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USACE Freshwater Harmful Algal Bloom Research and Development Initiative

Developing and delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.



ERDC/EL SR-24-1

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Aquatic Nuisance Research Program (ANSRP)

ERDC/EL SR-24-1
February 2024

USACE Freshwater Harmful Algal Bloom Research and Development Initiative

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TABLE OF CONTENTS

Abbreviations iv
Preface v
Problem Statement 01
Strategic Focus Area: HAB Prevention 05
Strategic Focus Area: HAB Detection 09
Strategic Focus Area: HAB Management 13
Moving Forward Together 16
HAB Research Gaps and Needs 17
Thank You 19
References 20
Appendix 1. Project Factsheets 22
Appendix 2. HAB R&D Community Engagement 100

LIST OF FIGURES

Figure 1. 01
Figure 2. 02
Figure 3. 02
Figure 4. 03
Figure 5. 04
Figure 6. 05
Figure 7. 06
Figure 8. 06
Figure 9. 07
Figure 10. 08
Figure 11. 09
Figure 12. 09
Figure 13. 10
Figure 14. 11
Figure 15. 12
Figure 16. 13
Figure 17. 14
Figure 18. 15

ABBREVIATIONS

ADA	*Anabaena/Dolichospermum/Aphanizomenon*
ANSRP	Aquatic Nuisance Species Research Program
BGSU	Bowling Green State University
BMP	Best management practice
cHAB	Cyanobacterial harmful algal blooms
chl-a	Chlorophyll-a
CMS	Coastal Modeling System
CR	Caloosahatchee River
CyAN	Cyanobacteria Assessment Network
CyanoSTUN	Cyanobacterial Suppression Through Ultraviolet-Light-C Neutralization
DIG	Digoxygenin
DO	Dissolved oxygen
eDNA	Environmental DNA
ERDC	US Army Engineer Research Development Center
EWQD	Enterprise Water Quality Database
fDOM	Fluorescent dissolved organic matter
FLREC	Fort Lauderdale Research and Education Center
FY	Fiscal year
HAB	Harmful algal bloom
HABHRCA	Harmful Algal Bloom and Hypoxia Research and Control Act
HABITATS	Harmful Algal Bloom Interception, Treatment and Transformation System
HAWQS	Hydrologic and Water Quality System
HRP	Horseradish peroxidase
H_2O_2	Hydrogen peroxide
ID&Q	Identification and quantification
KDHE	Kansas Department of Health and Environment
LBMT	Light-Based Mitigation Technology
LLD	Lower limit of detection
MC	Microcystin
MOI	Multiplicity of infection
NASA	National Aeronautics and Space Administration
NBOT	Nanobubble ozone technology
NCCOS	National Centers for Coastal Ocean Science
NEDIS	National Environmental Satellite, Data, and Information Service
NEPA	National Environmental Protection Act
NOAA	National Oceanic and Atmospheric Administration
N:P	Nitrogen-to-phosphorus ratio
NWK	USACE Kansas City District
OSU	Ohio State University
PC	Phycocyanin
qPCR	Quantitative PCR
RAFT	Research on Algae Flotation Techniques

ABBREVIATIONS

R&D	Research and development
ROV	Remotely operated vehicle
SAJ	USACE Jacksonville District
SFWMD	South Florida Water Management District
SHA	Sandwich hybridization assay
SWT	USACE Tulsa District
TAMUG	Texas A&M University Galveston
TiO_2	Titanium dioxide
TSS	Total suspended solid
UAS	Unmanned aircraft system
USACE	US Army Corps of Engineers
USDA-ARS	US Department of Agriculture's Agricultural Research Service
USEPA	US Environmental Protection Agency
USGS	US Geological Survey
UV	Ultraviolet
WLEB	Western Lake Erie Basin
WQ	Water quality
WRDA	Water Resources Development Act
WRDP	Water Resource Development Projects

PREFACE

This study was conducted for the US Army Engineer Research and Development Center–Environmental Laboratory (ERDC-EL) under AMSCO Code 008284.

The work was performed by the Office of the Technical Director and the Environmental Processes Branch of the Environmental Processes and Engineering Division, ERDC-EL. At the time of publication, Ms. Broke Steevens was branch chief; and Mr. Warren Lorentz was division chief; and the lead technical director was Dr. Elizabeth Ferguson . The deputy director of ERDC-EL was Dr. Brandon J. Lafferty, and the director was Dr. Edmond J. Russo Jr.

COL Christian Patterson was commander of ERDC, and Dr. David W. Pittman was the director.

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USACE FRESHWATER HARMFUL ALGAL BLOOM RESEARCH AND DEVELOPMENT INITIATIVE

Harmful Algal Blooms (HABs) represent a significant and costly threat to our nation's economy and natural resources. This report outlines the progress of the US Army Corps of Engineers–Engineer Research and Development Center (USACE-ERDC) in delivering scalable technologies for prevention, early detection, and management of HABs to reduce HAB event frequency, severity, and duration.

Problem Statement

Freshwater HABs are defined generally as an overgrowth of algae that harms the environment and economy. HABs often give water a blue-green or green soupy appearance (Figure 1),* but brown or red water discoloration may also occur. HABs impact ecosystems in a variety of ways, including the production and release of potent neurotoxins, hepatotoxins, cytotoxins, and gastrointestinal toxins, which directly impact human and wildlife health.[1] Although some HABs do not produce toxins, all HABs degrade aquatic ecosystems by shading out submerged vegetation and disrupting aquatic food webs. Decay of HAB biomass can also cause prolonged decreases in dissolved oxygen (hypoxia), which can kill fish and harm benthic macroinvertebrate communities.[2,3]



Figure 1. Examples of HABS at USACE reservoirs: Marion Reservoir (*left*) and Milford Lake (*right*; photo by Marvin G. Boyer). HABs can cause closures of boat ramps and recreation areas.

The devastating effects of freshwater HABs occur across multiple ecoregions, from large lakes (e.g., Finger Lakes in New York and Lake Okeechobee in Florida) to large inland waterways (e.g., Ohio River), costing US communities approximately $1B between 2010 and 2020.[4] A 2011 HAB event in Lake Erie caused the city of Toledo to lose potable water access for more than two days, costing an estimated $71M in economic losses.[5] A similar event recurred three years later, costing an estimated $65M in economic losses.[6,7] In 2015, a HAB event on the Ohio River persisted for over a month along 700 miles of the waterway (Figure 2). HAB events such as these are a symptom of nutrient pollution, which causes an estimated $1B/year in recreation and tourism revenue loss.[8] Warm-

* Most freshwater HABs are caused by photosynthetic microbes called cyanobacteria, which are sometimes informally referred to as blue-green algae.



Figure 2. Ohio River near Maysville, Kentucky, in September of 2015.

ing temperatures are expected to increase these impacts over time.[9,10]

HABs are particularly impactful to USACE, which manages vast waterways that provide a variety of services to the nation, including navigation, flood risk reduction, recreation, and fish and wildlife management, as well as potable water supply (Figure 3). A recent survey of USACE waterway projects found HAB event frequency had increased between 2004 and 2017, and by 2020 47% of USACE districts had been impacted by HABs (Figure 4).[11] Federal policy identifies HABs as one of the "most scientifically complex and economically damaging aquatic issues" posing a "significant challenge to the ability to safeguard the health of the nation's coastal and freshwater ecosystems."[12] The 2017 reauthorization of the Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA) underscored HABs as a national priority, acknowledged the need for HAB research, and added USACE as the 13th member agency of the HABHRCA Interagency Task Force.

USACE-ERDC was directed to develop and demonstrate technologies that will reduce HAB impacts across the nation. The Water Resources Development Act of 2018 (WRDA 2018, Sec. 1109) authorized USACE to implement a 5-year research initiative focused on scalable technologies for HAB detection, prevention, and management intended to reduce HAB frequency and effects on our nation's water resources. Funding to support implementation was first appropriated in fiscal year (FY) 2019 and was executed under the Aquatic Nuisance Species Research

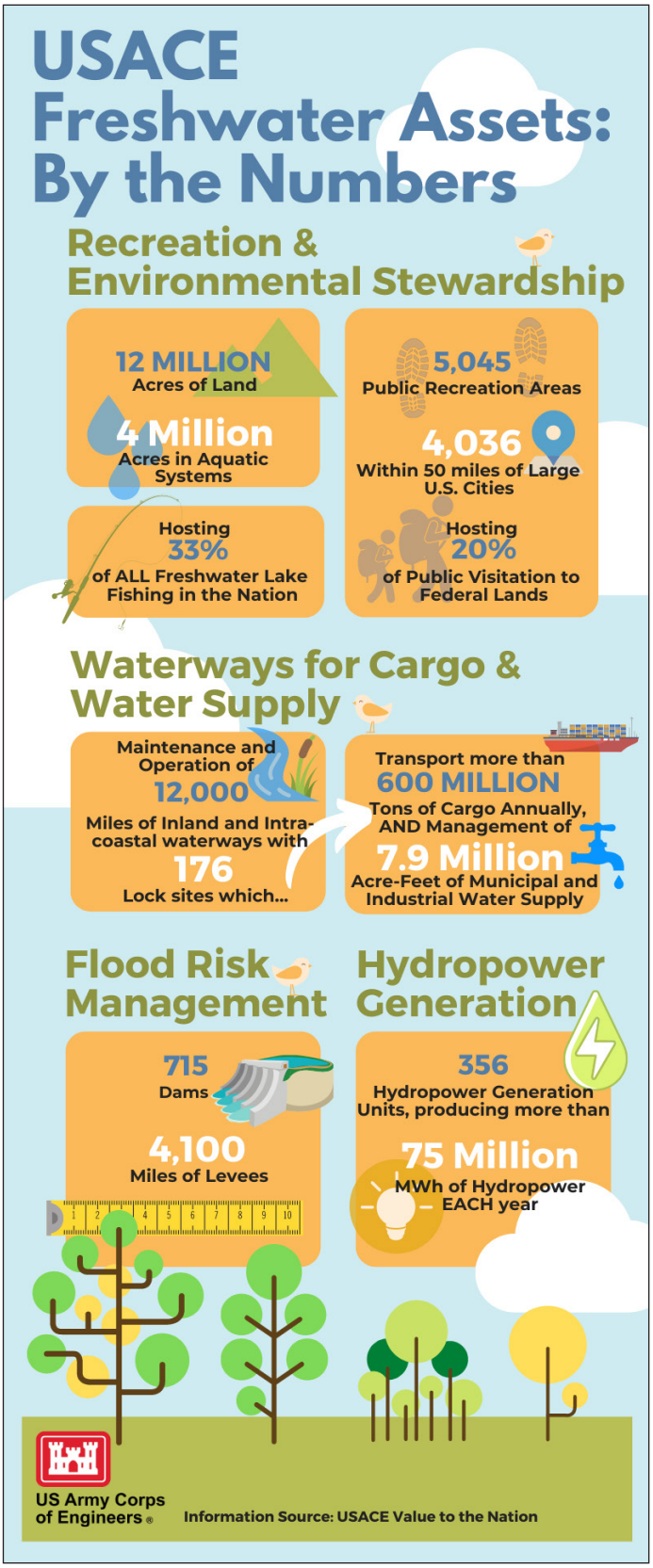


Figure 3. USACE freshwater assets from USACE Value to the nation.

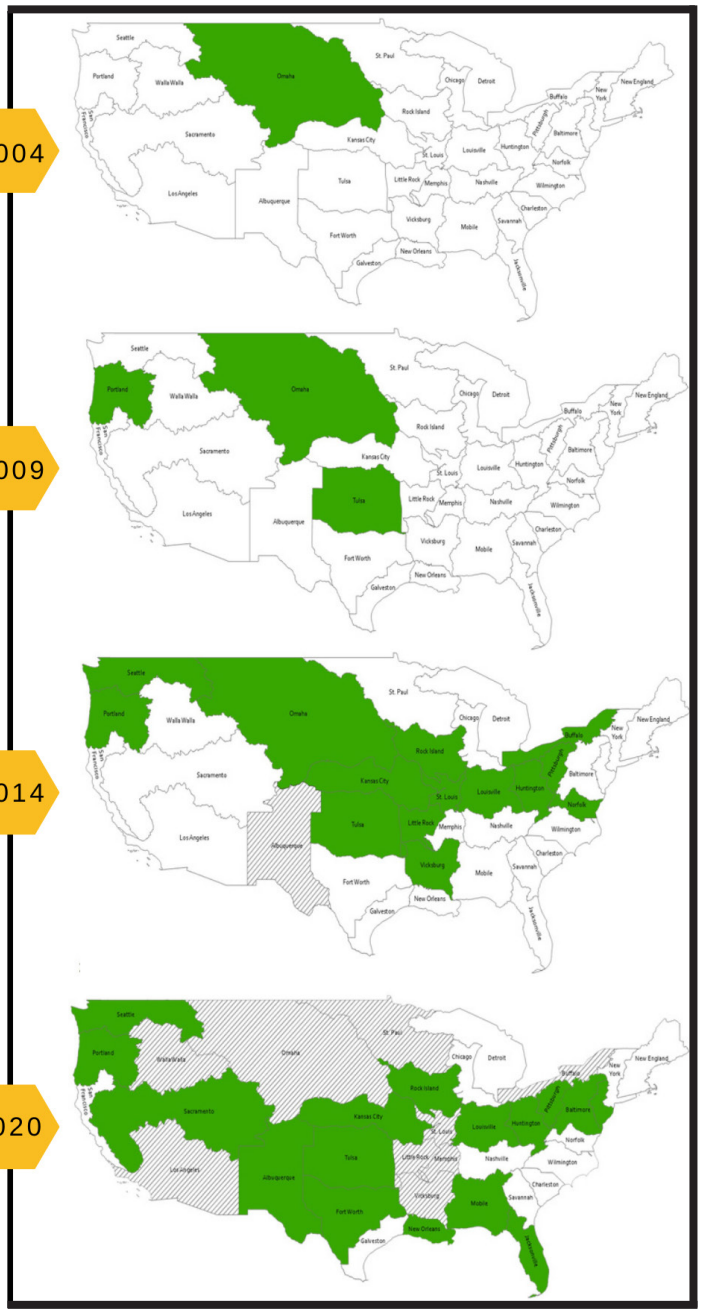


Figure 4. USACE districts reporting HAB events from 2004 to 2020. Green fill indicates a district reported a HAB event in the given year. White fill indicates that no HAB event was reported in the given year. Hatched fill indicates that no report was filed by a district in the given year (HAB events may have been experienced, but they were not reported).

Program (ANSRP). In addition to WRDA 2018, WRDA 2020, Sec. 128, authorized "[a] demonstration program to determine the causes of, and implement measures to effectively detect, prevent, treat, and eliminate, harmful algal blooms associated with water resources development projects."* The WRDA 2020–authorized program was intended to demonstrate field ready HAB prevention, detection, and management technologies to both benefit HAB impacted water resource development projects and to generate verified technology cost and performance data in support of technology transfer and informed technology use.

This special report commemorates the USACE Freshwater HAB Research and Development (R&D) Initiative and the progress made by USACE, ERDC, and partners during its 5-year implementation (Figure 5). Research highlights are organized by strategic focus areas: HAB prevention, detection, and management. The report concludes with a summary of ongoing HAB research that will continue beyond FY23, pending availability of funds, and a summary of remaining HAB research needs identified by USACE and partners. Appendix 1 includes factsheets for all projects initiated as part of the WRDA 2018–authorized USACE Freshwater HAB R&D Initiative as of report publication; factsheets are updated periodically and posted to our website. We would like to thank our USACE district, federal, state, and local partners for their participation in multiple workshops and listening sessions hosted over the past 5 years, which shaped the development and delivery of the work summarized in this report. Appendix 2 includes workshop summaries.

For additional information on the USACE-ERDC Aquatic Nuisance Control Research Program, please visit https://ansrp.el.erdc.dren.mil/HAB.html.

* https://www.congress.gov/116/plaws/publ260/PLAW-116publ260.pdf.

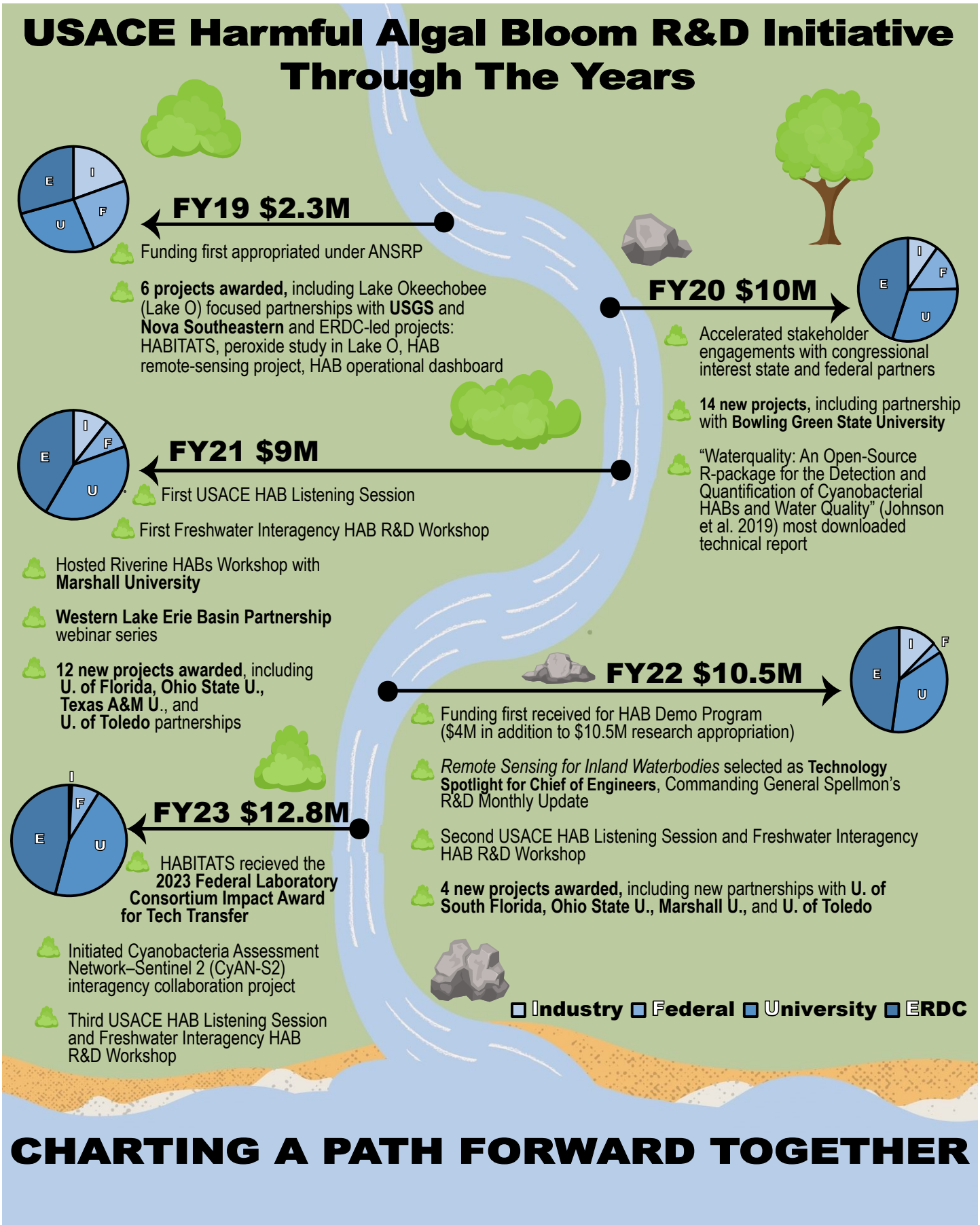


Figure 5. Graphic timeline depicting the USACE HAB R&D Initiative's progress each year from FY19 to FY23. (HABITATS is the Harmful Algal Bloom Interception, Treatment and Transformation System.)

Strategic Focus Area: HAB Prevention

Factors influencing freshwater HAB events include excessive nutrient (e.g., nitrogen and phosphorous) loading from varied sources including high-density septic discharge and agricultural runoff, poor water circulation, temperature, and extreme hydrologic or climatic events. USACE guidance indicates "water quality can be considered as an output of an ecosystem restoration project. However, projects or features that would result in treating or otherwise abating pollution problems caused by other parties where those parties have or are likely to have a legal responsibility for remediation or other compliance responsibility shall not be recommended for implementation."[13] Accordingly, ERDC's HAB prevention research primarily emphasized novel preventative treatments and engineering solutions versus nutrient reduction–related water treatment because water quality is only implicitly included under the USACE Civil Works mission. Partnerships with federal and state agencies that have water quality missions are key to understanding HAB event drivers and developing technologies to prevent them. Several HAB prevention focused projects are highlighted below.

