Office of Research and Development

Safe and Sustainable Water Resources Research Program

Research Area 4:

Assessment and Management of HABs

BOSC SSWR Subcommittee Meeting on Nutrients and HABs

December 1-2, 2021

Office of Research and Development

BOSC Meeting SSWR Nutrients and HABs
December 1-2, 2021

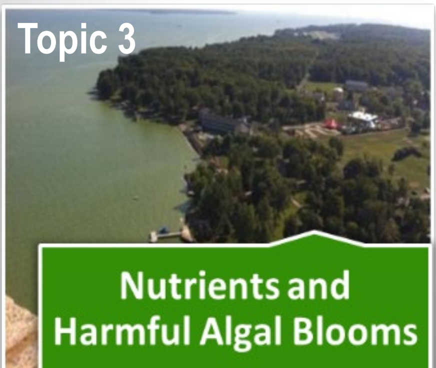
HABs Background and Research Overview

Nicholas Dugan, Ph.D.
Center for Environmental Solutions and Emergency Response (CESER)

HABs Overview

- Freshwater HABs occur when, most commonly, cyanobacteria become dense or toxic enough that it results in negative environmental or health effects.
- Cyanobacteria have the potential to produce toxins and other irritating metabolites.
- The most commonly reported cyanotoxins in the U.S. are microcystins, nodularins, cylindrospermopsins, anatoxins, and saxitoxins.
- Exposure is usually through direct contact with contaminated water, ingestion of contaminated water, or potentially from inhaling contaminated aerosols or from eating contaminated fish.

ORD's Safe and Sustainable Water Resources (SSWR) Research Program



HABs Research Legislative Drivers

- HAB and Hypoxia Research and Control Act
- Clean Water Act
- Safe Drinking Water Act

Research Area Coordination Team

- **Topic Lead:** Anne Rea, ORD
- **Coordinator:** Scot Hagerthey, ORD (Brenda Rashleigh)
- **Program Office (Office of Water):** Lesley D'Anglada, OST and Katie Foreman, OGWDW
- **Region:** Rochelle Labiosa, R10
- **State:** Robert Newby, New Jersey
- **Output Leads (ORD):** 1 Elizabeth Hilborn (CPHEA), 2 Nick Dugan, (CESER), 3 Blake Schaeffer (CEMM)

ORD's Nutrients and HABs Research

Research Area 4: Assessment and Management of HABs

Research Area 5: Science to Support Nutrient-Related Water Quality Goals

Research Area 6: Nutrient Reduction Strategies and Assessment

Program/Region/State Research Needs

- **Tools** to predict toxic bloom occurrence, characterize bloom development, increase the effectiveness of cyanotoxin monitoring techniques.
- **Economic analyses** of HAB/cyanotoxin impacts and source water protection activities.
- **Evaluate management actions** in watersheds and source water reservoirs to help prevent and mitigate HABs and to support guidance on treatment technologies for HABs/cyanotoxins.
- **Epidemiological and toxicological studies** on existing and emerging cyanotoxins for aquatic life and human health.
- **New threshold information synthesis** on toxin, toxin mixtures, and novel exposure pathways for humans and domestic and wild animals can inform guidance to adequately communicate the risks of HABs events.
- **Ambient water sensors** to help determine which practices are best suited to reduce nutrient loadings and lead to reduced HABs.

HABs Research

Research is organized into three subareas:

1. Assessing Adverse Health Outcomes from Exposure to HABs

2. Research to Support Managing HABs and Their Impacts in Ambient and Drinking Water

3. Developing Tools to Support HABs Risk Characterization and Assessment



Assessing Health Outcomes

Overview:

- The toxicity and epidemiology of HABs need better characterization.
- ORD is assessing potential impacts using multiple whole organism toxicity studies, in vitro, primary human liver cells, and tissue model approaches.
- Results of this work can support the work done by Program Offices and Regions in the development of guidelines and tools such as health advisories and water quality criteria, response and risk communication during HAB events, fish consumption advisories, and the designation of aquatic life and environmental health thresholds.

Research Products:

Assessing the impact of HABs and their associated toxins on human health.

Assessing the impact of HABs and their associated toxins on animal health.

Exploring the chemical, physical, and biological characteristics of HABs associated with adverse health outcomes.

Assessing Health Outcomes

Research Highlights:

Human health impacts

- Evaluating the effects of cyanobacteria on human 3D skin tissue models.
- Evaluating health effects among residents that are associated with the presence of cyanotoxins in their drinking water.

August, 2014 Lake Erie CyanoHAB—Toledo, OH drinking water intake

CyanoHAB in Ohio

- Characterizing and providing dose-response assessment of toxicity from inhalation of aerosols containing cyanotoxin.
- Comparing toxicity of ten microcystin congeners—liver toxins with various potencies and mechanisms of action—found in U.S. freshwater.

Characteristics associated with adverse health outcomes

- Developing a method to detect cyanotoxins in fish tissue; informs the development of fish consumption advisories.

Supporting the Management of HABs and Their Impacts

Overview:

- Develop and disseminate basic science and best practices to mitigate HABs.
- Reduce the risk of ecosystem harm, recreational exposure, and the ingestion of contaminated drinking water.
- Inform the development of water body management plans, design and purchase of supplies and equipment, and the operation of drinking water treatment facilities.

Research Products:

Exploring source water interventions that could mitigate HABs.

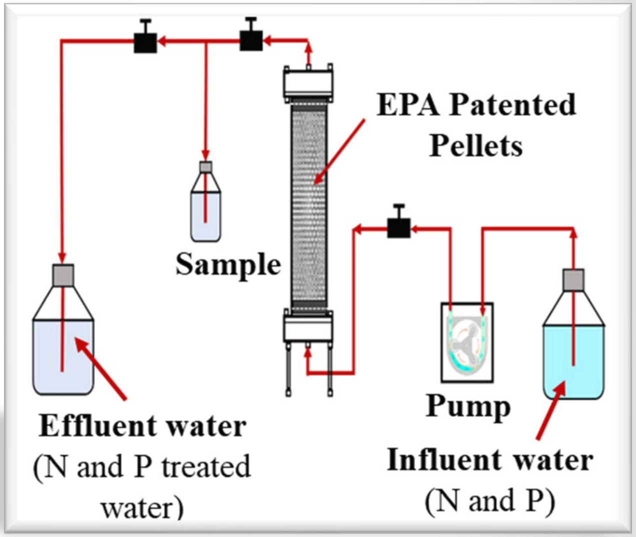
Researching drinking water treatment options to reduce the risk of ingestion exposure.

Supporting the Management of HABs and Their Impacts

Research Highlights:

Source water

- Test biological bloom control measures.
- Use metabolic signals to guide the timing of bloom control measures.
- Develop and test a low cost engineered media to remove nitrogen and phosphorous from wastewater.



