobacterial community

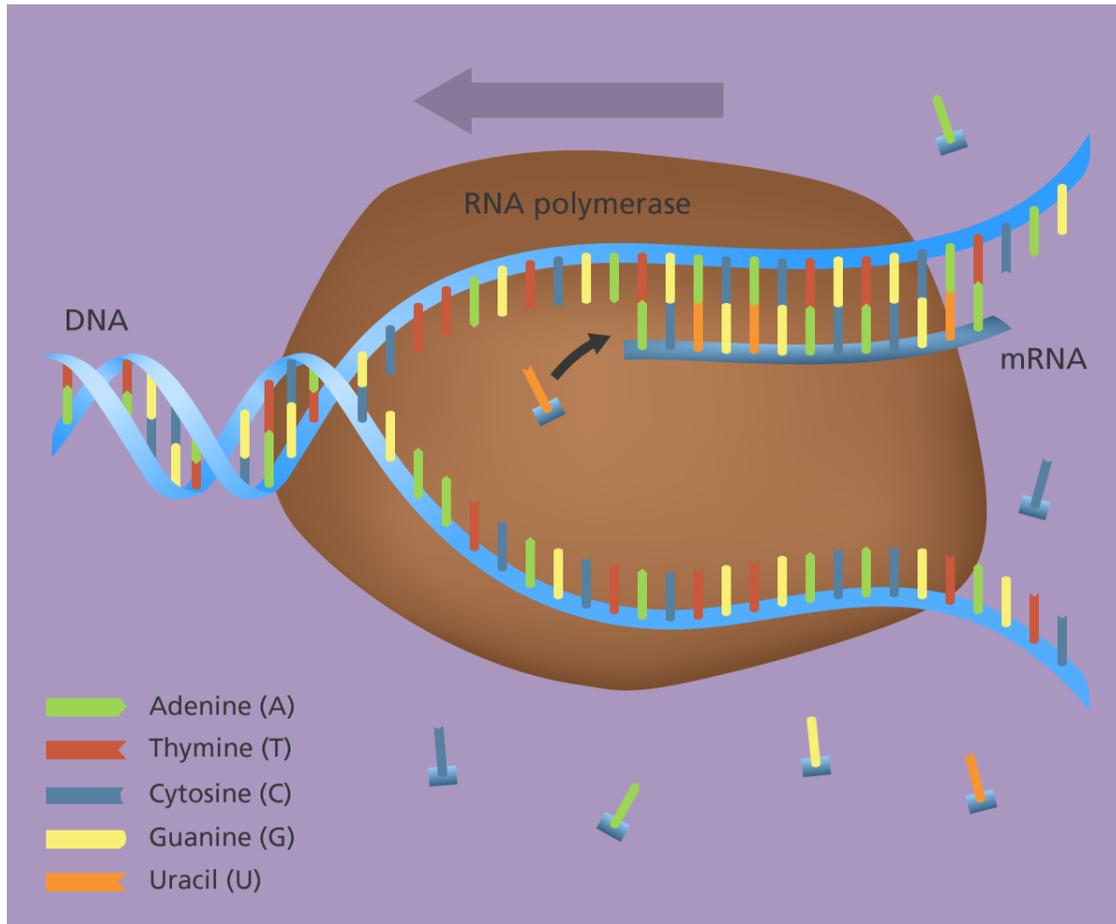


S=surface, M=Middle, B=bottom- Three sites were sampled vertically along the water column during bloom and non-bloom periods