Identification and preventative treatment of overwintering cyanobacterial cells in sediments (ERDC, Factsheet 20). Cyanobacteria cells can "overwinter" in sediments, become active under favorable conditions and then potentially perpetuate HAB events in subsequent seasons.[14] Dr. Andrew McQueen and his team at ERDC exposed sediment containing overwintering cells to copper- and peroxide-based US Environmental Protection Agency (USEPA) registered algaecides to determine if treatment in late winter or early spring could reduce HAB risks during the bloom season (typically May–October). Solid peroxide-based algaecides were most effective in minimizing cell density and phycocyanin concentrations during lab studies. Results from an 80-acre pond field demonstration were consistent with bench-scale observations, with average cyanobacteria cell densities remaining low May–October post-treatment compared to an adjacent control (Figure 6). Project results provide novel, preventative chemical strategies to reduce HAB severity.[15]

Ultrasound as a source water reservoir management strategy to control cyanobacteria blooms (Ohio State University, Factsheet 35). Ultrasound is often marketed as a HAB prevention and management strategy but key questions remain about how and why it works or does not work. Dr. Linda Weavers and her team at Ohio State University (OSU) designed a series of experiments to determine how ultrasound exposure impacts cyanobacteria cells. Results of controlled, small-scale laboratory experiments indicated *Microcystis* sp. undergo rapid sonoporation during ultrasound exposure, with algal cells becoming permeable in a matter of minutes. Additional experiments are underway to determine if similar results are achievable in field-deployed systems (Figure 7), and to assess whether ultrasonic signals can be tuned over space and time and whether sonoporation may leave cyano-



Figure 6. Effective control of overwintering cyanobacteria in sediments, with bloom formation successfully mitigated 6 months after winter treatment in 80 acre treatment (*right*) versus control (*left*).



Figure 7. Floating ultrasonic unit deployed at a demonstration site, including a submerged ultrasonic device (*arrow*), solar panels to power the device, and bird deterrents being utilized as solar panel protection.

bacteria more susceptible to lower dosage algaecide treatment. Results will be used to develop practical end-user guidance, enabling optimized ultrasound technology use alone and in combination with algaecide.

Light-based mitigation technology for the reduction of HABs (ERDC, Factsheet 7). Germicidal ultraviolet light (UV-C) can, under specific exposure conditions, inactivate cyanobacterial cells and neutralize their toxins without the use of chemicals. Here, a team led by ERDC's Mr. Taylor Rycroft identified the UV-C doses required to suppress harmful cyanobacteria for specific periods of time and then used this information to design and construct the ERDC CyanoSTUN (Cyanobacterial Suppression Through Ultraviolet-Light-C Neutralization) vessel (Figure 8). The team conducted a field trial with the CyanoSTUN in Massachusetts during the summer of 2023. Field study outcomes will inform CyanoSTUN design enhancements and implementation of UV-C-based HAB prevention and management strategies across the nation.

Mitigation of HAB toxins using deployable 3D-printed photocatalytic devices (ERDC, Factsheet 17). Photocatalytic titanium dioxide (TiO_2) nanoparticles represent an appealing targeted and reusable HAB-mitigation strategy.[16] TiO_2 reacts with sunlight to produce short-lived free radicals, which target and breakdown harmful algal bloom toxins but dissipate before long-term, nontarget species impacts can occur. Challenges include the rapid settling of TiO_2 particles out of the photoactive zone—which reduces effectiveness—and the undesirability of releasing free nanoparticles into the environment. ERDC's Mr. Al Kennedy and team solved these challenges by immobilizing TiO_2 particles into deployable, retrievable 3D-printed structures that can be customized for site-specificity. Lab demonstrations showed that 3D-printed TiO_2 degrades HAB toxins 12 times faster than UV light alone.[17] Treatment times can be decreased with surface modifications and more intense light (Figure 9). Protocols for an optimized TiO_2-based treatment will be available to USACE districts pending final publication of an associated

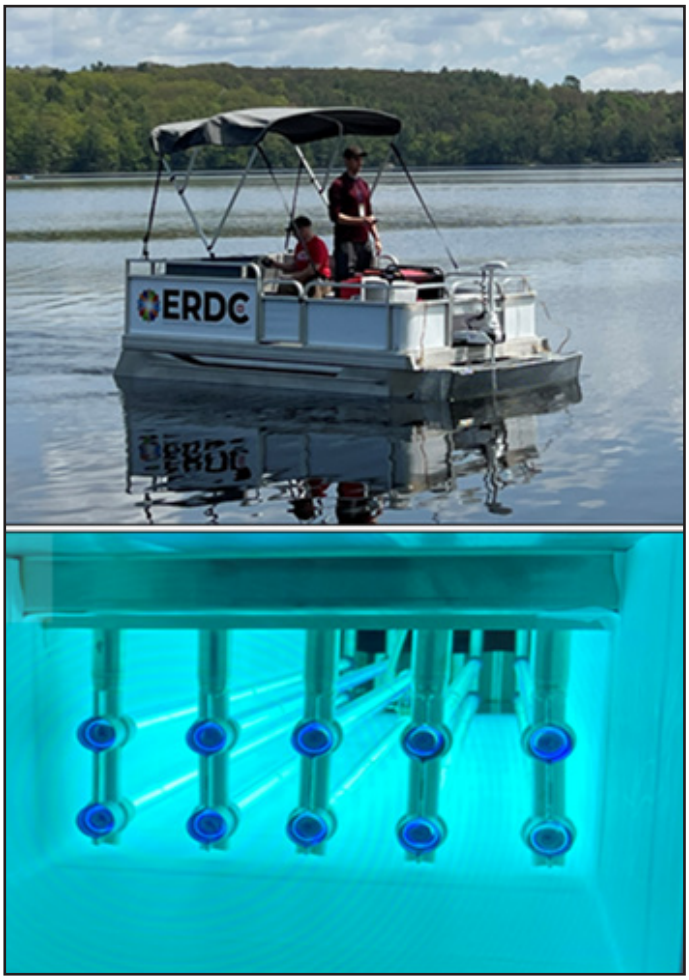


Figure 8. *Top right*, ERDC CyanoSTUN vessel during operation; and *bottom right*, UV-C reactor mounted beneath the CyanoSTUN pontoon platform.

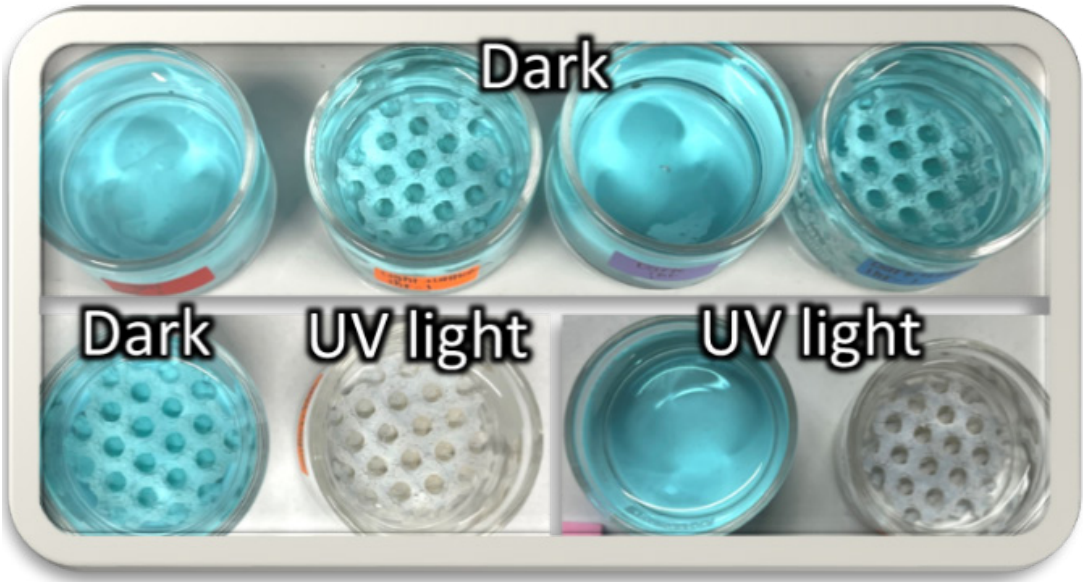


Figure 9. 3D-printed TiO_2 structures are fabricated using a disk and lattice design to increase surface area and improve HAB mitigation capability. Experimentation indicates that UV light further improves this technology, as shown by clearing of the blue liquid and associated absorbance visualized on the TiO_2 structure.

ERDC Technical Report. The workflow requires inexpensive 3D printers and TiO_2 filament for small-scale deployment. Future industry-scale deployment could be achieved through public-private partnerships using 3D printer farms or traditional manufacturing.

Factors critical to long-term lake and reservoir management: relationships between land use, nutrient loading, inflows, HABs, and anoxia (Texas A&M University, Factsheet 27). Many of the lakes in the southcentral US are thermally stratified systems that experience annual mixing, eutrophication, and recurrent HABs. User-friendly modeling tools are needed to make data-driven decisions for reservoir operations and long-term HAB management within these systems. This requires a better understanding of the factors driving recurrence. Dr. Dan Roelke and team investigated how varied land-use practices, seasonal nutrient loading (magnitude, type, and source), and hydraulic residence times influence HABs, hypoxia, and microbial functioning within these water bodies. Environmental data (e.g., genetic and water quality) collected from 20 reservoirs was used to quantify relationships among these factors as they pertain to HABs. The data is being used to deliver a user-friendly, quantitative modeling tool nonexperts can use to make informed water management decisions that minimize long-term HAB risks within these systems. Collaborators at ERDC's Environmental Lab, Dr. Marissa Kneer and team, are providing further insight into the importance of internal nutrient loading from sediments on HAB recurrence in these reservoirs using intact sediment core experiments to determine the magnitude of sediment nutrient loading during seasonal anoxia and resuspension events (Factsheet 18).

Operational strategies for HAB management in inland reservoirs (ERDC, Factsheet 29). A dynamic interplay exists between water flow, water quality, and HABs, making it difficult to anticipate how reservoir-based operational decisions impact HAB management. Using routine monitoring data and the patterns observed therein, Dr. Jodi Ryder and team were able to identify water quality and hydrodynamic parameters most indicative of blooms at several managed lakes. This information was subsequently leveraged to develop a statistical model that assesses likelihood of bloom formation within a reservoir in the short term, and to assess various operational flow changes for potential bloom-mitigating effects. The produced model will prove useful in decision-making processes, and allows water resource managers the powerful ability to observe operational changes (and associated impacts) within a model framework before enacting changes.

Coupling lake, watershed, and estuarine models to better understand the role of engineered

freshwater discharges in driving the severity, location, and timing of HABs (University of Florida, Factsheet 37). USACE faces challenges in satisfying responsibilities for reservoir discharge in HAB-impacted systems. Using coupled lake, watershed, and estuarine models, Dr. David Kaplan and team are investigating the role freshwater discharges play in HAB severity, location, and timing in order to identify optimal discharge scenarios that minimize HAB risk to receiving waters while also meeting requirements for flood risk management and for maintenance of essential flow to ecologically critical habitats. This project aims to develop data- and model-driven guidance for engineered releases and for watershed management based on an improved understanding of the interactive effects of discharges, watershed flow and nutrient deliveries, phytoplankton community dynamics, and river/nearshore hydrodynamics (Figure 10). While this effort is focused on Lake Okeechobee and the Caloosahatchee River watershed, findings can be used to similarly inform reservoir management decisions nationwide.

Investigating redirection of Detroit River flow to reduce HABs in Western Lake Erie Basin (USACE Detroit District, ERDC, and University of Toledo Collaboration, Factsheet 4). This collaboration leverages the hydrodynamic modeling expertise of USACE-ERDC and existing modeling tools to simulate the hydrodynamic effect of conceptual in-water features to determine if dilution scenarios may be feasible. Outcomes of this limited-scope study will help stakeholders consider and potentially refine the concept for further study, pending results.

Developing scalable HAB prevention technologies in the Ohio region (OSU, Factsheet 2). Sustainable and scalable solutions for HAB prevention require a reduction in nonpoint nutrient sources that contribute to bloom formation—a challenging endeavor. To address this need, OSU's Dr. Michael Brooker and his team partnered with the US Department of Agriculture's Agricultural Research Service (USDA-ARS) to evaluate USDA approved best management practices (BMPs) for nutrient management on legacy-phosphorous fields. Efforts leveraging existing networks (Pilot Watershed and Public-Private Partnership) are ongoing to identify agricultural sites of interest where drainage samples will be tested to quantify impacts of BMPs and determine—via modeling of obtained results—ways to improve BMPs to achieve improved nutrient reduction outcomes locally and at watershed scales.

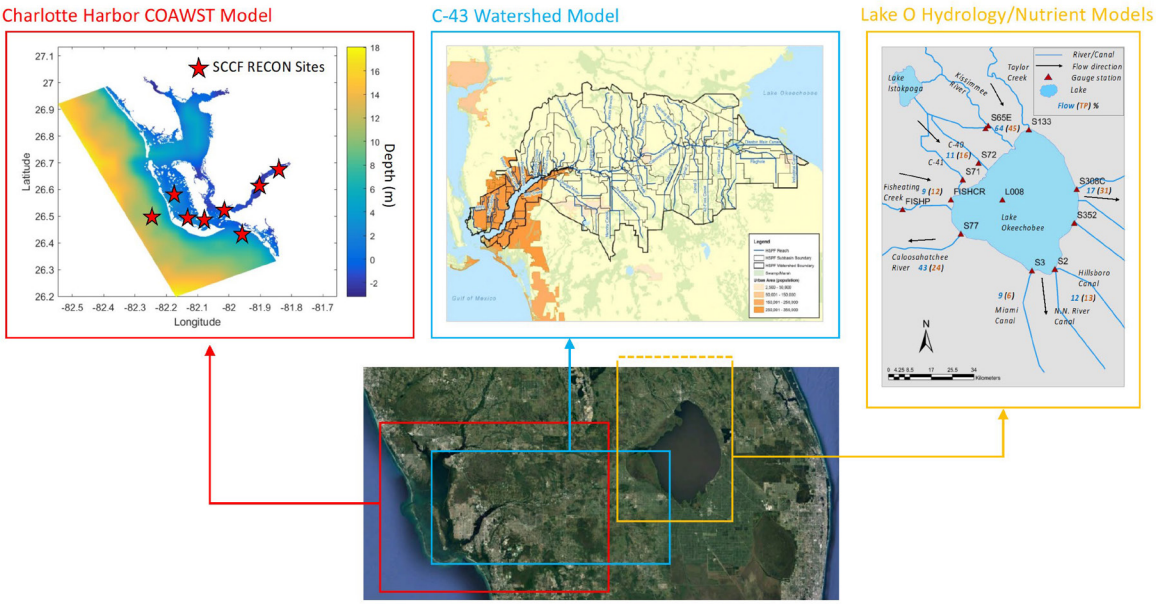


Figure 10. To better understand the role freshwater discharge plays in HAB formation, researchers are combining data and models spanning from Lake Okeechobee to the Caloosahatchee River Estuary. The effort uses lake, watershed, and coastal hydrodynamic modeling to assess how lake operation decisions affect estuarine circulation, residence time, and water quality, including particle tracking within the Caloosahatchee River.

Strategic Focus Area: HAB Detection

Rapid HAB detection capability, which includes identification and quantification (ID&Q) of cyanobacteria species and their toxins present in freshwater samples, is critical for effective risk management. Current methodologies for both species and toxin analysis are costly and/or have long analysis times, which delays critical risk management decision-making. Research by ERDC and partners in the HAB detection focus area emphasizes rapid ID&Q of both cyanobacteria and toxins using innovative technologies. Examples include field sensors for rapid water sample toxin analysis as well as remote sensing for rapid HAB assessment (Figure 11).



Figure 11. USACE-ERDC researchers and partners working at field sites to develop rapid, quantitative methods for HABs and HAB toxin detection, which is key for effective HAB risk management.

Remote sensing and related tools for HAB surveillance and informed monitoring (ERDC, Factsheets 15, 25, 26). Numerous environmental monitoring and detection challenges can be met with remote-sensing platforms (satellites and airborne systems). Imagery collected by these technologies are being investigated to assess the presence/absence, severity, and extent of HABs via spectral indicators, which can be correlated to HAB-associated pigments (e.g., chlorophyll a and phycocyanin; Figure 12). ERDC researchers, Ms. Molly Reif and Dr. Richard Johansen and partners, are aiming to improve the detection capabilities and accuracy of these monitoring technologies by (1) investigating whether cyanobacteria genera can be discriminated using their unique spectral signatures in lab and field;[18–20] and (2) evaluating locally optimized spectral algorithms[21] for broad use across large geographic extents and varying factors, including field data acquisition methods, geographic regions, and seasonality. Decision support software tools that build on foundational remote-sensing research have also been developed and delivered through HAB R&D efforts. They provide methods to rapidly detect water quality indicators of HABs, ultimately supporting proactive (verses reactive) monitoring and management strategies for USACE-managed lakes and reservoirs. The tool series includes a comprehensive R software package: waterquality; a streamlined Python-based toolbox: waterquality for ArcGIS Pro;[22] and an Esri web viewer: HAB Explorer (Figure 13). Each

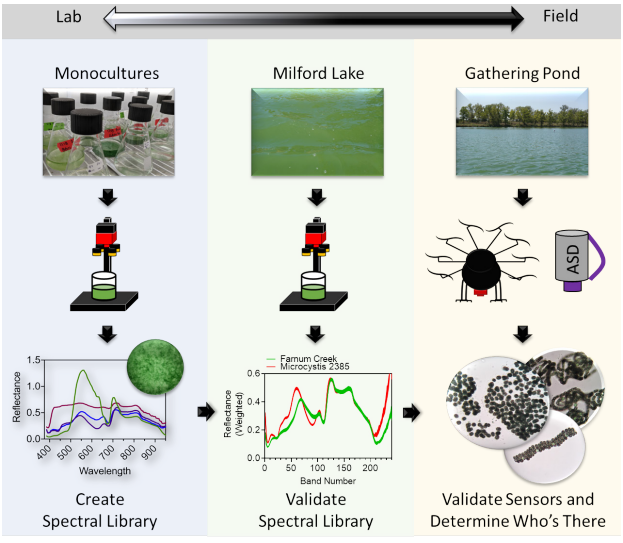


Figure 12. Remote-sensing HAB detection capabilities were improved through the development of cyanobacteria-specific spectral libraries, which were combined with sensor-based platforms (and associated algorithms) before validation in-field at USACE-managed Milford Lake (Kansas).

tool was designed with specific technical transfer considerations and accommodates a broad, end-user base with variable skill sets and monitoring needs. Technical trainings, demonstrations, and solicited end-user feedback led to tool optimization, enhanced functionality, and increased usage. End products (e.g., maps, graphs, and statistical analyses) equip managers with a suite of monitoring options that aid near real-time characterization of inland waterbody conditions and assists with critical HAB management decisions. In total, these tools enable translation of complex data into meaningful, decision-supporting information without the need for specialized

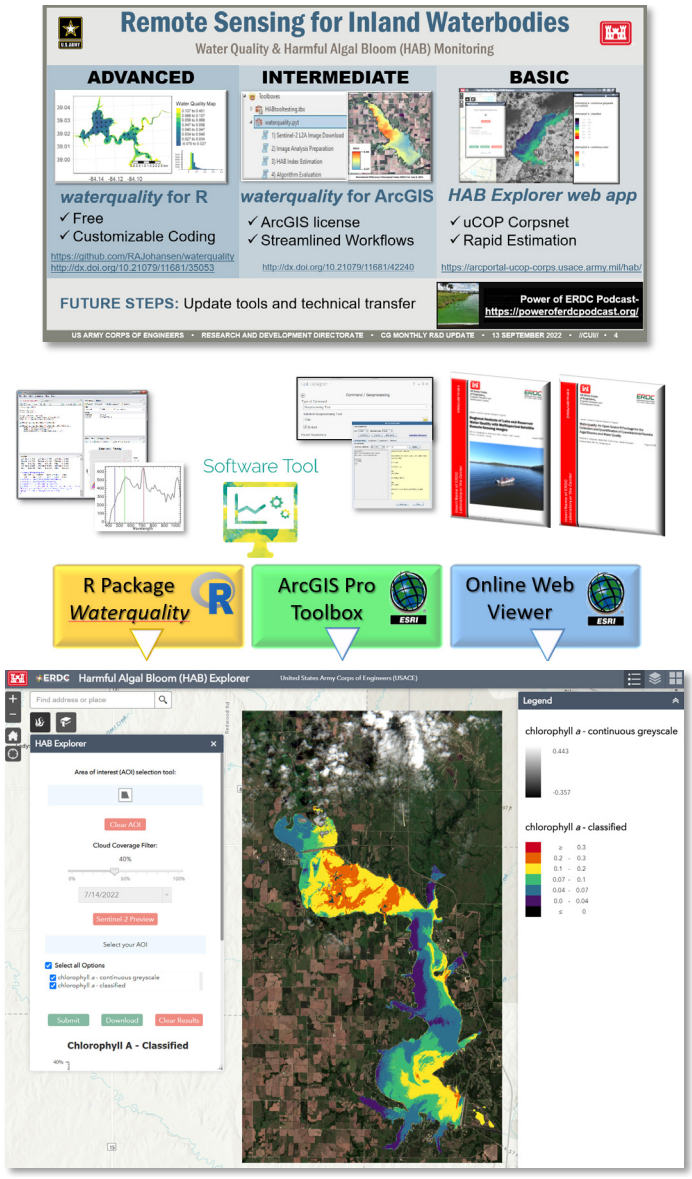


Figure 13. ERDC-developed packages for processing and interpreting remotely sensed HAB data include an advanced, intermediate, and basic option: waterquality for R, waterquality for ArcGIS, and the HAB Explorer web app (respectively).

expertise. ERDC's remote sensing–based HAB detection products have been extremely well received by USACE; the associated technical report titled "Waterquality: An Open-Source R Package for the Detection and Quantification of Cyanobacterial Harmful Algal Blooms and Water Quality"[23] was the most downloaded ERDC publication in 2020.