Evaluation of N and P removal media for water and wastewater

Drinking water treatment

- Evaluate how algicide exposure affects cyanobacterial responses to water treatment.
- Develop an accessible laboratory procedure to screen the best powdered activated carbon (PAC) and best optimal dose.
- Evaluate efficient methods of quantifying toxins in water treatment waste streams.

Tools to Support HABs Risk Characterization and Assessment

Overview:

- HAB environmental risk characterization quantifies environmental drivers, such as nutrients and temperature, that impact algal biomass and occurrence of toxins.
- Research strategy ranges from single point samples, to high frequency sensors, to satellite imagery.
- Results to characterize the onset, temporal frequency, spatial extent, and magnitude of HABs in rivers, streams, lakes, and reservoirs to help prioritize limited resources.

Research Products:

Conducting monitoring to improve HAB assessment and characterization.

Developing HAB models, software, and tools.

Characterizing the development, intensity, and spatial extent HABs.

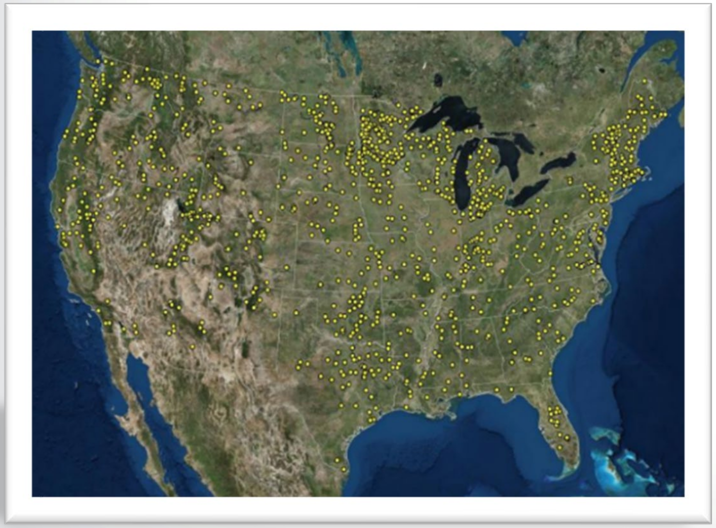
Tools to Support HABs Risk Characterization and Assessment

Research Highlights:

Models, software, and tools

Created the CyAN app from collaborative work with NASA, NOAA, and USGS to quickly inform decisions regarding recreational and drinking water safety related to cyanobacterial blooms. Available as an Android app and currently developing CyANWeb for all web-based devices.

Satellite data is used to map the location of cyanobacterial blooms.



Monitoring

Developed method for early detection of algal toxin production using qPCR.

HABs characterization

Machine learning models combined with genomic and environmental drivers to predict toxins and biomass.

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HAB Toxicity

Elizabeth Hilborn, Ph.D.

Center for Public Health and Environmental Assessment (CPHEA)

Assessing Risk: Toxicity

Health Effects of HABs:

Toxicology:

- Compared toxicity of ten microcystin congeners with mouse model—liver toxins with various potencies and mechanisms of action— found in U.S. freshwater.
- Evaluated the effects of eight microcystin congeners on primary human liver cells.
- Will characterize and providing dose-response assessment of toxicity from inhalation of aerosols containing cyanotoxin.

August 2014 Lake Erie CyanoHAB
Toledo, OH drinking water intake

- Evaluated toxicity of golden algae and cyanobacteria toxins to aquatic organisms.
- Evaluated the effects of cyanobacteria on human 3D skin tissue models.

Assessing Risk: Adverse Observed Effects

Health Effects of HABs:

Human health effects associated with cyanobacteria and cyanotoxin exposures.

- Effects associated with recreational exposures.
- Effects associated with drinking water exposures (Output 4.3 cross link).

Investigating phenomena potentially associated with adverse health outcomes.

- Developing a method to detect cyanotoxins in fish tissue; informs the development of fish consumption advisories.
- Working to characterize aerosols associated with cyanobacteria blooms.

EPA
United States Environmental Protection Agency

Assessing Risk: Toxicity

Acute Effects of Ten Microcystin Congeners

Acute toxicity effects of microcystin congeners dosed orally in mice

Issue: Need mammalian toxicity data on microcystin congeners

Approach:

Part 1: At 7mg/kg, mice given single oral dose of one of 10 microcystin congeners.

- Monitor clinical signs; necropsy
- Determine comparative toxicity

Relative toxicity based on mammalian in vivo:
LA>LR>LY>YR>LW>LF>WR>RR

Part 2: Mice dosed orally. Dose-response acute toxicity of MCLR, MCLA, MCRR, MCLY, MCYR.

Results used to determine NOAELs and LOAELs

Status: Chernoff et al. Toxins (Basel). 2020 Jun 18;12(6)

Chernoff et al. Toxins (Basel). 2021 Jan 24;13(2)

Future Plans: Comparison of 10 microcystin congeners given IP; facilitate research for mode of action; subacute toxicity of anatoxin-*a* in mice.

Effects of Microcystin Hydrophobicity on Cytotoxicity

Acute toxicity effects of microcystin congeners in primary human hepatocytes

Issue: Data gaps in toxic effects of microcystin congeners on human cells.

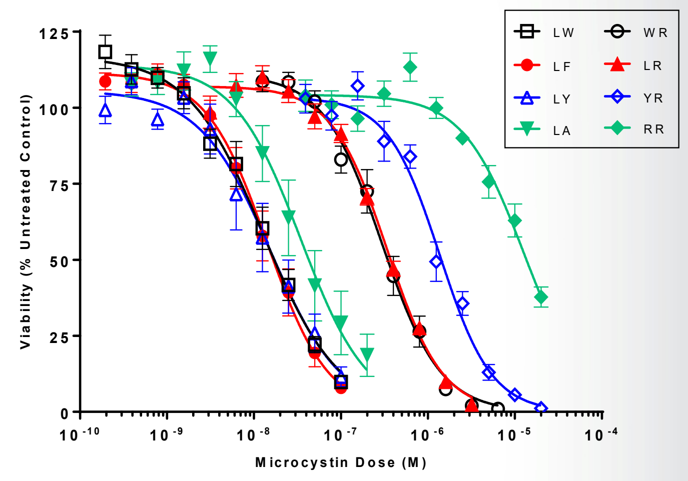
Approach: Compare toxicity of multiple hydrophobic and hydrophilic microcystin congeners in human hepatocytes

- Determine EC50s of eight microcystin congeners through cell viability testing.
- Evaluate the effects of congeners on reactive oxygen species (ROS), protein phosphatase (PP2A) activity, and glutathione (GSH) activity.
- Assess the effects of congener hydrophobicity on cytotoxicity.

Status/Ongoing Activities:

- Results presented at International Conference on Harmful Algae and International Society for the Study of Xenobiotics conferences.
- Manuscript in preparation.
- Ongoing toxicity testing of microcystin congeners in human 3D liver cultures.

