The Influence of Turbidity, Light Intensity, and Temperature Stress on Microcystin-Production by Toxic Cyanobacteria in Oligotrophic Systems and Batch Experiments

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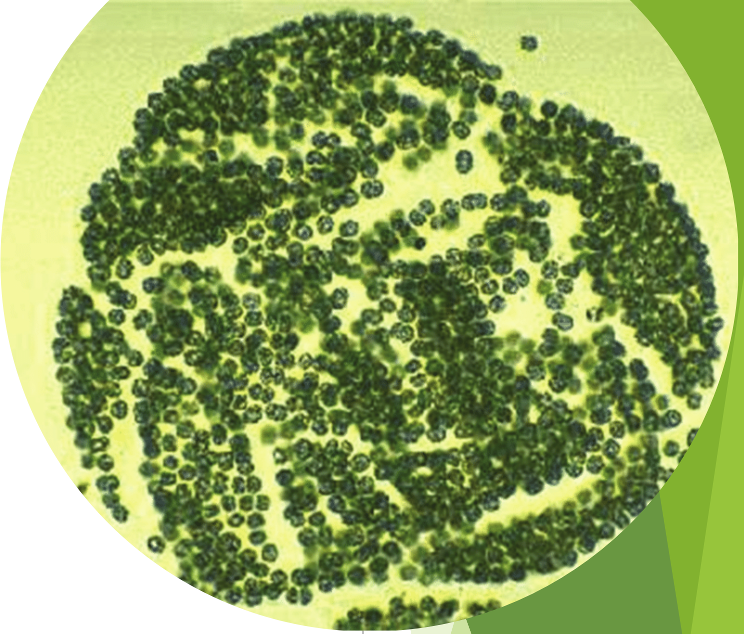
Project Overview

- Toxin-producing cyanobacterial harmful algal blooms (HABs) are being observed for the first time in Duluth-Superior Harbor (DSH)
- Barker’s Island Beach (BIB) has experienced two toxic blooms in the summer/autumn of the last two years
- Water quality data was collected June-October 2022 to identify potential environmental triggers for toxin production
- Light intensity, turbidity, and temperature identified as potential stressors
- Batch experiments will be conducted on toxic cultures to study the relationships between toxin production and triggers
- Field studies will be conducted to validate experimental results

What are Cyanobacterial HABs?

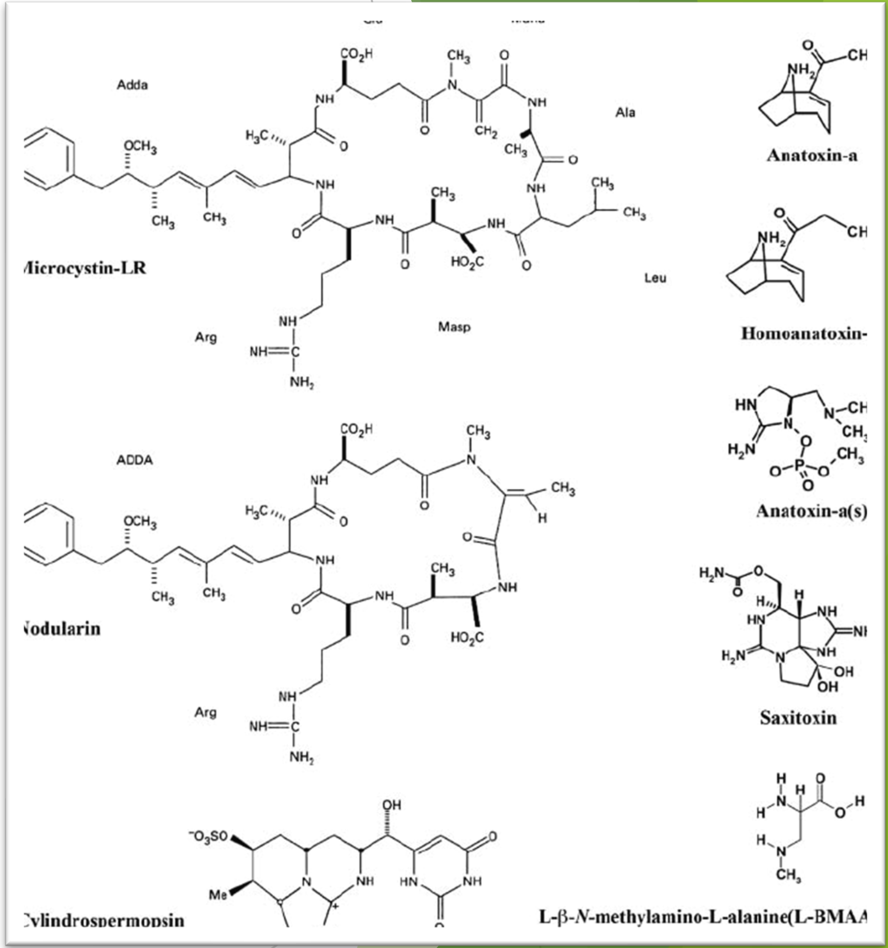


- HABS comprised primarily of cyanobacteria
- Phylum of gram-negative bacteria
- Oldest photosynthetic organisms and only photosynthetic prokaryotes – responsible for creating the planet's oxygen-rich atmosphere
- Prefer warm, shallow, stratified, eutrophic waters and sunny conditions
- Can release a range of toxins under stress
- Can aggregate to form large, unsightly surface "blooms" that pose significant economic, human health, and ecological risk