Cyanobacteria Assessment Network: pilot study with Sentinel-2–derived chlorophyll data (CyAN-S2) (Interagency collaboration, Factsheet 32). The Cyanobacteria Assessment Network (CyAN) has provided cyanoHAB-relevant satellite data and cyanobacteria products to support safe recreational and drinking water across more than 2,000 lakes throughout the United States. CyAN was initiated in 2015 and successfully achieved Application Readiness Level 9 for sustained use in decision-making in 2023. Stakeholders have requested a higher spatial- and temporal-resolution product for improved cyanoHAB detection to better serve both the public and federal agencies. Researchers from across five federal institutions (USACE, USEPA, US Geological Survey [USGS], National Aeronautics and Space Administration [NASA], and National Oceanic and Atmospheric Administration [NOAA]) aim to meet this request by developing a national chlorophyll product using Sentinel-2 satellite imagery. FY23 activities include a USACE-funded interagency pilot study using data sets (in situ and satellite) from across three priority regions encompassing a total of 212 lakes, including Harsha Lake (Ohio), Lake Okeechobee (Florida), and Detroit Lake (Oregon). Results will be published in FY24 and set the stage for an interagency collaboration to develop, validate, and deliver a national Sentinel-2–based CyAN.

Predictive models and efficient data-processing tools (NOAA National Centers for Coastal Ocean Science [NCCOS] and ERDC, Factsheets 24, 28). The HAB Nowcast/Forecast project, led by NOAA-NCCOS's Dr. Richard Stumpf and team, predicts HAB location and intensity within Lake Okeechobee near-term (from 1 to 5 days), and incorporates the hydrodynamic circulation and environmental drivers to predict likelihood that a bloom will intensify

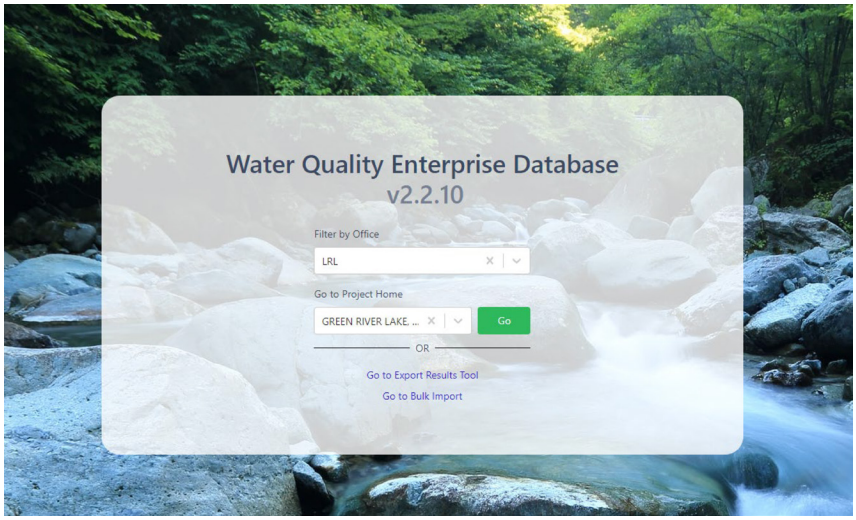
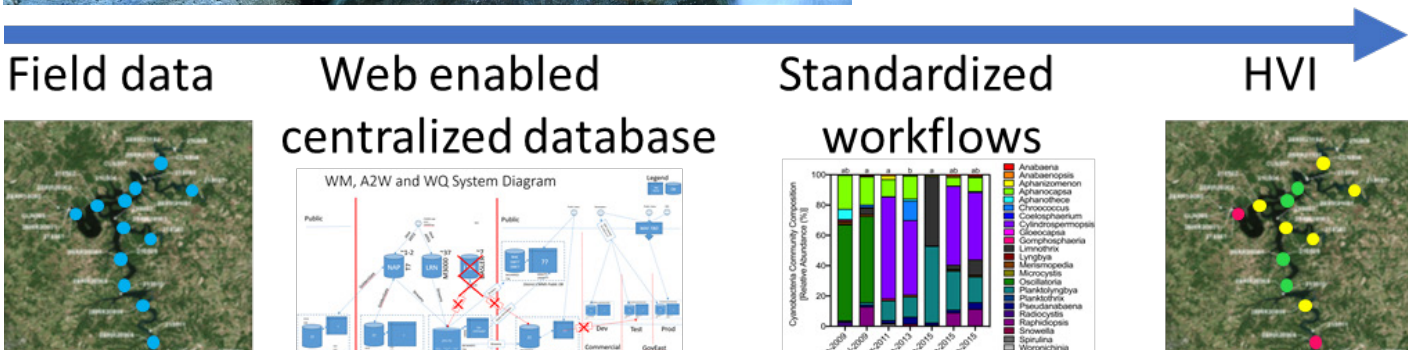


Figure 14. The Water Quality Enterprise Database is accessible through a web-portal (*left*) and provides users with a HAB vulnerability index (HVI) based on statistical/modeling workflows incorporating historic and user-input data.

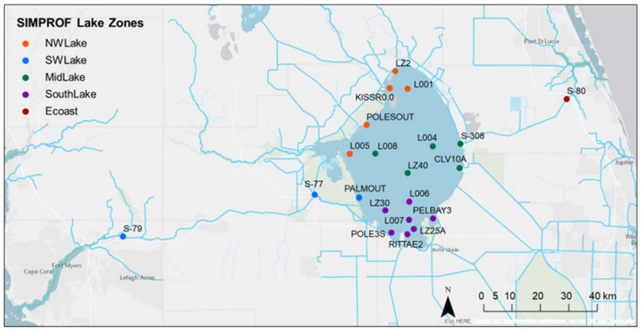


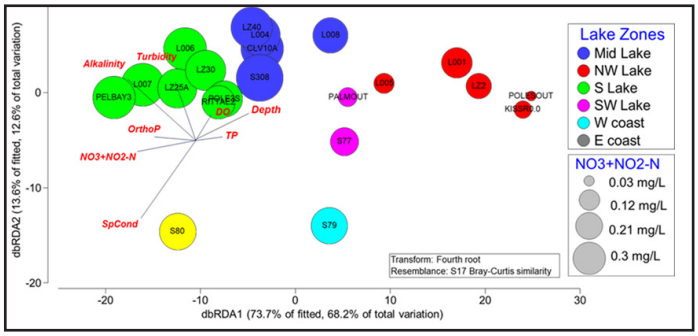
or decline over weeks to a month (Factsheet 24). The HAB Nowcast/Forecast tool incorporates multiple data types (satellite, water quality, and weather) and identifies trends within and relationships among them. Stakeholder engagement has ensured the tools meet end-user needs and expectations. Although the HAB Nowcast/Forecast tool is specific to Lake Okeechobee, the approach taken to develop it is nationally relevant and could potentially be adapted to meet similar modeling needs across numerous USACE-managed reservoirs. In a separate project, ERDC's Dr. Jodi Ryder and team have developed new tools and HAB-specific functionalities within the USACE-wide Enterprise Water Quality Database (EWQD), including statistical analyses, standardized data extraction tools, search functions, and dashboard views to make HAB-related water quality data analysis and interpretation more efficient and available to end-users with various expertise (Figure 14, Factsheet 28).

Rapid field detection of HAB-forming genera (Bowling Green State University, Factsheet 3). Expedited methods are needed for on-site monitoring of freshwater HAB-forming cyanobacteria and the toxins they produce. Researchers led by Dr. George Bullerjahn of Bowling Green State University (BGSU) developed a rapid test capable of identifying and quantifying prevalent HAB-causing cyanobacteria in a single water sample at the same time. The multiplexed test employs genera-specific molecular technologies (sandwich hybridization assays [SHAs] targeting 16S rRNA[24]). To enable on-site use by non-expert personnel, the molecular technologies are combined with fluorescent/colorimetric probes and field-portable instrumentation. Results are easily interpretable, providing information to discriminate among seven of the most common bloom-forming cyanobacteria and to quantify their biovolumes. The new tool is designed to provide near real-time actionable results, thus reducing delays associated with critical data acquisition. ERDC's Dr. Ping Gong and his team have developed new primers for quantifying genes associated with common cyanobacteria (Factsheet 9) and have used these new primers to help BGSU in their SHA probe design and validation. Such collaborative efforts are accelerating delivery of HAB detection technologies to support rapid decision-making and response.

Active early detection and diagnosis of HABs with scalable biological treatment strategies (University of Toledo, Factsheet 30). Near real-time HAB diagnostics including novel sensors and rapid molecular tools can provide critical information to water managers. This study led by University of Toledo will develop and validate cutting edge HAB diagnostics to support improved water management decision-making and will also evaluate effectiveness of multiple algaecides on HAB impacted water; thus developing a robust data set linking novel HAB diagnostics with treatment performance. One exciting and novel aspect of this project is that the extensive molecular microbial community characterization data generated, which will include information on both the algae and the viruses that target the algae, may be able to identify molecular markers that can serve as early warning of toxin release, thereby providing advance notice to the public and to water treatment plant operators that additional toxin removing treatment may be required.

Profiling microbial community composition and functions in the Lake Okeechobee watershed (Nova Southeastern University and USGS, Factsheets 19 and 33). Teams led by Drs. Jose Lopez and Viviana Mazzei conducted monthly field surveys to study the spatiotemporal patterns, functions, and drivers of microbial assemblages coupled with in situ mesocosm experiments to test microbial community response to artificially elevated NO_3, NH_4, urea, and PO_4 and their potential as HAB "drivers" (Figure 15).They found that phytoplankton assemblages are strongly spatially structured in the Lake Okeechobee system and dominated by cyanobacteria including species capable of producing microcystin, cylindrospermopsin, anatoxin, and saxitoxin. Distance-based linear models revealed that NO_3 explained the greatest proportion of the spatial variation individually (45%) and the addition of total phosphorous, turbidity, conductivity, dissolved oxygen (DO), alkalinity, depth, and orthoP cumulatively explained 92% of the total spatial variation. In mesocosms, also dominated by cyanobacteria, NO_3 and NH_4 resulted in significantly higher algal cell, chl-a, and phycocyanin concentrations after 72 hours of daily nutrient dosing. Molecular techniques revealed that treatment with NH_4 and urea induced upregulation of photosynthesis, metabolism, and other processes associated with cell growth. Upregulation of factors involved in phage defense and genome rearrangement were also observed. The large cache of genomic data resulting from this effort provides important baseline information to fill previous knowledge gaps and provides context for future comparisons and possible HAB predictions.





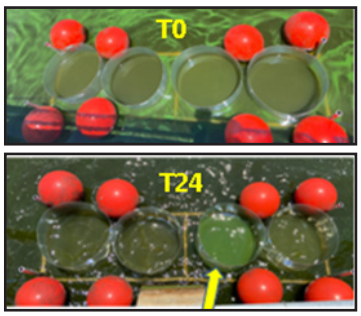


Figure 15. Drivers of HABs and associated biological patterns were investigated using a combination of monthly field surveys and in situ nutrient enrichment mesocosms using molecular, biological, and traditional taxonomic methods. *Top left*, distinct lake zones identified based on phytoplankton assemblage similarity where taxa were identified morphologically. *Top right*, ordination describing the results of the distance-based linear models. *Left*, mesocosm experiments at the S-79 lock on the Caloosahatchee River after nutrient additions of phosphate, ammonia, urea, and zero control at two time points (0 and 24 h). Note that a large microcystis bloom was occurring outside the mesocosm cylinders in May 2021.

Strategic Focus Area: HAB Management

Developing and demonstrating cost-effective HAB management technologies, which can be rapidly deployed and are both scalable and applicable nationwide, is a key research focus area. Management technologies include chemical, physical, and biological based means to remove, destroy, or neutralize cyanobacteria cells and the toxins they produce. Each approach has its advantages and disadvantages. Chemical approaches such as algaecides reduce the abundance of cyanobacteria cells but not always their toxins. Physical approaches involve using floating booms or skimmers to physically remove HAB biomass from a waterbody, or can involve the addition of flocculants to sink cyanobacterial cells out of productive water column depths. Biological approaches vary widely in their mechanism and maturity level. A governing principle of the USACE approach to delivering scalable HAB management technologies is ensuring each research project produces performance data relevant to multiple ecoregions to guide optimal technology use for maximum HAB risk reduction.

HABITATS: Harmful Algal Bloom Interception, Treatment, and Transformation System (ERDC, Factsheet 22). The Harmful Algal Bloom Interception, Treatment, and Transformation System (HABITATS), developed by Dr. Martin Page and team,[25] is a scalable three-step process for physically removing surface blooms of cyanobacteria and their entrained nutrients from inland waters. Upon intercepting algae-contaminated water (via booms and skimmers), it uses dissolved air flotation and ozonation to clean the water while concentrating the algae. The concentrated algal biomass is transformed into biocrude fuel while destroying any toxins using hydrothermal liquefaction. Through multiple field demonstrations in Florida and New York, HABITATS was optimized for water treatment performance and fuel conversion to enable energy-neutral and carbon-positive operations.[26] Over 2 million gal of lake water were cleaned, yielding over 1,400 gal of concentrated algal biomass, which is enough to generate 70 gal of biocrude fuel. Deployable trailer-mounted and shipboard prototypes have been developed (Figure 16). HABITATS received the 2020 USACE Innovation of the Year Award and the 2022 Technology Impact Award from the Federal Laboratories Consortium.



Figure 16. HABITATS is a rapidly deployable system for managing large HABs. The system, available in shipboard (*top*) or truck and trailer onshore modules (*middle right*), concentrates algal biomass (*bottom left*) and destroys toxins while turning algae into resources like fuel (*bottom right*) and fertilizer.

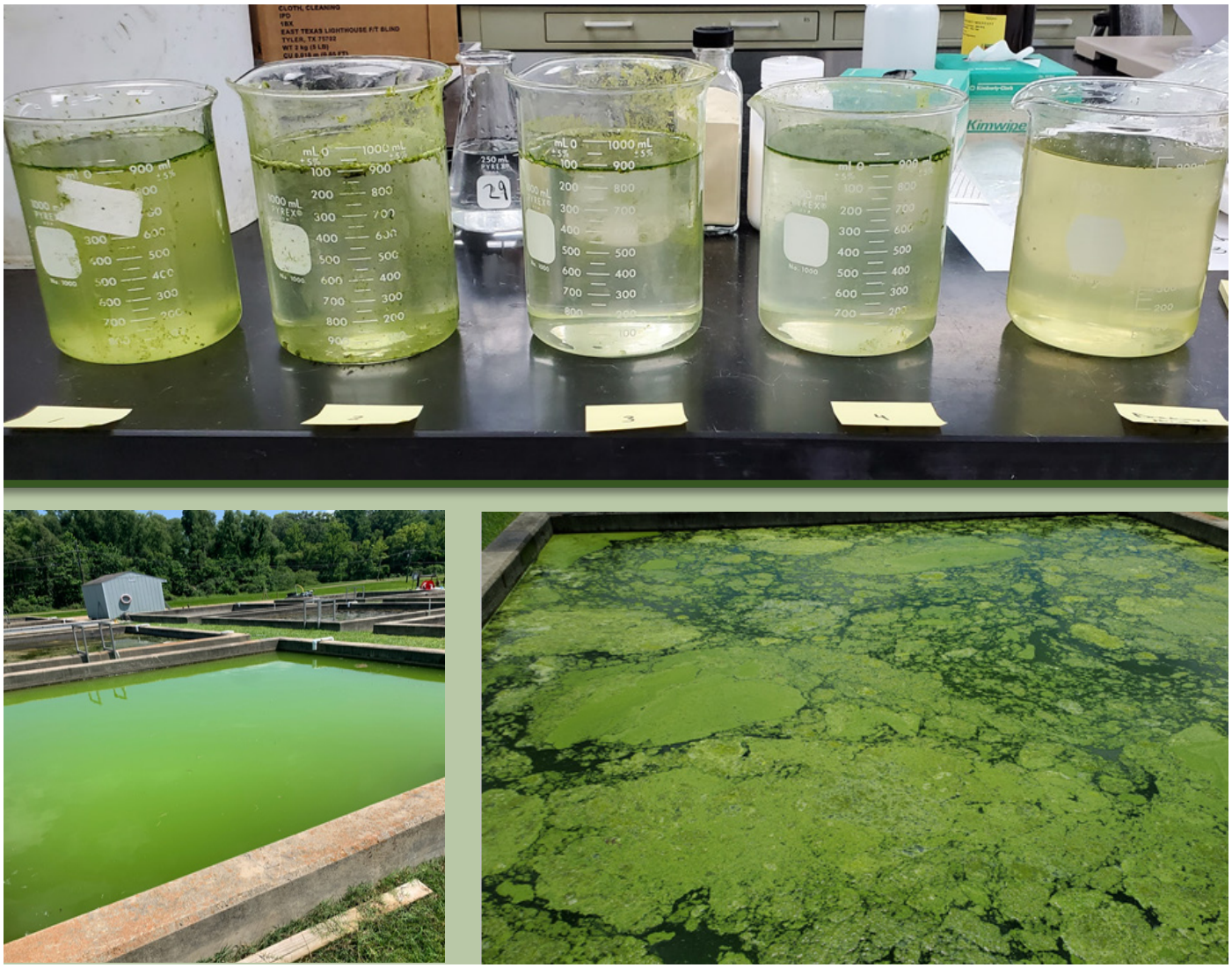


Figure 17. Research on algae flotation techniques (RAFT) combines a novel, ERDC-developed flocculant with microbubble technology to rapidly surface-concentrate algae and increase ease of HAB management. *Top*, samples from each demonstration test pond characterizing the varying concentrations and growth patterns of microalgae even when exposed to similar environmental conditions; from *left to right*, are samples from test ponds 1, 2, 3, 4, and a rainwater overflow basin. Each test pond was located within 3 ft of the others and experienced almost identical environmental and growth conditions. *Bottom*, an algal bloom within a test pool pre-RAFT (*left*) and post-RAFT (*right*) treatment.

Research on Algae Flotation Techniques (RAFT) (ERDC, Factsheet 5). Algal harvesting can be done efficiently at larger scales when blooms are concentrated near the water surface. Yet in some eutrophic systems, HABs are often dispersed throughout the water column, making physical removal difficult. Methods that induce cyanobacteria flotation (and concentration) are needed to aid harvesting efforts. Using an experimentally optimized approach, ERDC researchers led by Mr. Clinton Cender developed a rapid and highly effective method for stable surface concentration of algal biomass. The three-stage process uses environmentally benign materials; specifically, simulated polysaccharides in aqueous solution, aqueous flocculants, and mechanically produced microbubbles to (1) entrap algal cells and air, (2) separate algal biomass from water, and (3) induce buoyancy, respectively. Field demonstrations indicate in situ surface flotation can be achieved in HAB-impacted waters within 30 min of treatment given adequate chemical delivery and adequate dispersion (Figure 17).

