



High Frequency Monitoring of cyanoHABs Dynamics

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Prediction of cyanoHAB/toxin events

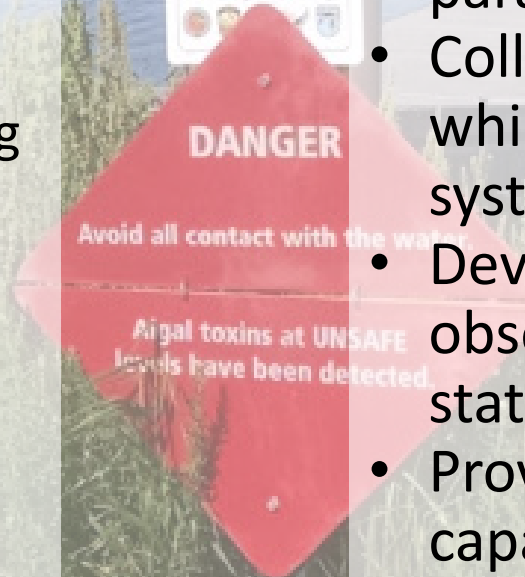
Questions

- Which biotic/abiotic factors drive cyanobacterial communities and cyanotoxin production?
- Are there environmental or molecular measures which can indicate impending blooms and cyanotoxin production?
- How does sampling frequency impact the picture provided by monitoring?
- What are the challenges and opportunities of reservoir hydrology in HAB dynamics and management?
- Goal

Reactive->Proactive Risk Management

Study Approach

- Leverage a variety of monitoring parameters
- Collect data/samples at a frequency which can capture variability of the system
- Develop relationships between observed parameters and bloom status
- Provide near-term predictive capabilities to focus cyanotoxin monitoring





HAB monitoring tools

HF Physico-chemical

- Water Quality
 - Temp
 - pH
 - ORP
 - Sp Cond
 - Turbidity
 - Dis Oxygen
 - TOC
 - DOC
 - NO₃-N
 - UV-Vis spectra
- PAR
- Weather



Wet Chemistry

- Total Nitrogen
- NO₂-NO₃
- NO₂
- Total NH₄
- Total Phosphorous
- Total Reactive Phosphorous



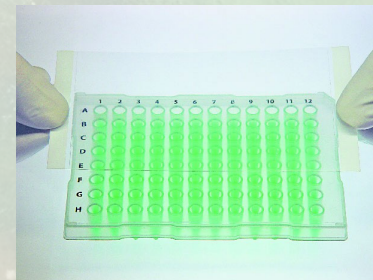
Phototroph Dynamics

- *In-vivo* Fluorescence
 - Phycocyanin
 - Chlorophyll
 - Other pigments
 - Diatoms
 - Cryptophyta
- Microscopic enumeration



Molecular Markers

- Next Gen Sequencing
 - 16S rRNA gene
 - 18S rRNA gene
 - Cytochrome oxidase
 - Metagenome
 - Metatranscriptome
- qPCR/RT-qPCR assays
 - Toxin specific gene assays



Cyanotoxin Analysis

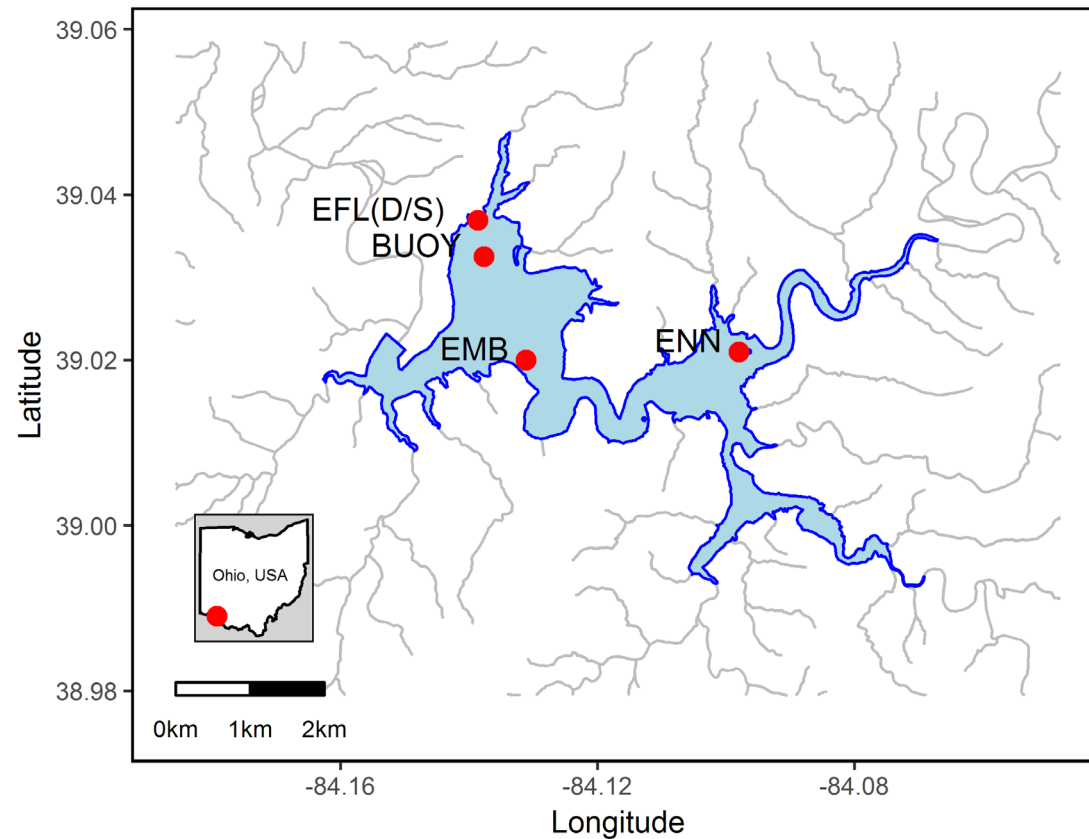
- ELISA
 - MC-ADDA
- LC-MSMS
 - MC congeners
 - Cylindrospermopsin
 - Anatoxin-a
 - MMPB