Future Plans: Assessing cyanotoxins in human 3D colon cultures.



Viability (EC50)

LW ≈ LF ≈ LY > LA > WR ≈ LR > YR > RR

More Toxic | Less Toxic

Hydrophobicity (Amino Acids)

LF ≈ LW > LY > LA > WR = LR > YR > RR

More Hydrophobic | Less Hydrophobic

Effects of Cyanobacteria Human Skin Tissue

Normal Human 3D skin tissue model exposed to cyanobacteria

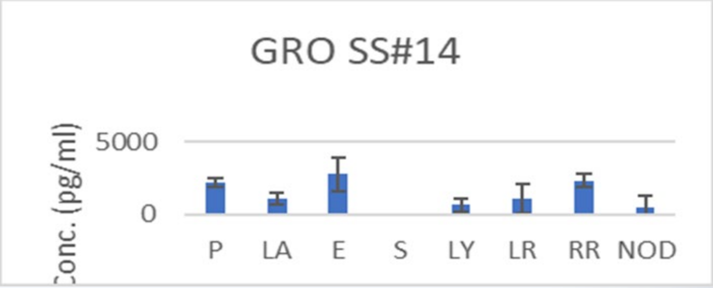
Issue: Dermal effects of exposure to cyanobacteria is limited.

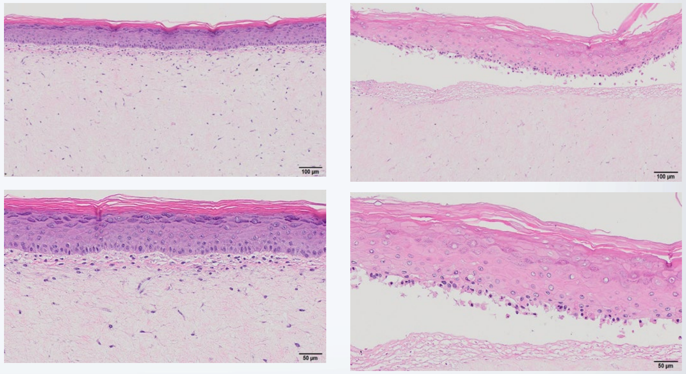
Approach: Evaluate the inflammatory effects of cyanobacteria and their components to normal human skin tissue using an in vitro 3-D (organoid) normal human skin tissue model.

- Conducting a validated skin irritation test.
- Skin will be exposed to microcystin congeners, cyanobacteria, environmental water samples.
- Will assess cell proliferation/viability; histological changes; cytokines/Lymphokines/soluble receptors; gene expression (mRNA) profile.

Status/Ongoing Activities: Ongoing characterization of the toxicological and immunological responses of normal human skin following exposure to cyanobacteria and their metabolites.

GRO cytokine/chemokine production of skin tissue following exposure to microcystin





Normal skin tissue **Damaged skin tissue**

Toxicity of Cyanotoxin Aerosols

Dose-response assessment of toxicity from inhalation of aerosols containing cyanotoxin

Issue: Need experimental inhalation exposures to cyanotoxins.

Approach:

- Expose mice to aerosols containing cyanotoxin within an AEC.
- Examine toxicity of microcystins and anatoxins in nasal cavity, liver, respiratory epithelium.
- Characterize inflammatory response of serum proteins.
- Identify biomarkers using RNA sequencing.
- Develop dose-response model using identified biomarkers quantified by gene expressions for a few toxin species.


Aerosol Exposure Chamber (AEC)

Status/Ongoing Activities:

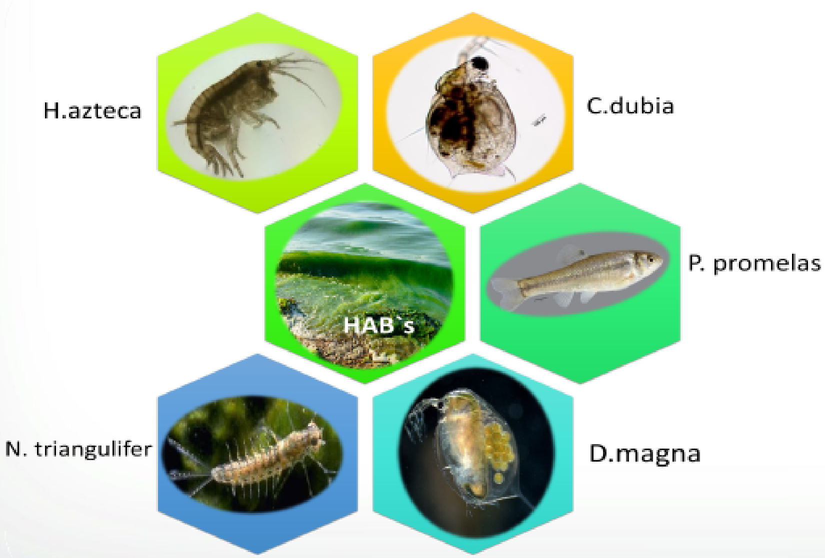
- Experiment: Ongoing and expecting to complete some results within FY22.
- Completed: Chamber test, protocol approval, decon test, in-house sequencing test, pipeline setup of RNA sequence analysis.

Aquatic Ecotoxicity of Cyanotoxins and Algal Toxins

Acute and chronic toxicity testing

Issue: Little information about toxicity to aquatic organisms.

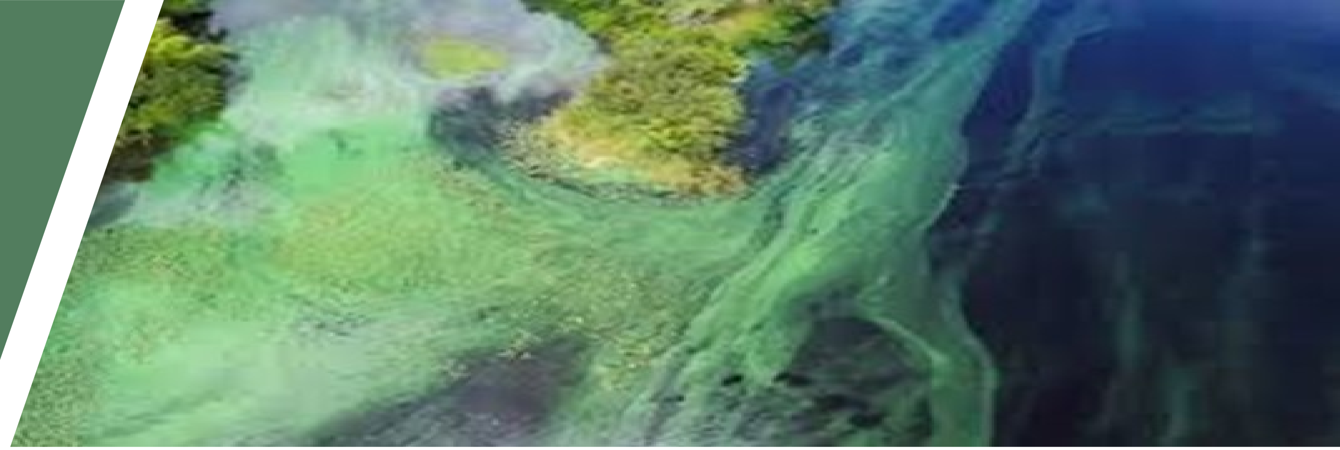
Approach: Conducted acute and chronic toxicity tests with *C. dubia*, *P. promelas*, *H. azteca*, and *N. triangulifer* by exposing them to microcystins and to golden algae (*P. parvum*).