Efficacy of algaecides and novel ozone nanobubble technology on prevention and management of HABs (OSU, Factsheet 23). Increased monitoring has broadened our knowledge of HABs, revealing that blooms are not limited to summer months, but instead occur year-round. In fact, cyanotoxin concentrations can be greatest in the fall and winter when water

temperatures are much cooler, and when traditional control methods—like algaecides—are often ineffective. Heather Raymond and her team aimed to remedy this cold-water HAB challenge by evaluating the effects of multiple USEPA approved algaecides verses an innovative nanobubble ozone technology (NBOT) for HAB control and toxin destruction across varying temperature regimes. Experiments were conducted in the lab, in controlled mesocosms (Figure 18), and in full scale field trials. Algaecides had variable and reduced efficacy at lower temperatures and a greater impact to nontarget organisms as compared to NBOT. NBOT deployment trials at HAB-impacted recreational lakes and beaches led to a reduction in local advisories.

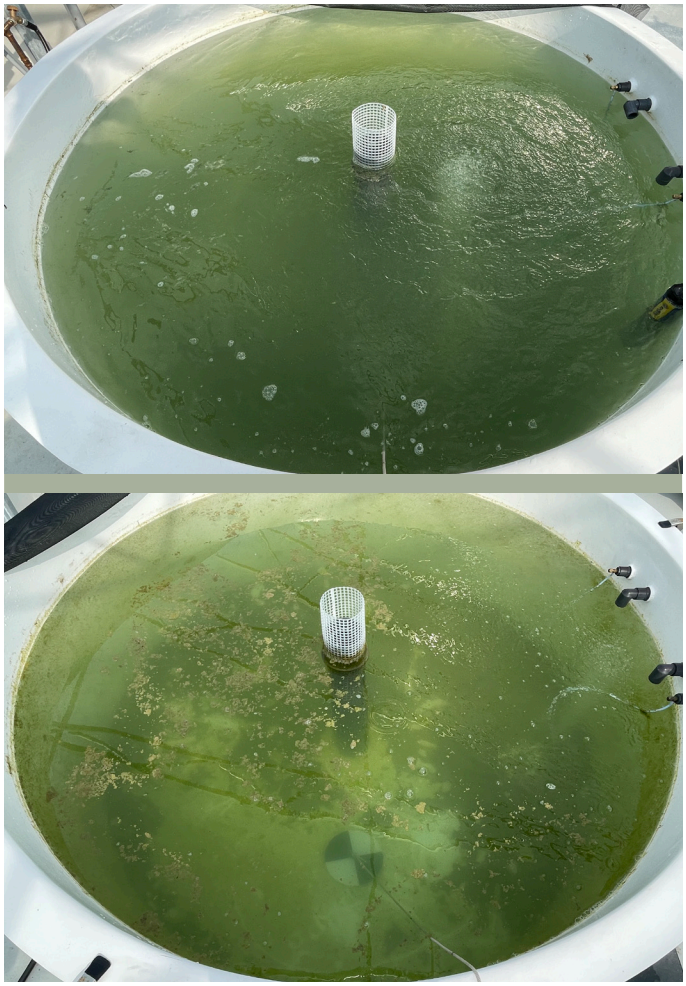


Figure 18. Tank-based mesocosm trials employing NBOT show that water clarity increases in HAB-impacted waters within 48 hours of treatment: *top*, control tank (no treatment); and *bottom*, the response to high-dose NBOT treatment.

Novel biological controls for mitigating HABs (ERDC, Factsheets 9, 13). Biotechnologies leveraging target-specific, biologically derived materials could replace existing nonspecific chemical control technologies and reduce risks to nontarget organisms. Materials of interest for HAB mitigation include cyanocidal compounds and cyanophages. Cyanocidal compounds produced by some bacteria in HAB-impacted waters can inhibit cyanobacterial growth and are hypothesized to play a natural role in bloom dynamics (Factsheet 13). Cyanophages are viruses that exclusively infect cyanobacteria, ultimately disrupting the life cycle of HAB-forming species via lytic infections (Factsheet 9).[27] Several technical challenges exist to successful deployment of these innovative technologies, including the influence of biotic and abiotic environmental conditions, development of host resistance, difficulties in material production, and scalability for field application. Researchers at the forefront of this cutting-edge field are making great strides in this arena through benchtop- and mesocosm-scale experiments, and are setting the stage for advancements of novel biotechnologies that combat freshwater HABs in an environmentally benign fashion.

Bacterial remediation of microcystin-HAB toxins (ERDC, Factsheet 16). Methods conventionally used in water treatment operations (e.g., boiling and chlorination) do not successfully destroy toxins associated with HABs. In an effort to remedy this issue and reduce toxin exposure risks, ERDC researchers developed a bacteria-produced enzyme with the capacity to naturally degrade the potent and extremely common HAB toxin, microcystin (MC)–LR. The derived enzyme was evaluated for usefulness in a variety of real-world matrices, and resulted in an effective MC-degrading product that can be safely administered to small-scale blooms to reduce toxicity. The product has been submitted for a patent. Additional efforts are underway to improve scalability through the identification of industry partners.

Moving Forward Together

Preceding sections of this document highlight projects delivered between FY19 and FY23 during the WRDA 2018-authorized HAB research initiative. Although significant progress was made during the 5-year initiative, there are many additional opportunities to address HABs. The following sections of this document describe HAB research and demonstration activities that could continue in FY24, pending availability of funds. A summary of remaining HAB R&D gaps identified during FY23 stakeholder engagements is also provided. Finally, this special report concludes with USACE-ERDC's expression of deepest gratitude for the creativity, flexibility, and commitment of our partners in working together to deliver scalable solutions that reduce the severity and frequency of freshwater HAB impacts to our nation's communities.

Continuing HAB R&D. University collaborations initiated in FY22 and FY23 that may have additional work continuing in FY24 involve sustainable HAB prevention, detection, and management; for example, University of Toledo partners will validate early detection sensors, develop nature-inspired biological treatments for cyanobacteria and toxins, and demonstrate these capabilities at field scale (Factsheet 30). Researchers at Marshall University are developing predictive models for HABs in riverine systems, which will enable hypothesis testing to elucidate HAB drivers in flowing freshwater systems and enable strategic management (Factsheet 36). Two new FY23 university-led projects are developing a comprehensive assessment of the economic benefits of using existing and emerging technologies to prevent and manage freshwater HABs and will develop and demonstrate a generalizable approach that will help translate site-specific HAB modeling to other ecoregions of interest. Finally, new ERDC-led HAB research projects were selected for funding in FY23. Projects include establishing user-friendly tools to support improved HAB detection via remotely sensed monitoring, modeling, prediction, and toxin detection (lead researchers: Molly Reif, Clinton Cender, Todd Steissberg, Jodi Ryder, Andrew McQueen, and Luke Gurtowski), and exploring the development of novel HAB prevention and management technologies (lead researchers: Martin Page, Ping Gong, Al Kennedy, and Justin Puhnaty). Factsheets for these new FY23-start projects will be posted to our website in early 2024.

HAB Demonstration Program. The WRDA 2020–authorized HAB Demonstration (Demo) Program supports field-ready and scalable HAB prevention, detection, and/or management technology demo projects at or near Water Resource Development Projects (WRDPs) impacted by HABs to (1) reduce HAB impacts to WRDPs, and (2) generate defensible cost and performance data to support technology transfer and guide informed technology usage at other sites. Appropriated funds were received to initiate the HAB Demo Program in FY22. USACE Implementation Guidance[28] required development and approval of a Programmatic National Environmental Protection Act (NEPA) Document for the HAB Demo Program, which was obtained on 25 October 2023. We anticipate awarding multiple HAB Demo Program projects in FY24; see www.grants.gov for funding opportunity announcements and our website for the latest news on this and other ongoing activities.

HAB Research Gaps and Needs

The following section describes HAB research gaps and needs identified during stakeholder community engagements. Pending the availability of funds in FY24 and beyond, we will work together with our partners to close these gaps and move toward delivery of scalable freshwater HAB solutions to minimize the frequency and severity of HAB impacts to our nation's water resources.

HAB Prevention Need: Identifying and mitigating the role of sediments in HAB formation. Sediments may contribute to bloom formation because they (1) are a source of nutrients to freshwater systems and can become biologically available when exposed or disturbed and (2) harbor "resting" cyanobacteria cells, which can become active and "seed" or contribute to genetic diversity within a bloom, potentially making a bloom more resilient, longer in duration, and more toxic. The extent to which these two mechanisms contribute to HAB events is an evolving topic in need of further research. This includes the timing of any potential contributions from these factors over a bloom event (i.e., do sediment nutrients contribute to bloom formation, or do they contribute later in the season, impacting bloom duration and toxicity?). Projects that contribute to advancing knowledge in these two topic areas, along with system-specific methods that can be efficiently employed at field scale to identify the relative contribution of these two factors to bloom formation, are needed.

Value to USACE: Sediment management is central to the USACE inland water resources mission as it pertains to navigation, flood risk management, and ecosystem restoration. Fully understanding how sediments contribute to nutrient cycling and how potential "resting" cells influence and contribute to blooms throughout the course of the year could be critical to predicting and managing these events.

HAB Prevention Need: Understanding HAB drivers in freshwater rivers, lakes, and reservoirs. Understanding the environmental or anthropogenic factors that may drive HAB occurrence, duration, intensity, and other critical pieces of data required to control these occurrences will help natural resource managers make decisions (e.g., best management practices for land, water resource management at reservoirs, public engagement and notices, and so forth). Additionally, there is a need to improve early warning capabilities for both HAB occurrences and HAB toxicity. Projects are needed that advance the understanding of HAB responses to ecosystem conditions, develop predictive modeling to identify potential HAB formation and toxicity, and identify additional points of vulnerability and transport of established HABs across ecosystems within defined ecoregions. Leveraging R&D will contribute to the goal of rapidly improved predictions and reduced uncertainty and will provide natural resource managers with the knowledge and tools to predict and minimize HAB effects.

Value to USACE: Understanding the natural and anthropogenic drivers of HAB occurrences and toxicity is critical to prediction, prevention, and controlling and minimizing their effects. As previously mentioned, the "prevention" portion of this research will be conducted in conjunction with federal, state, and local resource managers because their inputs are crucial to tackling watershed-wide challenges. USACE-ERDC's engagements with interagency partners at workshops, HABHRCA meetings, new formal partnerships, and others currently under development indicate that this path is achievable.

HAB Detection Need: Validation of remote-sensing approaches for early cyanoHAB detection and an expanded suite of remote-sensing capabilities. Satellite imagery can identify phycocyanin

in freshwater. However, pigments are not a reliable predictor of biological processes relevant to bloom response or management. There is need to have targeted, robust sampling campaigns to ground truth remote sensing such that HABs can be positively identified earlier and on a smaller scale and to provide real-time data to natural resource managers. Related needs include 24/7 unmanned HAB species detection across the nation (air, land, and water), integrated detection tools (drones, sensors, eDNA, databases, weather, social media, etc.), and expansion of our current ability to rapidly identify and respond to removal of and recovery from HAB events. One area of interest includes expanding upon the current work using unmanned aircraft systems (UAS) or drones to detect and monitor cyano-HABs and other invasive species of concern.

Value to USACE: Many USACE water resource managers are already using UAS and other state-of-the-art remote-sensing technology. However, data is limited, and resolution is poor. The long-term goal is that USACE-ERDC will partner with federal and state entities working in this arena to leverage data, tools, and funding to build a robust network beneficial to all partners for detecting HAB-forming environmental conditions prior to bloom formation and providing early positive identification and resolution of bloom events.

HAB Prevention Need: Improved freshwater monitoring in support of early warnings and early treatment to reduce HAB frequency, size, and impacts. Creating cutting-edge sensors to identify HABs and their toxins in real time and at low concentrations in the environment is another critical need in the detection focus area. Rapid and early response to HAB events is critical to managing impacts; improved detection capability is key to R&D efforts. USACE districts have highlighted the importance of having real-time information regarding HABs. Data detailing the cyanobacteria involved, toxins produced, and observed concentrations of each would improve risk management during bloom season and enable critical outreach and coordination with state and local officials.

Value to USACE: As stewards of over 400 natural resource projects across the nation, USACE has a responsibility to manage these resources and to provide for the safety of the ecosystem, water supply, and users. Accurate early warning systems can provide trusted guidance to natural resource managers in the field and contribute to well-informed decisions based on sound science and near real-time data.

HAB Management Need: Ecoregion-specific guidance for optimized use of HAB management technologies, alone and in combination. Physical, chemical, and biological algae treatments each could effectively reduce HAB impacts and risk at field scales. However, algae type, algae abundance, and system- and ecoregion-specific environmental parameters govern the cost-effectiveness of each at field scales alone or in combination. Lab and field-scale studies are necessary to produce meaningful system-specific performance data and to optimized use of HAB management technologies alone and in combination. Examples of HAB management technologies include biological, bioinspired, and molecular biology–based tools; slow-release mechanisms of HAB-neutralizing or HAB-preventing agents; cavitation; or other nonchemical additive management approaches. Projects that produce data-driven and technology performance–based decision support guidance for resource managers will be prioritized. The aforementioned WRDA 2020–authorized HAB technology demonstration program would serve as a logical steppingstone to bring the scaled-up technologies to the end user. Performing field demonstrations can be time consuming and costly depending on the technology, size, type of waterbody, and other factors. However, field demonstrations are critical for full technology transition to field practitioners.

Value to USACE: As progress continues in the prediction and prevention of HABs, managing HAB events will remain a nationwide need. USACE needs a wide array of management strategies, including nonchemical methods, at its disposal. Bringing the current R&D being developed under the ANSRP program through field demonstration is critical to ensure the technology and methods are effective under dynamic environmental conditions and that end users have sound science-based confidence regarding when and how to use HAB management tools most effectively.

Thank You

Delivery of scalable freshwater HAB solutions is impossible without partnering with federal, state, and local resource agencies—including all entities invested in the successful understanding and control of HABs in freshwater systems. Partnerships with academic and industry subject matter experts will continue to be a critical component of the USACE Freshwater HAB R&D Initiative. Our partners bring knowledge, opportunities, and authorities required to tackle this national challenge. ERDC will continue to share and leverage R&D data and knowledge and will endeavor to minimize duplication of research efforts. ERDC is committed to tackling the tough science and engineering challenges required to deliver scalable HAB prevention, detection, and management solutions to our nation's freshwater resources through close partnerships and teamwork. Many sincere thanks to our internal and external partners for all the great work accomplished since the USACE Freshwater HAB R&D Initiative was initiated in FY19!

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Appendix 1. Project Factsheets

Integrating Modeling Tools and Observations for Prediction and Management of HABs in the St. Lucie Estuary and Watershed

Lead: Mauricio Arias, PhD, PE, University of South Florida

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Managing harmful algal blooms (HABs) in the St. Lucie Estuary and Watershed is a complex problem that requires the integration of fundamental knowledge about ecosystem structure and function with state-of-the-art statistical and mechanistic models.

Objective

The primary objective of this effort is to generate actionable knowledge and develop a decision support tool that will allow managers to better predict and manage HABs in the St. Lucie Estuary and Watershed at multiple temporal scales. This project will integrate related efforts ongoing for Lake Okeechobee (Lake O) and Chattahoochee Estuary System to form a comprehensive HAB management tool for Lake O and receiving waters.

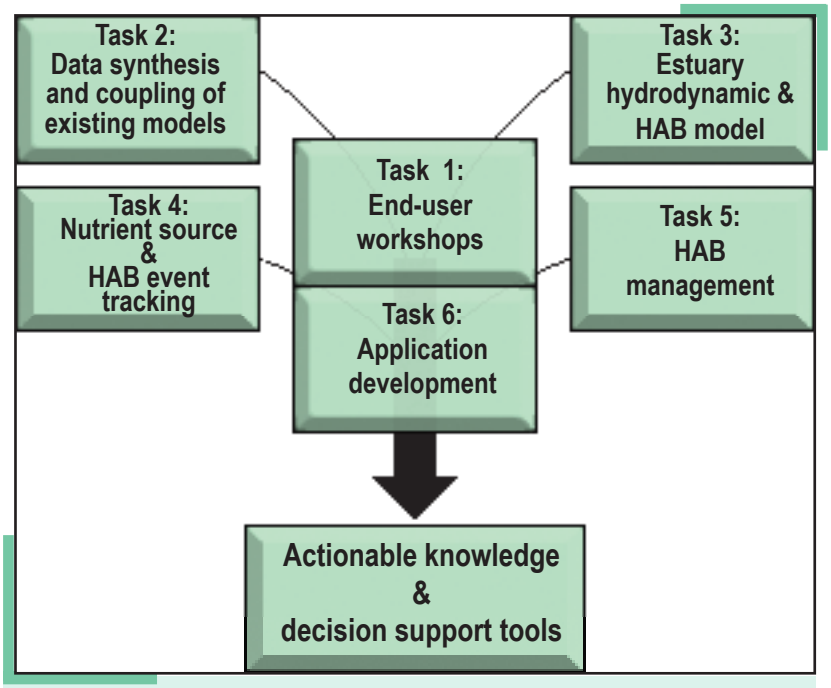


Figure 1. *Conceptual diagram of project tasks.*

Approach

The project will be organized around multistakeholder workshops to engage the project team with resource managers and decision-makers, synthesize critical information, leverage existing and new state-of-the-art biophysical models, and create an integrated data-driven modeling platform for prediction and management of HABs.

Partnership/Leveraging Opportunities

This work will leverage multiple collaborations and other work units including:

- Partnership with the University of Florida's Center for Coastal Solutions
- Partnership with the South Florida Water Management District, Coastal Ecosystems Section
- Collaboration with the ongoing USEPA-funded project "Temporal and Spatial Optimization of Existing and Emerging Nutrient Management Technologies and Practices for Control of Harmful Algal Blooms"
- Collaboration and coordination with the ERDC-funded project "Coupling Lake, Watershed, and Estuarine Models to Better Understand the Role of Engineered Freshwater Discharges in Driving the Severity, Location, and Timing of Harmful Algal Blooms"

Integrating Modeling Tools and Observations for Prediction and Management of HABs in the St. Lucie Estuary and Watershed

Lead: Mauricio Arias, PhD, PE, University of South Florida

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Major Milestones

Date	Milestone
FY22	Eleven water quality monitoring campaigns
FY22, Q2	Project kickoff meeting and end-user workshop
FY22, Q2	Attendance and presentation at in-progress review meeting
FY22, Q3	Second end-user workshop
FY22, Q3	Attendance and presentation at interagency workshop
FY22, Q4	Annual Report
FY23	Draft Final Report
FY24	Final Report

Value to USACE Mission

By integrating modeling tools and observations, the project will provide a more comprehensive understanding of the factors that contribute to HABs and how they can be mitigated. This will help USACE to more effectively carry out its mission of managing and maintaining the nation's water resources for the benefit of the public.

Developing Scalable HAB Prevention Technologies in the Ohio Region

Lead: Michael Brooker, The Ohio State University

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Harmful algal blooms (HABs) have become more frequent and severe across the United States. Agriculture contributes a major portion of nutrients, like phosphorus, that feed these blooms and best management practices (BMPs) are needed to reduce nutrient loads. An accurate estimation of BMP performance quantified through monitoring of field-scale BMPs is needed so that the optimal solutions can be identified for management at watershed and regional scale.

Objective

Farmers will be recruited to adopt BMPs and allow edge-of-field monitoring on their agricultural lands. Novel BMPs and designs will be identified and applied in construction on the recruited farmers' properties. The impacts of these BMPs will be assessed through water quality monitoring. The results from this will be used to assess the management needs at the regional scale required to mitigate future HABs.

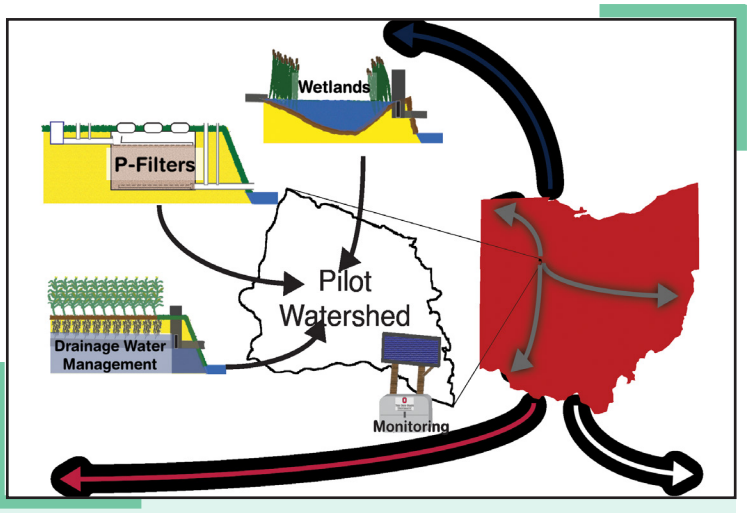


Figure 1. *Conceptual diagram*

Approach

Through existing partnerships and efforts, farmers will be recruited to adopt BMPs. This study will focus on identifying fields with greater risk of nutrient loss. Upon finding cooperative farmers, BMPs featuring novel designs will be constructed and instrumented with automated water sampling equipment. Samples from these fields will be analyzed to assess the impact of the BMPs on nutrient losses. Data from this study will be used to inform how these practices are represented within watershed models.