Lake Harsha, Clermont County, OH

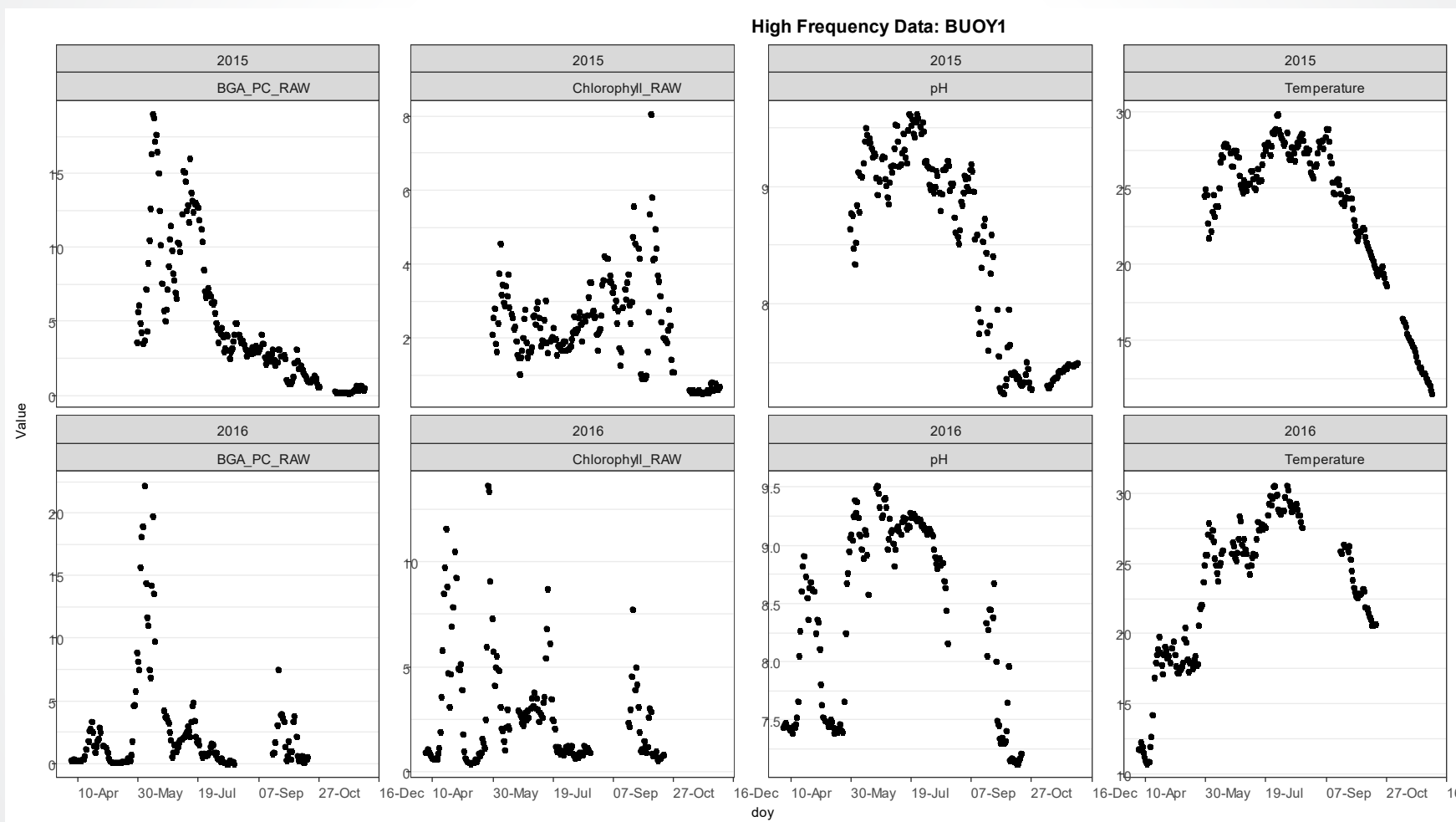
William H. Harsha Reservoir



- Watershed Area
 - 342 miles²
- Summer Pool Elevation
 - 733 ft
- Summer Pool Area
 - 2000 acres
- Water Quality Data since 2012 at 3 week intervals