Status/Ongoing Activities:

- LC50s could not be calculated because no lethality found up to 75 ug/L total microcystins.
- *N. triangulifer* most sensitive to *P. parvum* extract.
- Outstanding: Develop method to measure prymnesin (toxin).

United States Environmental Protection Agency



Assessing Risk: Adverse Observed Effects

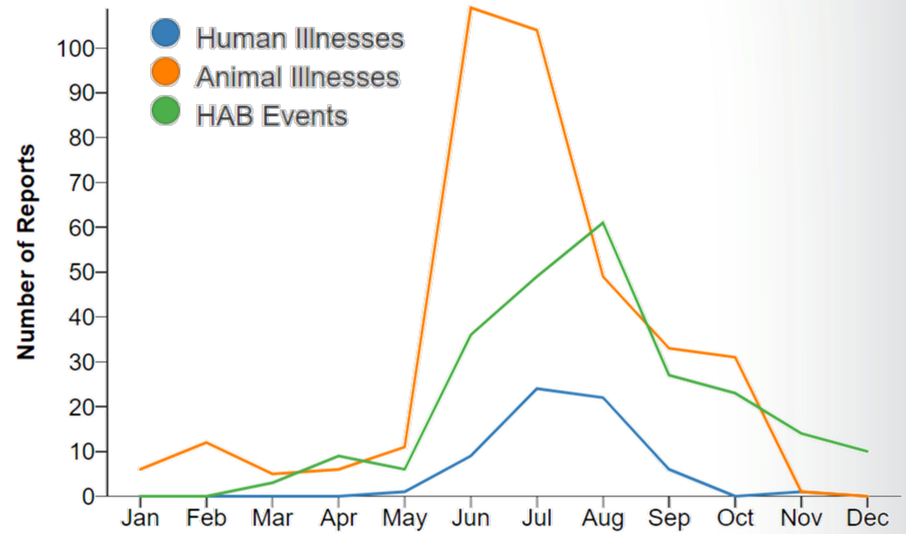
Characterize HAB-Associated Health Effects

Surveillance for HAB-associated illness

Issue: People and animals are exposed to poorly characterized aquatic environments.

Approach: Collaborate with Centers for Disease Control and Prevention One Health Harmful Algal Blooms System.

- Characterize human and animal illnesses.
- Characterize environmental evidence.
- Characterize acuity and health care usage.



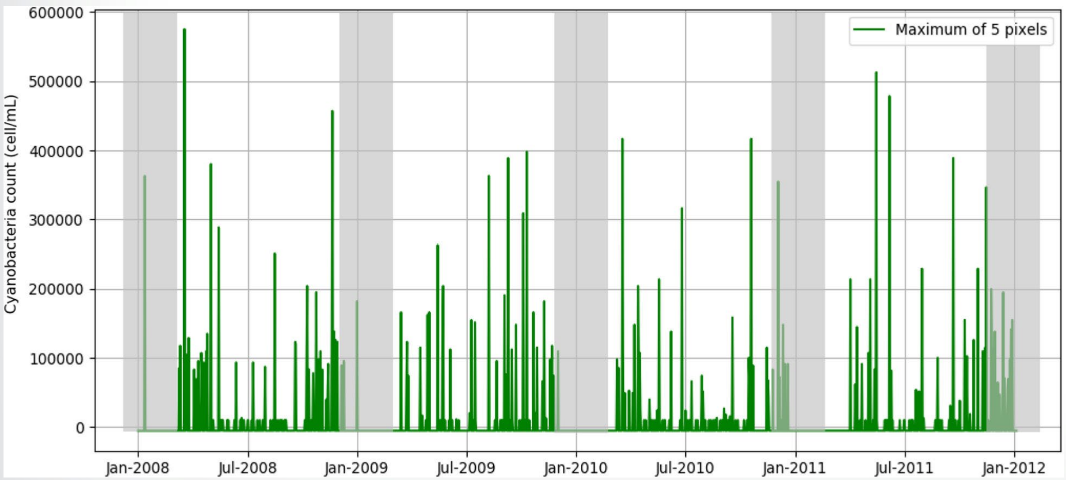
Status/Ongoing Activities:

- First two years of surveillance summarized and published.
- MMWR December 18, 2020/69(50);1889–1894

Characterize HAB-Associated Health Effects

Illness associated with satellite-determined cyanobacteria blooms

Issue: People uncommonly reported as exposed to cyanobacteria and cyanotoxins in drinking water.



Approach: Identify remotely-sensed cyanobacteria blooms near drinking water intake.

- Characterize human illnesses reported from emergency room visits among population served.
- Control for weather, temperature, time, air pollution, day of week.
- Increased ER visits for respiratory illness were detected two days after bloom at intake.

Status/Ongoing Activities:

Published: Wu et al. Environmental Health 20:83. 2021

EPA United States Environmental Protection Agency

Assessing Risk: Investigating phenomena potentially associated with adverse health outcomes

Characterization of Cyanotoxin Aerosols

Assessment of ambient aerosolized microcystins

Issue: Human nasal exposure to microcystins has been documented–little evidence for ambient aerosolized toxin concentrations.

Approach: Assess aerosolized microcystins adjacent to blooming ponds.

- Select ponds with differing microcystin concentrations.
- Monitor water quality and air daily over ten days.
- Measure local weather conditions.
- Analyze air filters using different methods.

Status/Ongoing Activities:

- Sample collection is complete.
- Analysis is ongoing.

Characterization of Toxins in Fish Tissue

Assessment of toxins in fish tissue

Issue: Cyanobacteria toxins are detected in different species of fish.

Approach: Adapt a total microcystin method used for water to tissues using 2-methoxy-3-methyl-4-phenylbutyric acid (MMPB) derivatization method.

- Spike fish homogenates with microcystin standards and assess spike recovery.
- Test this method on fish collected from water bodies experiencing algal blooms.

Status/Ongoing Activities:

- MMPB method appears to be performing well in a variety of spiked fish tissues, spanning a range of lipid content.
- Samples of fish collected from blooming water bodies in FL, UT, NJ, and CT were analyzed with the MMPB method.
- Detection of total microcystin were found in fillets from fish collected in FL, UT, and CT.