Major Milestones

Deliverable	Description
Publications	**Journal Article:** Scott et al. 2023. "Techno-Economic Analysis of Phosphorus Removal Structures." *Environmental Science & Technology* 57 (34): 12858. https://doi.org/10.1021/acs.est.3c02696. **Journal Article:** Brooker et al. In review. "Quantifying Phosphorus Loads from Legacy-Phosphorus Fields." *Journal of Environmental Quality*. **Undergraduate Thesis:** M. Kacica. 2023. "Estimating the Benefits of Legacy Phosphorus Fields for Targeted Best Management Practices." The Ohio State University. https://kb.osu.edu/handle/1811/102733. **Undergraduate Thesis:** E. Ebersbach. 2023. "Impact of Soil Texture on Phosphorus Loss from Legacy-P Fields." The Ohio State University. https://kb.osu.edu/handle/1811/102810.

Developing Scalable HAB Prevention Technologies in the Ohio Region

Lead: Michael Brooker, The Ohio State University

USACE Harmful Algal Bloom Research & Development Initiative

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Major Milestones (cont.)

Deliverable	Description
Outreach	**Presentations:** • S. Francis. 2023. "Combating Legacy Phosphorus: Phosphorus Removal Structures for Subsurface-Drained Agricultural Fields." Presented at the International Association for Great Lakes Research, May 11, Toronto, Ontario, Canada. • J. Martin. 2023. "Targeting Phosphorus Removal Structures to Legacy Phosphorus Sources." presented at the American Ecological Engineering Society, June 6, Tampa, FL. • M. Brooker. 2023. "Do Legacy-P Agricultural Fields Discharge Greater Nutrient Loads?" Presented at the American Ecological Engineering Society, June 6, Tampa, FL. • M. Brooker. 2023. "Do Legacy-P Agricultural Fields Discharge Greater Nutrient Loads?" Presented at Lake Erie Public Webinar, hosted by the Environmental Climate Change Canada, June 20, (virtual). • J. Martin. 2023. "Assessing the Value of a Public-Private Partnership to Target Best Management Practices to Legacy-Phosphorus Fields." Presented at the SWCS Conference, August 8, Des Moines, IA. • J. Martin. 2023. "Working with the Agricultural Community to Focus Practices for Watershed Phosphorus Reductions." Presented at the SWCS Conference, August 8, Des Moines, IA. **Posters (presented at the OSU Denman Forum and 2023 AEES Meeting):** • M. Kacica. 2023. "Approaching Phosphorus Reduction Targets by Managing Legacy Phosphorus Fields." • E. Ebersbach. 2023. "Impact of Soil Texture on Phosphorus Loss from Legacy-P Fields." **Field Days:** • J. Martin. "Water Quality Research." Presented at the Field to Table Field Day Event hosted by A. Dean, Bryan, OH.
Field Sites	**A wetland-phosphorus filter system** was constructed and instrumented with water quality monitoring equipment in Montpellier, Ohio. https://h2.ohio.gov/project/montpelier-wetland-restoration.

Partnership/Leveraging Opportunities

This work will support and leverage coinciding research projects like (1) the "Developing Public-Private Partnerships (PPPs) to Target Legacy Phosphorus Fields to Increase Water Quality and Availability" research funded by the USDA-NIFA AFRI program; (2) the "Pilot Watershed: Shallow Run, Hardin County Ohio" project funded by the USDA-NRCS RCPP program; and (3) the "Beneficial Implementation of Byproducts to Reduce P Loss from Agricultural Land" project funded by the ODHE HABRI program.

Rapid, Portable, and Multiplexed Detection of Freshwater HAB-Forming Genera

Lead: George S. Bullerjahn, Bowling Green State University

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Cyanobacterial harmful algal blooms (cHABs) and their management have a costly impact on freshwater resources of the United States. Detection, treatment, and mitigation of blooms is a large task, especially since blooms rapidly change composition seasonally. cHABs can alter composition in short time frames in which some management efforts cannot match. Consequently, both scheduled and opportunistic monitoring is challenging if results are unavailable for several days. Laboratory methods such as microscopy and qPCR used for detection and analysis of cHABs can also be cost prohibitive.

Approach

The use of new molecular techniques has led to faster, easier, and more reliable cHAB quantification. The sandwich hybridization assay (SHA) is one of these techniques, having already been utilized for marine phytoplankton. SHA is a tool that incorporates reverse complementary hybridization of two oligonucleotide probes; one of which is specific to a desired taxonomic rank, the "capture" probe, and the other being more general to a broader group of organisms, the "signal" probe. The capture probe is carefully designed to hybridize to 16S rRNA of the target organism(s) and discriminate against outgroups. The signal probe contains a detection method that is often a digoxygenin (DIG) label recognized by an antibody linked to horseradish peroxidase (HRP), thereby yielding a chromogenic signal.

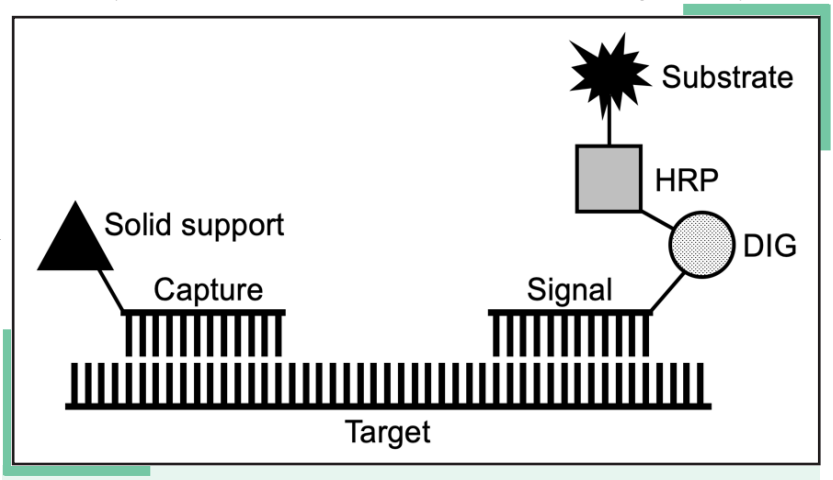


Figure 1. *The principle of SHA. The capture and DIG-labeled signal probes hybridize to reverse complement regions of the target sequence. Then, the HRP antibody binds to the DIG and substrate, producing a measurable color change.*

In the past year, we have developed and successfully tested oligonucleotide probes specific for species of the following toxic, bloom-forming genera: *Raphidiopsis* (*Cylindrospermopsis*), the ADA clade (*Anabaena/Dolichospermum/Aphanizomenon*) and Planktothrix (Table 1). SHAs with our culture collection have shown that each probe successfully hybridizes only to lysates of the appropriate target genus. In development of a multiplexed detection method, these probes are being used in concert with the previously available Microcystis probe developed by Dianne Greenfield's group.

Probe	Target	Sequence	Reference
CYL186	*Cylindrospermopsis* spp.	5'-biotin-[C9x3]-ACGCCTTGGTGGTCCCTTA-3'	This study
PLX594	*Planktothrix* spp.	5'-biotin-[C9x3]-GTTATAGCCCAGCAGAGCG-3'	This study
EUB338	All bacteria	5'-DIG-[C9]-GCWGCCWCCCGTAGGWGT-[C9]-DIG-3'	Daims et al. 1999
ADA724	ADA clade	5'-biotin-[C9x3]-CACAGCTCGGGTCGATACGA-3'	This study
CYA762	All cyanobacteria	5'-DIG-[C9]-CGCTCCCCTAGCTTTCGTC-[C9]-DIG-3'	Schönhuber et al. 1999

Table 1. *Capture and signal probes developed this year.*

Rapid, Portable, and Multiplexed Detection of Freshwater HAB-Forming Genera

Lead: George S. Bullerjahn, Bowling Green State University

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Assessing SHA Probe Performance

Standard curves yielded an environmentally relevant dynamic range for cHAB biovolumes, as seen using the ADA probe against homogenates of *Dolichospermum circinale* CS-541/06 (Figure 2). Similar results were obtained using the other probes developed thus far.

Adaptation to the LightDeck Platform

The current SHA is a plate-based assay, yielding a colorimetric signal following a reaction with an HRP-conjugated digoxygenin antibody. For a rapid field assay, modifying the protocol to the fluorescence-based LightDeck system affords a means to develop a multiplexed SHA, employing cartridges printed with multiple genus-specific capture probes. Pilot experiments with cartridges printed with the ADA724 probe demonstrates hybridization and fluorescence detection from the signal probe (Figure 3). These data are a proof of concept that the LightDeck platform can be adapted to the SHA, using the capture probes developed in this study.

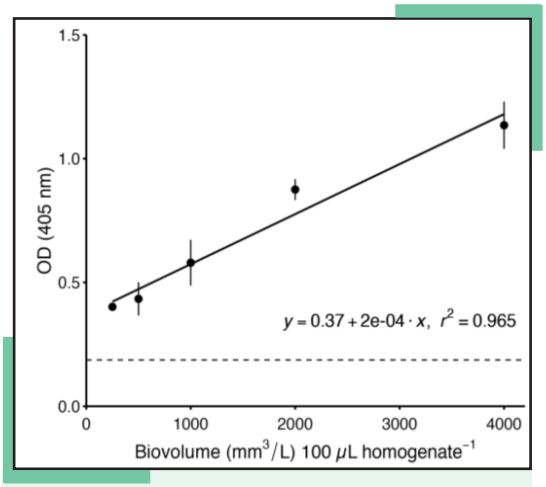


Figure 2. *Linear response of ADA724 and D. circinale CS-541/06*

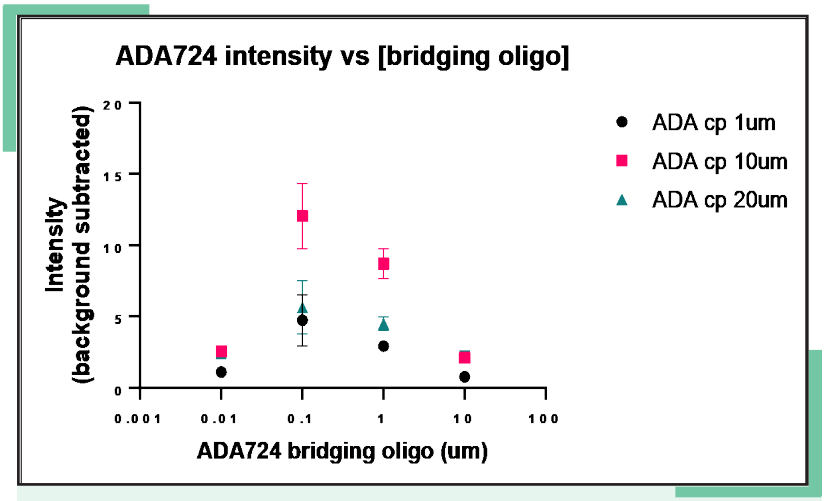


Figure 3. *Fluorescence signal from the printed ADA capture probe hybridized with a complementary Cy5 fluorescent signal probe*

Future Goals

The next steps include testing the SHA response with cultures at different growth stages, testing the specificity of the probes in mixed cultures, and finally, field testing of the SHA on bloom biomass obtained from the field. Knowing that the technology is now working, additional probes targeting benthic strains known to produce neurotoxins (e.g., *Microcoleus spp.*) are also a high priority.

Investigating Redirection of Detroit River Flow to Reduce Harmful Algal Blooms in Western Lake Erie Basin

Lead: Tim Calappi, Detroit District, Timothy.J.Calappi@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Harmful algal blooms (HABs) in the Western Lake Erie Basin (WLEB) pose a significant threat to the ecosystem and human health. Approximately 12 million people in the US and Canada utilize the WLEB as a source of drinking water. HABs have increased significantly over the past 15 years in the WLEB in large part due to nutrient inputs delivered from the Maumee River.

Objective

Diverting nutrient-poor Detroit River water towards the Maumee River outlet has potential to dilute nutrient concentrations in the WLEB and thereby reduce HAB severity and extent. The objective of this project is to use computer models to simulate Detroit River water diversion and assess predicted range of nutrient dilution factors that could theoretically be achieved in the WLEB.

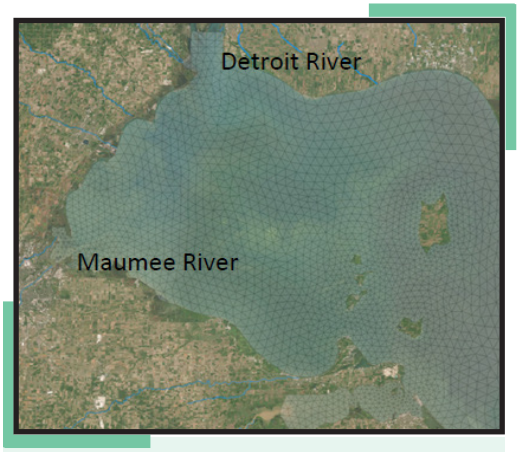


Figure 1. *Satellite image of the study area.*

Approach

Circulation in the WLEB will be investigated using a combination of new and existing hydrodynamic models. Due to the shallow nature of the WLEB, waves are an important part of determining large scale circulation patterns. A Coastal Modeling System (CMS) Wave grid will be developed and used in combination with the circulation model to evaluate three alignment alternatives that would divert Detroit River water towards the Maumee River outlet.

Partnership/Leveraging Opportunities

USACE is interested in partnerships and research that advances the reduction of HABs in the WLEB and the Great Lakes. This study examines a unique approach that could be used in conjunction with existing management efforts to reduce HAB impacts to the WLEB and inform strategic investments in the region. Many of the current HAB management efforts are focused on nutrient reduction within the watershed. While those efforts are vitally important to reducing HABs, this study looks at another innovative approach to address HABs by redirecting flow via a physical structure to dilute nutrient concentrations in the WLEB. In addition, the modeling efforts conducted can be shared with other stakeholders and modified for the needs of future project proposals that require an understanding of water movement and waves.

Major Milestones

Date	Milestone
Jun 2023	Bathymetric data processing
Jul 2023	Develop existing conditions model and increase resolution
Aug 2023	Model calibration
Sep 2023	Wave modeling
Nov 2023	Develop alternative alignments
Dec 2023	Modeling results analysis; focused cost analysis
Jan 2024	Final report complete and shared with stakeholders

Value to USACE Mission

The Environmental Lab has a variety of ongoing research and development efforts nationwide to evaluate and deliver scalable HAB management solutions. This study will provide stakeholders in the WLEB important information that can be used to inform future research and HAB management decisions moving forward.

Research on Algae Flotation Techniques (RAFT)

Lead: Clinton Cender, ERDC, Clinton.J.Cender@usace.army.mil

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Problem

Algae harvesting can be done efficiently at larger scales when algal blooms are concentrated near the water surface. The harvesting approach becomes less efficient and scalable when the algae are dispersed throughout the water column, which is common in some eutrophic water bodies.

Objective

Develop a scalable method for inducing cyanobacteria flotation that can be efficiently and safely applied to natural water bodies. Concentrating the algae would allow for huge gains in scalability of HAB mitigation through more efficient physical removal.

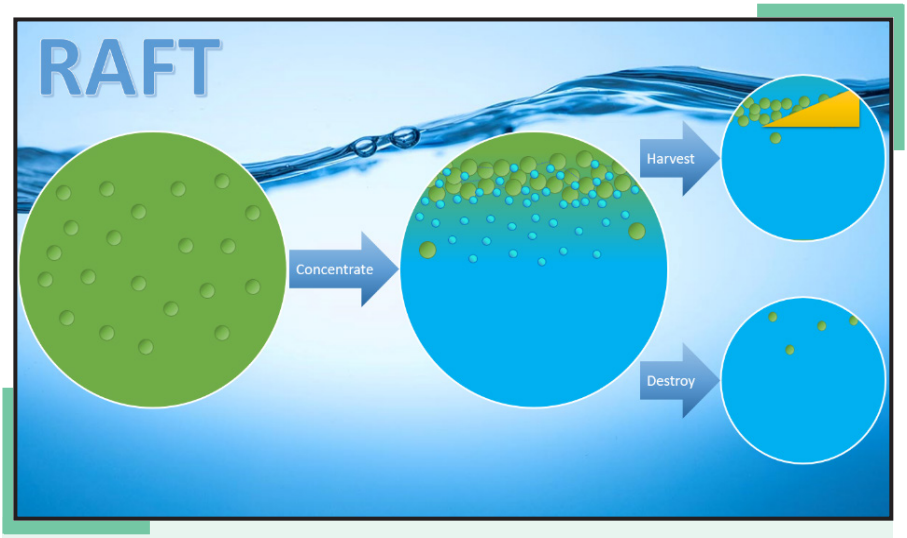


Figure 1. *RAFT algae flotation objective and concept.*

Approach

Certain cyanobacteria strains produce polymeric substances that help them aggregate while trapping dissolved oxygen, resulting in increased flotation. The RAFT team is studying environmentally benign chemicals that can enhance these natural aggregation and flotation mechanisms. The algae flotation will be accelerated further by the application of mechanically produced nanobubbles that synergize with the chemical aggregation process. Iterative benchtop studies on the interaction of microalgae with simulated polymeric substances led to the optimization of a three-stage treatment. First, the polysaccharide xanthan gum was homogenized in algae. A cationic polyacrylamide was then used to destabilize the emulsion charge and adsorb the algae to thpolysaccharide. Finally, microbubbles infused beneath the mucus entrapped coagulated algae, creating a buoyant, stable layer at the water's surface. The benchtop methodologies were translated and scaled for demonstration in four 14,000 gal. test ponds seeded with algae and environmentally grown to bloom conditions. The treated test ponds demonstrated significant and rapid algae biomass surface accumulation measured as phycocyanin. The uniformly dispersed algae were initially measured at five relative fluorescence units and after treatment the surface concentrated algae measured 30 relative fluorescence units.



Figure 2. *Test ponds.*

Results

The 2022 RAFT field demonstration resulted in significant, and rapid, surface concentration of the present cyanobacteria and Chlorophyta. Microbubble dissolved air flotation after chemical treatment began generating the surface biomass within a 30 min timeframe. In situ flotation similar to that observed during the demonstration may be achievable with adequate chemical delivery and dispersion throughout the harmful algal bloom.

Research on Algae Flotation Techniques (RAFT)

Lead: Clinton Cender, ERDC, Clinton.J.Cender@usace.army.mil

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Figure 3. *Observed surface biomass accumulation.*

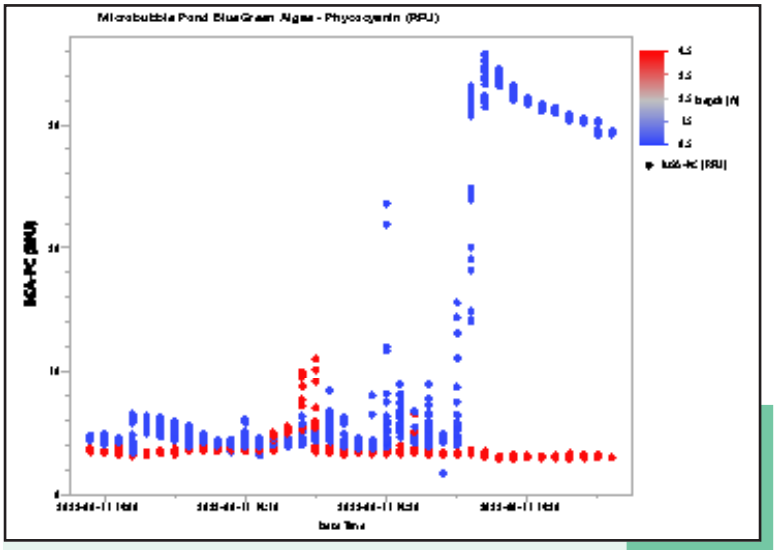


Figure 4. *RAFT phycocyanin data during treatment across depths.*

Major Milestones

Deliverable	Description
Publications	**Tech Report.** Cender et al. 2023. *Rapid Algae Flotation Techniques.* ERDC TR-23-6. Vicksburg, MS: US Army Engineer Research and Development Center. **Tech Report.** Cender et al. In prep. *Pilot Scale Optimization: Research on Algae Flotation Techniques (RAFT).* ERDC TR. Vicksburg, MS: US Army Engineer Research and Development Center. **Journal Article.** Cender et al. In prep. "Microalgae Flotation: Manipulating Surface Concentrations with Mucilage Entrapped Air. International Water Association." *Water Research.*
Products	**Patent.** US Utility Patent Application. "Combination of Cationic Polyacrylamide and Xanthan as Flocculation Agents for Harmful Algal Blooms." USPTO Ser. No.: 18/130,344
Demonstrations	Pilot-scale algae flotation system demonstration and optimization. August 2022. ERDC HQ concrete experimental pond test bed. Vicksburg, MS.