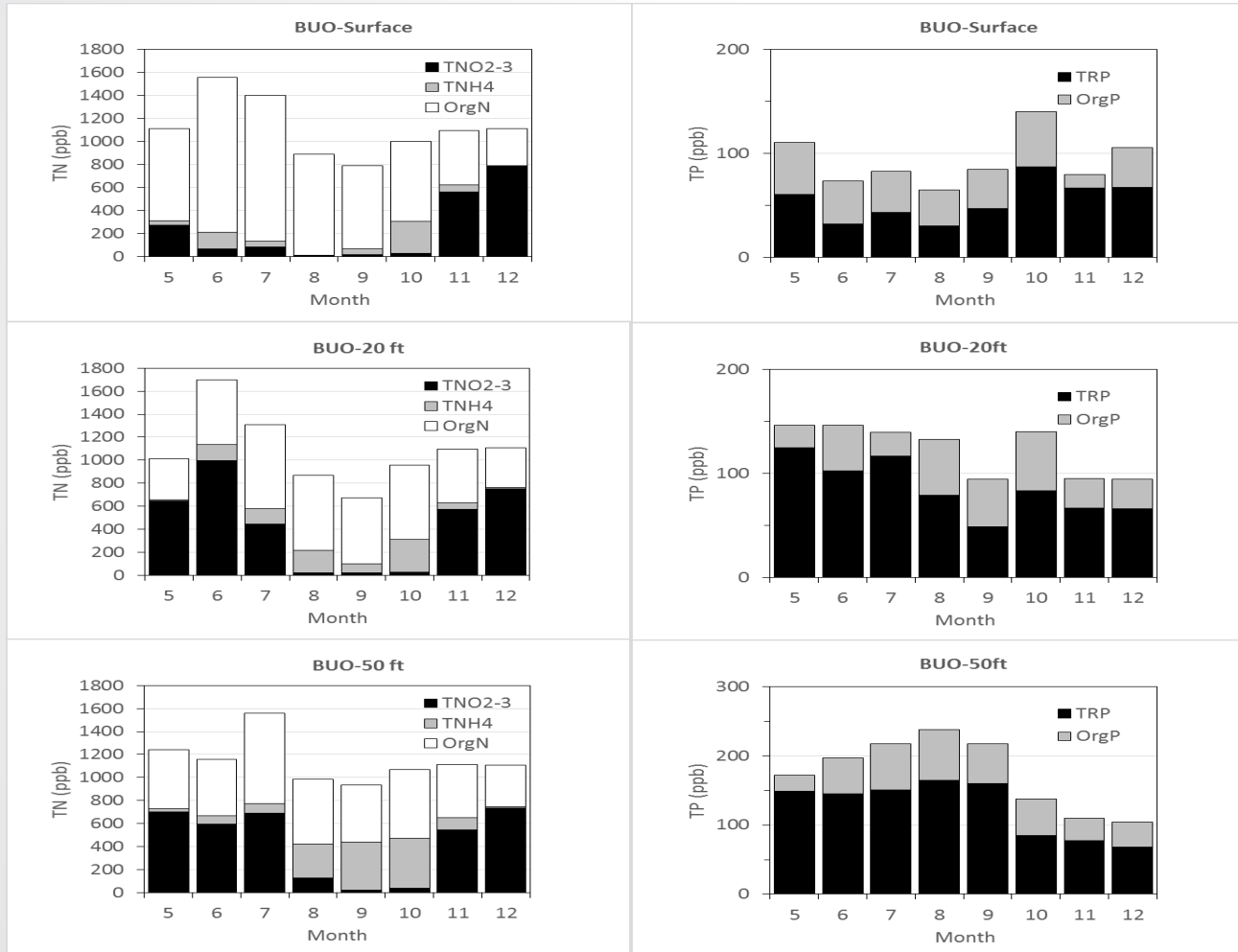


High frequency parameters





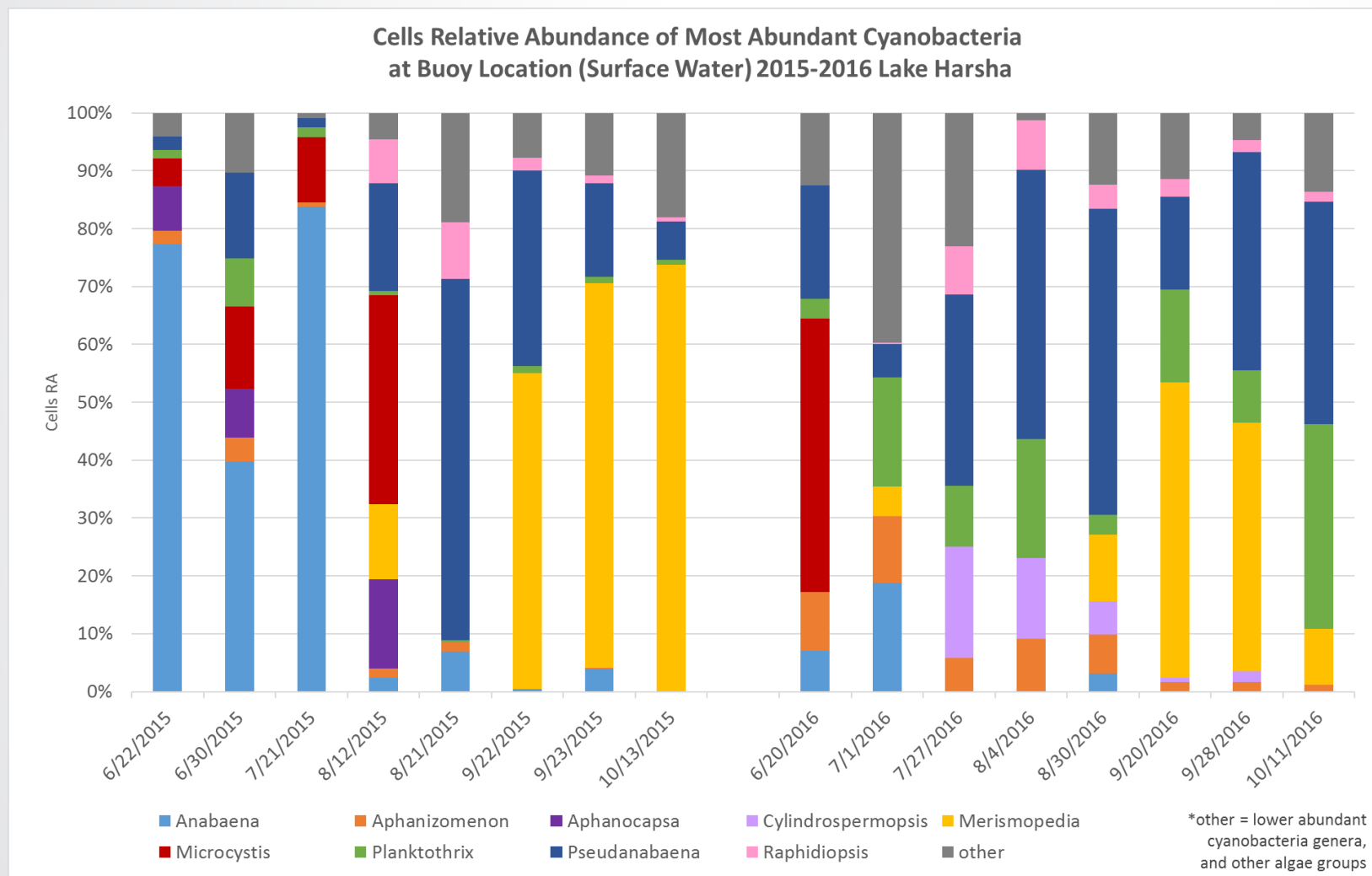
Nutrients



- Interior plateau ecoregion Targets
 - TN = 688 ppb
 - TP = 34 ppb
- Lake Harsha lake is well above these levels most of the time especially for TP.
- Depth profiles among sampling sites suggest the hypolimnion as a source of NH_4^+ and TRP
 - Concentrations increase until fall turn over
- Inorganic nutrients deplete in the surface waters by early summer, while organic fractions tend to dominate, presumably as live algae.



