Managing CyanoHABs and Cyanotoxins in Freshwater systems: Current Research

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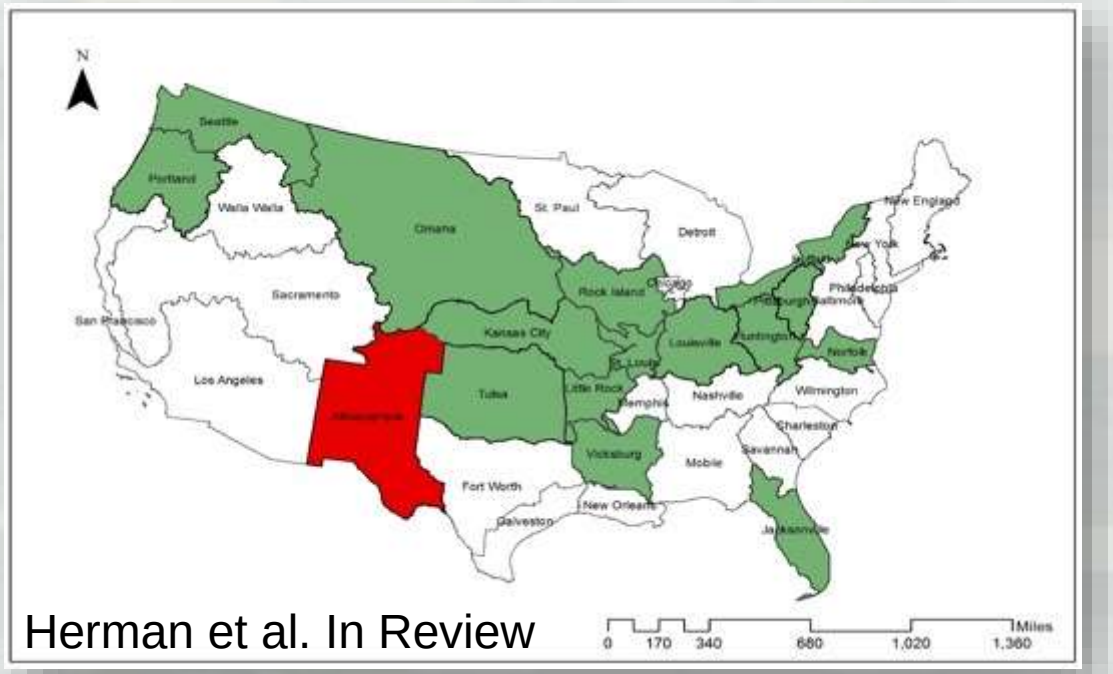
Harmful Algal Blooms (HABs) and the USACE

- The USACE manages over 400 freshwater lakes to provide a variety of services including recreation, fish and wildlife management, and potable water supplies
- Cyanobacteria, the most common group of freshwater harmful/nuisance algae, have been documented in 43 of the continental united states.
- Additionally, a 2014 USACE-wide survey identified cyanobacteria and golden algae as the predominant bloom-forming groups in Corps operated reservoirs.



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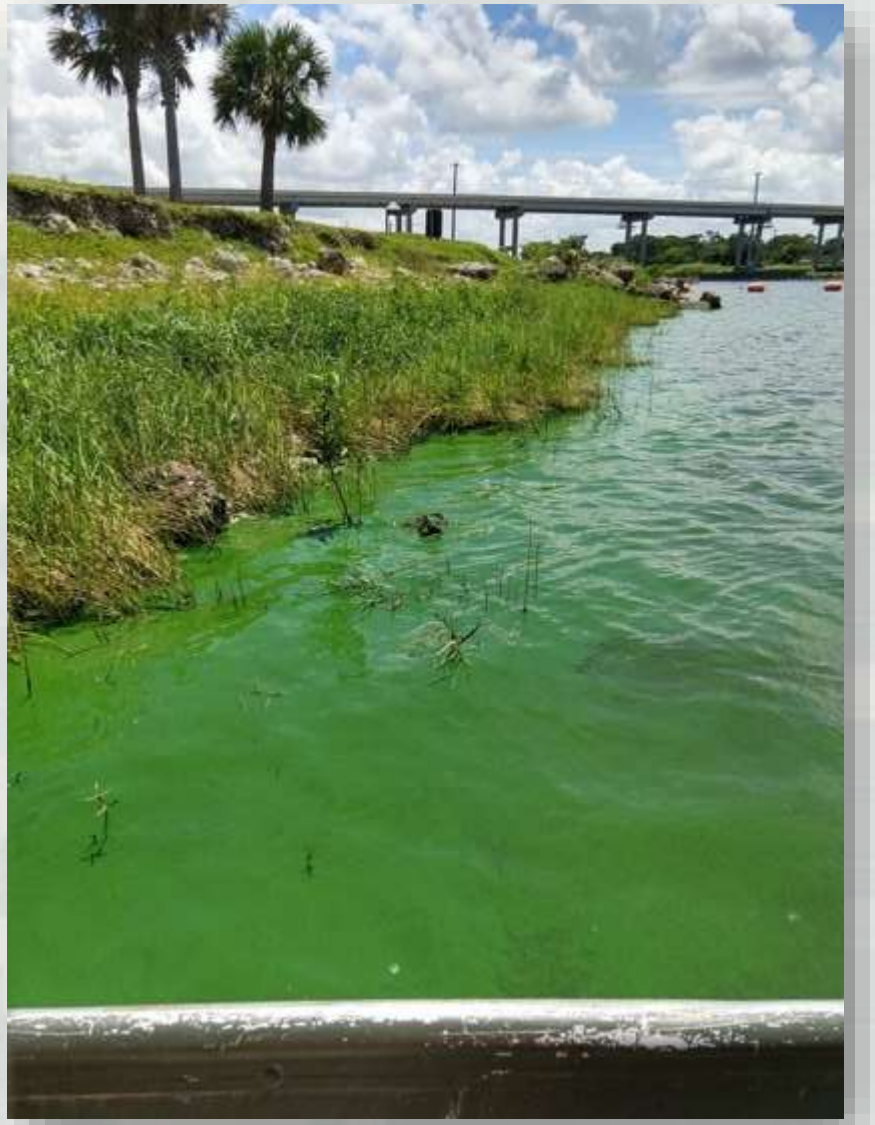
- The survey also showed a recent increase in the frequency of HAB events at USACE Districts.
- As such, ERDC HAB research and service capabilities are rapidly expanding.

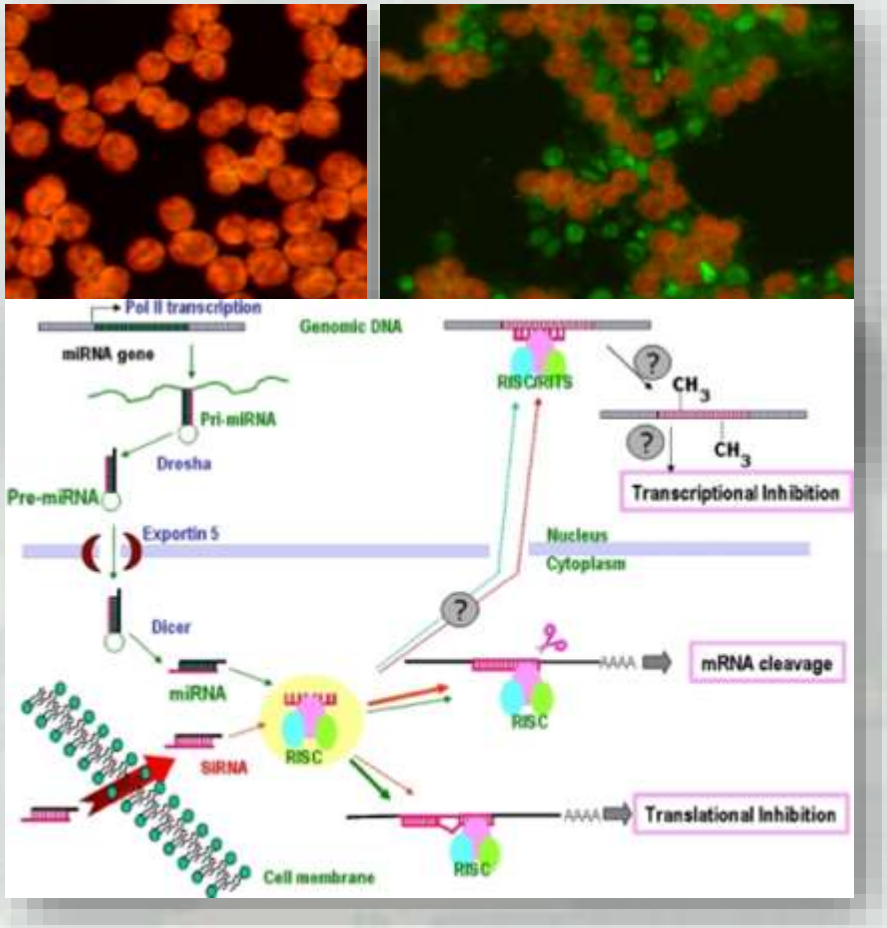
ERDC Support…

- **Technical Areas**
  - Water Quality
  - Detection
  - Nutrient Reduction
  - Risk Assessment
  - Management
- **Ongoing Research Projects**
- **Previous Research Projects**
- **Reimbursable services**



Ongoing HAB Research Projects

- **Detection**
  - Using HAB indicator estimation in small inland waterbodies: Remote sensing-based software tools to assist with USACE water quality monitoring
- **Management**
  - Scalable algaecide studies
  - Small regulatory RNAs for the control of HABs - an environmentally benign approach
  - HABITATS – Harmful Algal Bloom Interception, Treatment and Transformation
  - Operational strategies to control HABs