Partnership/Leveraging Opportunities

This work leveraged ongoing field studies within USACE's Aquatic Nuisance Species Research Program, such as HABITATS, which included collaborations with the New York State Department of Environmental Conservation and the Florida Department of Environmental Protection. In addition, commercial products are being evaluated, modified, and optimized in the RAFT study.

Value to USACE Mission

The motivation driving the RAFT project is the need to collect and remove HABs that have begun forming in greater frequency across national freshwater bodies more effectively. Creating a surface concentrated HAB will allow existing and new mitigation techniques to make bold steps in efficiency and scope.

Environmental Genetic Reconnaissance for Monitoring and Managing HABs

Lead: Sheena Feist, ERDC, Sheena.M.Feist@usace.army.mil
Co-PI: Richard Lance, ERDC, Richard.F.Lance@usace.army.mil

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Problem

Recurrence and severity of harmful algal blooms (HABs) are increasing due to several factors (e.g., human practices and climate change). Sensitive, robust methods that allow for early and rapid HAB detection are needed. Among the suite of HAB detection tools available, a powerful option exists in genetics-based approaches utilizing environmental sampling, or environmental DNA (eDNA). The eDNA field has rapidly matured in recent years, with several new and improved methods available for use in HABs. A synthesis of these recent methods—and comparisons of benefits and/or tradeoffs of each—are missing from the literature.

Approach

We conducted a literature review of recent eDNA methods and their application for freshwater HAB detection and monitoring. In total, we reviewed 14 papers with a specific focus on two HAB species of significant concern (*Microcystis aeruginosa* and *Prymensium parvum*). The review provides a synthesis of available HAB eDNA methods and associated in-field instrumentation: quantitative PCR (qPCR), PCR-free isothermal amplification, and high-throughput sequencing (i.e., metabarcoding). Each technology was assessed for deployment suitability across different scenarios and ability to meet varying HAB monitoring and management objectives. A companion white paper gleans from the review; it details strategic future directions for eDNA-based HAB surveillance and eDNA-informed HAB management.

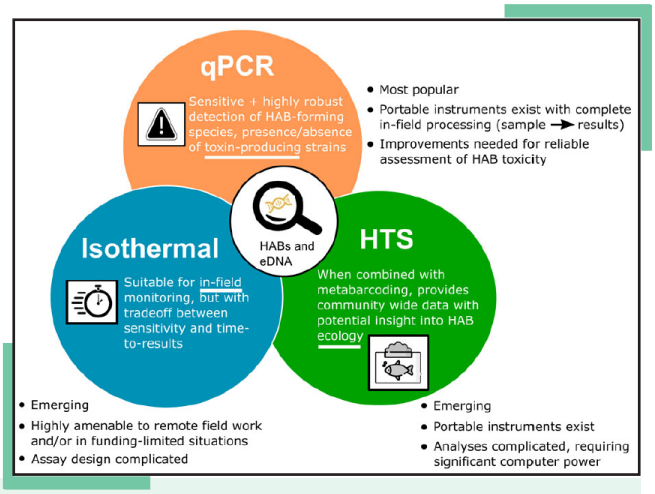


Figure 1. *eDNA methods available for HABs, reproduced from separate author's publication.*

Major Milestones

Deliverable	Description
Publications	**Journal Article:** Feist and Lance. 2021. "Genetic Detection of Freshwater Harmful Algal Blooms: A Review Focused on . . . *M. aeruginosa* and *P. parvum*." *Harmful Algae* 110:102124. https://doi.org/10.1016/j.hal.2021.102124.
Documents	**White Paper.** Feist and Lance. 2021. "HAB Environmental Genetic Reconnaissance: State of the Science and Future Strategies."

Value to USACE Mission

eDNA methods are extremely sensitive, reliably specific, expedient, and cost-effective. As such, eDNA-based detection of HAB-forming species results in several potential advantages as compared to other HAB monitoring technologies. These advantages translate to early detection, large scalability of efforts, and capacity to provide data potentially useful for predicting bloom formation and/or informing bloom treatments. Data can also improve our understanding of the ecological impacts of HABs, including impacts to high-risk aquatic systems housing economically important species.

Light-Based Mitigation Technology for the Reduction of HABs

Co PI: Elizabeth Gao, ERDC-CERL, Elizabeth.J.Gao@usace.army.mil
Co PI: Taylor Rycroft, ERDC-EL, Taylor.E.Rycroft@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Current physical, chemical, and biological technologies for managing harmful algal blooms (HABs) have unintended adverse impacts on the surrounding ecosystem, limited deployability and scalability, and often require personnel to come into close proximity of HABs.

Objective

Validate and optimize the performance of an ultraviolet (UVC) light–based mitigation technology that does not require the use of chemicals, and assess its potential for integration with remotely operated vehicle (ROV) for safe and effective, large-scale HAB mitigation.

Approach

(1) Perform bench scale studies to verify and optimize the UVC dose requirements for inactivation of cyanobacteria and degradation of cyanotoxins as a function of ambient water quality; (2) Evaluate the photodegradation of cyanotoxin microcystin-LR (MC-LR); (3) Assess whether degradation of MC-LR by UVC radiation produces harmful byproducts; (4) Evaluate and demonstrate the performance of a UVC-enabled ROV at pilot scale; and (5) Assess required enhancements, scalability, applicability, and environmental impact of the UVC-enabled ROV.



Figure 1. *ERDC CyanoSTUN.*

Results

The ERDC CyanoSTUN (Cyanobacterial Suppression Through Ultraviolet-C Neutralization) was developed and system tested (Figures 1 and 2). Modelling results suggested that CyanoSTUN can suppress a 1-acre bloom at a depth of 7 in. in 10 hr, assuming 60% UVC transmittance within the bloom. Lab studies showed that UVC can transform toxic MC-LR into nontoxic photolytic products (Figure 3).

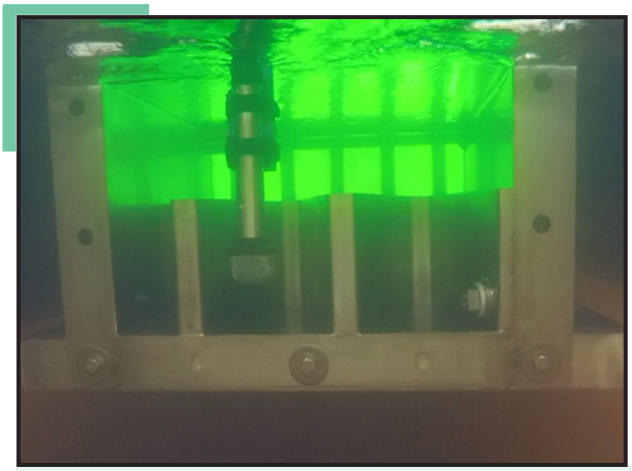


Figure 2. *Testing CyanoSTUN's UVC reactor.*

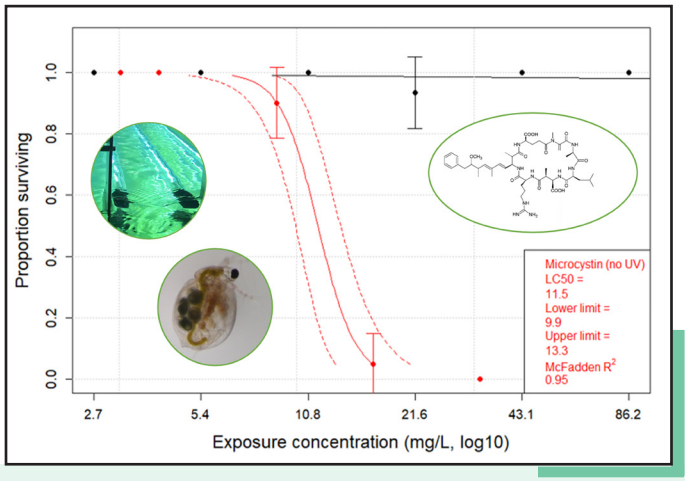


Figure 3. *UV_{254} transforms toxic MC-LR into non-toxic photolytic products in laboratory.*

Light-Based Mitigation Technology for the Reduction of HABs

Co PI: Elizabeth Gao, ERDC-CERL, Elizabeth.J.Gao@usace.army.mil
Co PI: Taylor Rycroft, ERDC-EL, Taylor.E.Rycroft@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

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Major Milestones

Deliverable	Description
Publications	**Journal article:** Moores et al. In review. "Reduced toxicity of microcystin after photolytic treatment." *Water Environment Research*. **Tech report:** Rycroft et al. In prep. "CyanoSTUN: An Ultraviolet-Enabled Boat for the Suppression of Cyanobacterial Harmful Algal Blooms." ERDC TR. **Special report:** Rycroft et al. In review. "Guidance for USACE Water Managers on Using ERDC's CyanoSTUN for Suppression of Cyanobacterial Harmful Algal Blooms." ERDC SR.
Products	**CyanoSTUN (Cyanobacterial Suppression Through Ultraviolet-C Neutralization):** An agile pontoon boat equipped with a wastewater-grade UV-C reactor that irradiates raw HAB-contaminated surface water with UVC germicidal light (254 nm). No chemicals are added to the water. There is no pretreatment other than an aluminum screen with 0.5 in. round holes. The UV reactor is mounted to the bottom of a pontoon boat platform such that target surface water is not removed from the waterbody; treatment occurs in situ where the top 7 in. of the water column passes through the UV reactor as the boat traverses the surface. The speed of the boat is expected to range (e.g., between 0.3 and 1.3 mph), depending on the UV transmittance of the HAB-contaminated water; and water residence time as it flows through the channel reactor is expected to range between 4 and 14 s. We expect to be able to drive the boat at a rate of 0.94 ft/s (0.64 mph) to suppress 1 acre of a bloom at 7 in. depth in 10 hr, assuming a 60% UV transmittance within the bloom. (Developed by Taylor Rycroft, Chris Donnelly, and Zach Tomberg). **Patent:** Lead inventor: Taylor Rycroft. Utility patent application filed at USPTO 3QFY23.
Demonstration	**Field Demonstration** of ERDC's UV-Enabled Boat. Location Worcester, MA. 4QFY23.

Partnership/Leveraging Opportunities

This project will integrate with the Aquatic Nuisance Species Research Program project entitled "Mitigation of HABs Toxins Using Deployable 3D Printed Photocatalytic Devices" by using 3D-printed devices as a polishing step to reduce microcystin concentrations after UV-C stuns the cyanobacterial cells.

Value to USACE Mission

CyanoSTUN may give USACE water managers confronted with HABs the ability to rapidly and safely "pause" a bloom for an extended period, or destroy it entirely. The technology may also enable effective inactivation of cyanobacteria and reduction of cyanotoxins without causing deleterious, off-target ecological effects.

Rapid Response and Management of HABs through Peroxide-based Algaecide Studies

Co-PI: Kurt Getsinger, ERDC, Kurt.D.Getsinger@usace.army.mil
Co-PI: Benjamin Sperry, ERDC, Bejamin.P.Sperry@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Cyanobacteria harmful algal blooms (cHABs) greatly impair water resources on Lake Okeechobee. Impacts are seen in water quality, fish/wildlife habitat, recreation/tourism, and property/canal development. A rapid response, using algaecides, would provide direct control of cHABs.

Objective

Evaluate the performance of an operational-scale, peroxide-based algaecide treatment to control cHABs in the Lake Okeechobee Watershed. Monitor for changes to aqueous toxin levels pre- and posttreatment. Provide guidance and best management practices for operational applications of PAK 27 for cHAB control.

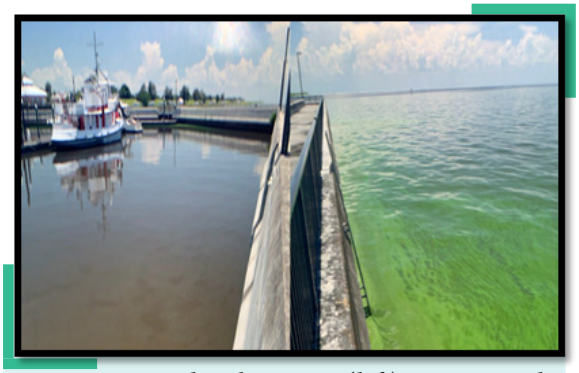


Figure 1. *Treated with PAK 27 (left) vs Untreated (right) waters on Lake Okechobee.*

Approach

Field demonstrations (operational-scale and in situ mesocosms) were conducted to verify the use of PAK 27 peroxide-based algaecide for a rapid-response tool to quickly control cHAB blooms in selected sites on Lake Okeechobee. Treatments were applied once target organisms reached bloom conditions. Pre- and posttreatment evaluations were conducted to determine level of bloom control (efficacy) and quantity/quality of potential cHAB toxins in the water column.

Major Milestones

Date	Milestone
Publications	**Tech Report:** Sperry, Benjamin P., et al. 2023. *Field Demonstration of a Peroxide-Based Algaecide for Harmful Algal Bloom Control in Lake Okeechobee.* ERDC/EL TR-23-7. Vicksburg, MS: US Army Engineer Research and Development Center.
Demonstrations	**In situ mesocosm study:** Pahokee Marina using PAK 27 algaecide. FY19. **Field-scale rapid response demonstration:** Pahokee Marina using Pak 27 algaecide. FY20.

Partnership/Leveraging Opportunities

This work leveraged multiple collaborations and other work units including the USACE Jacksonville District (USACE-SAJ), the Florida Agriculture & Consumer Services, and the University of Florida.

Value to USACE Mission

Development of peroxide-based algaecide management strategies provide USACE with tools to rapidly reduce occurrence/acreage of blooms, which can mitigate health and environmental damages in marinas, lakeshore embayments, residential canals, spillway gates/locks, beaches, campgrounds, and parks where water flows can be mitigated.

Cyanophage Treatment Development for Mitigating Freshwater Cyanobacteria HABs

Lead: Ping Gong, ERDC, Ping.Gong@usace.army.mil
Co PI: Christopher Waechter, US Bureau of Reclamation

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Harmful algal blooms (HABs) are an environmental problem of increasing concern because they directly affect public health and environmental quality. Viruses that exclusively infect cyanobacteria, cyanophages, are perhaps the most abundant, but also the most underutilized, biological resource on Earth. It is believed that cyanophages hold great promise and can be harnessed to mitigate cyanoHABs in a species-specific fashion, thanks to their host specificity.

Objective

Develop a novel mitigation biotechnology by exploring the lytic life cycle of cyanophages.

Approach

The first step was to isolate, identify, and characterize cyanophages from environmental samples collected from HABs-affected lakes. Methods were developed to infect cyanobacterial cultures with cyanophage, optimize biotic and abiotic conditions for inducing lytic life cycle, and quantify the disruption of cyanobacteria caused by cyanophage infection.

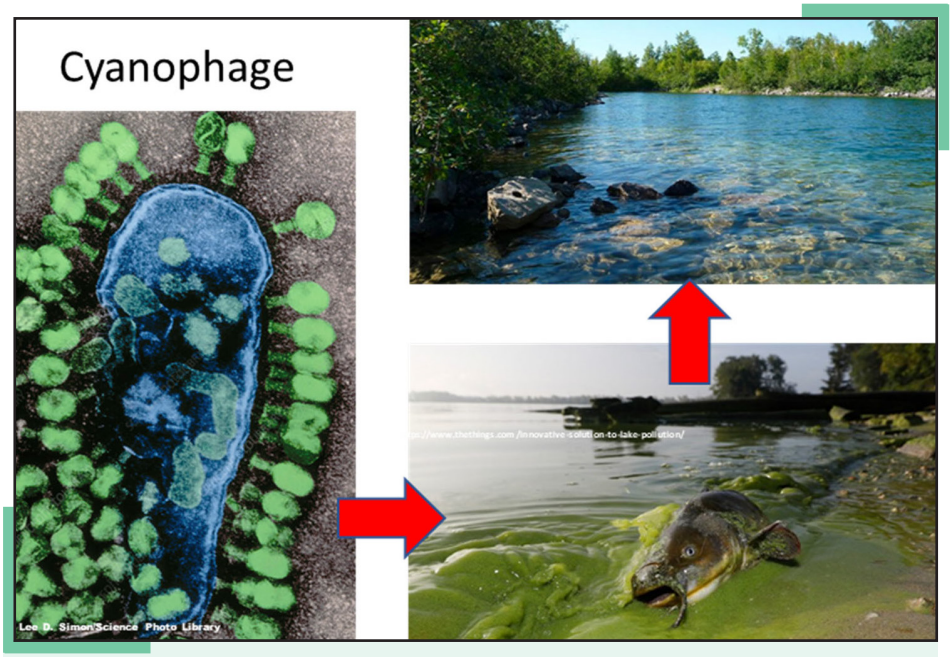


Figure 1. *Cyanophage-based biotechnology (left) to be developed for restoring HAB-impacted lakes and reservoirs (bottom) to balanced, HAB-free state (top).*

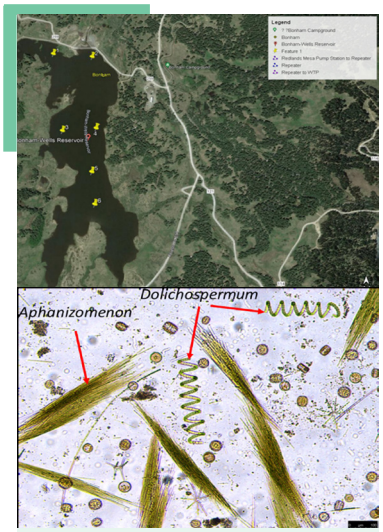


Figure 2. *Sampling sites in Bonham Reservoir, Colorado (top), and microscopic cyanobacteria identification (bottom).*

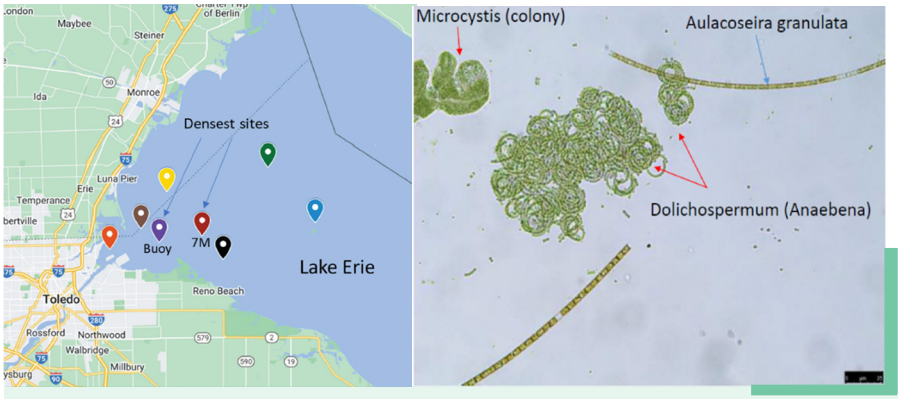


Figure 3. *Sampling sites at Lake Erie near Ohio (left), and microscopic image of isolated cyanobacteria (right).*

Cyanophage Treatment Development for Mitigating Freshwater Cyanobacteria HABs

Lead: Ping Gong, ERDC, Ping.Gong@usace.army.mil
Co PI: Christopher Waechter, US Bureau of Reclamation

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Results

A wide range of multiplicity of infection (MOI) can be considered for cyanophage-based HAB control. When the cyanophage/cyanobacteria ratio is higher than 1:10,000, the HAB may be controlled.