Phytoplankton dynamics

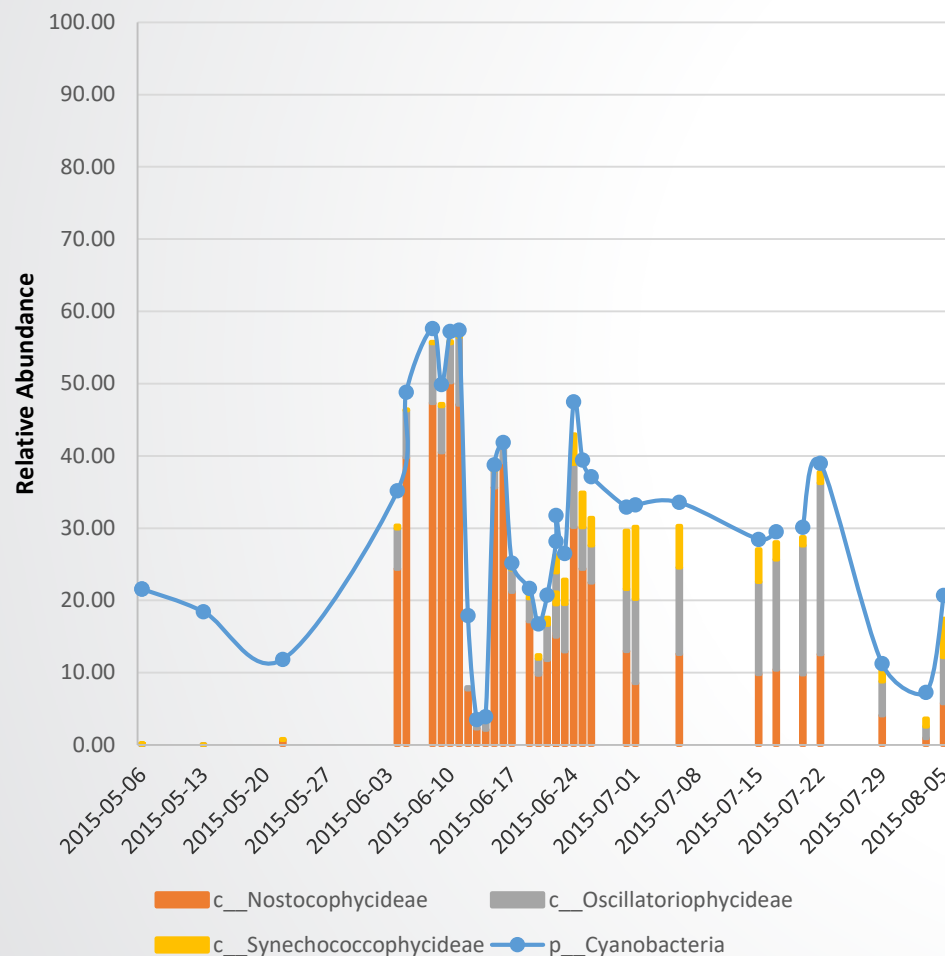


- 2015 and 2016 summer cyanobacteria relative abundances
- Cyanobacteria community is diverse and changes as season progresses
- Inter-annual differences are significant