POC: Ping Gong (Ping.Gong@usace.army.mil)

Previous HAB Research Projects

- **Detection / Water Quality**
  - Field-based detection using hyperspectral imaging
    - *Analysis of historic water quality from bloom and non-bloom lakes*
    - *Microscopic counts of algae/cyanobacteria*
    - *Rapid molecular methods for cyanobacteria/cyanotoxins*
- **Management**
  - Hydrodynamic cavitation for the management of cyanobacteria blooms
- **Nutrient Reduction**
  - Reducing phosphorous loading using iron sorption mechanisms

3 h of flow resulted in rust

POC: Carina Jung (Carina.M.Jung@usace.army.mil)

HAB Reimbursable Services

- **Water Quality**
  - Routine water quality monitoring program
  - Surface water quality modeling
- **Detection**
  - Analytical cyanotoxin quantitation/detection (ELISA and LC/MS)
  - Hi-Sensitivity flow cytometry for identification/enumeration
- **Risk Assessment**
  - Site-specific HAB risk framework
  - Ecological risks of chemical algaecides and treatment efficacy
  - Ecological and human health risks from algal toxins

HAB indicator estimation in small inland waterbodies: Remote sensing-based software tools to assist USACE WQ monitoring

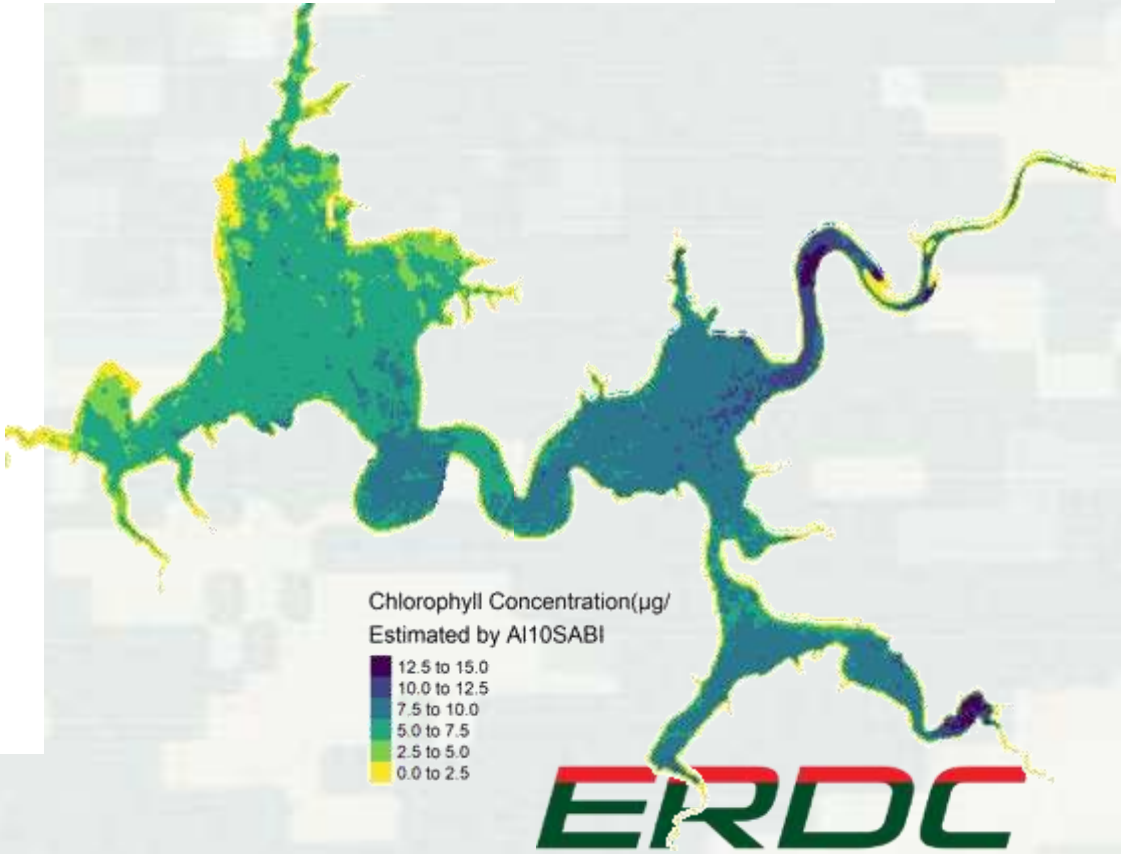
POC: Molly Reif (Molly.K.Reif@usace.army.mil)

Purpose: The USACE has the challenge of monitoring hundreds of inland lakes and reservoirs that cover vast geographic areas. Limited resources can lead to reactionary responses to HAB outbreaks.

Results: Software tools are needed to assist with remote monitoring and prediction of HABs.

Benefits:

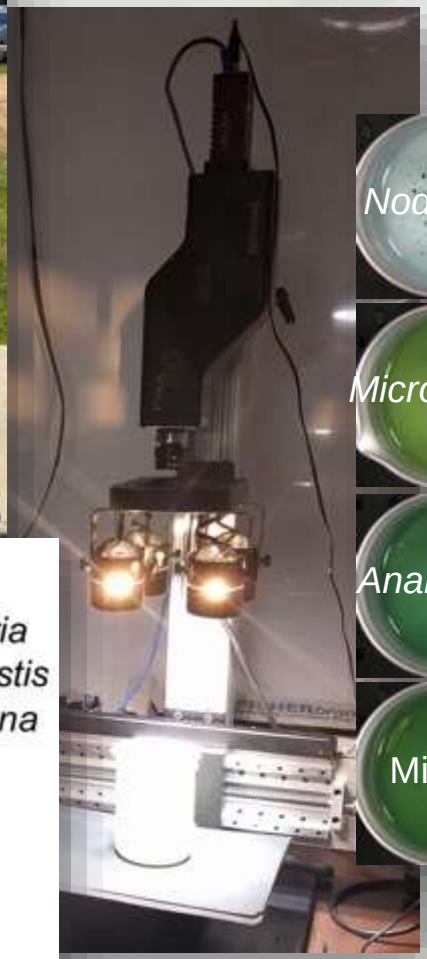
1. Hands-on R software package for developing image-based, relative abundance maps of HAB indicators (Univ of Cincinnati collaboration)
2. ArcGIS workflow tool, representing a more streamlined approach with pre-set options for HAB indicator estimation
3. A web-based viewer for limited but easy access to HAB indicator estimations, using constrained image types and algorithms

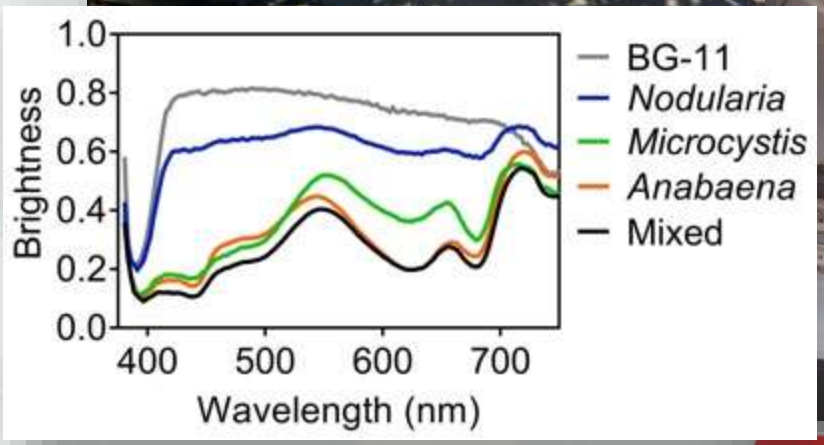


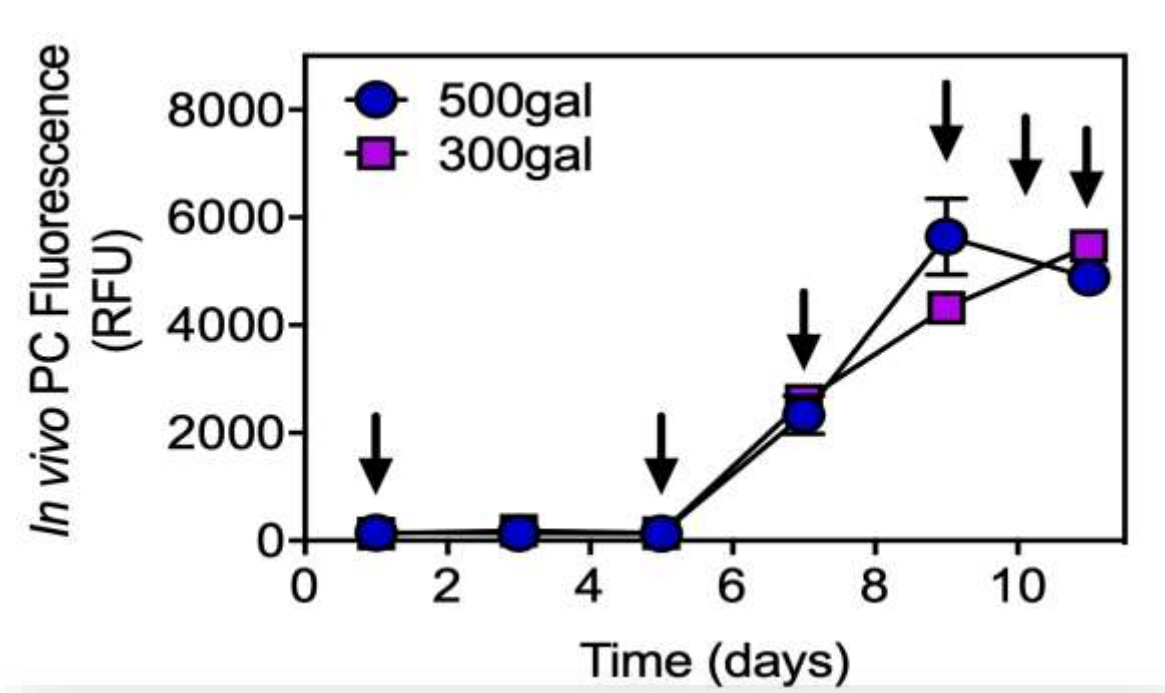
Aerial Detection using Hyperspectral Imaging