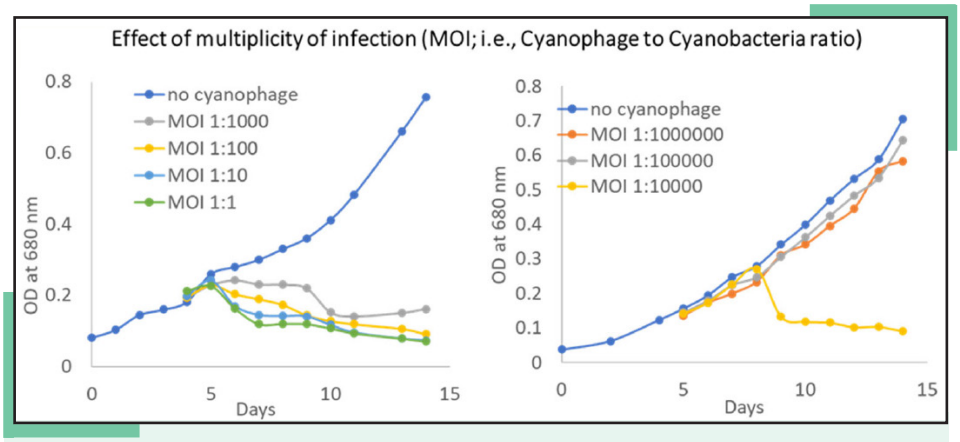


Figure 4. *Determination of multiplicity of infection for lytic phage Ma-LMM01 to its host M. aeruginosa NIES-298.*

Major Milestones

Deliverables	Description
Publications	**Journal Article:** Grasso, et al. 2022. "A Review of Cyanophage-Host Relationships: Highlighting Cyanophages as a Potential Cyanobacteria Control Strategy." *Toxins* 14:385. https://doi.org/10.3390/toxins14060385. **Tech Report:** "Physical/Chemical Induction of Lytic Cyanophage Life Cycle." ERDC TR (in prep).

Partnership/Leveraging Opportunities

This work leverages multiple collaborations and other ERDC work units, including "Small Regulatory Ribonucleic Acids for the Control of Harmful Algal Blooms," "Rapid, Portable, and Multiplexed Detection of Freshwater Harmful Algal Bloom-Forming Genera," and "Development of a Near Real-Time Field Test Kit for the Rapid, Simultaneous Detection, and Quantitation of High Priority Toxic Cyanobacteria."

Value to USACE Mission

This project should result in the development of a novel, cost-effective, species-specific, and environmentally benign technology for HAB treatment in lakes and reservoirs. Such a technology would greatly benefit HAB management at USACE lakes and reservoirs.

Novel Graphene-Based Composite Materials for Management of HABs

Lead: Luke Gurtowski, ERDC, Luke.A.Gurtowski@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Harmful algal blooms (HABs) caused by cyanobacteria and other algal species annually cause water quality concerns in domestic waterways throughout the Nation that damage human health, environmental security, and various industries, including tourism and fishing. Current solutions for HABs are limited. Therefore, sustainable technologies must be developed and tested to ensure management of HABs to protect water quality nationwide for prolonged use and welfare.

Objective

This effort aimed to evaluate the efficacy of a materials-based approach for management of HABs. Innovative polymer composites were fabricated using graphene-based materials and chitosan, a derivative of the world's second-most abundant biopolymer, chitin. Removal capacities and rates for various algal species and toxins related to HABs were analyzed following introduction to materials developed for this study to understand interactions and fully determine performance for this application.

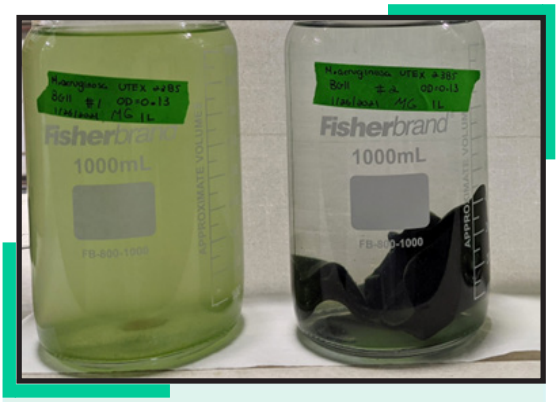


Figure 1. *(Left) Untreated sample, and (right) treated sample.*

Approach

Evaluations were conducted at various scales with novel technologies prepared for HAB management strategies. Individual solutions containing different algal monocultures, including freshwater species with varying degrees of toxicity, were assessed while in contact with composite materials developed utilizing a patented wet-casting technique in addition to related base materials as comparative treatment technologies. Toxins produced by certain algal cultures were also tested following introduction to the technologies included in this study to analyze their removal performances. These assessments were conducted initially at laboratory scale, and favorable technologies were further tested at small pilot scales.

Major Milestones

Deliverables	Description
Publications	**Journal Article:** Zetterholm, S.G. et al. 2022. "Graphene-Mediated Removal of Microcystin-LR in Chitosan/Graphene Composites for Treatment of HABs. *Chemosphere* 300:134583. **Master's Thesis:** Zetterholm, S.G. 2021. "Chitosan Graphene Composite Fabrication and Characterzation for Treatment of Harmful Algal Blooms and Toxins." University of Southern Mississippi.

Partnership/Leveraging Opportunities

This work leveraged multiple collaborations and other work units, including Rice University, the University of Southern Mississippi, and the University of Mississippi.

Value to USACE Mission

This project will ultimately provide cost-efficient technologies that can be deployed to manage HABs and mitigate their effects. Furthermore, this project will advance the knowledge base of capabilities for this application to promote further development efforts.

Near Real-Time Field Test Kit for Simultaneous Detection and Quantificaiton of High Priority Toxic Cyanobacteria

Lead: Ping Gong, ERDC, Ping.Gong@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Many cyanobacterial species produce a wide variety of cyanotoxins that can sicken humans and kill fish and other freshwater zooplankton. There is a lack of field-deployable and portable tools capable of simultaneous detection and quantification of multiple cyanotoxin-producing genera.

Objective

Develop and validate a multiplexed test kit to rapidly identify and quantify the seven most common cyanotoxin-producing cyanobacterial genera. The test kit includes real-time quantitative PCR (qPCR) primer sets and Sandwich Hybridization Assay (SHA) capture/signal probes that are compatible with the MBio® system.

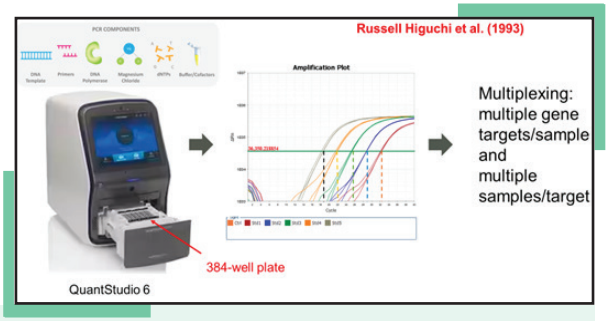


Figure 1. *Scheme of realtime quantitative polymerase chain reaction (qPCR) allowing detection of multiple cyanobacterial genera by developing genus-specific primers.*

Approach

In collaboration with Bowling Green State University (BGSU), we employed a bioinformatics approach (e.g., multiple sequence alignment, phylogenetic tree construction, and entropy calculation) to design genus-specific qPCR primers and SHA capture probes that can distinguish up to seven cyanobacterial genera (*Microcystis, Microcoleus, Lyngbya, Planktothrix, Cylindrospermopsis, Synechocystis,* and the ADA clade [*Aphanizomenon & Dolichospermum*]) from each other and from other nontarget species/genera. The SHA capture probes, zip code tags/antitags, signaling probes (designed by BGSU team), and qPCR primers (designed by ERDC team) are both screened and validated using pure cultures and environmental samples. Validated probes will be transformed to fit to MBio® system by LightDeck Diagnosis Inc.



Figure 2. *Anticipated final product of a SHA-based sensor cartridge that can produce readout in a portable MBio® LightDeck™ diagnostic platform (collaborative work with BGSU).*

Primer set	Lowest C_t for nontarget species	Single-peak melt curve?	Primer efficiency (mean ± SD)	R^2 value > 0.99?
Genus-specific	n = no. of strains	Target genus/clan (n = no. of strains × repeats)		
ADA⁺	28 (n=8)	Yes	97 ± 8 (n=2×2)	Yes
Microcoleus #1⁺	29* (n=9)	Yes	88 ± 6 (n=1×2)	Yes
Microcystis #2⁺	26 (n=7)	Yes	91 ± 4 (n=2×1+1×2)	Yes
Synechocystis #2⁺	30** (n=9)	Yes	112 ± 23 (n=1×2)	Yes
ADA⁺⁺	31 (n=8)	Yes	88 ± 1 (n=1×2)	Yes
Microcystis⁺⁺	31 (n=7)	Yes	86 (n =1×1)	Yes
Planktothrix⁺⁺	15 (n=9)	No	67 (n=1)	No
Synechocystis⁺⁺	28 (n=9)	No	93 (n=1)	Yes
Cyanobacterial universal		Cyanobacterial strains (n = 10)		
LF1/LR1⁺	26	Yes	97 ± 4	Yes
LF2/LR2⁺	18	Yes	98 ± 5	Yes
JF2/JR3⁺	28	Yes	97 ± 4	Yes
BD16SF1/R1⁺⁺	18	No	103 ± 17	No
Commercial set⁺⁺⁺	8	No	91 ± 30	No

⁺Designed in Present Study; ⁺⁺Literature reported; ⁺⁺⁺Commercially available; **Lyngbya* amplified at 18 cycles; ***Microcystis* amplified at 19 cycles

Table 1. *Testing results of genus-specific and cyanobacterial universal qPCR primers targeting 16 SrRNA gene in 10 cyanobacterial strains.*

Results

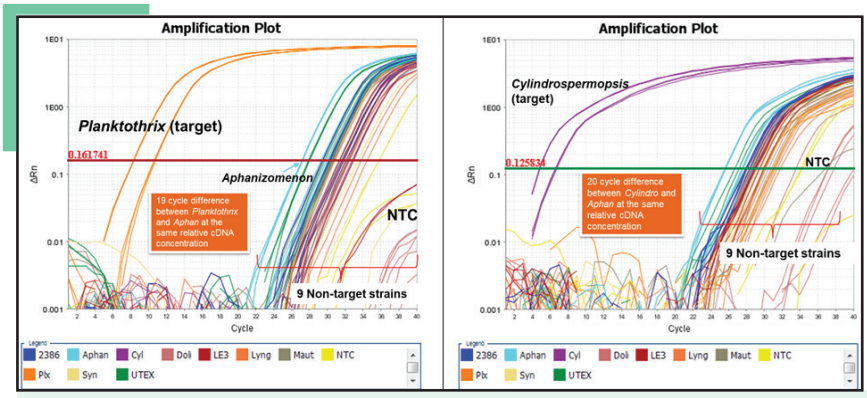
(1) Four sets of de novo designed qPCR primers specifically targeting 16S rRNA in ADA clade, *Microcystis*, *Microcoleus*, and *Synechocystis* not only met the performance evaluation criteria but outperformed their literature-reported counterparts; (2) two sets of universal qPCR primers (LF1/LR1 and JF2/JR3) demonstrated better performance than literature-reported and commercial primers; (3) we also developed good-quality genus-specific primer sets for *Planktothrix* and *Cylindrospermopsis*; (4) we independently validated the four SHA capture probes; (5) we will use qPCR assays to validate SHA results obtained by BGSU team.

Near Real-Time Field Test Kit for Simultaneous Detection and Quantificaiton of High Priority Toxic Cyanobacteria

Lead: Ping Gong, ERDC, Ping.Gong@usace.army.mil

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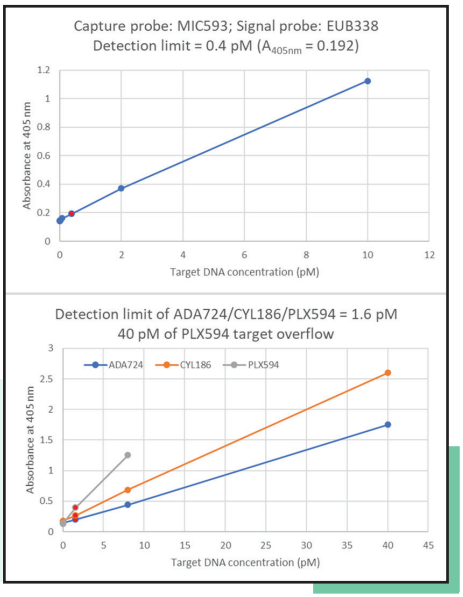


Figure 3 (*Above*). *Testing results for two new good-quality qPCR primer sets targeting Planktothrix and Cylindrospermopsis, showing separation of their respective target strain from nontarget cyanobacterial strains.* **Figure 4 (*Right*).** *ERDC team's independent validation of four SHA capture probes MIC593, ADA724, CYL186, and PLX594 developed by BGSU team targeting four genera/clade: Microcystis, ADA clade, Cylindrospermopsis, and Planktothrix, respectively.*

Major Milestones

Date	Milestone
FY21, Q1	Literature review and de novo design of genus-specific capture probes
FY21, Q2	Review and design signal probes and zip code tags
FY21, Q3	Screening the probes using lab cultures belonging to 7 target genera
FY21, Q3	RNA extraction method optimization
FY21, Q3	Field collection of HAB samples
FY21, Q4	Development of genus-specific qPCR assays for cell number calibration
FY21, Q4	Data analysis and reporting
FY22, Q1	Revise and submit review article on SHA
FY22, Q2–3	Test and validate genus-specific qPCR primers
FY22, Q3	Design and test new genus-specific capture probes and signal probes
FY22, Q4	Optimize SHA protocol
FY23, Q1–2	Validate the SHA probes and qPCR primers

Partnership/Leveraging Opportunities

This work will leverage multiple collaborations and other work units including *Small regulatory ribonucleic acids for the control of harmful algal blooms*, and *Rapid, portable and multiplexed detection of freshwater harmful algal bloom-forming genera.*

Learn about other EL research areas, including
Aquatic Nuisance Species
Aquatic Plant Control
Ecosystem Management and Restoration

Find ERDC on the web at:

Integrated Aptamer-Based Electrochemical Biosensor Platform for Rapid Detection of Freshwater Cyanotoxins

Lead: Dr. Karl J. Indest, ERDC, Karl.J.Indest@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Cyanobacteria harmful algal blooms (HABs) are a worldwide problem and cyanotoxins are a priority concern for US inland waterways. Microcystins (MCs) are the most reported freshwater toxins and are potent hepatotoxins. A key tool in the effective management of HABs and their toxins is the ability to rapidly determine the presence/concentration of toxins in waterways.

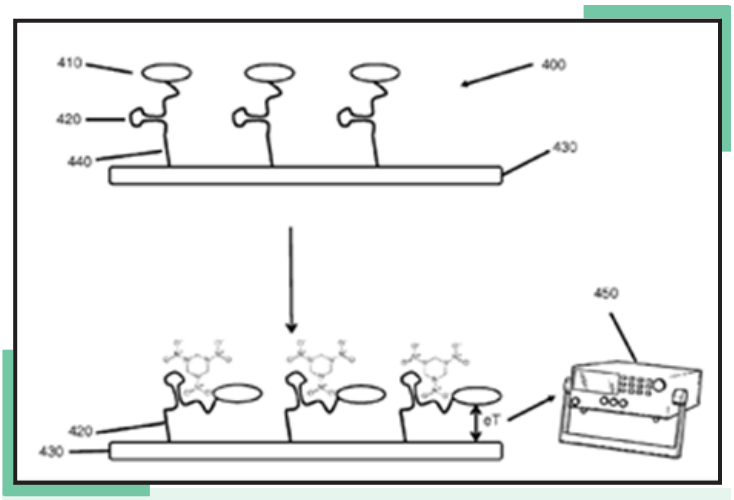


Figure 1. *Conceptual illustration from US Patent 20170107515 A1 illustrating the integration of DNA based aptamer sensing elements with an impedance based detection platform.*

Objectiv

This project aimed to develop a field portable technology based on DNA aptamer sensing elements integrated into an electrochemical based platform (Aptasensor) to enable rapid assays to detect the presence of multiple freshwater cyanobacterial toxins in a single water sample. Current methods rely on complex procedures require time-consuming sample preparation and laboratory instrumentation, which result in delays in critical management and mitigation strategies.

Approach

Establish the lower limit of detection (LLD) for multiple DNA aptamers across 3–4 log toxin concentrations using a custom electrochemical platform consisting of DNA aptamers covalently attached to a gold electrode (Aptasensor). Assess Aptasensor specificity against target and non-target toxins as well as sensor performance after repeated use. Assess performance parameters of Aptasensor using impedance spectroscopy and square wave voltammetry. Evaluate aptasensor electrochemical platform compared to standard methods to determine efficacy. Assessment of sensitivity of two published MC-LR aptamers (AN6 and HC1) was performed using traditional electrodes and square wave voltammetry.

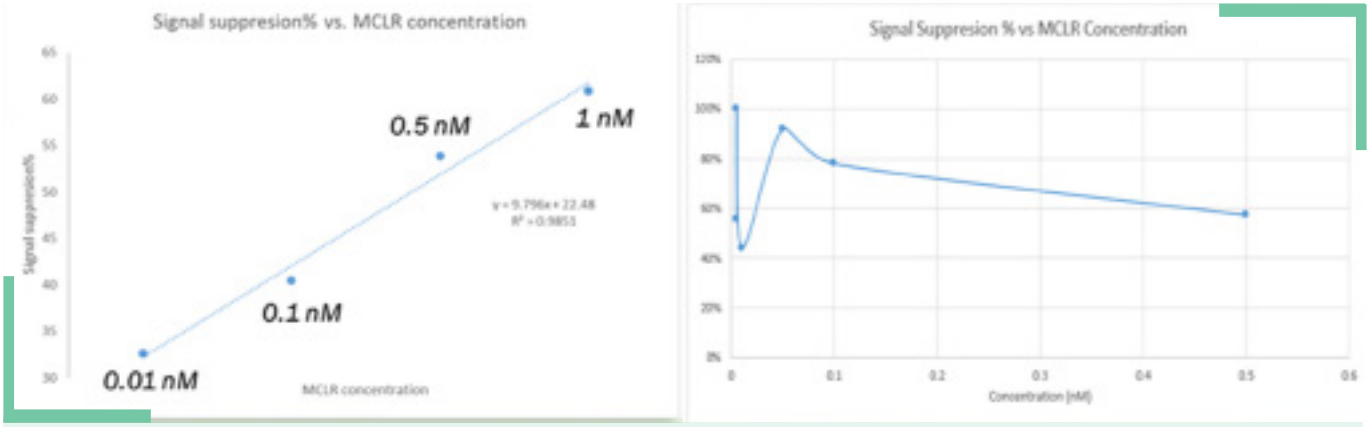


Figure 2. *Initial traditional electrode square wave voltammetry sensitivity trial for (a) AN6 and (b) HC1.*

Integrated Aptamer-Based Electrochemical Biosensor Platform for Rapid Detection of Freshwater Cyanotoxins

Lead: Dr. Karl J. Indest, ERDC, Karl.J.Indest@usace.army.mil

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Results

Initial trials indicated a dose-dependent response of the Aptasensor using known toxin standards with sensitivity in the picomolar range, effectively reaching lower than the LLDs for standard cyanotoxin detection methods. However, variability was observed in repeated trials. Additionally, methodology for these trials proved to be time intensive with a completion time of three days. These obstacles may be overcome with further method development in future funded efforts. There are relatively new technologies available to rapidly assess the presence of cyanotoxins within water using fluorescently labeled antibodies on chip. These technologies have been developed to detect specific cyanotoxins on the nanomolar scale, which fall within the EPA standards for recreational water for current bloom events and offer a viable alternative to DNA aptamer based sensing technologies.

Major Milestones

Deliverable	Description
Publications	**Tech Report:** Fernando et al. In review. "Evaluation and Investigation of an Integrated Aptamer-Based Electrochemical Biosensor (Aptasensor) Platform for Rapid Detection of HAB Toxins." ERDC TR.

Partnership/Leveraging Opportunities

Project work leverages findings from a previous ERDC Army Basic research project that identified RNA aptamers capable of binding nitroaromatic compounds and resulted in US patent 20170107515A1. In addition, this work leverages research on testing and evaluating DNA aptamers integrated into electrochemical detection systems for their ability to bind small chemicals.

Value to USACE Mission

This research aimed to develop and demonstrate a process or technology for the reduction of HAB events through early and rapid detection of cyanotoxins. The technology developed in this project demonstrates potential scalability of the process or technology to encompass a field relevant HAB event.

A Novel Biological Control Approach for Cyanobacteria

Lead: Dr. Karl J. Indest, ERDC, Karl.J.Indest@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Harmful algal blooms (HABs) are a worldwide problem and HABs associated cyanotoxins are a priority concern for US inland waterways. USACE district and division managers lack targeted, effective tools for combatting problematic cyanoHABs (cHABs) in their respective regions. Short-term mitigation strategies for the effective management of HABs and their toxins are needed like target-specific novel cyanocidal chemical products.