Source: Wisconsin Public Radio

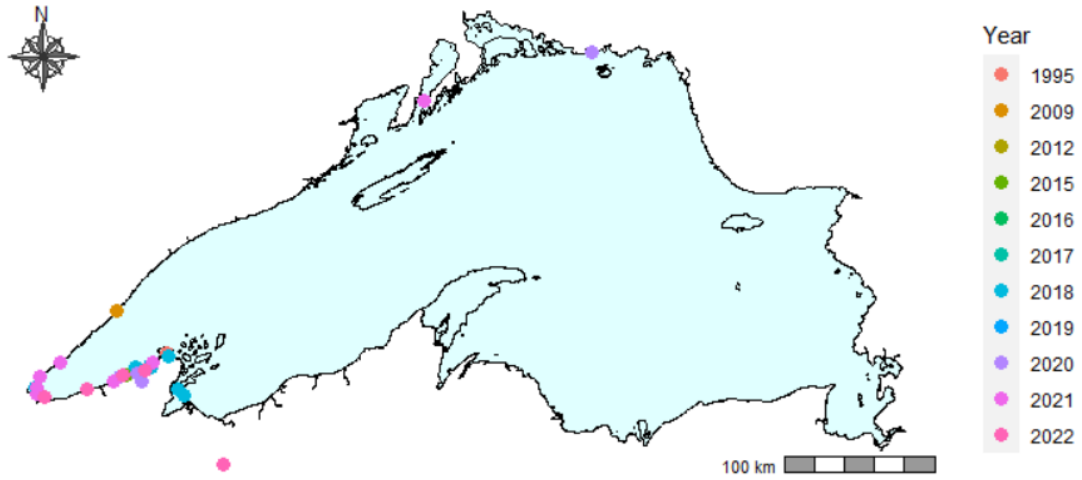
Cyanobacterial Toxins (cyanotoxins)

- Toxic compounds released by cyanobacteria as secondary metabolites
  - Not required for growth, development, or reproduction
  - Released during times of oxidative stress (grazing, WQ changes, etc.)
  - Toxic to humans and animals - known to bioaccumulate in crops and fish
- Most common is Microcystin
  - MC-LR
  - Synthesized by a range of species
  - Potent hepatotoxin and possible human carcinogen
  - Responsible for the deaths of several dogs, livestock, and fish



The Problem

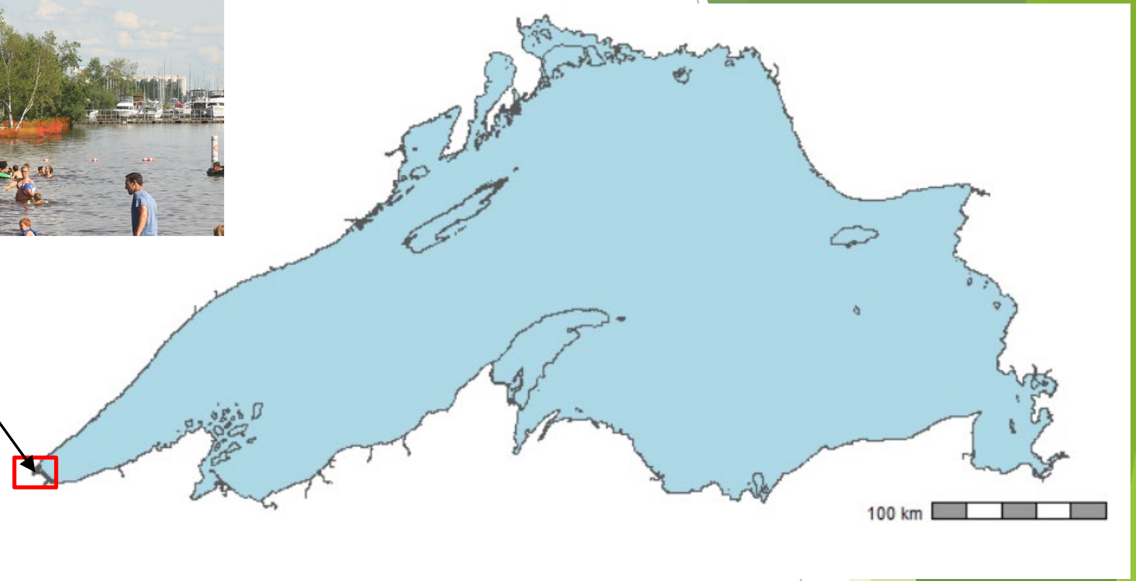
- Cyanobacterial HABs are increasing in frequency + intensity + toxicity
  - Global warming
  - Increased nutrient input
  - Increased human activity
  - Altered precipitation events (heavy rain, drought, etc.)
- Frequent occurrence in the Great Lakes since the 60s
  - Primarily observed in Lake Erie, Green Bay, Saginaw Bay
- Recent occurrence in Lake Superior is a phenomenon
  - Cold + Oligotrophic
  - Numerous algal blooms reported along the southwestern part of the lake along the Wisconsin nearshore in the last several years
  - Generally localized and very small in comparison to inland lakes – not as extensive as Lake Erie



Data: Lake Superior Nearshore Monitoring Group (LaFrancois, Benesh)