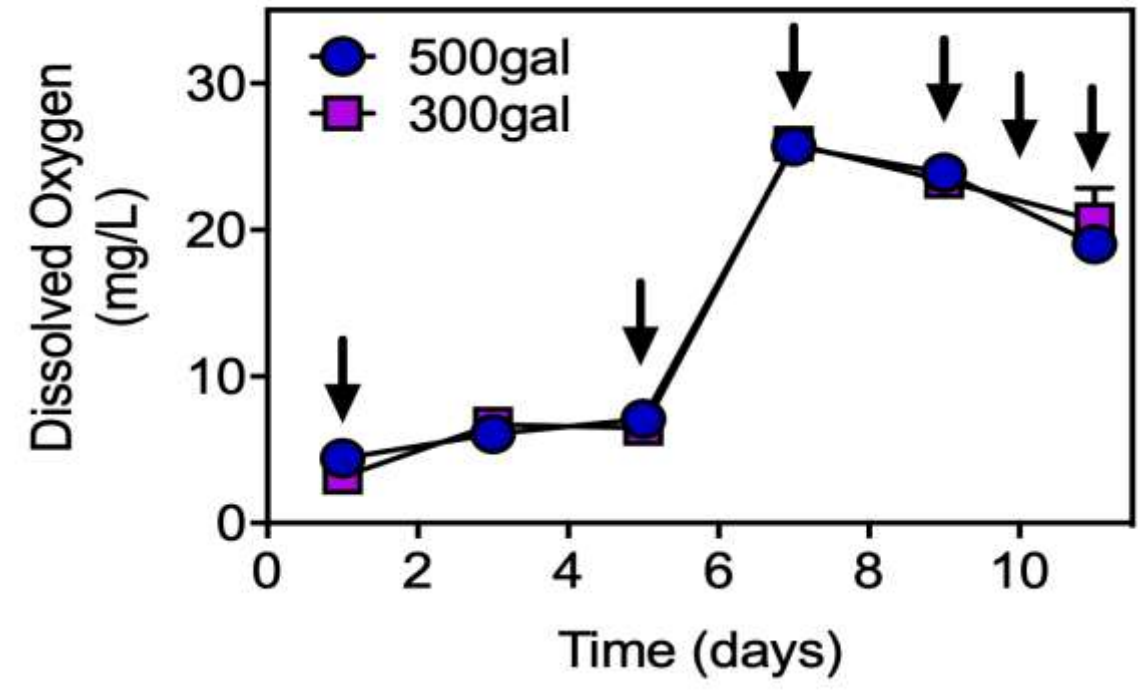
POC: Kaytee Pokrzywinski (Kaytee.Pokrzywinski@usace.army.mil)

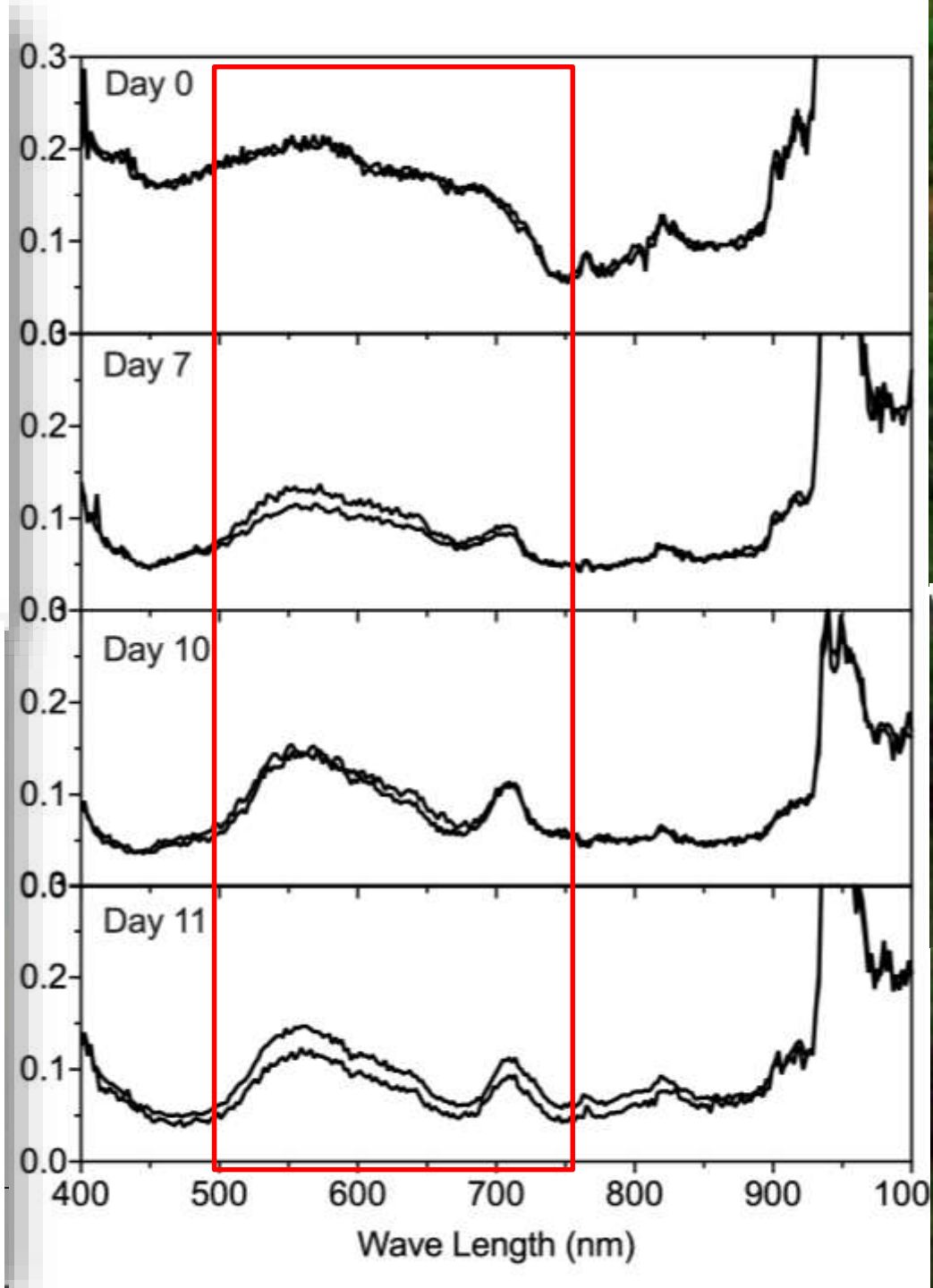
- **Purpose:** Develop new lab and field-based (lake wide) sensors for early HAB detection and monitoring. Guide point sampling efforts.
- **Results:** Unique hyperspectral signatures can be obtained from cyanobacteria, spectral shifts are associated with changes in nutrient and toxin status.
- **Benefits:** Guidance for point sampling, can reduce the amount of grab samples required and help direct where sampling should occur. Meso-scale linkage of spectra to toxin status can allow assessment of bloom extent and associated risks.