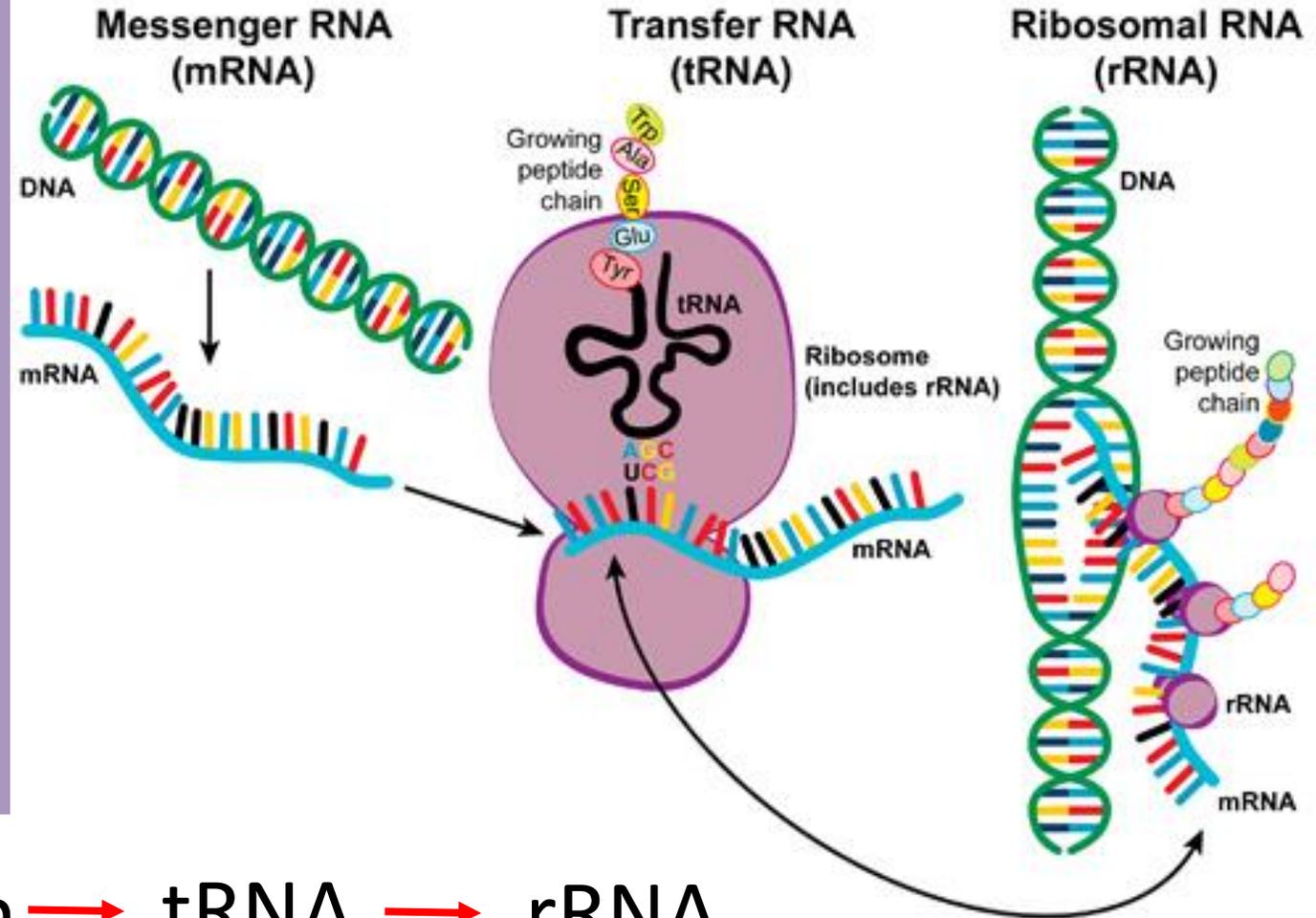
Overall, at the phylum level, the vertical distribution of different phyla was nearly uniform. There were some differences in the relative abundances of cyanobacteria. In general, cyanobium dominated in bloom period and planktothrix during non-bloom. In Provo Bay, Cyanobium dominated at the surface and bottom but, cyanobium and Aphanizomenon were equally present in the middle of the water column. We are still analyzing this data in terms of its significance to water depths at each site.