Toxicity of Cyanobacteria Species (*Microcystis aeruginosa*)

Environmental conditions affect cyanobacteria physiology and toxicity

Issue: Toxins are released as bacteria age and degrade.

Approach: Compare *Microcystis* cells from culture and from lake under different conditions using flow cytometry, microscopy, and hyperspectral imaging.

Status/Ongoing Activities:

- Fluorescence indicator of viability.
- Detecting Microcystis physiology modifications with fluorescence equipment.

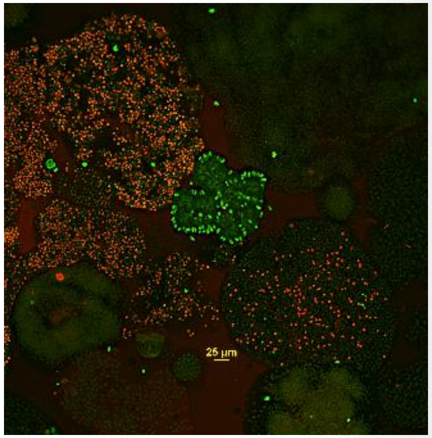
Future research

- Link fluorescence response with toxin release
- Study Microcystis toxicity with lab techniques

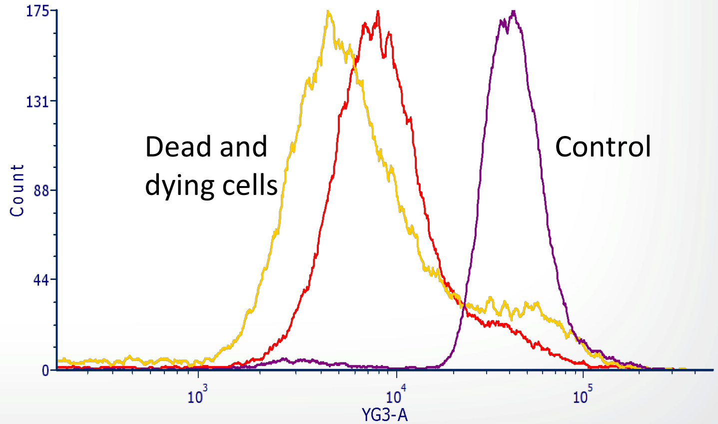
Fluorescence Microscopy

Lake Microcystis

Death & Dying (dull green)



Flow Cytometry *Microcystis* culture



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Predicting and Forecasting HABs

Blake Schaeffer, Ph.D.

Center for Environmental Measurement and Modeling (CEMM)

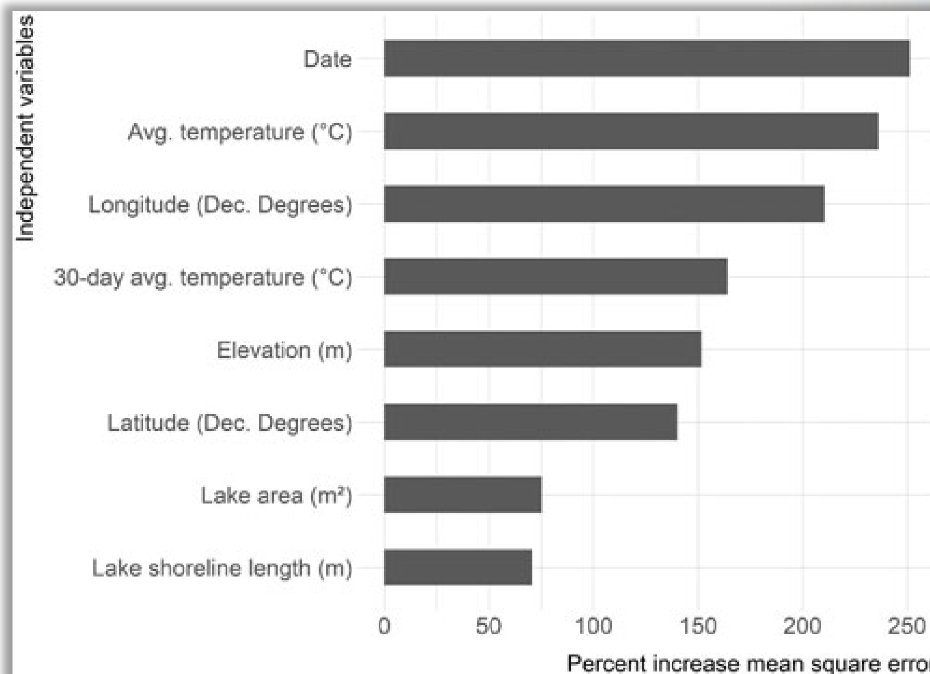
Predictive Model of Lake Photic Zone Temperature

Issue: Warming lake temperature is linked to cyanobacteria bloom dynamics.

Approach: Random forest model of lake photic zone temperature.

Status/Ongoing Activities:

- Kreakie et al. 2021. *Frontiers in Environmental Science,* 451.
- Ongoing: Back casted daily lake photic zone temperature (1983-2017).
- Future: Forecast changes with various climate change predictions.

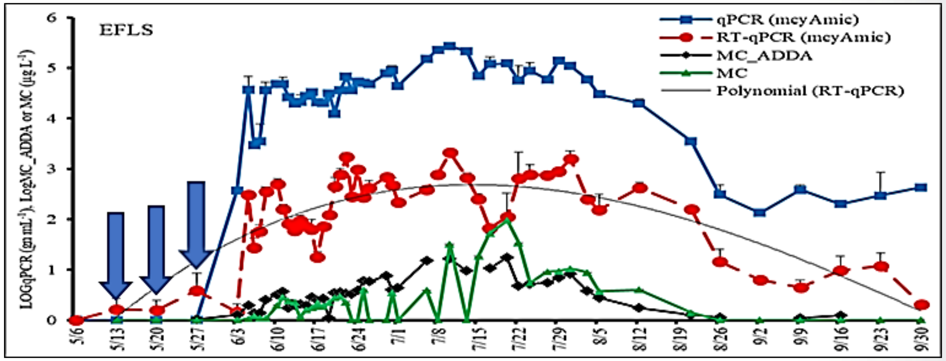


Variable importance plot for lake photic zone temperature.

Molecular Approaches for CyanoHABs and Toxins

Issue: Methods needed to characterize and predict cyanoHAB dynamics and associated toxins in the context of phytoplankton ecology.

Approach: DNA sequence-based characterization, RNA-sequence based function analysis and qPCR/RT-qPCR based quantification and early warning of cyanotoxin production.



Early signals (blue arrows) and correlations of mcy gene abundance and transcripts with microcystin concentrations.

Status/Ongoing Activities:

- Three published manuscripts.
- Ongoing: Three manuscripts in preparation.
- Future: Long-term prediction and validation of early warning of toxins and threshold of molecular biomarker signals.