U.S. Environmental Protection Agency

The Setting



- **Barker's Island Beach (BIB)**
  - Popular recreational destination in DSH
  - Surrounding land use primarily commercial and residential
  - Closed in September 2021 for toxin bloom (microcystin > 8 $\mu g \cdot L^{-1}$)
    - Primarily *Dolichospermum lemmermannii*

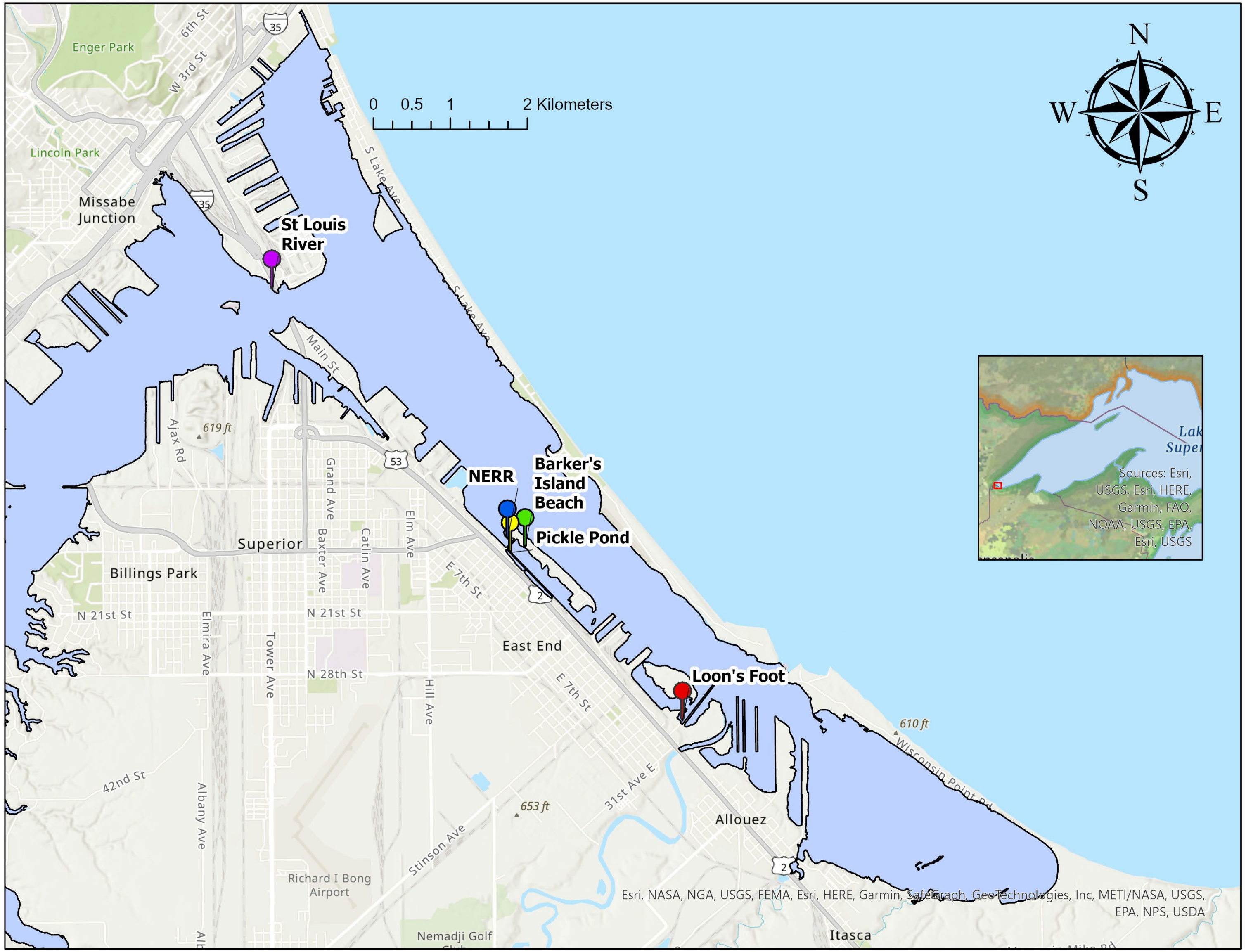
Source: Wisconsin Public Radio

The Big Question

- What is it about the water quality of the Duluth-Superior Harbor that is causing toxic bloom formation?
  - Which water quality factors are associated with BIB blooms?
  - What types of conditions are correlated with toxin synthesis and release?
  - Do lab experiments translate effectively to field observations?