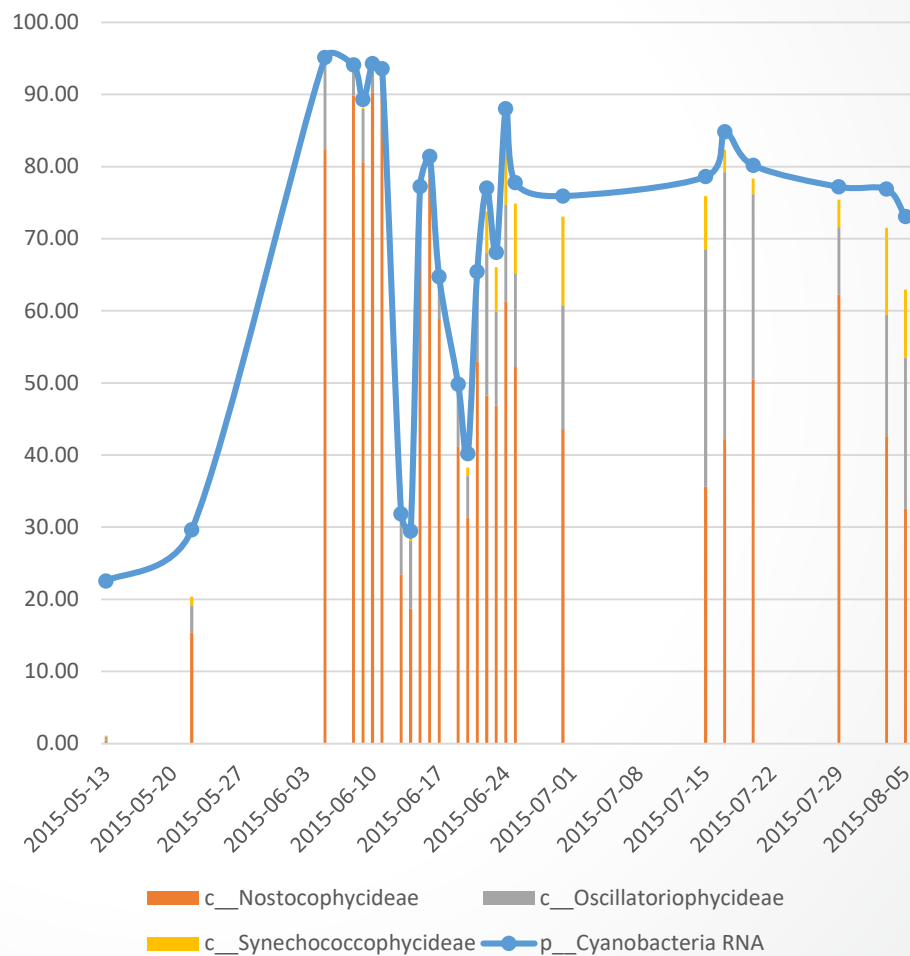


16S rDNA and rRNA sequencing

BUOY rDNA 2015



BUOY rRNA

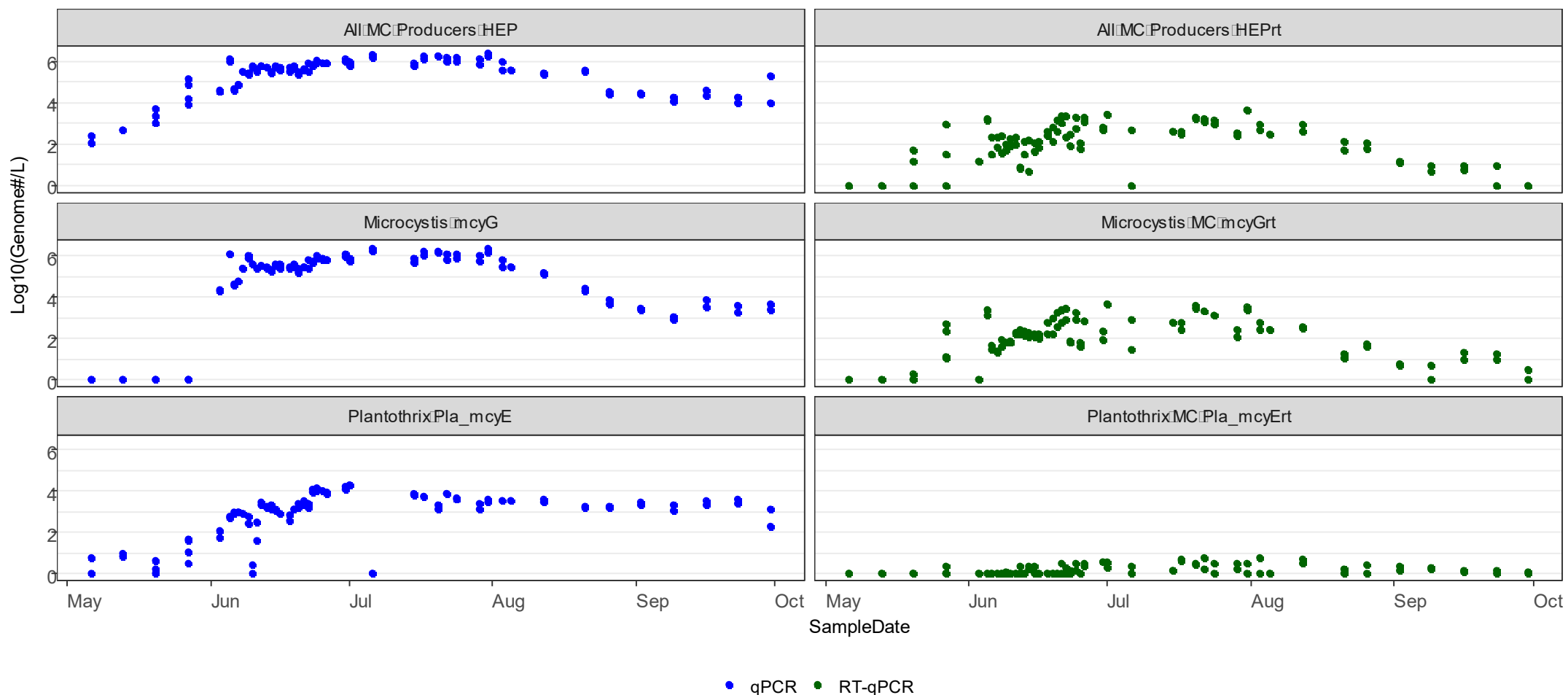


k__Bacteria
p__Cyanobacteria
c__Chloroplast
o__Chlorophyta
f__Chlamydomonadaceae
o__Stramenopiles
o__Cryptophyta
o__Euglenozoa
o__Haptophyceae
o__Streptophyta
c__Nostocophycideae
g__Aphanizomenon
g__Cylindrospermopsis
g__Dolichospermum
g__Trichormus
g__Anabaena
c__Oscillatoriothycideae
g__Microcystis
g__Phormidium
g__Planktothrix
g__Snowella
c__Synechococcophycideae
g__Pseudanabaena
g__Synechococcus