Gene expression



Ribonucleic acid (RNA)

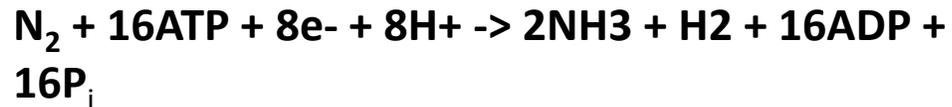


mRNA encodes information → tRNA → rRNA

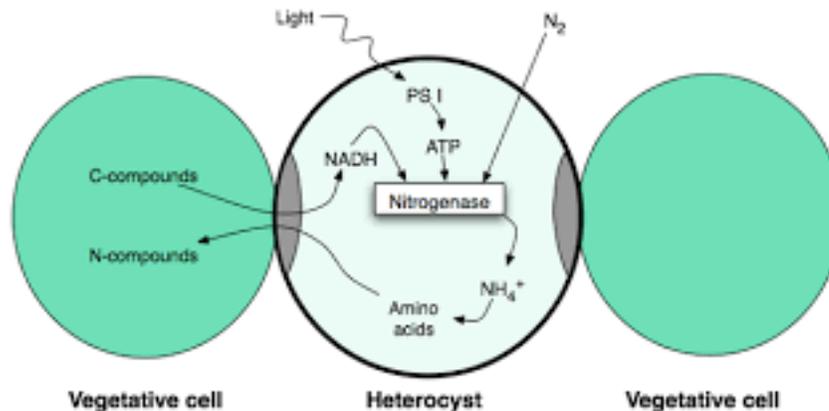
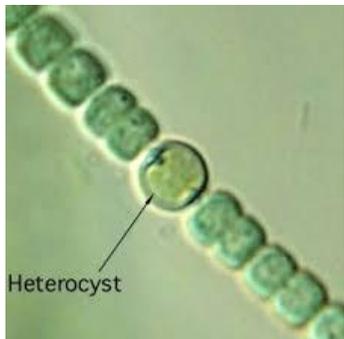
Specific features: N-fixers vs P transportation

N-fixers

N-fixation: most filaments form heterocyst, which conduct anaerobic N fixation.



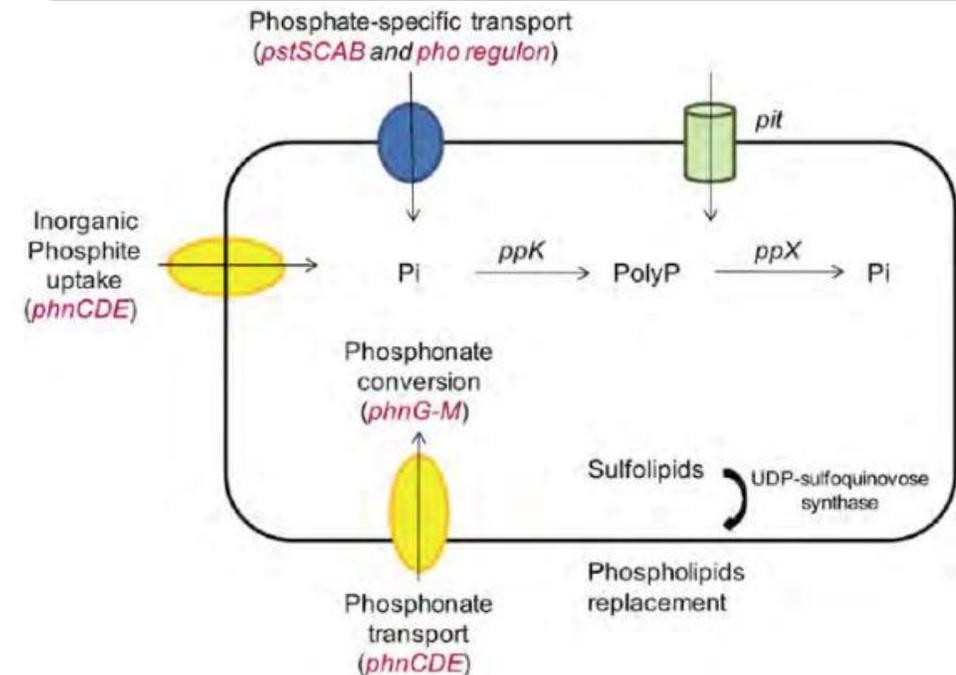
Encoding genes: Mo nitrogenase, a typical N-fixation enzyme, is encoded by *nifHDK*.