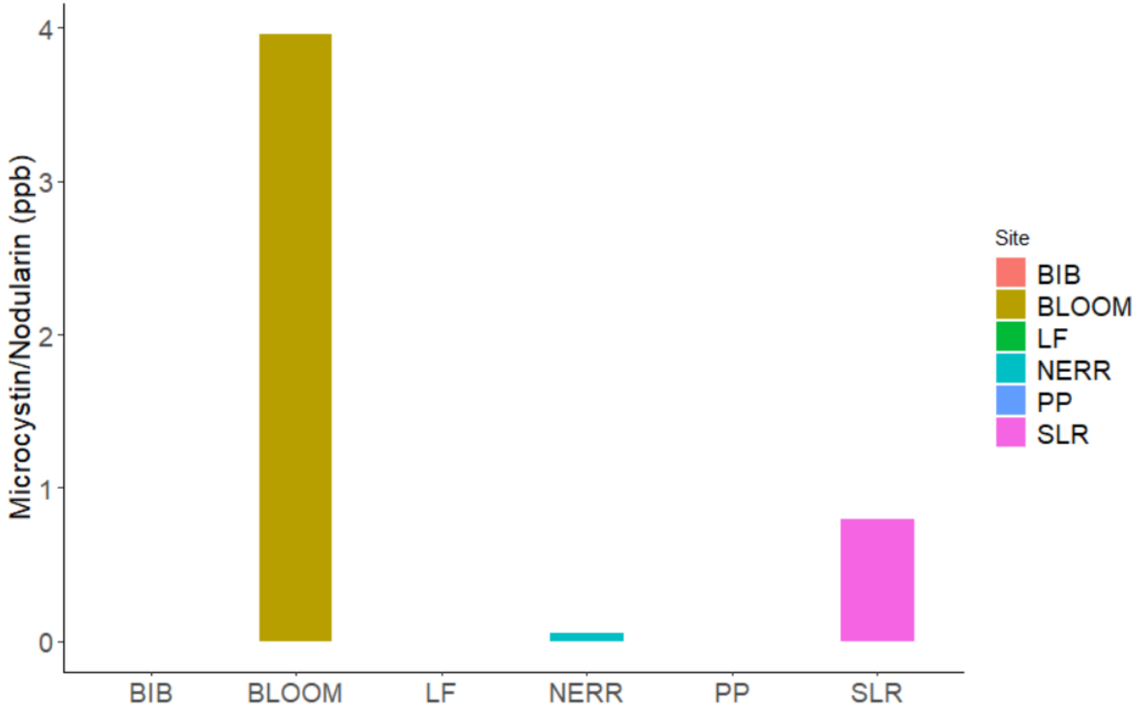
Collect Data

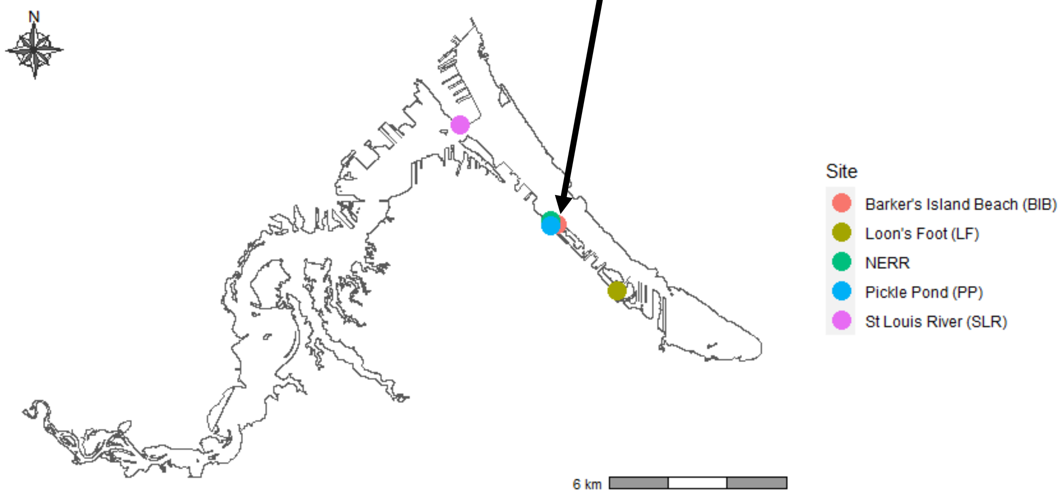
- 5 sites weekly to capture spatiotemporal variability June-Oct 2022
- Measured WQ characteristics
  - temperature, dissolved oxygen (DO), conductivity, pH, turbidity, PAR
- Measured chlorophyll a (Chl a) and phycocyanin (PCY) concentrations using handheld fluorometer
- Collected water samples just below surface when ± 5 $\mu g \cdot L^{-1}$ change in Chl a/PCY detected by fluorometer
  - Nutrients (TP, TN)
  - Cations/anions (Fe, K, Mg, Ca, Na, Si, Cl, SO4)
  - Chl a, PCY (extraction)
  - Microcystin/Nodularin (MCX)
  - Dissolved Organic Carbon (DOC)
  - eDNA for metabarcoding (bloom)