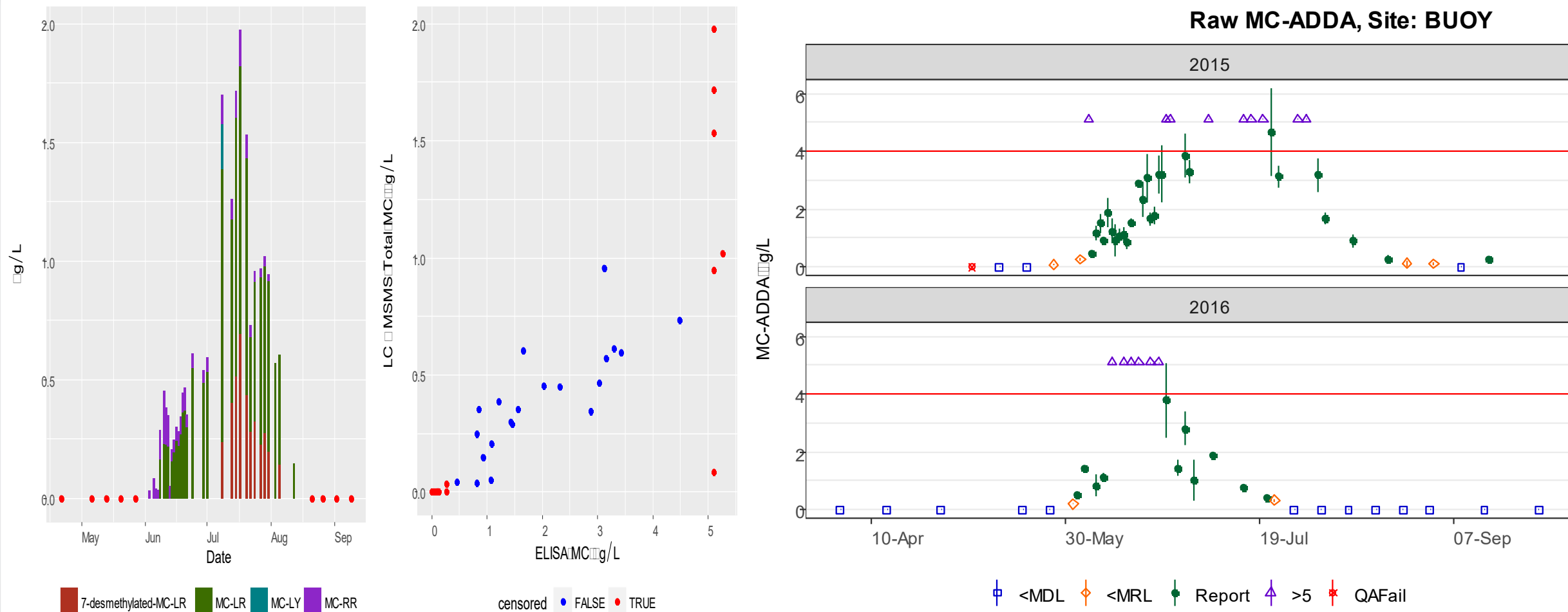
qPCR and RT-qPCR

2015 Molecular Markers





Cyanotoxin analysis



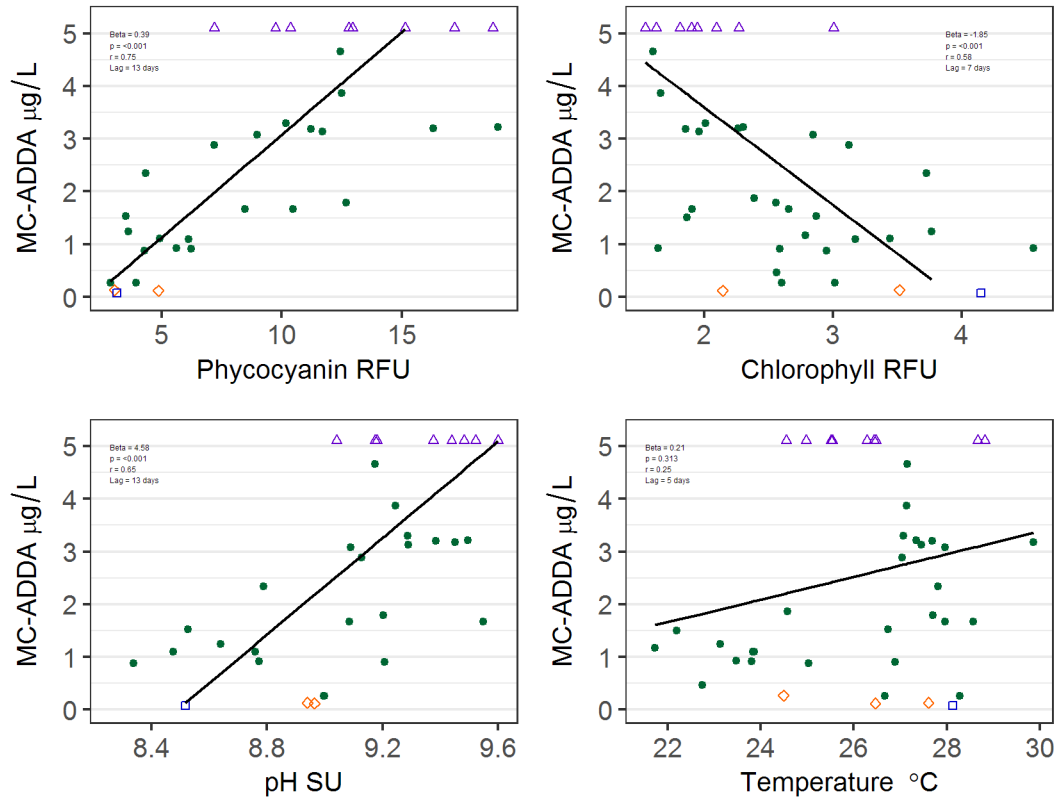
Nodularin, MC-YR, MC-HtyR, MC-RR, 3-desmethylated-MC-RR, MC-LR, MC-WR, 7-desmethylated-MC-LR, MC-HiLR, 3-desmethylated-MC-LR, MC-LA, MC-LY, MC-LW, MC-LF

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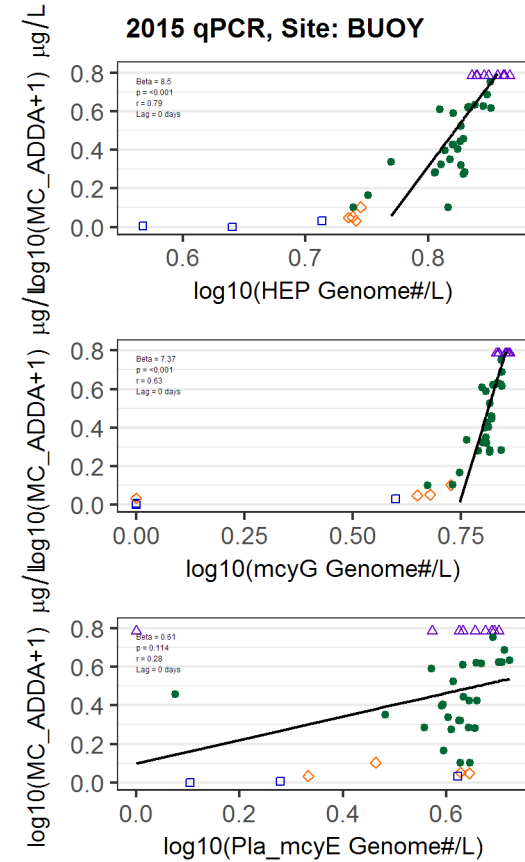
Relationships

2015 HF Parameters Lake Harsha, Site: BUOY



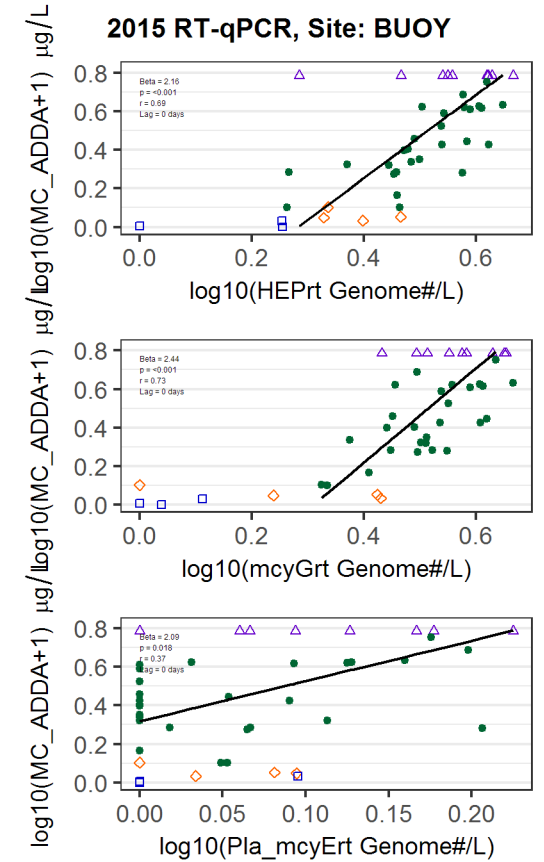
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2015 qPCR, Site: BUOY