Partners: EPA Regions 7 and 10, OR DEQ, WA DoE.

Inland Reservoir High Frequency Modeling

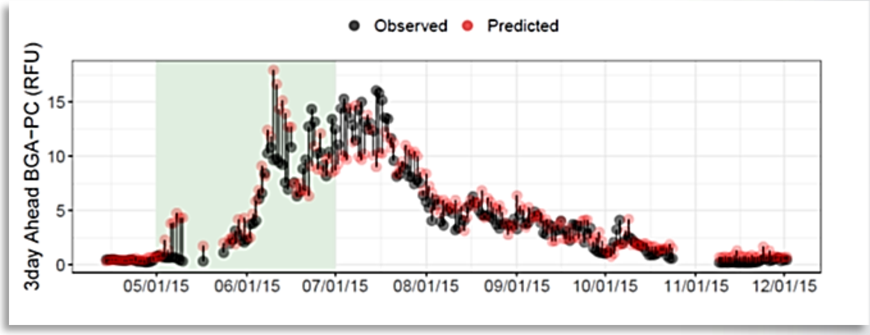
Issue: Managing the risk posed by CyanoHABs on inland reservoirs.

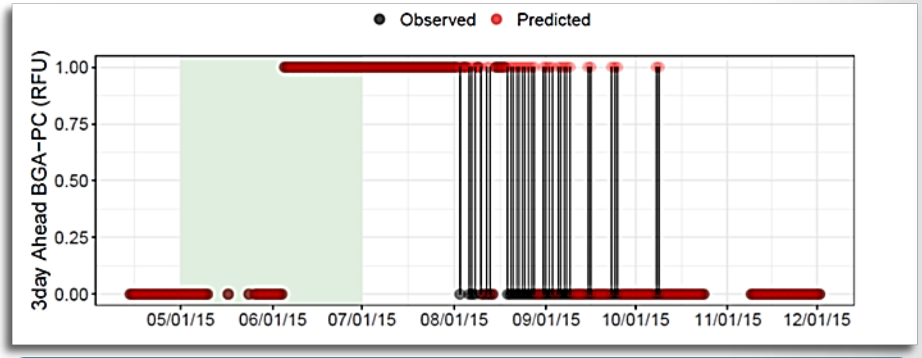
Approach: Use a high-frequency sonde and discrete data with gradient boosted regression and decision trees.

Status/Ongoing Activities:

- Manuscript in preparation.
- Ongoing: Continued model development.
- Future: Model access to partners and stakeholders, addition of weather, high-frequency nutrient data, and molecular markers.

Partners: Clermont County, OH, East Fork of the Little Miami River cooperative.





Model validation with the magnitude response represented on the top and a binary threshold approach on the bottom.

Ohio River Risk Characterization for CyanoHABs

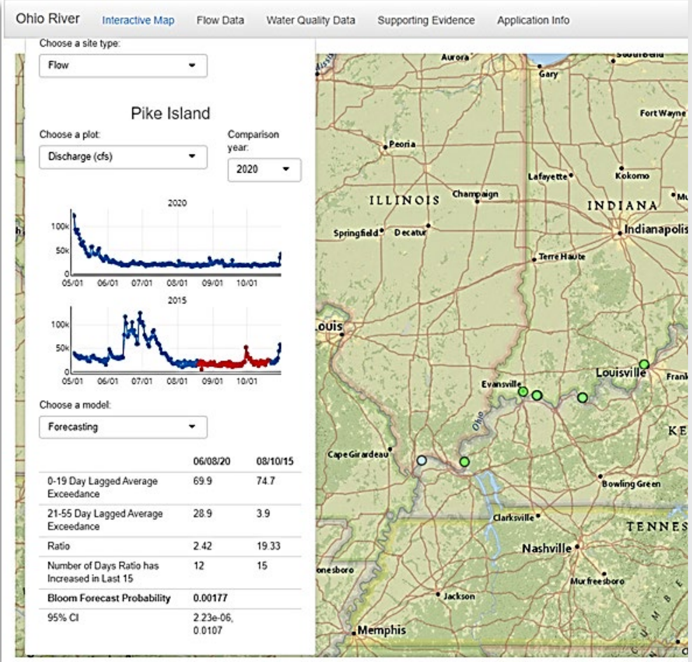
Issue: Managing cyanoHAB risk in the Ohio River.

Approach: Two probabilistic risk models, one for bloom forecasting and another for predicting bloom persistence.

Status/Ongoing Activities:

- Several presentations to partners, stakeholders, and scientific communities. Development of an online tool, manuscript in EPA clearance.
- Ongoing: Actively used to monitor and manage the Ohio River.
- Future: Update the models on an annual basis with new data for the river.

Partners: Ohio River Valley Water Sanitation Commission (ORSANCO) EPA Regions 3, 4 and 5, NWS-Ohio River Forecast Center, and USACE



Screen shot of interactive Shinyapp accessible by the QR code below.

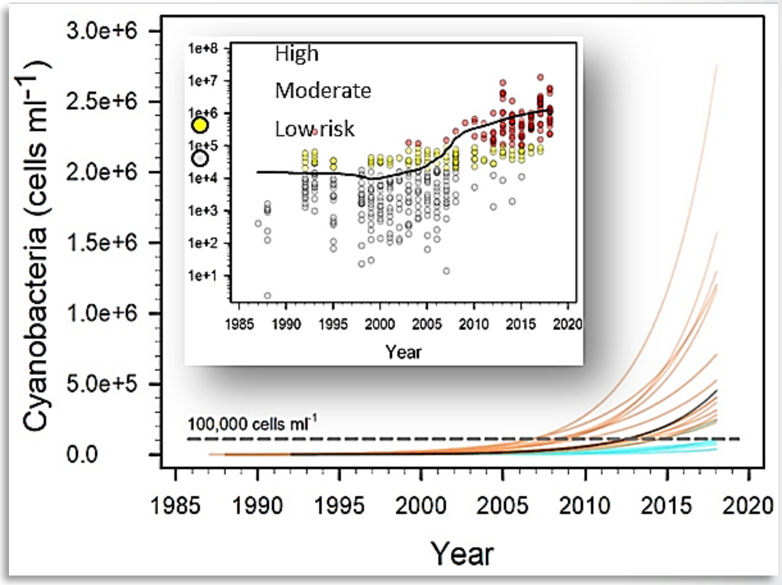
CyanoHABs in Warming Waters

Issue: How cyanHABs may change in response to climate.

Approach: 27–32 years of monitoring data from 20 temperate reservoirs located in Indiana, Kentucky, and Ohio.

Status/Ongoing Activities:

- Smucker et al. 2021. *Global Change Biology* 27: 2507–2519. Six presentations to stakeholders and scientific communities.
- Ongoing: Draft manuscript on satellite-derived estimates of cyanobacteria in reservoirs trends and relationships with field-based measures of eutrophication.
- Future: Remote sensing to examine frequencies and durations, and potential for fuller taxonomic and limnological analysis.