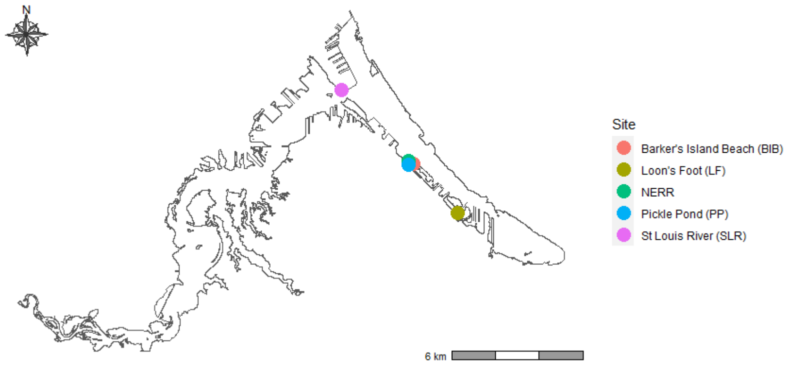
Toxic Bloom September 2022

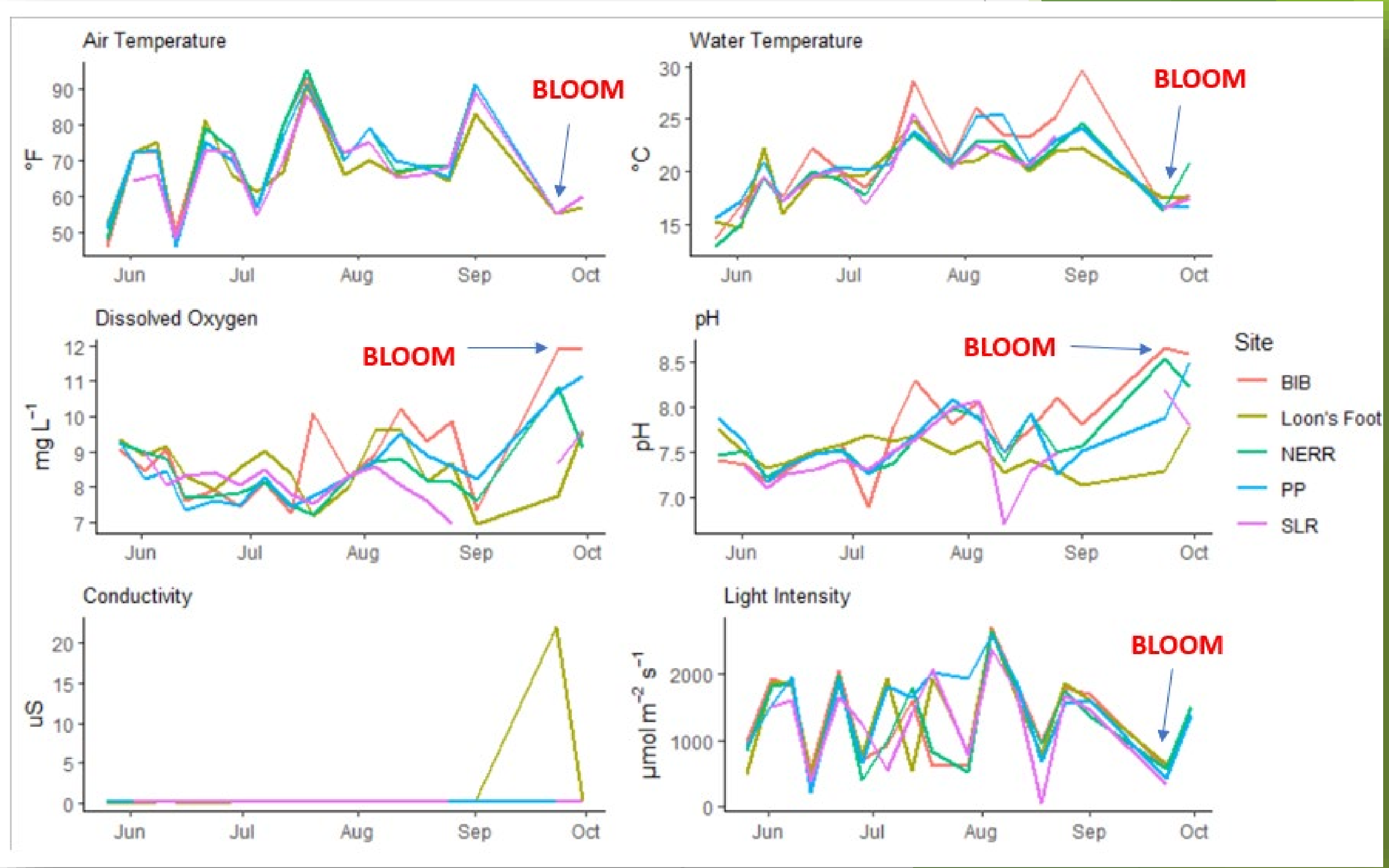
- Long skinny bloom along the shoreline of BIB
- Water samples collected for metabarcoding and culturing
- Microscope identified *Microcystis* and *Dolichospermum spp*
- MCX concentration of 3.96 μg·L^{-1} as verified by ELISA assay
  - No detectable MCX just outside bloom