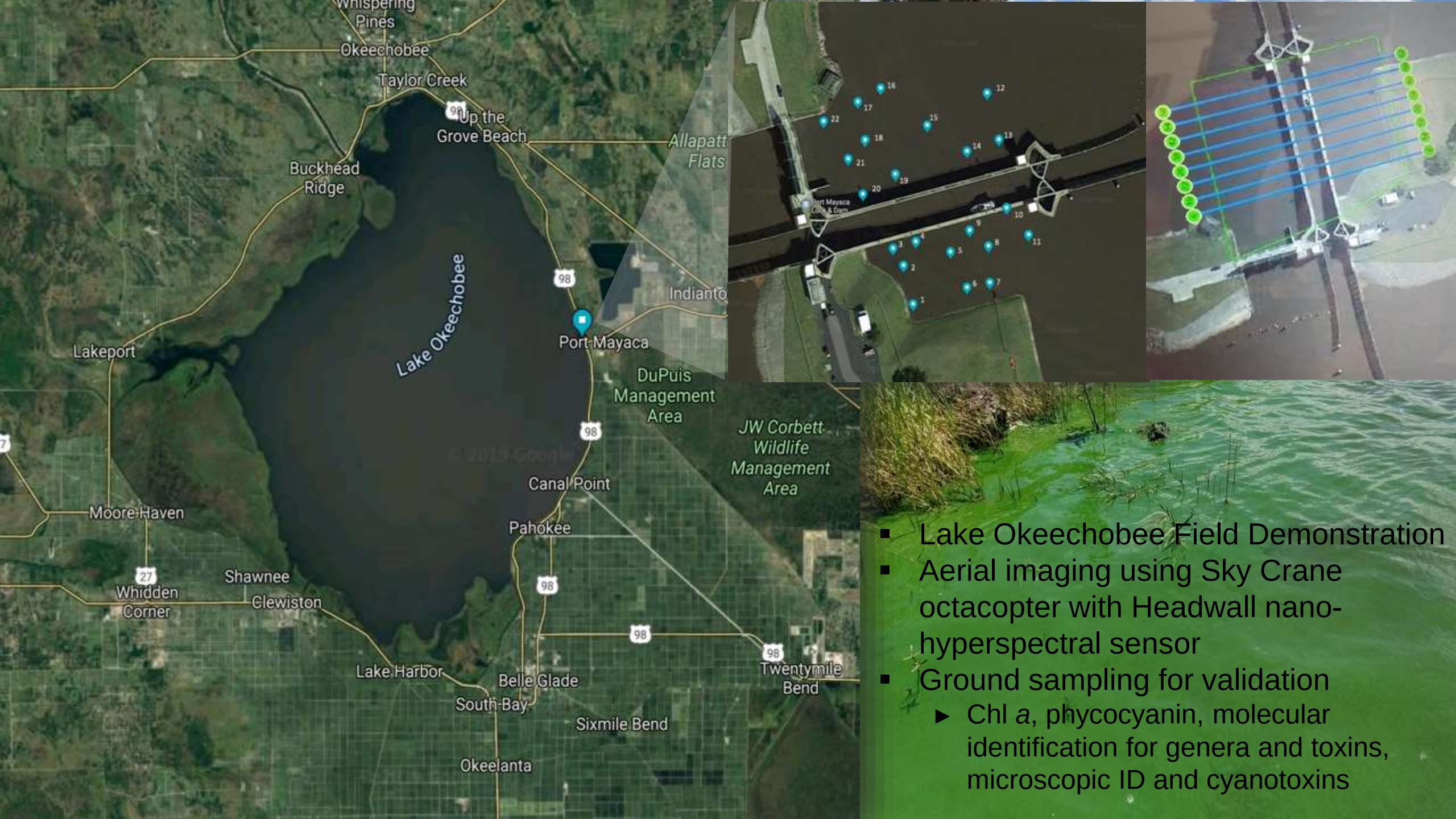




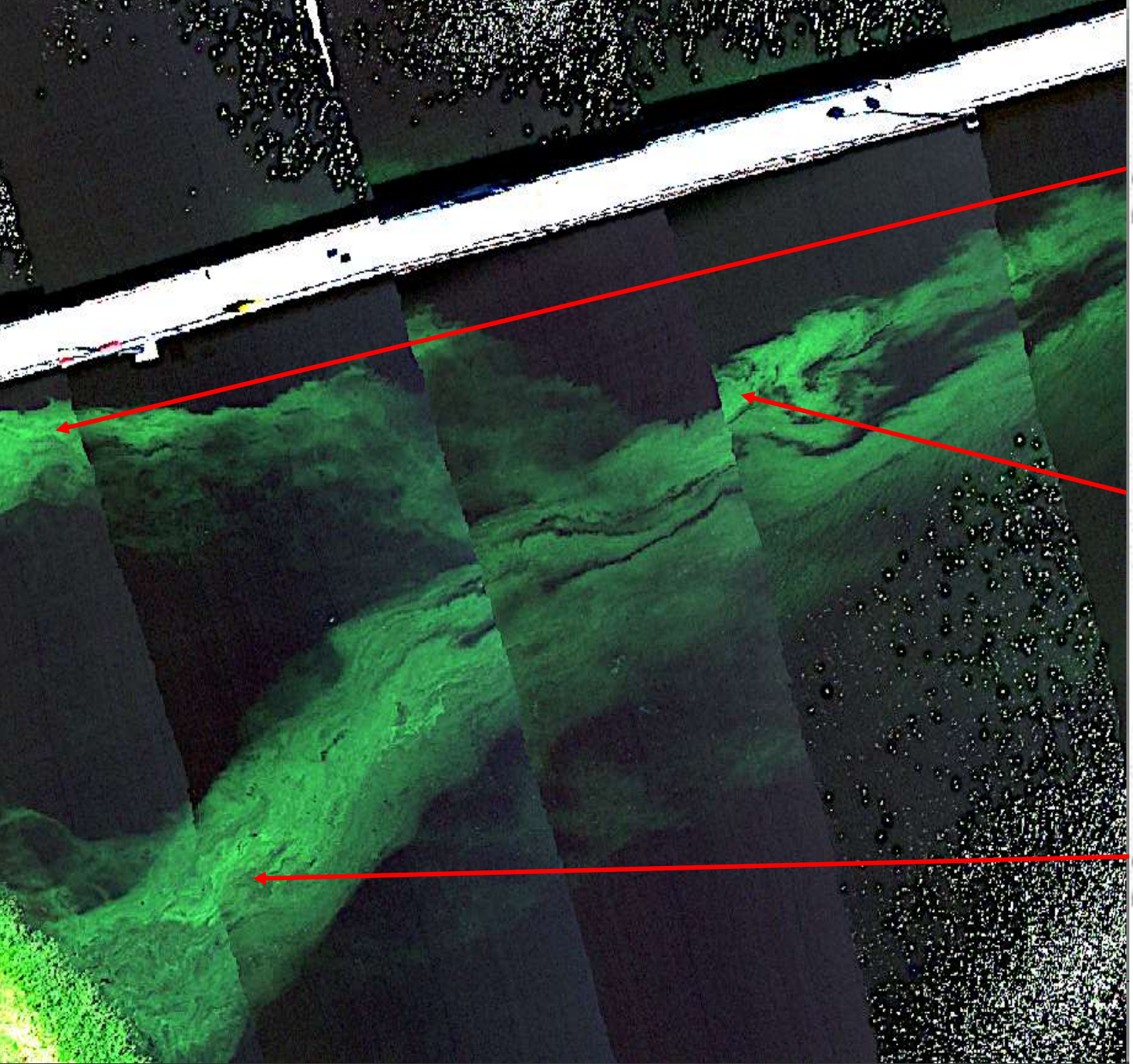

In vivo PC Fluorescence (RFU)
500gal
300gal
8000
6000
4000
2000
0
0
2
4
6
8
10
Time (days)

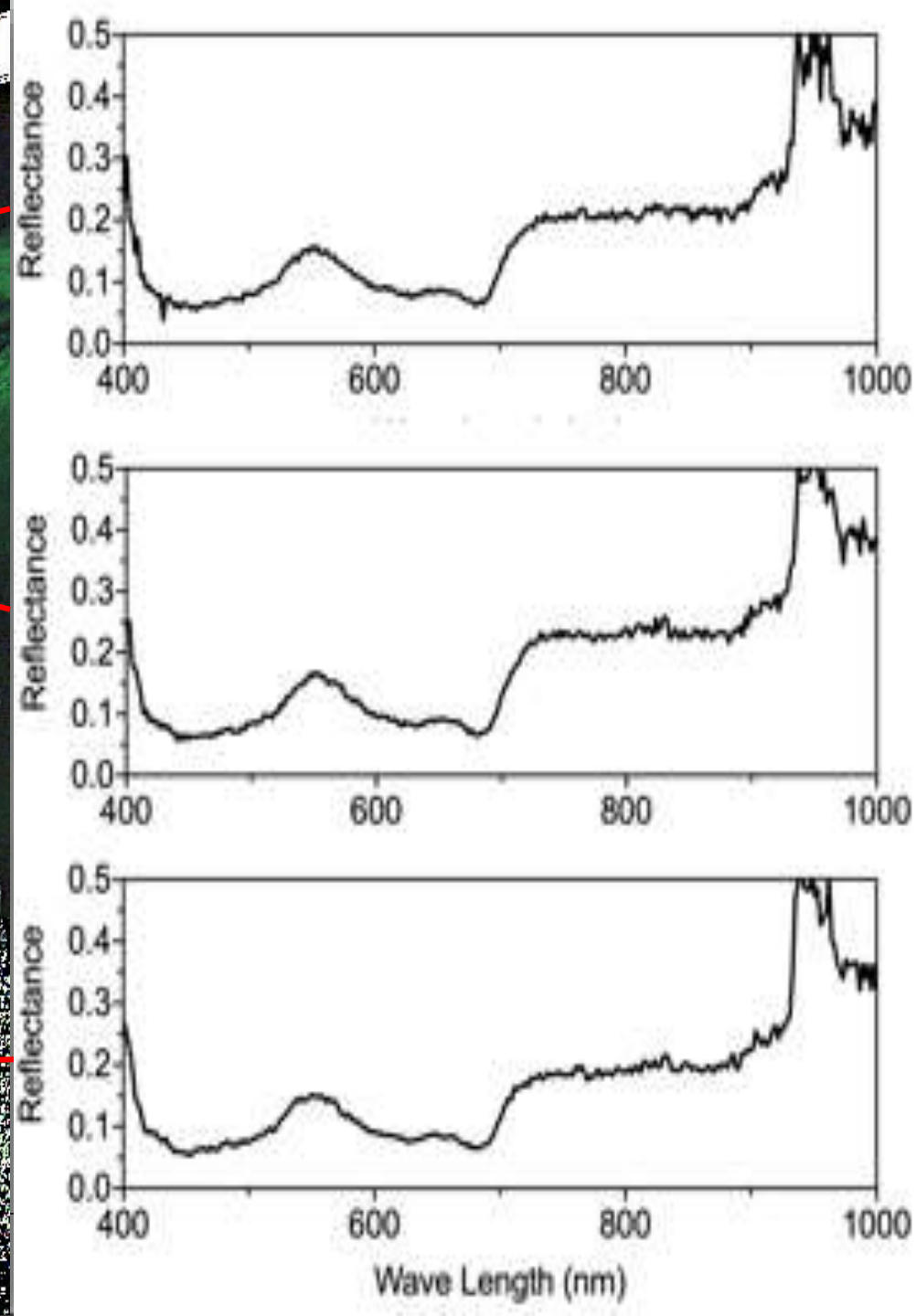

Dissolved Oxygen (mg/L)
500gal
300gal
30
20
10
0
0
2
4
6
8
10
Time (days)