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2015 RT-qPCR, Site: BUOY

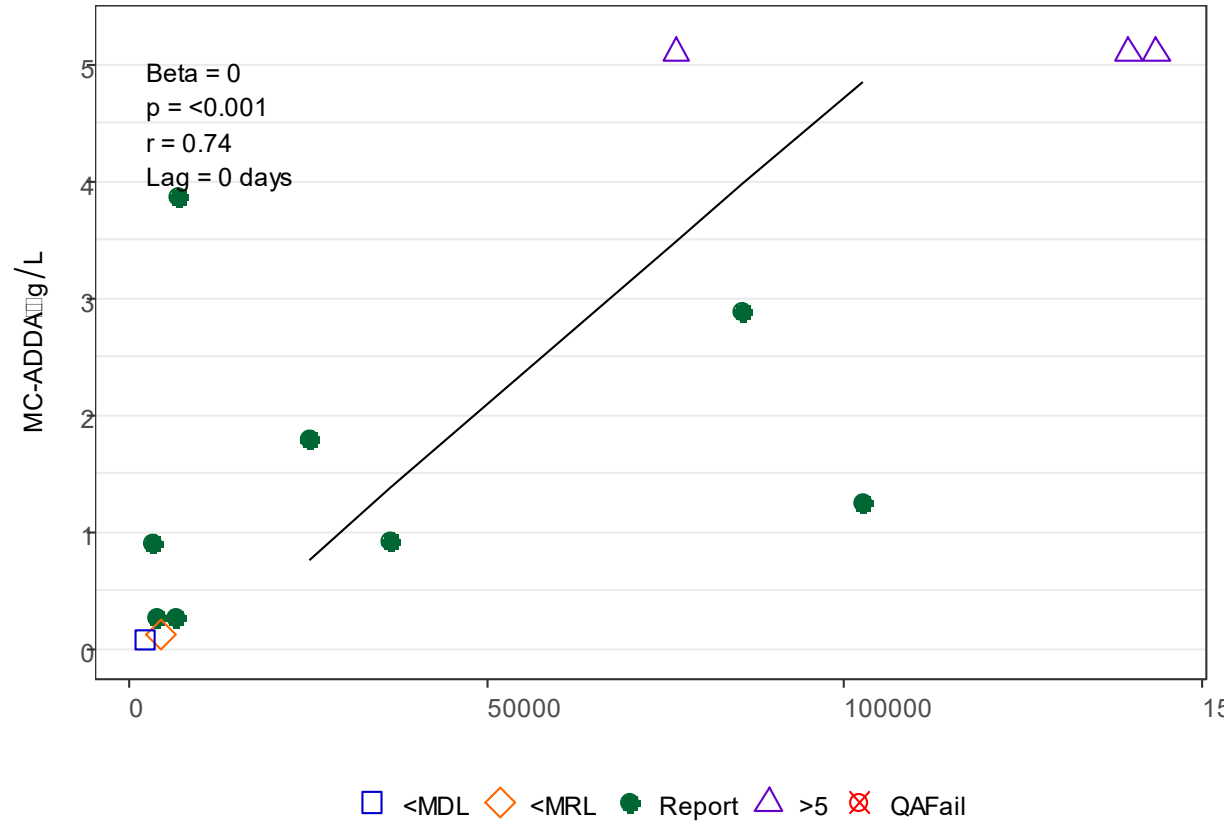


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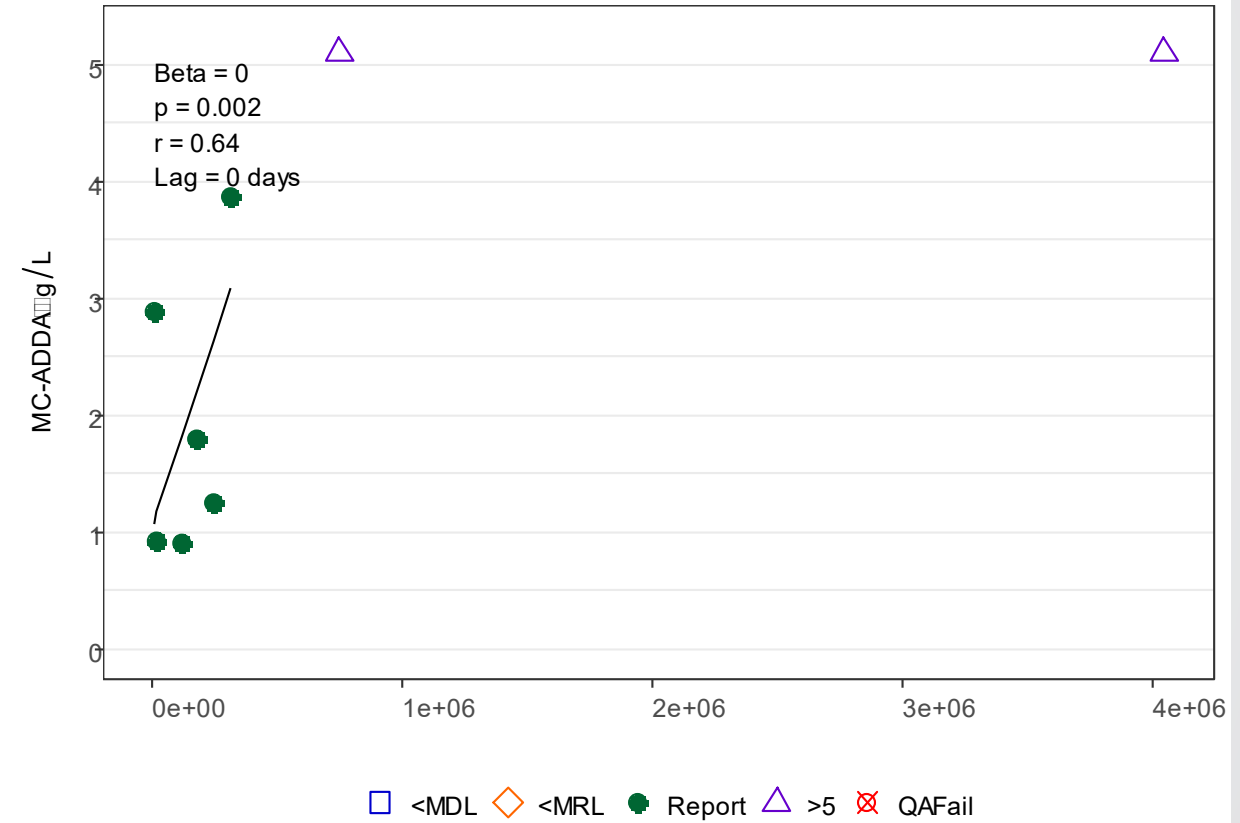