P affinity transporters

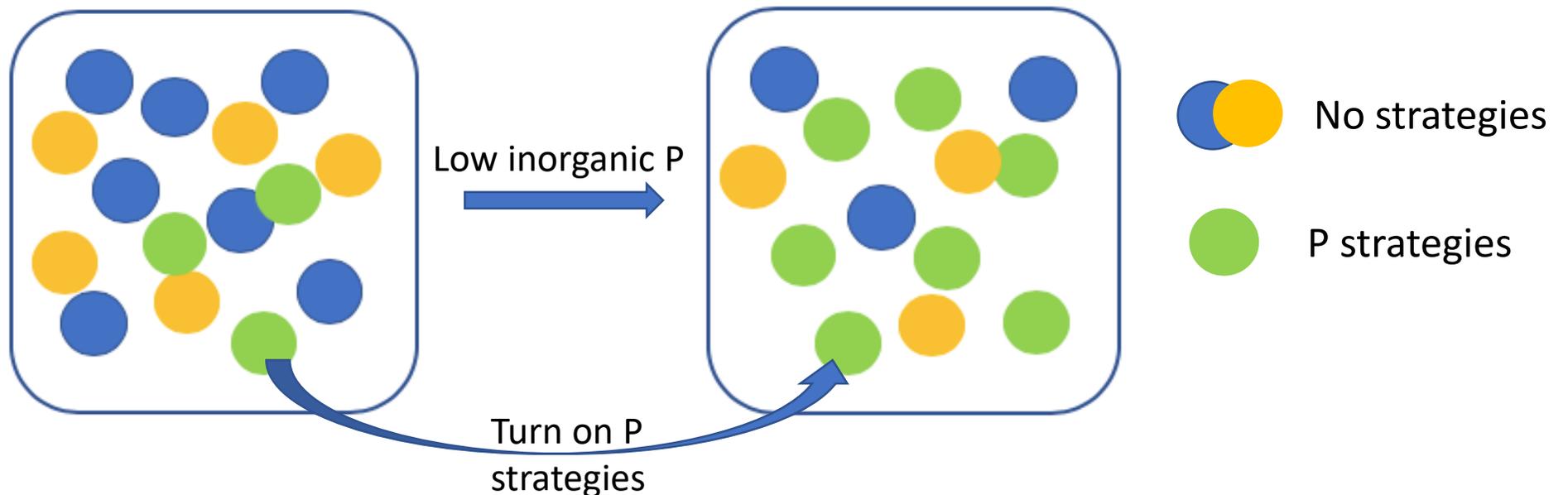
High affinity P assimilations: increase Pi transport under low ambient phosphate conditions.

Encoding genes: Typically Pho regulon and *pstS-ABC* type transporter.