Day 0
Day 7
Day 10
Day 11
0.3
0.2
0.1
0.0
400
500
600
700
800
900
100
Wave Length (nm)



- Lake Okeechobee Field Demonstration
- Aerial imaging using Sky Crane octacopter with Headwall nano-hyperspectral sensor
- Ground sampling for validation
  - Chl *a*, phycocyanin, molecular identification for genera and toxins, microscopic ID and cyanotoxins




Reflectance
0.0
0.1
0.2
0.3
0.4
0.5
400
600
800
1000
Wave Length (nm)

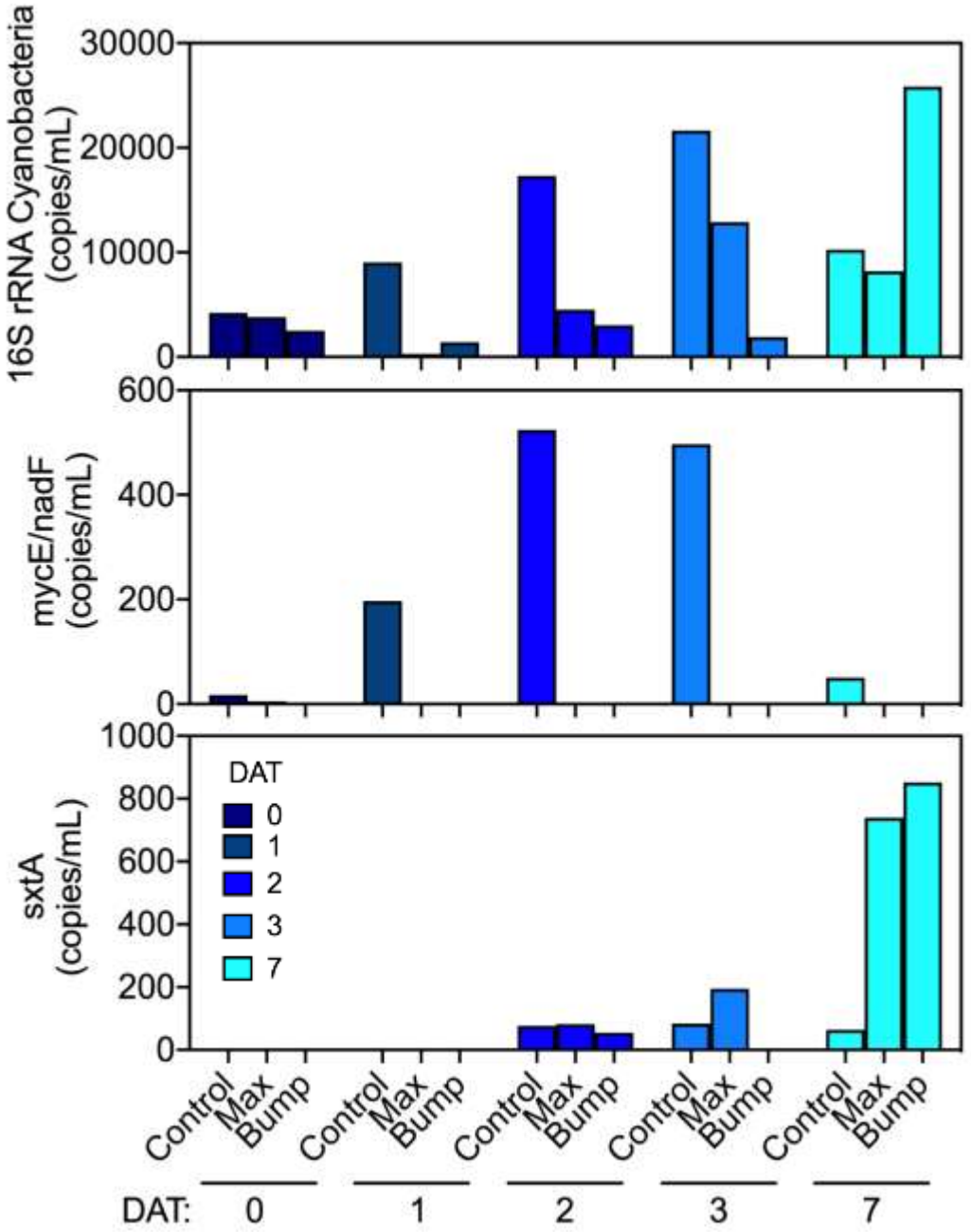
Scalable Algaecide Treatments

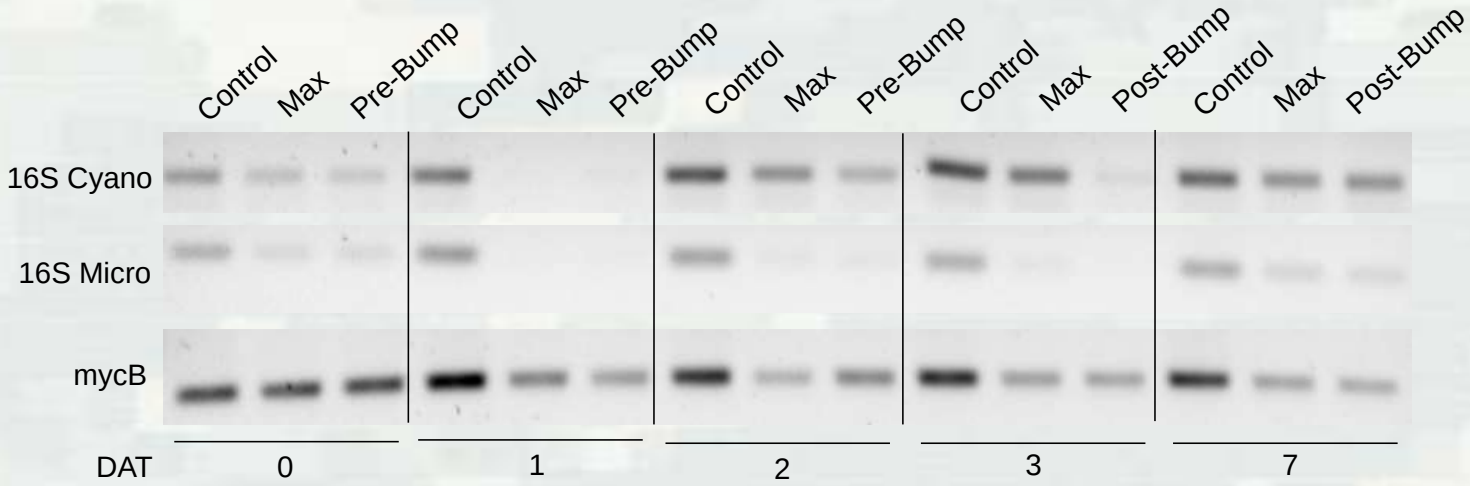
POC: Kurt Getsinger (Kurtis.D.Getsinger@usace.army.mil)

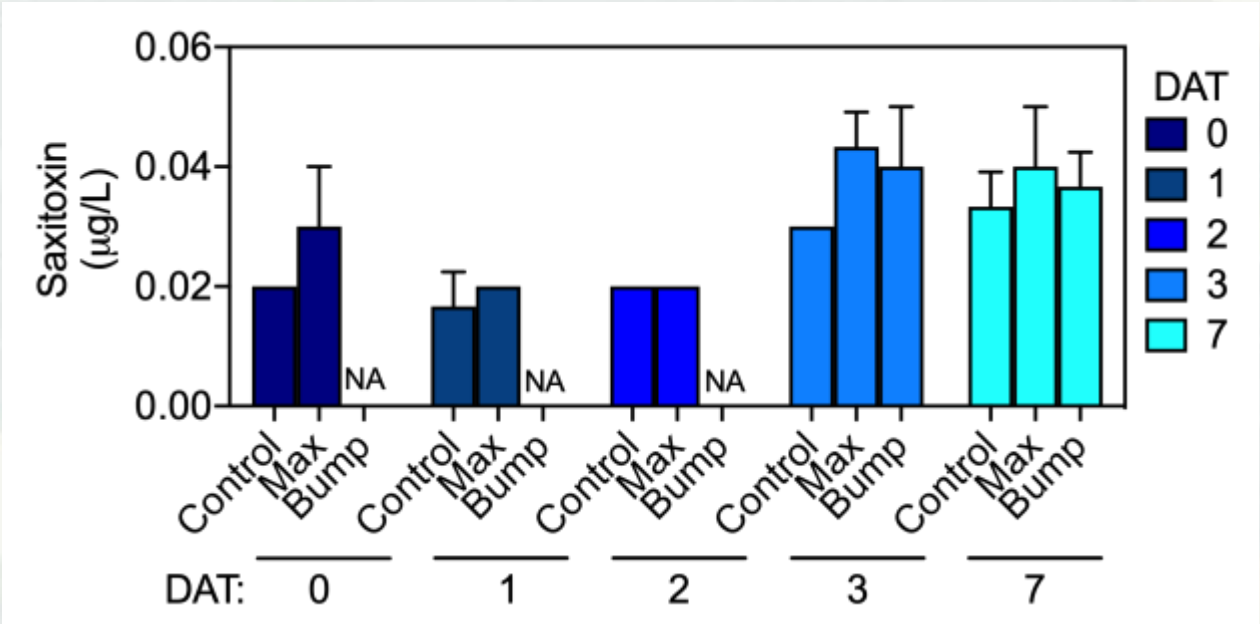
Purpose: Evaluate a peroxide-based algaecide to control cyanobacteria HABs in a small contained site on Lake Okeechobee

Results: Reduction in total cyanobacteria in treatments.