Analyze available data to identify potential stressors of toxin production



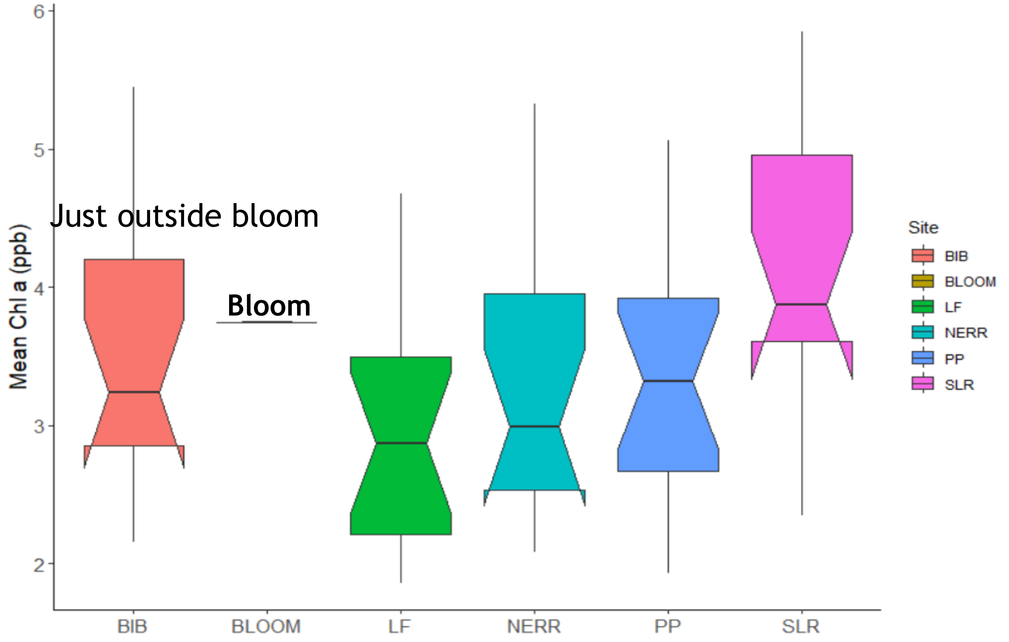


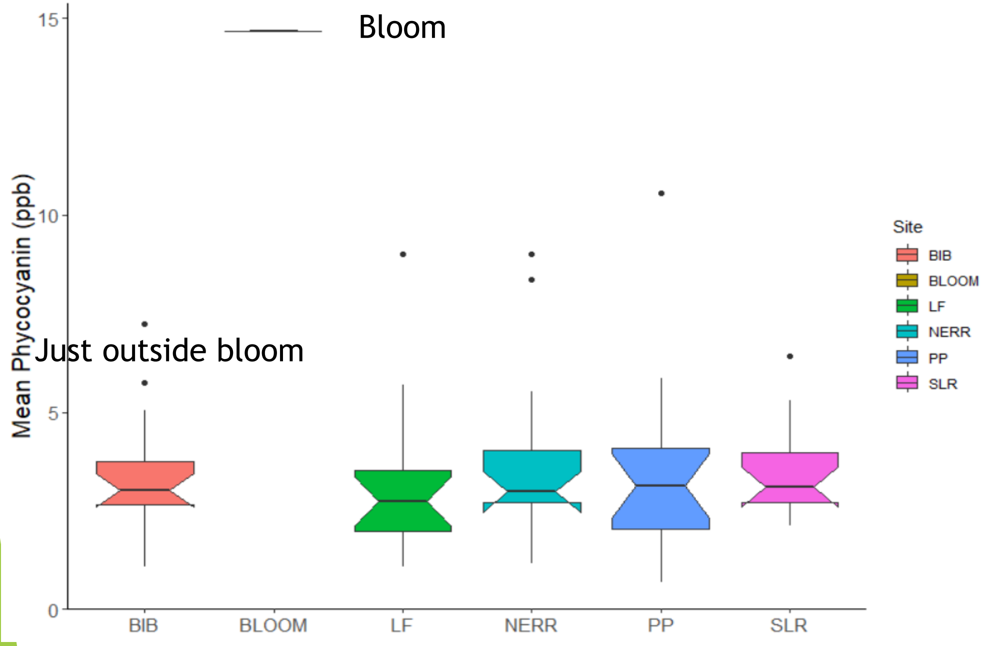
Antecedent Conditions:

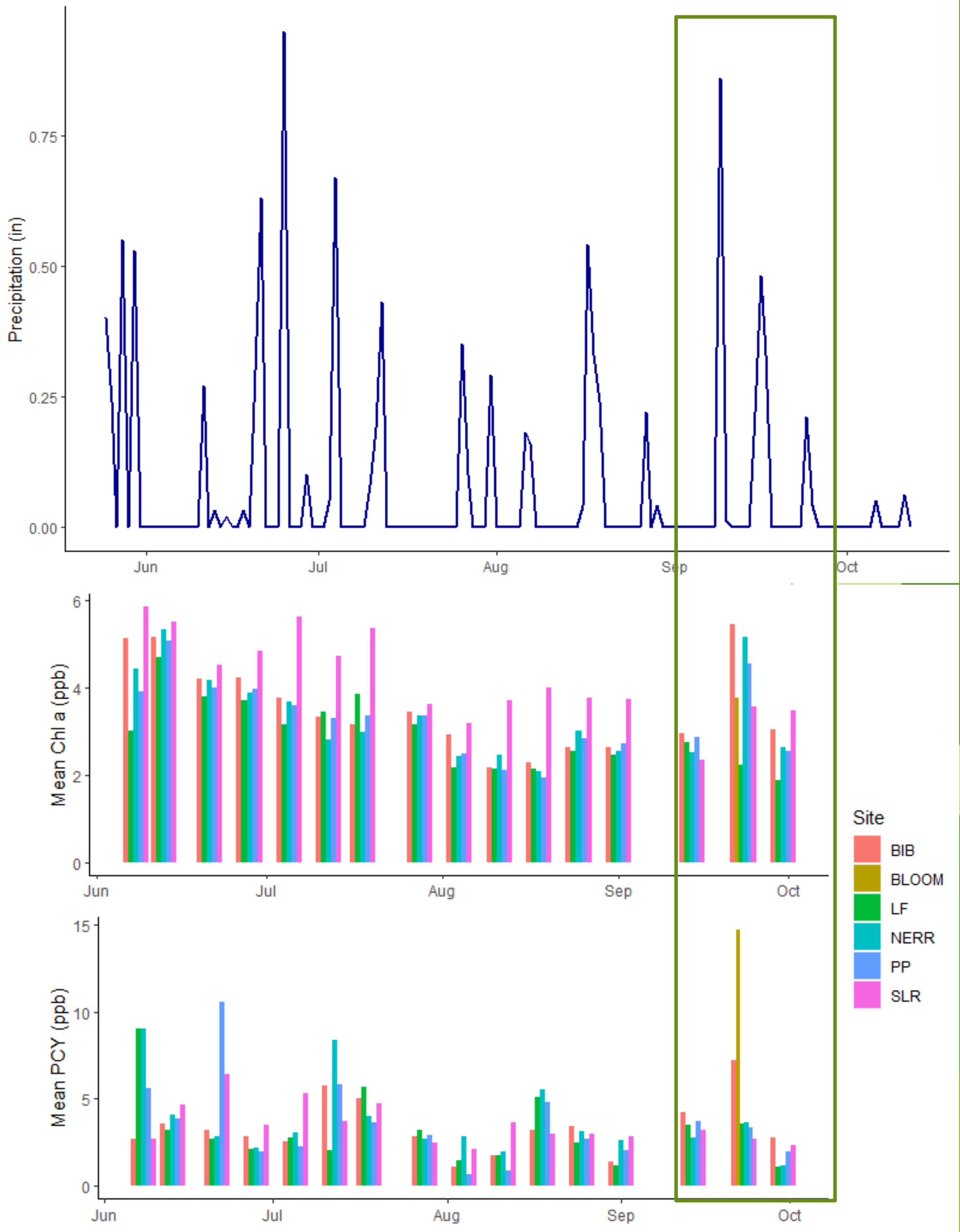
- High air temp
- High water temp
- Low DO
- High Light Intensity