Relationships

2015 *Anabaena* Cell Density Site BUOY



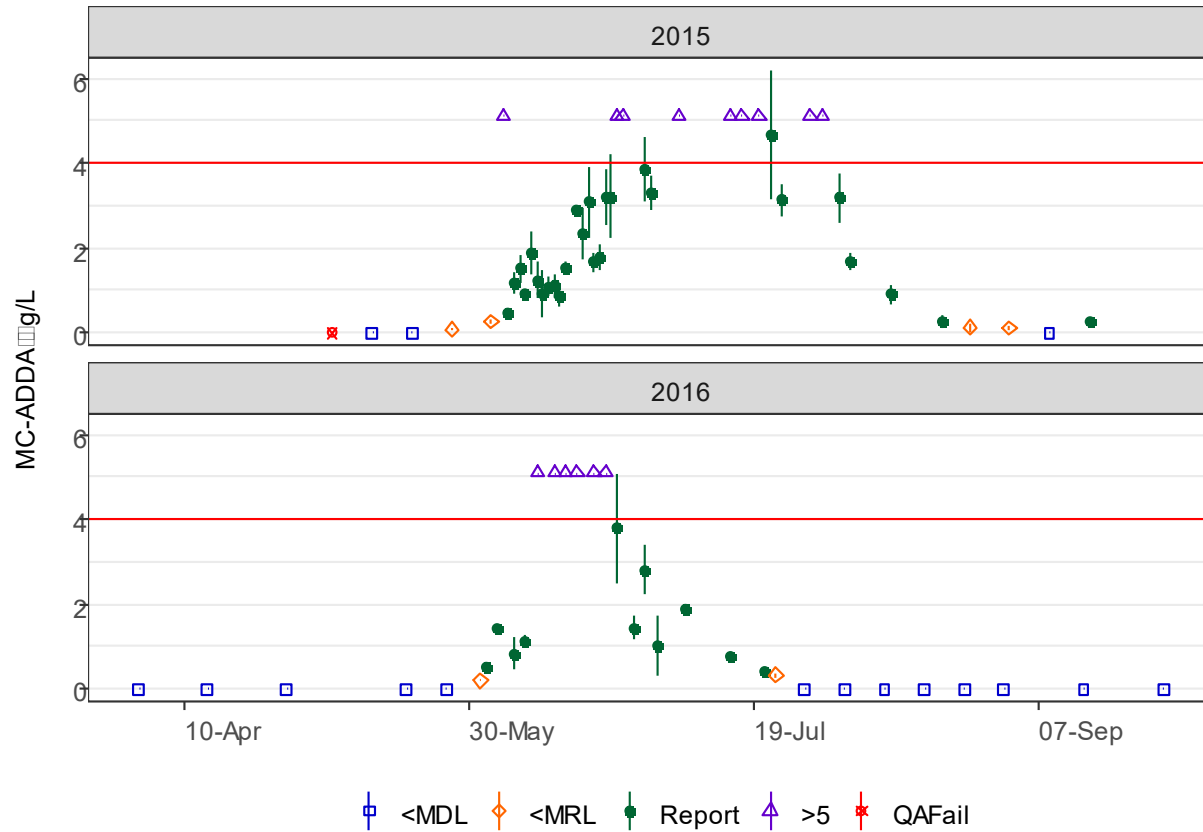
2015 *Microcystis* BioVolume Density Site BUOY



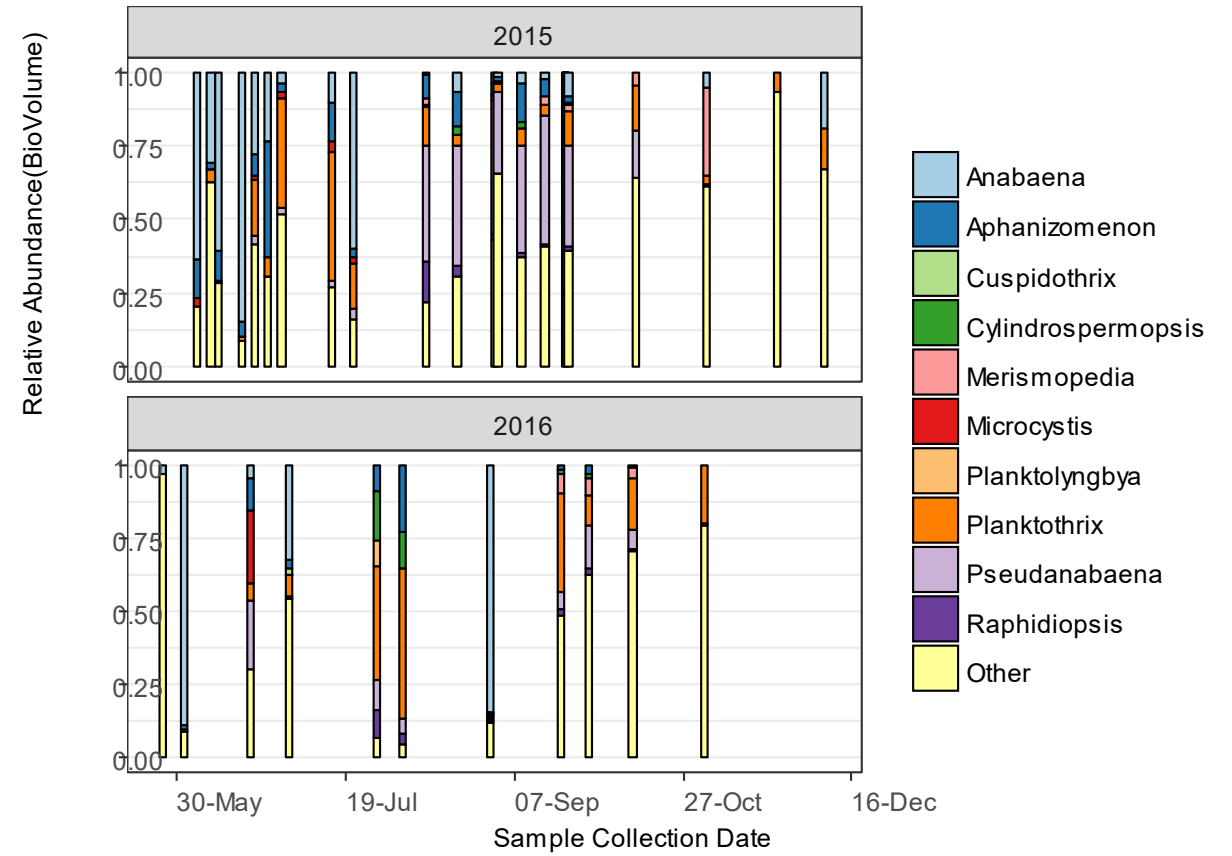


Relationships

Raw MC-ADDA, Site: BUOY



Cyanobacteria BioVolumeDensity Lake Harsh





Summary

- Lake Harsha is a eutrophic inland reservoir located in the interior plateau ecoregion
- A variety of tools were used to characterize the cyanobacterial community and environmental factors during the cyanoHAB events of 2014-16
- High resolution sampling has given insight into the relationship between indicator parameters of bloom status and cyanotoxin production
 - Taxa trends show Lake Harsha is a diverse system with multiple cyanotoxin producing groups
 - Cyanotoxin analytical techniques confirmed the presence of cyanotoxins over the course of the season
 - ELISA may be better for magnitude
 - LC-MSMS demonstrates diversity of MC congeners present
 - *in-vivo* fluorescence can provide useful information regarding HAB status
 - Molecular techniques indicate the occurrence of genes involved in cyanotoxin production and correlate well with cyanotoxin water column concentrations
- Evidence suggests *Microcystis* is the greatest contributor to microcystin production