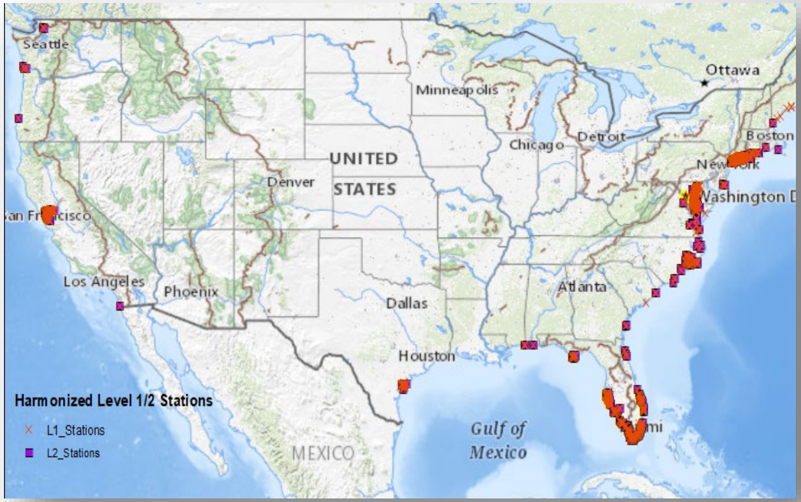
CyanoHAB high health risk events across 20 reservoirs rapidly increased within a decade at different rates and magnitudes.

Partners: U.S. Army Corps of Engineers Louisville District

Mapping CyanoHAB Probabilities in Coastal Systems

Issue: CyanoHABs detected in coastal systems in the U.S., with cyanotoxins in shellfish at levels of concern for human health.

Approach: Multi-density neural networks to estimate chlorophyll in estuaries from Sentinel-2 missions with to develop logistic models predicting probability of cyanoHABs.



Locations of paired chlorophyll to Sentinel-2 observations.

Status/Ongoing Activities:

- EstuarySat database of > 123,000 matched observations.
- CERF presentation and draft manuscript in progress.
- Ongoing: Evaluating atmospheric corrections and post-calibration of cyanoHAB metrics to measures related to human health thresholds.
- Future: Predictive logistic regression models published in EPA Estuary Data Mapper.

Partners: NEPs, NERRs

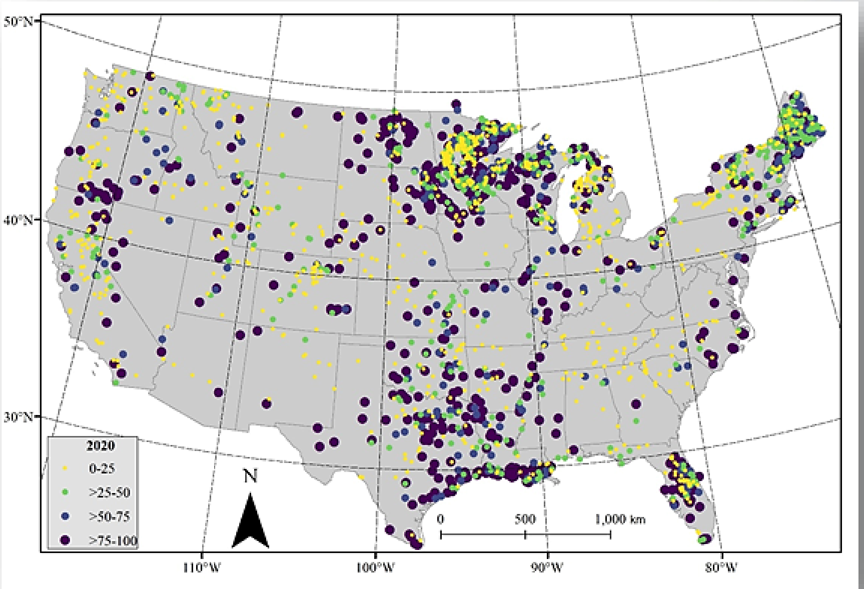
Cyanobacteria Assessment Network (CyAN)

Issue: Limited resources available to cover broad spatial and temporal scales to monitor cyanobacteria.

Approach: Satellites quantify spatial extent, temporal frequency, and magnitude.

Status/Ongoing Activities:

- 25+ publications, 200+ presentations, 85+ media events.
- Ongoing: 1 manuscript in review, and 4 in prep.
- Future: Potential for Sentinel-2 higher resolution chlorophyll.



2020 spatial extent of satellite detected cyanoHABs across <2,000 lakes. CyAN is accessible through the QR code.

Partners: EPA Office of Water and Regions 1-10, states, local groups

Modeling Anthropogenic and Environmental Influences

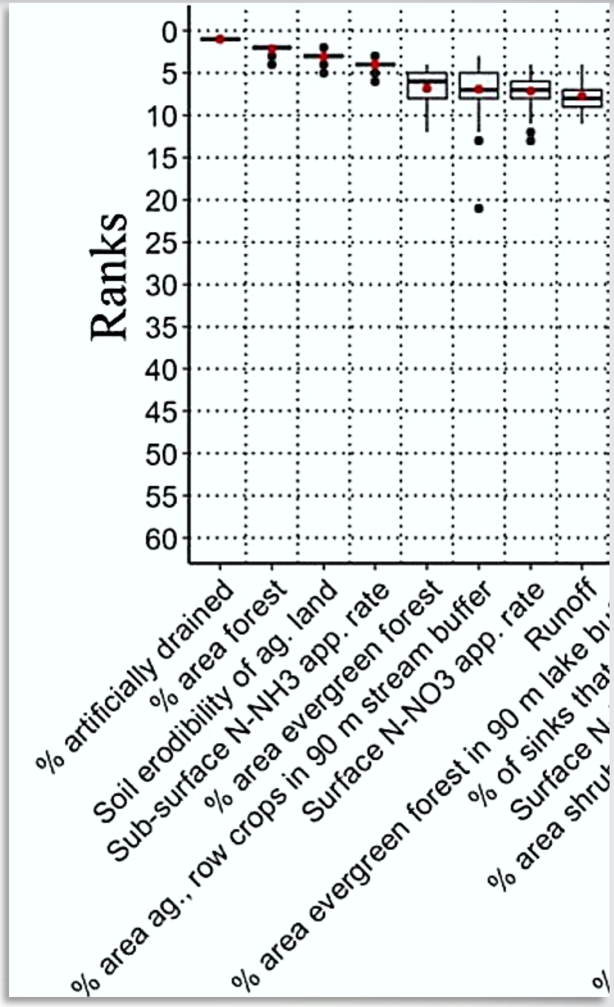
Issue: Identification of top drivers that either promote or deter cyanobacteria bloom development for 369 northcentral U.S. freshwater lakes or reservoirs.