Benefits: Assist resource managers in developing an effective and rapid response strategy to control blooms in the lake and connecting waterways


16S rRNA Cyanobacteria (copies/mL)
30000
20000
10000
0
mycE/nadF (copies/mL)
600
400
200
0
sxtA (copies/mL)
1000
800
600
400
200
0
DAT
0
1
2
3
7
Control
Max
Bump
DAT: 0 1 2 3 7


Control
Max
Pre-Bump
Post-Bump
16S Cyano
16S Micro
mycB
DAT
0
1
2
3
7


Saxitoxin (µg/L)
0.06
0.04
0.02
0.00
NA
DAT
0
1
2
3
7
Control
Max
Bump
DAT: 0 1 2 3 7

ERDC

Tiered Management

POC: Kaytee Pokrzywinski (Kaytee.Pokrzywinski@usace.army.mil)

Purpose: Develop a tiered management approach to assess efficacy in the absence of confounding variables.

Results: Integrate cost, feasibility, modeling and risk management into a decision support tool.

Benefits: Assist resource managers in routinely planning for and managing algal blooms in their districts.

Small scale screening

Meso-scale screening

Full-scale demonstration

Algaecide Decision Support - Future

POC: Kaytee Pokrzywinski (Kaytee.Pokrzywinski@usace.army.mil)

Purpose: Develop cyanoHABs-specific algaecide decision support tool to assist with rapid response.

Results:

- Screening of chemical algaecides against monocultures of cyanoHABs.
- Algaecides applied at various rates and densities to assess concentration limitations on efficacy.

	Chemical A	Chemical B	Chemical C	Chemical D
Microcystis	⊘	⊘	○	⊘
Anabaena	○	○	⊘	○
Aphanizomenon	○	○	⊘	○
Cylindrospermopsis	○	○	○	○
Oscillatoria	⊘	○	○	○
Planktothrix	⊘	○	○	○

Benefits:

- Results of these studies form the foundation of a targeted decision support tool for resource managers to rapidly respond to cyanoHAB events and assess risk.
- These results can also be incorporated into site-specific modeling efforts to pre-determine efficacy at a larger scale.

Harmful Algal Bloom Interception, Treatment, and Transformation System (HABITATS)

POC: Martin Page (Martin.A.Page@usace.army.mil)

INTERCEPTION

Selectively remove algae from the water, rather than treating all the water.

TREATMENT *AND* ***TRANSFORMATION***

Clarify and oxidize the water to allow for safe discharge back into the environment, and concentrate the algae into a thick paste to minimize waste volumes.

Recover resources from the concentrated algae while destroying any potential toxins.

Year 1	Baseline pilot study
Year 2	Optimized, integrated pilot study
Year 3	1 MGD demonstration

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Operational Strategies to Control HABs in Inland Reservoirs

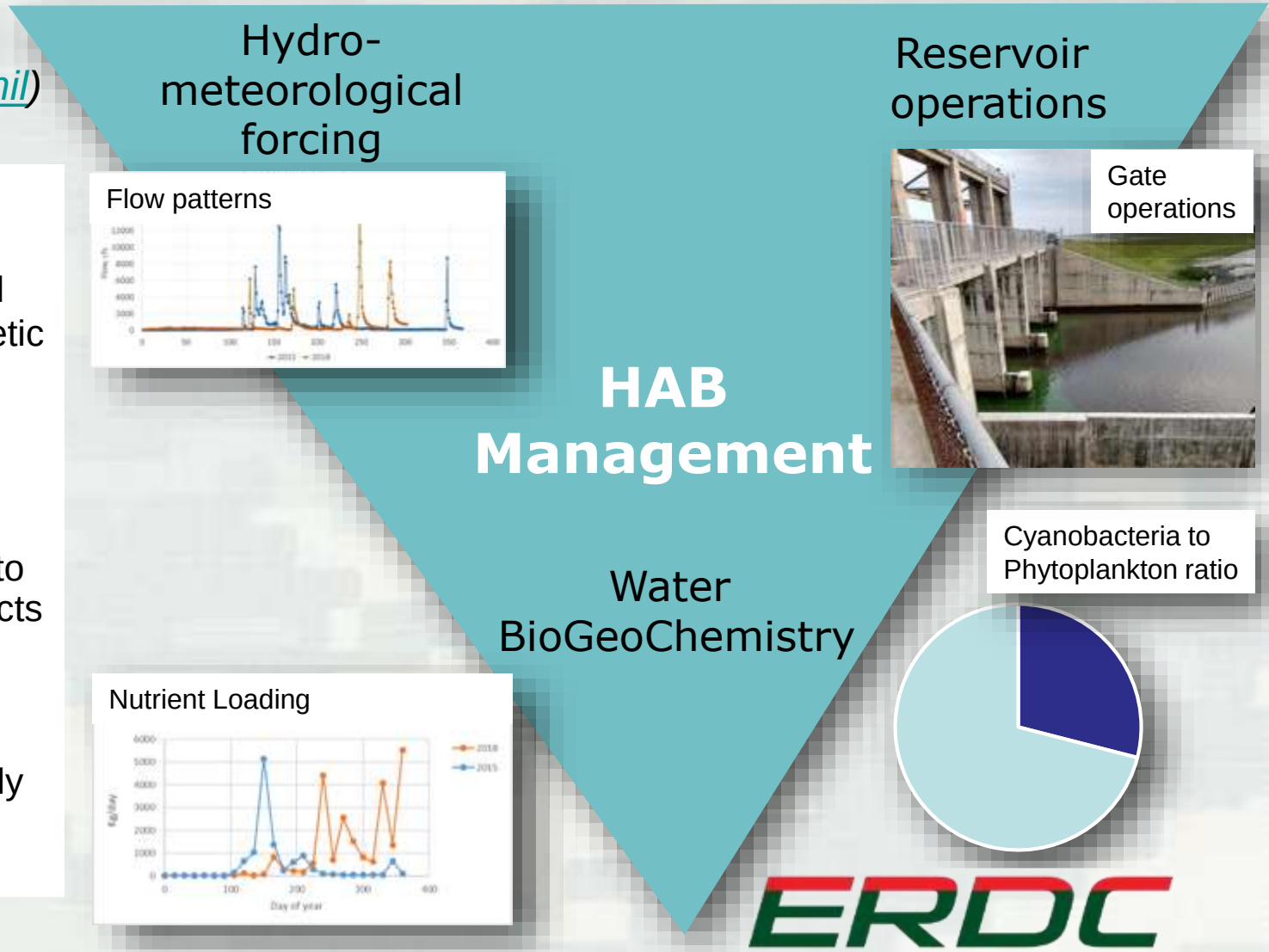
POC: Jodi Ryder (Jodi.L.Ryder@usace.army.mil)

Purpose: Provide guidance on operational management techniques and procedures. Ie withholding or release of water, the use of targeted flow strategies like horizontal flushing or hypolimnetic withdrawls, etc.

Results:

- A systematic historical study of the influence of reservoir control options on HABs.
- The development of modeling tools and protocols to allow reservoir managers to test the projected effects of operational changes.

Benefits: With increasing frequency of geographic distribution of HABs there is a need to understand what effects, if any, previous attempts to operationally manage HABs have been successful in reducing bloom extent, duration and expression of toxins.



Physicochemical Treatment of Cyanobacteria/Toxins by Hydrodynamic Cavitation

POC: Victor Medina (Victor.F.Medina@usace.army.mil)

Purpose:

- Assess hydrodynamic cavitation to remove cyanobacteria cells and toxins from water through the generation of microbubbles in contaminated water.

Results:

- Hydroxyl radicals were generated when microbubbles imploded.
- After 2 hours of cavitation, *Microcystis* and microcystins were reduced 48% and 68%, respectively.

Benefits:

- Hydrodynamic cavitation can be used to effectively treat cyanobacteria in a minimally invasive, energy efficient, cost effective way.
- This technology could be used effectively in problematic areas and small coves for spot-treating harmful algae.

Microcystis was cultured in tanks and treated for 2 hrs. Samples were collected every 30 min.

Parameters	Initial	Final	% Reduction
Turbidity	0.15	0.02	87
Chlorophyll *a*	9.60	0.50	95
Cell Count (10^8 cell/mL)	121	39	68
Microcystin	0.535	0.281	48