Bloom Conditions:

- Low air temp
- Low water temp
- High DO
- Low Light Intensity


Just outside bloom
Bloom
Mean Chl a (ppb)
2
3
4
5
6
BIB
BLOOM
LF
NERR
PP
SLR
Site
BIB
BLOOM
LF
NERR
PP
SLR

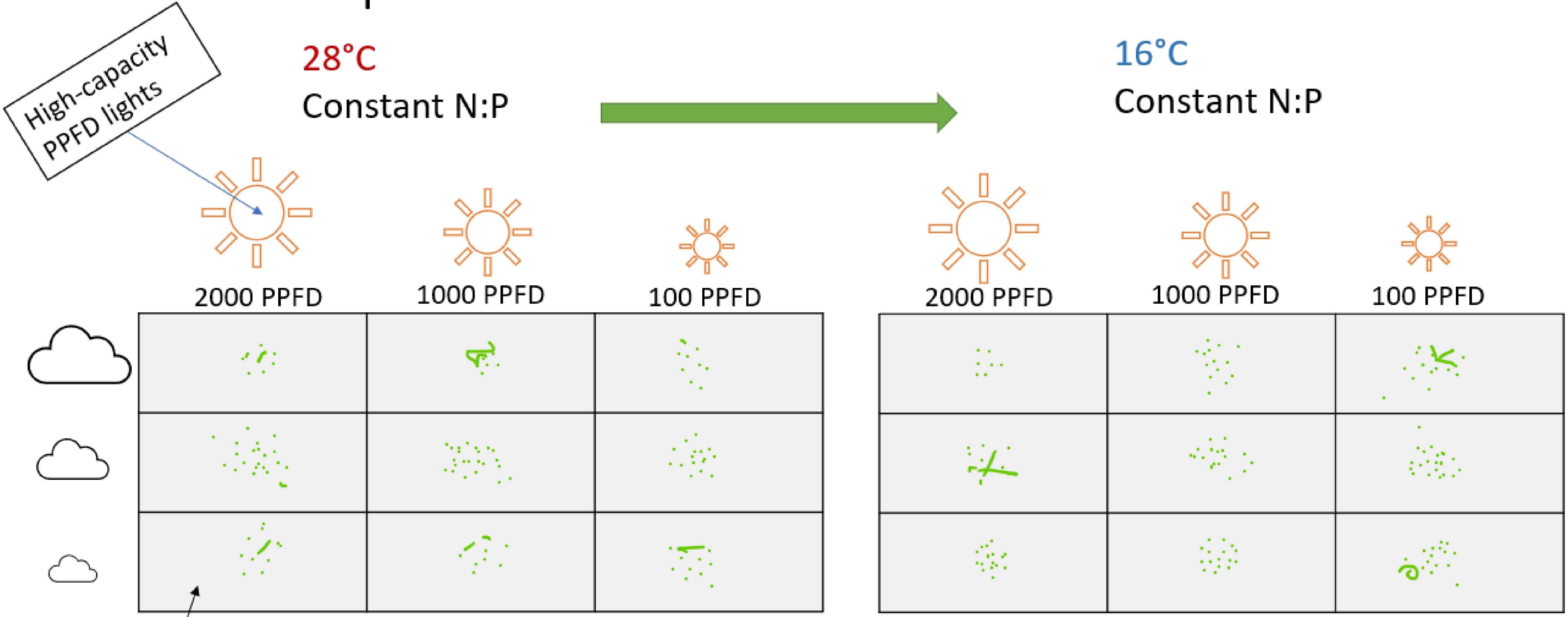

Bloom
Just outside bloom
Mean Phycocyanin (ppb)
0
5
10
15
BIB
BLOOM
LF
NERR
PP
SLR
Site
BIB
BLOOM
LF
NERR
PP
SLR