Approach: Identify primary drivers for cyanobacteria bloom development.

Status/Ongoing Activities:

- Iames et al. 2021. *Water Resources Research.*
- Future: Use of these techniques to rank primary drivers for cyanobacteria development in estuarine systems in USA.

Partners: EPA Office of Water



Distribution of predictor variable of importance.

Predict Risk from CyanoHAB and Microcystin Toxins

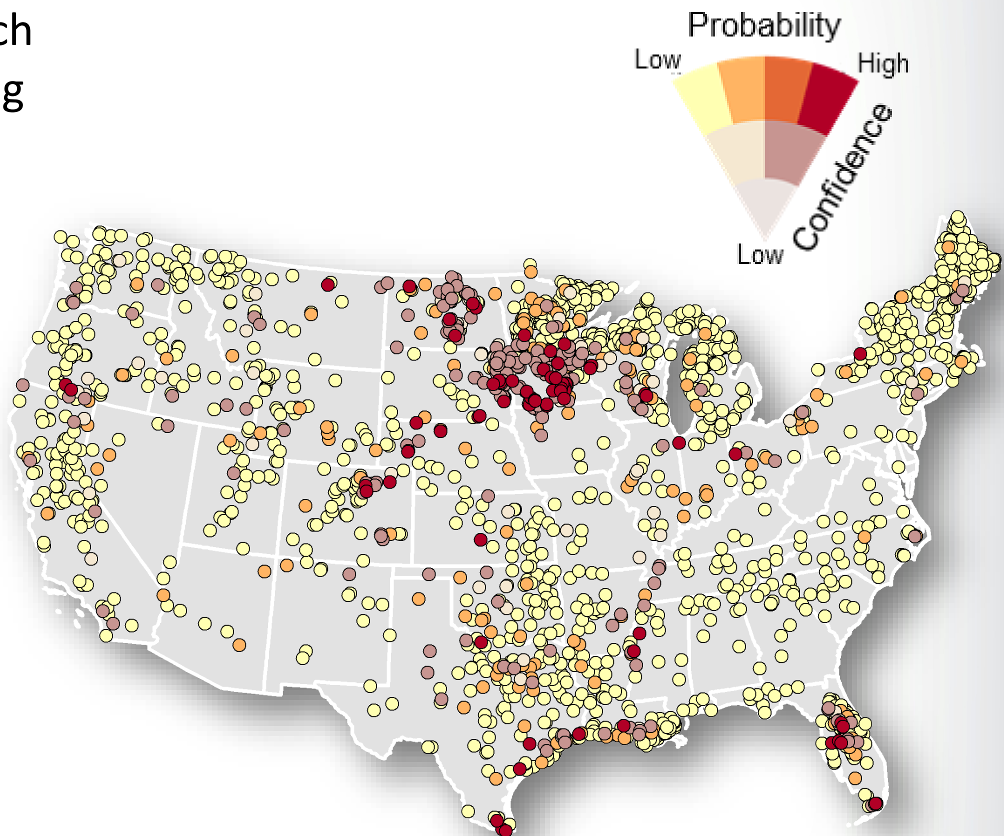
Issue: Tools are needed to prioritize which lakes are more likely to require monitoring for cyanoHABs.

Approach: Model the probability of exceeding thresholds for cyanobacteria and microcystin by combining satellite imagery and national field survey data for large lakes in the contiguous US.

Status/Ongoing Activity:

- Manuscript in EPA clearance.
- 17 external and internal EPA presentations.
- Future: Evaluate regional and local watershed drivers of cyanoHABs risk.

Partners: EPA Regions 10, 8, 7, and 4



Probability of exceeding 0.2 μg/L microcystin in 2,192 satellite-monitored lakes

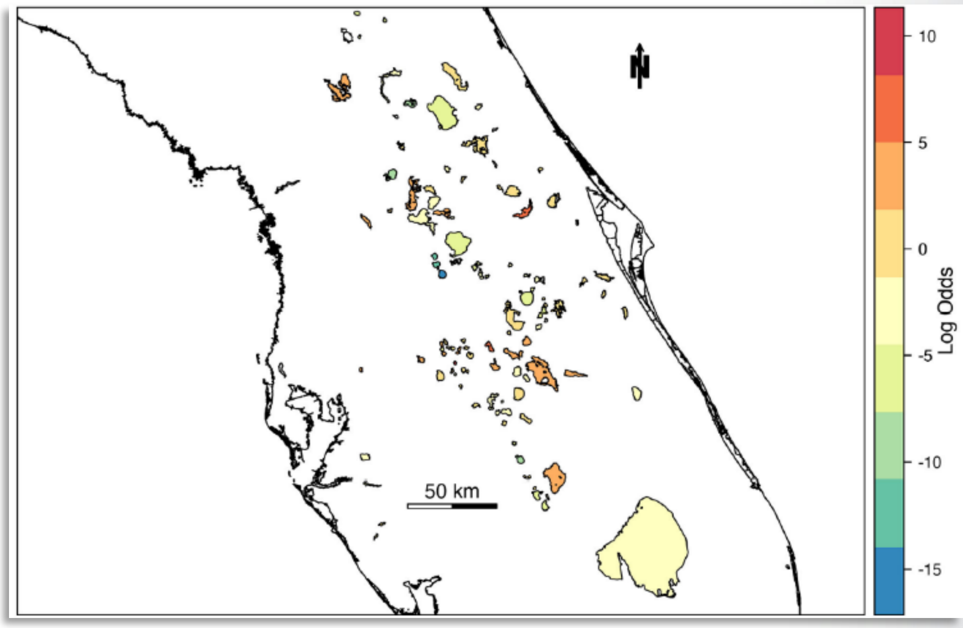
Forecasting CyanoHAB Biomass

Issue: Developing forecast capability remains a major priority.

Approach: Spatio-temporal Bayesian hierarchical model (INLA) with 88% accuracy based on holdout data.

Status/Ongoing Activities:

- Myer et al. 2020. Frontiers in Environmental Science.
- Ongoing: Apply model at a broader regional scale and assess performance.
- Future: Apply model at U.S. scale and assess performance.



Lakes with relatively higher log-odds of a high-risk cyanobacteria bloom are indicated with warm colors, while cool colors represent lakes with relatively lower log-odds of high-risk blooms.

Questions?

Charge Question 3

Harmful algal blooms are complex ecological processes that are affected by a variety of factors, including nutrient availability, water temperature, weather patterns, solar irradiation, limnology, and competing microorganisms. Much is still unknown regarding the human and environmental health effects of toxins produced during blooms.

What suggestion(s)/ recommendation(s) does the Subcommittee have on ORD's implementation of the HABs portfolios, particularly in

a) determining the toxicity of HABs, and

b) developing the capacity to forecast HAB events to prevent or mitigate exposure?