High-Sensitivity Flow Cytometry – In Development

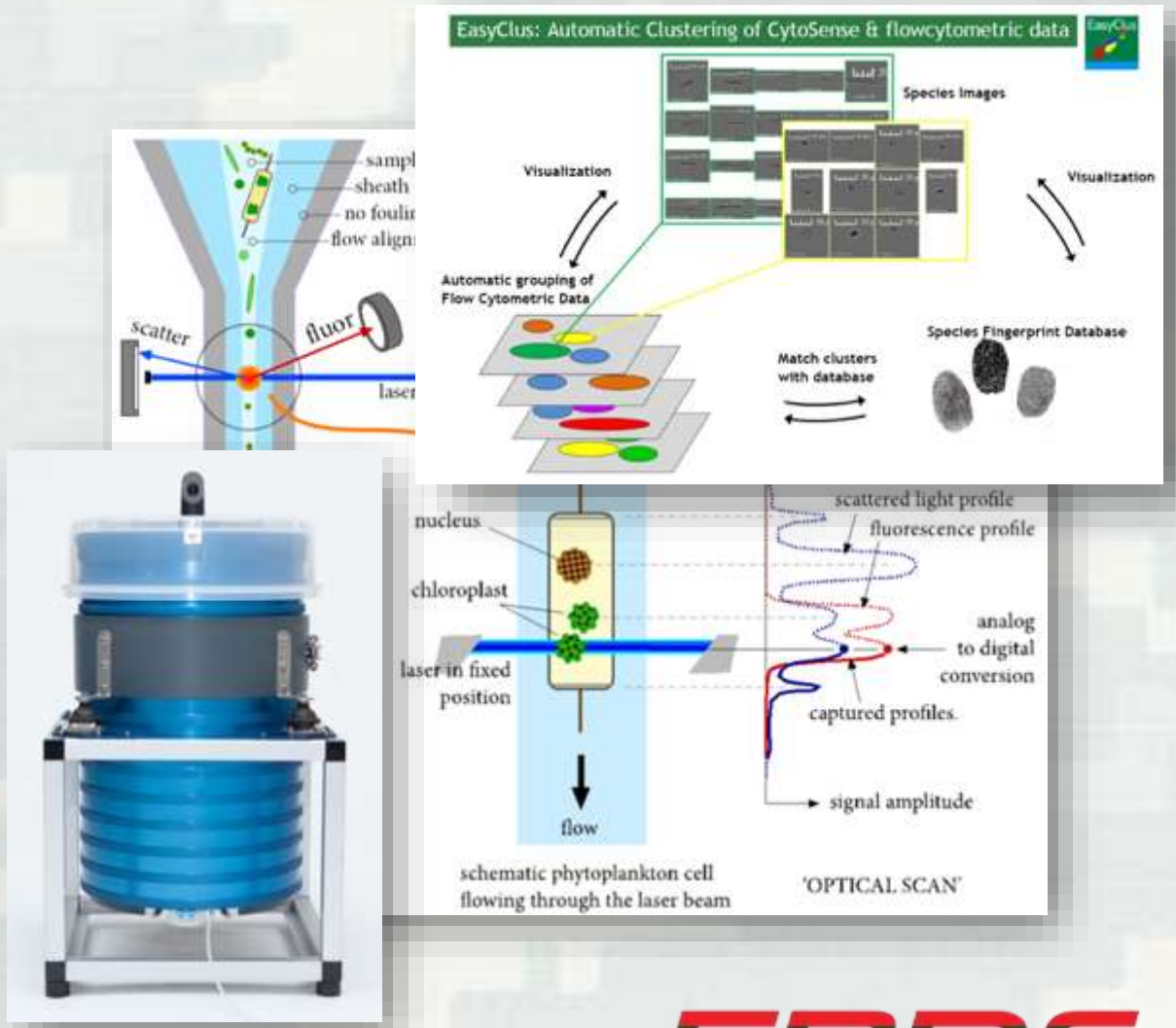
POC: Kaytee Pokrzywinski

Purpose: Develop rapid, routine monitoring network to screen for cyanobacteria.

Results:

1) Automated fingerprinting of phytoplankton communities, mostly cyanobacteria.
2) Does not replace taxonomic efforts streamlines monitoring for management.

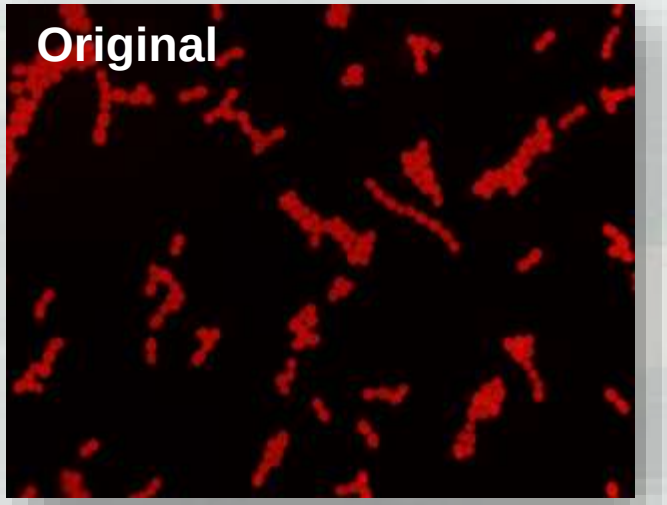
Benefits: Set total cyanobacteria and genus-specific thresholds for monitoring/management alerts. Allows for early detection and rapid response.

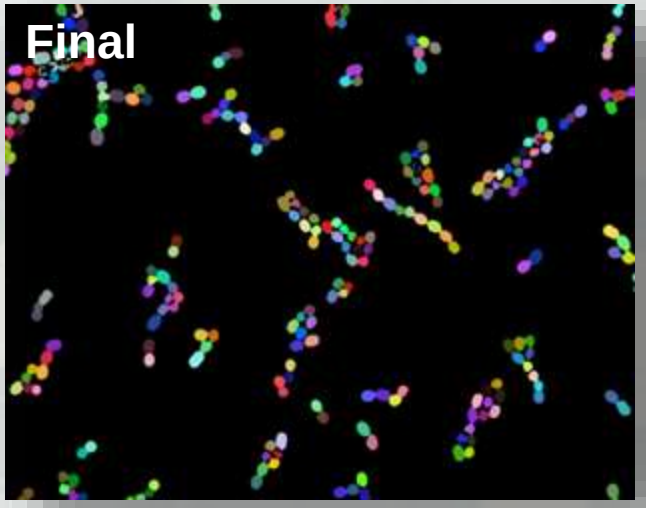


Automated Algae/Cyanobacteria Cell Count

POC: Kaytee Pokrzywinski (Kaytee.Pokrzywinski@usace.army.mil)

- **Purpose**: Develop method to count cyanobacteria to validate other detection strategies as filaments and colonies in cyanobacteria can make counting challenging.
- **Results:** Use R-programming to automate image counting of cells including filaments/colonies
- **Benefit:** Automated image processing method that can count cells/particles in minutes vs hours

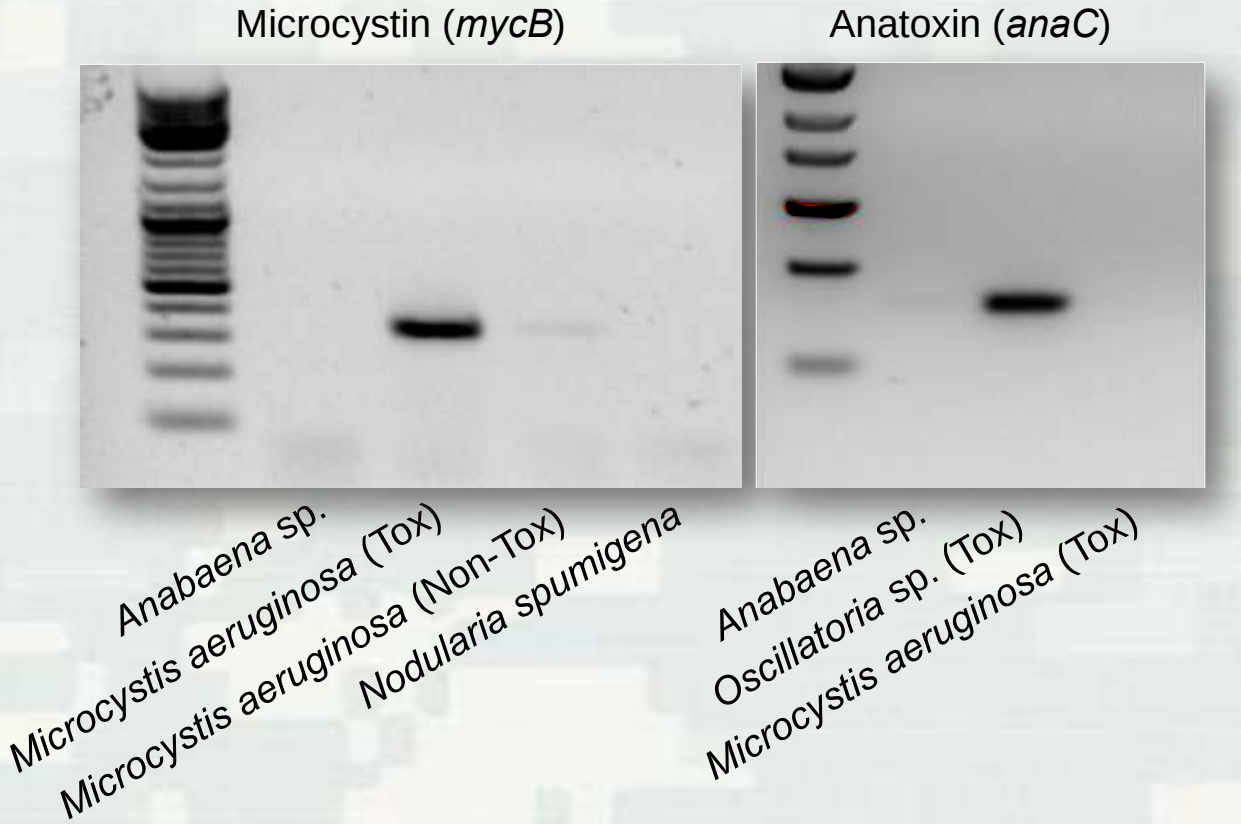




Molecular Identification of Cyanobacteria/Toxin Genes

POC: Kaytee Pokrzywinski (Kaytee.Pokrzywinski@usace.army.mil)

- **Purpose**: Develop laboratory and field-based rapid screening tools for cyanobacteria and associated toxins
- **Results:** PCR/qPCR methods targeting sequences specific to
  - toxin genes: microcystin/nodularin, cylindrospermopsin, saxitoxin and anatoxin.
  - 16S rRNA total cyanobacteria and genus-level – *Microcystis, Anabaena, Aphanizomenon, Oscillatoria, Nodularia, Cylindrospermopsis*
- **Benefits:** Rapid identification of cyanobacteria to genus-level and indication of potentially toxic blooms. Required for early detection and rapid response.