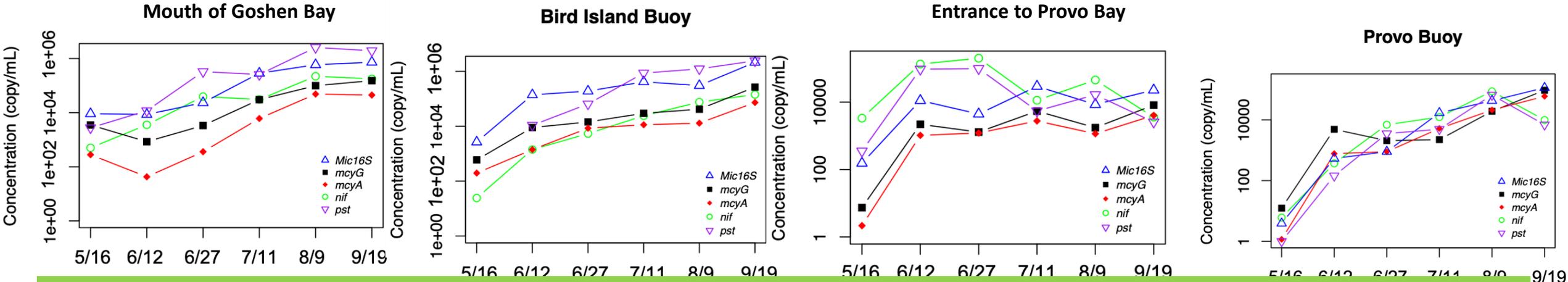
Common strategies for survival in low-phosphate environments

- Two common ways
 - Induction of high-affinity phosphate scavenging systems (*pstSCAB*)
 - Up-regulation of enzymes to hydrolyze DOP into phosphate (*phnCDE*)



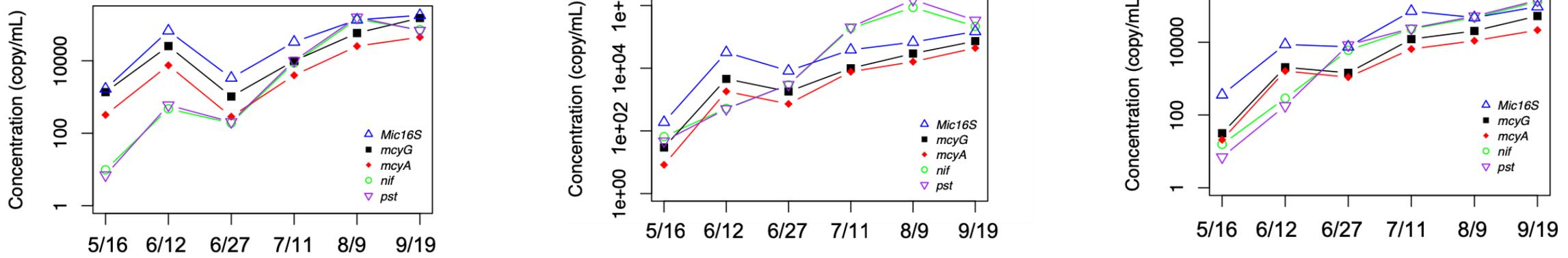
- N-fixation and P strategies can turn on together to overcome bloom season's limited nutrient conditions

Gene quantifications- Based on genomic DNA



Monitor different functional genes- based on genomic DNA (Not expression), Pst= High affinity P transportation uptake, Nif- N-fixing , mcyG=Microcycsin producing cluster, mcyA= microcystin cluster, Mic16S= Microcystin 16S.

In general, gene abundance based on quantitative PCR increased with increasing temperature at but Provo Bay Entrance site. This makes sense because the abundance of Cyanobacteria also increased with temperature at all sites.



Summary

- Generally, the bloom can be divided into three stages; initially, the lake was composed of *Cyanobium*; later on, filamentous cyanobacteria (mainly *Aphanizomenon* and *Dolichospermum*) altered its dominance from early June to August; finally, *Planktothrix* appeared in August and dominated the bacterioplankton in September.
- The metabolic analysis confirmed the activation of N-fixation gens and *Pho* regulon (including *pst* genes) for filamentous cyanobacteria under nutrient starvation (*negatively linked with nutrients*).
- The N fixed by filamentous cyanobacteria could be the potential N source supporting the growth and bloom of *Microcystis* and *Planktothrix*.
- The flourish of potential MC-producing species occurred in the meantime or after potential N-fixers