Precipitation (in)
0.00
0.25
0.50
0.75
Jun
Jul
Aug
Sep
Oct
Mean Chl a (ppb)
0
2
4
6
Jun
Jul
Aug
Sep
Oct
Mean PCY (ppb)
0
5
10
15
Jun
Jul
Aug
Sep
Oct
Site
BIB
BLOOM
LF
NERR
PP
SLR

Field Conclusions

- Toxic bloom occurred after notable drop in air and water temperatures + light intensity
- Hypothesis: Sudden changes in environmental conditions triggered toxin production by stressed cyanobacteria
  - Null hypothesis: no changes in MC production with changing conditions
  - Alternative Hypothesis: High light intensity, temperature drop, low turbidity = Highest MC production
- Batch experiments will attempt to isolate factors or combination of factors to pinpoint triggers of toxin production
  - Varying turbidities will be used to account for any other “unknown” factors and to mimic changes caused by precipitation events

Batch Experiments



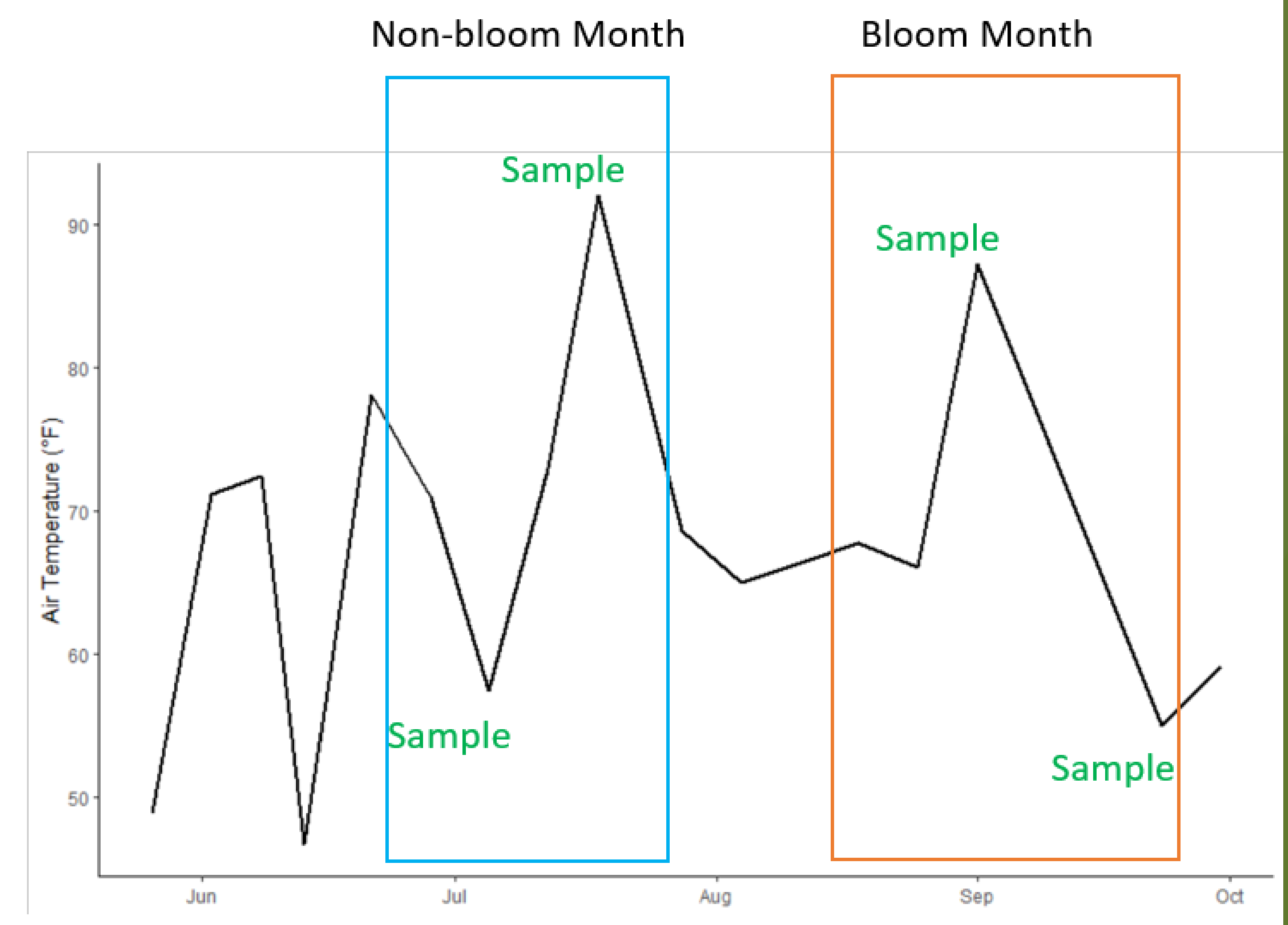
DSH Water (125mL, concentrated down to varying turbidities)

2 timepoints: 0 and 48 hours

- MCX (ELISA)
- Chl a/PCY (extraction/fluorometer)
- eDNA to track gene transcription of *mcyA-D genes (if feasible)*

Validate

- Compare field observations to batch experiments:
  - June – October 2023
  - Collect water samples at 3 discrete depths (surface, mid, bottom) – turbidity gradients
  - Collect on shady and sunny side of dock – light intensity
  - Collect during high and low temperatures during July (non-bloom month) and high and low temperatures in September (bloom month)
    - Sample daily after Labor Day until bloom is observed
  - Sample after major precipitation events (if feasible)



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