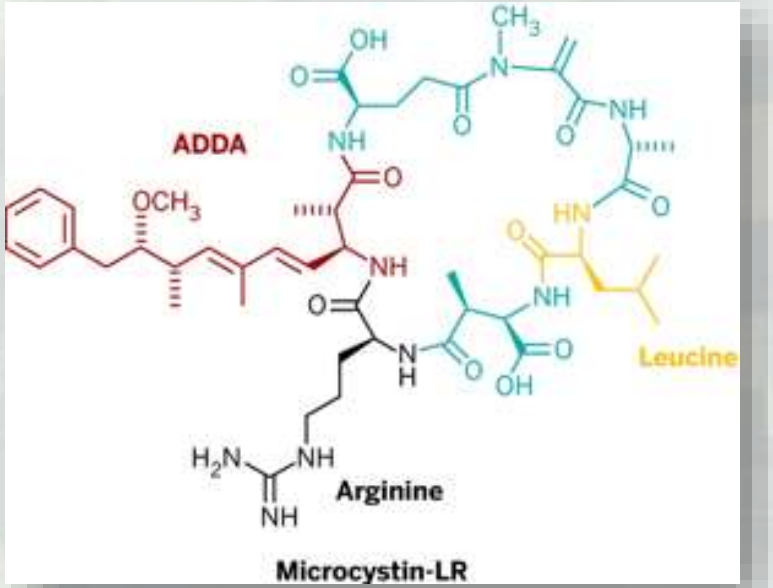


Reimbursable Service – From Previous Research Project

Analytical Cyanotoxin Detection/Quantitation

POC: Lee Moores (Lee.C.Moores@usace.army.mil)

- **Purpose:** Develop in-house analytical capabilities for detecting cyanotoxins to promote faster sample processing.
- **Results:**
  - Enzyme linked immunosorbent assays (ELISAs) for microcystins/nodularins, anatoxin-A, saxitoxins and cylindrospermopsin.
  - LC/MS/MS in development for microcystins/nodularins, saxitoxins, anatoxins and cylindrospermopsin (in development).
- **Benefits:** Rapidly (< 2 days) determine if a cyanobacteria bloom is toxic and how treatment can impact toxin release.



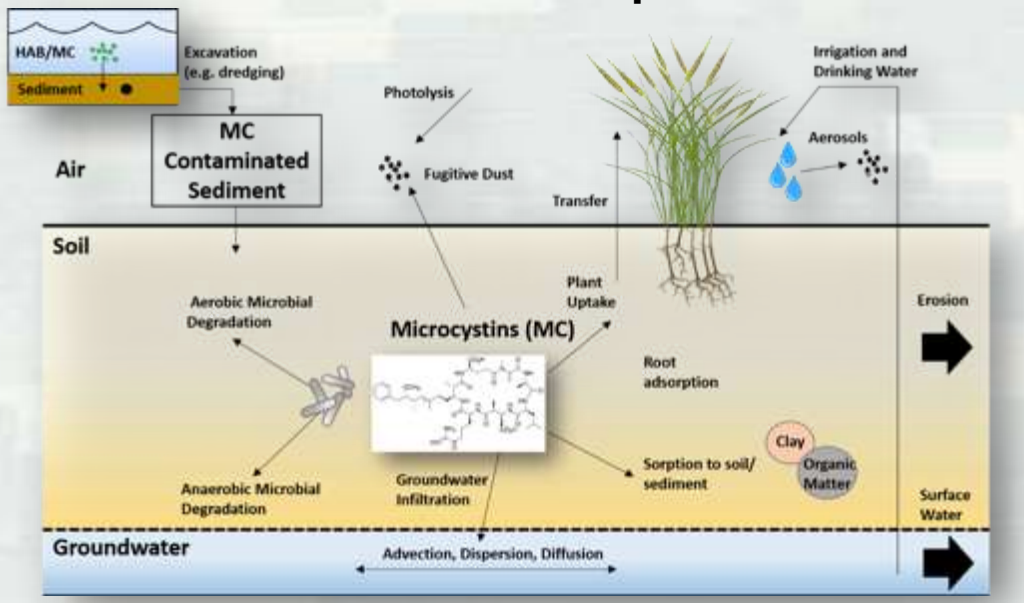
Reimbursable Service– In Development

Risk Assessment

POC: Andrew McQueen (Andrew.D.Mcqueen@usace.army.mil)

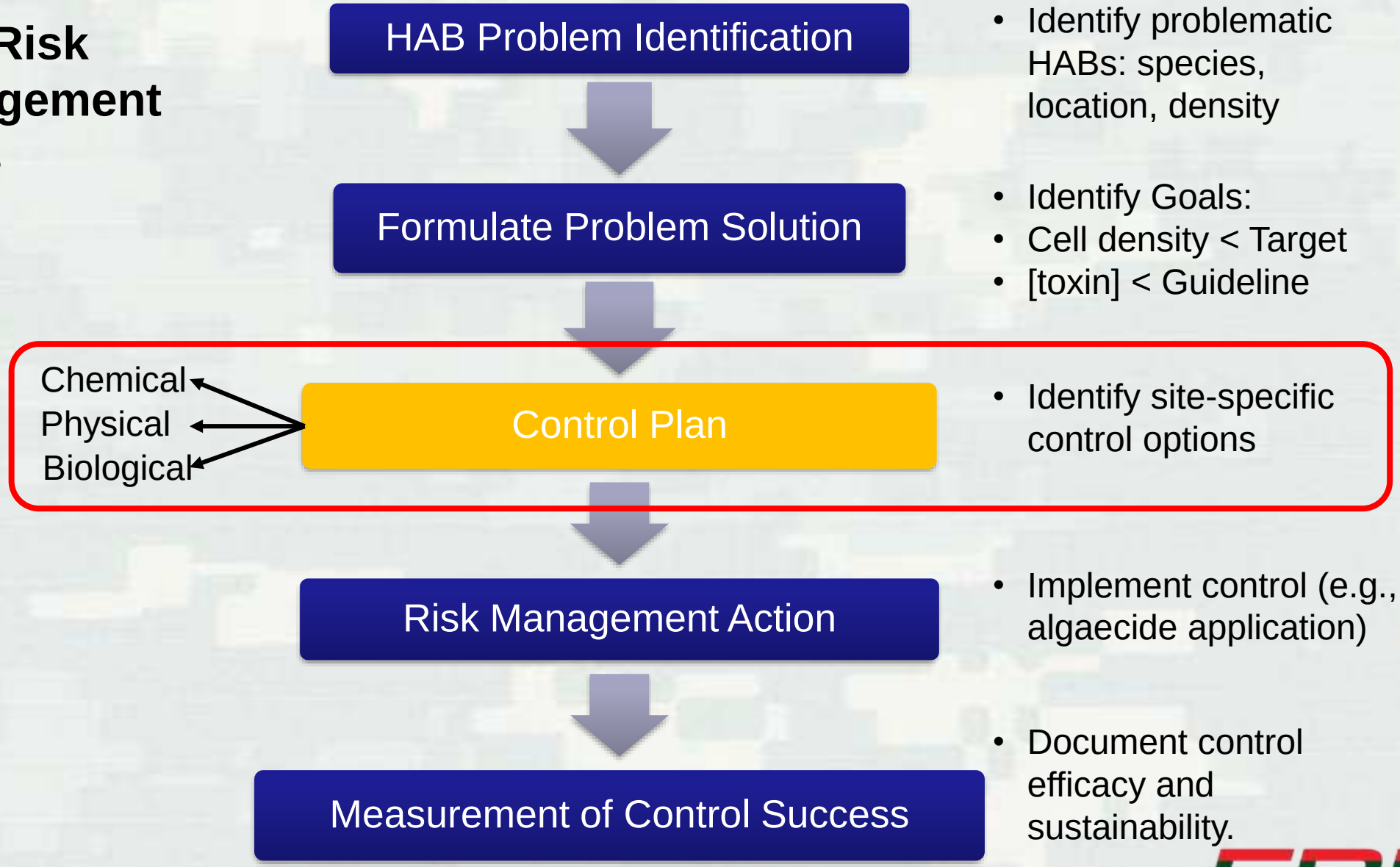
- **Treatment Efficacy**
  - Identify potential treatment solutions
  - Provide site-specific algal response data
- **Ecological Risks/ Toxicity Testing**
  - Define risks to fish and invertebrates
  - Define margin-of-safety for non-target organisms
- **Algal Toxins**
  - Technical support for ecological and human health risk
- **Risk Communication**

Fate and Transport of Toxins



McQueen et al. 2019

HAB Risk Management Steps



- Identify problematic HABs: species, location, density
- Identify Goals:
- Cell density < Target
- [toxin] < Guideline
- Identify site-specific control options
- Implement control (e.g., algaecide application)
- Document control efficacy and sustainability.

Conclusions

- There is an increase in frequency and duration in freshwater HAB events
- HAB research at ERDC has been rapidly expanding; advancing our capabilities, technical areas and research facilities.
- Focused on issues directly facing the Corps and often receive input from districts and customers.
- Technical areas include:
  - Water quality (modeling and monitoring)
  - Detection (molecular methods to remote sensing)
  - Nutrient reduction
  - Risk assessment
  - Management (physico-chemical, biological and chemical)
- Primarily aimed at applied research for early detection and rapid response.

Contact Information

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Carina Jung	*Email: Carina.M.Jung@usace.army.mil*	*Phone: (601)634-7247*