Objective

To identify a novel cHAB control method utilizing a bacterially derived compound(s) demonstrating both cyanocidal activity and class-level specificity that will provide USACE districts, water resource managers, and other interested stakeholders with a proven, effective alternative to less discriminatory cHAB control methods. Biological control through bacterially derived bioactive compounds remains a relatively untapped research area in cHAB mitigation, with the potential for highly specific cyanobacteria control.

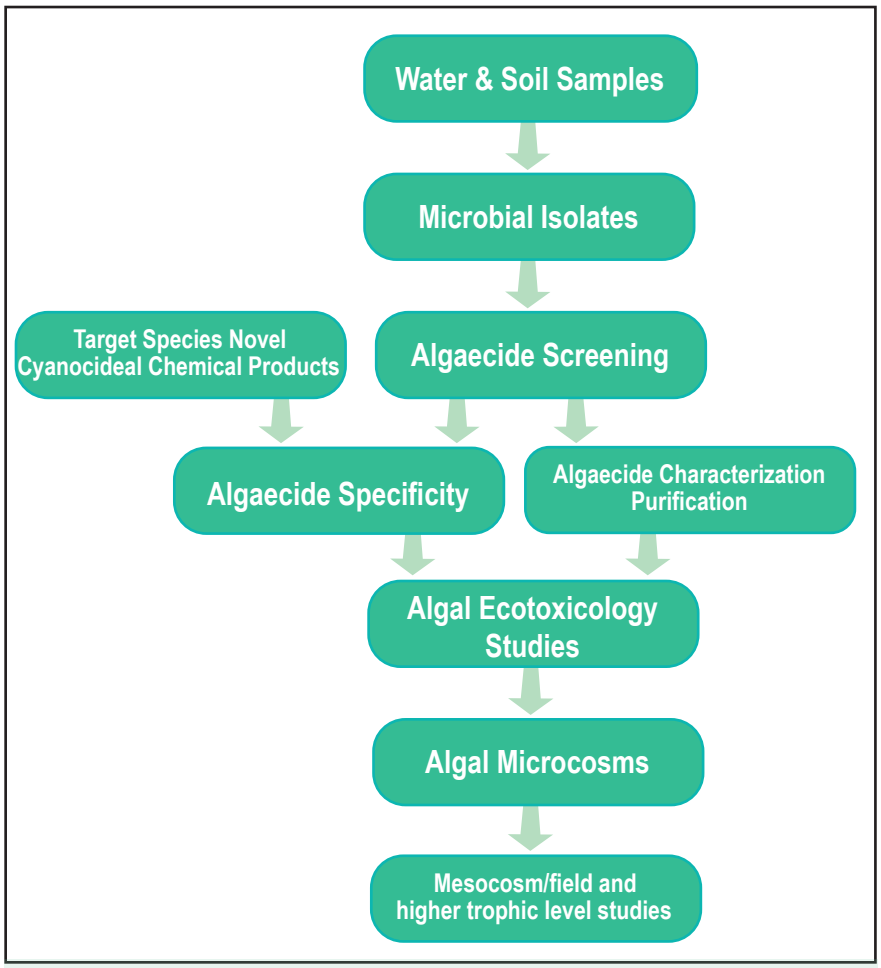


Figure 1. *Project approach to screening and indentifying natural cyanocide compounds.*

Approach

In FY21, identified and evaluated experimental bacterial cyanocide compounds that achieve 30% reduction in biomass in 72 h in model organisms and in standard screening assays on various cyanobacteria monocultures. Screened and identified aquatic/terrestrial bacteria for secondary metabolites able to inhibit algal growth in liquid/semisolid media. In FY22, there were continued algaecide evaluations on broad phytoplankton-based species and efforts to determine potential effects on nontarget species via zooplankton toxicity studies. Research efforts included partial purification and characterization of bacterial algaecide compounds. In FY23, microcosm community impact evaluations of bacterial algaecide compounds were performed.

A Novel Biological Control Approach for Cyanobacteria

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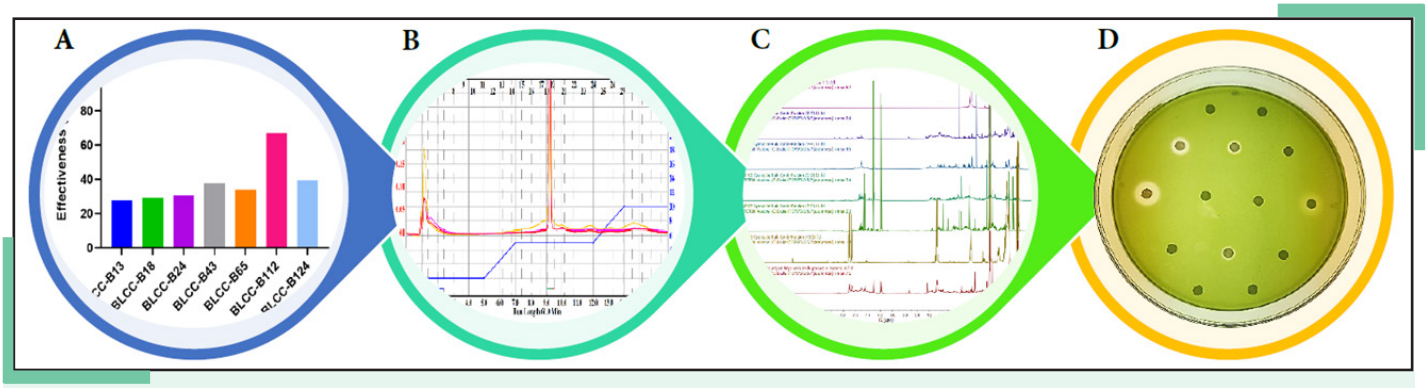


Figure 2: *Flow chart of the methods used to identify new cyanocide compounds: (a) bacteria strains were tested on Microcystis sp. and those that reduced the algal biomass by 30% were used for downstream experiments; (b) candidate bacterial strains were extracted and extracts analyzed using flash chromatography; (c) purified standards were run on a Nuclear Magnetic Resonance (NMR) instrument to help identify unknown compounds; (d) bacterial extracts were screened for cyanocide activity represented as zones of clearing on a cyanobacterial lawn plate.*

Major Milestones

Deliverable	Description
Publications	**Journal Article:** Lefler et al. 2022. "Draft Genome Sequences of Bacteria with Cyanocidal Potential Isolated from Eutrophic Waters Associated with Cyanobacterial Harmful Algal Blooms." *Microbiology Resource Announcements*. https://doi.org/10.1128/mra.00205-23. **Journal Article:** (In Prep) Lefler et al. "Efficacy and Toxicity of Bacterial Derived Cyanocidal Compounds on Target and Nontarget Species."

Partnership/Leveraging Opportunities

This work will leverage a collaboration with the University of Florida's Fort Lauderdale Research and Education Center (FLREC) via Dr. Dail Laughinghouse. Currently, this project supports a part-time ORISE scientist under the direction of Dr. Laughinghouse. In addition, FLREC is a unique laboratory resource that cultures and maintains several environmentally relevant HABs and other algal species needed for successful execution of this work unit.

Value to USACE Mission

USACE District and Division managers lack targeted, effective tools in combatting problematic localized cHABs in their respective regions. Target-specific novel cyanocidal chemical products offer short-term mitigation strategies for effective management of HABs and their toxins.

In Situ Evaluation of Peroxide Treatments Applied to HABs

Lead: Richard Johansen, ERDC, Richard.A.Johansen@usace.army.mil
Co-Lead: Alan Katzenmeyer, ERDC, Alan.W.Katzenmeyer@usace.army.mil

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Problem

Improved monitoring of rapid-response harmful algal bloom (HAB) treatments (i.e. peroxide-base algaecides) is needed because traditional methods do not provide the spatiotemporal coverage to evaluate the efficacy of these applications.

Objective

Demonstrate a suite of complementary monitoring approaches for districts to incorporate into their HAB treatment efforts. Evaluate the efficacy and longevity (up to 72 h) of singular peroxide-based treatment for rapid response to HABs.

Approach

Develop approaches for rapid response monitoring and treatments by applying multiple sensors with varying spatial and temporal resolutions (satellite imagery, sondes, grab samples, etc.) to better serve district personnel with varying expertise levels or financial limitations. Additionally, this work will evaluate the efficacy and longevity (up to 72 h) of Pak-27, a peroxide-based algaecide, for use as a rapid response to HABs.



Figure 1. *Experimentation setup in the laboratory.*



Figure 2. *Numerous technologies and approaches were utilized to intensively monitor the efficiency and longevity of a peroxide-based treatment. A monitoring screen (top) a YSI Sonde (Middle; Left is closeup of bulkhead, right is full device), and an Ecomapper (bottom).*

In Situ Evaluation of Peroxide Treatments Applied to HABs

Lead: Richard Johansen, ERDC, Richard.A.Johansen@usace.army.mil
Co-Lead: Alan Katzenmeyer, ERDC, Alan.W.Katzenmeyer@usace.army.mil

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Major Milestones

Deliverable	Description
Publications	**Tech Note:** Katzenmeyer et al. In prep. "Conceptual Workflow for Data Collection and Field Plan for Deployment of Scaled In Situ Sensors." ERDC TN. **Special Report:** Johansen et al. In prep. "A Review of Sensor-Based Approaches for Monitoring Rapid Response Treatments of cyanoHABs." ERDC SR. **Tech Report:** Johansen et al. In prep. "Multimodal Approach to Monitoring a Perox-ide-Bbased Rapid Response Treatment of a Harmful Algal Bloom." ERDC TR.
Products	**Data and Code Repository:** https://public.git.erdc.dren.mil/RJohansen/hab_peroxide_evaluation
Demonstrations	**Peroxide Treatment (Pak 27) of HAB:** July 2021. Marion Reservoir, Kansas.
Marketing Video	**Overview of ongoing HAB research at EL:** A high level overview of ERDC field-based sensor capabilities for water quality and HAB detection and monitoring.

Partnership/Leveraging Opportunities

This work will leverage multiple collaborations and other work units, including the Kansas Department of Health and Environment (KDHE) and USACE district partners (Tulsa and Kansas City) will assist in peroxide-based treatment while also providing complementary species and toxins data collection. There is potential to collaborate with the project *UAS Survey to Support EPA Region 7 HAB Monitoring* (PI: Reif), as unmanned aircraft system (UAS) imagery collected at the same time/location could benefit both projects. Lastly, there is potential to leverage work done for the project *Comprehensive satellite-based algorithms for broadscale cyanoHAB detection and monitoring* (PI: Johansen) as water quality data and image products from the peroxide treatment monitoring work unit could be used to support regional algorithm assessment.



Figure 3. *Field research*

Value to USACE Mission

This project is intended to lead to the development of improved monitoring for rapid-response HAB treatments as well as a singular peroxide-based treatment for HABs, which will better serve district personnel with varying expertise levels or financial limitations.

Comprehensive Satellite-Based Algorithims for Broadscale cHAB Detection and Monitoring

Lead: Richard Johansen, ERDC, Richard.A.Johansen@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Satellite-derived algorithms to estimate cyano-harmful algal blooms (cHABs) water quality indicators tend to be locally optimized for a particular study, limiting the use of satellite-based remote-sensing approaches across geographies and bio-physical conditions.

Objective

Improve the accuracy and estimation of satellite-derived cHAB algorithms under varying conditions to aid district managers in their monitoring efforts.

Approach

Aggregate recent (2019–2022) HAB/water quality data from across the entire continental United States with coincident remote-sensing imagery to evaluate the performance of eight Sentinel-2 algorithms for the quantification of HAB-associated pigments (chl-a and phycocyanin). Develop a baseline data set to demonstrate the ability of these simple algorithms to be utilized as regional or global algorithms across the multiple variants: water quality parameter (chl-a, phycocyanin), data acquisition method (i.e., sonde, lab, etc.), geographic region, and seasonality.

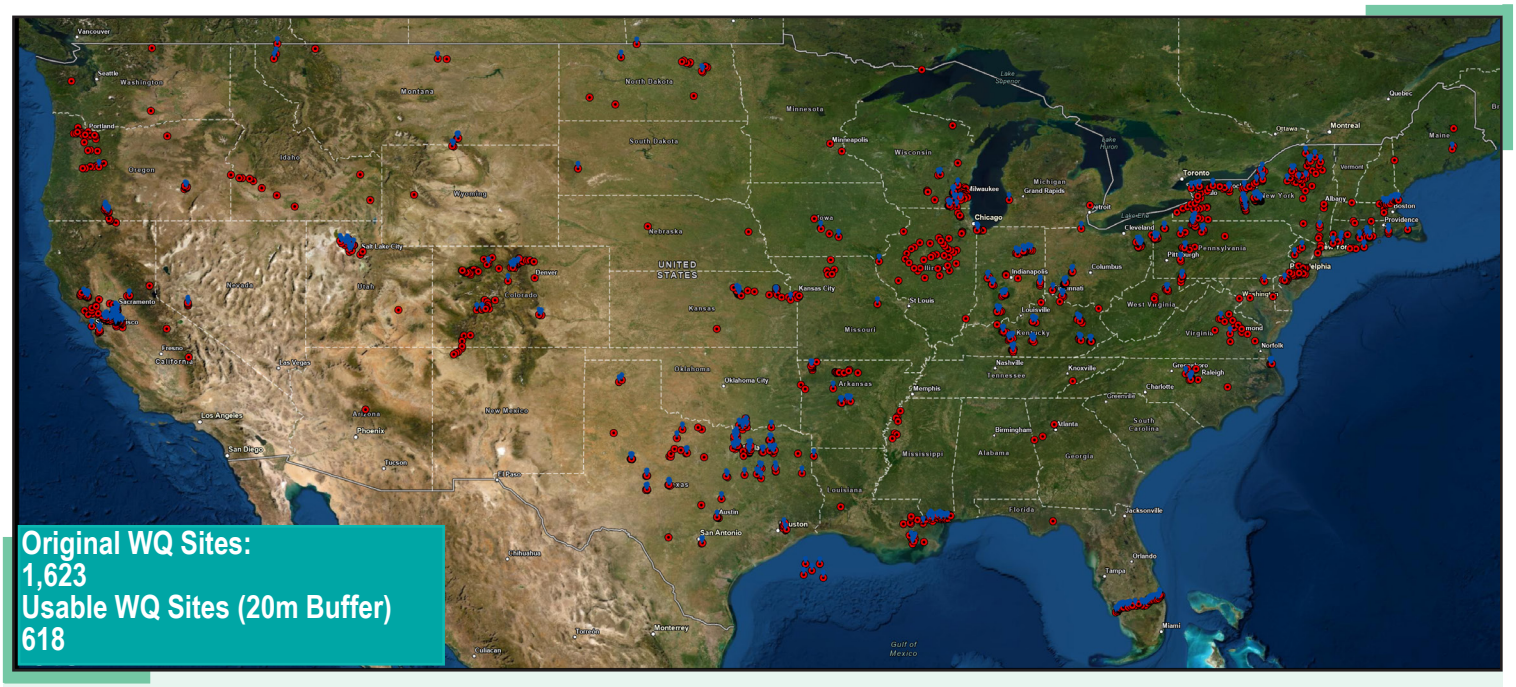


Figure 1. *After preprocessing, a total of 618 water quality (WQ) sites were selected to be matched up with coincident remote-sensing imagery from 01 January 2019 to 31 May 2022. Each algorithm was developed from pixel spectra, which correlated to a specific ground sample site location and date, and then was evaluated using k-means cross validation.*

Comprehensive Satellite-Based Algorithims for Broadscale cHAB Detection and Monitoring

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Results

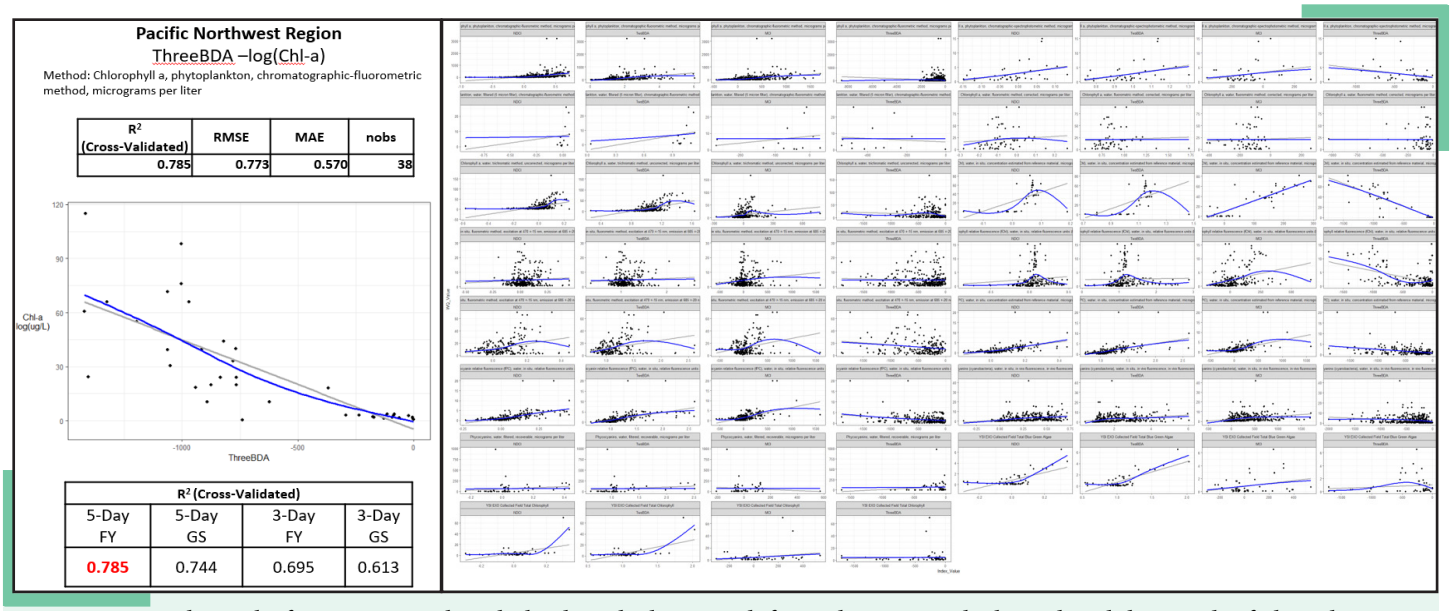


Figure 2: *Sample results from iterating though the described approach for each variate, which produced thousands of algorithm performances for all possible combinations.*

Major Milestones

Deliverable	Description
Publications	**Special Report:** Johansen et al. 2023. *A Review of Empirical Algorithms for the Detection and Quantification of Harmful Algal Blooms Using Satellite-Borne Remote Sensing.* ERDC/EL SR-22-2. https://hdl.handle.net/11681/44523. **Journal Article:** Johansen et al. In prep. "Spatiotemporal assessment of Sentinel-2 algorithms for broad-scale detection of HABs utilizing Google Earth Engine." *Ecological Indicators.*
Products	**Project data and code:** GitLab: https://public.git.erdc.dren.mil/RJohansen/regional-hab-algorithms
Tech Transfer	**Webinar:** "Selecting Satellite-Based Algorithms for Detection and Estimation of HABs" (Pending)

Comprehensive Satellite-Based Algorithims for Broadscale cHAB Detection and Monitoring

Lead: Richard Johansen, ERDC, Richard.A.Johansen@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

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Partnership/Leveraging Opportunities

This work will leverage ongoing field studies within USACE's Aquatic Nuisance Species Research Program, such as HABITATS, which includes collaborations with the New York State Department of Environmental Conservation and the Florida Department of Environmental Protection. In addition to government collaborations, commercial products are being evaluated, modified, and optimized in the rapid algae flotation technology (RAFT) study.

Value to USACE Mission

This effort intends to aid district managers in HAB monitoring efforts by providing technical guidance on the selection of the most appropriate spectrally derived algorithms and helping to transform district water quality monitoring practices, reducing the need for specialized remote-sensing skills or geospatial backgrounds.

Bacterial Remediation of Microcystin HAB Toxins

Lead: Carina M. Jung, PhD, ERDC, Carina.M.Jung@usace.army.mil
Co-Lead: Lyndsay Carrigee, PhD, ERDC, Lyndsay.A.Carrigee@usace.army.mil

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Problem

Cyanobacteria containing toxins have been documented in most states and are a priority concern for inland waterways. Microcystins (MCs) are the most reported freshwater toxins, with approximately 100 analogues and are potent hepatotoxins. Conventional methods for water treatment (boiling, chlorination, and UV treatment), are unsuccessful at destroying these toxins. Naturally occurring bacteria can typically degrade MC toxins through the mlrABCD cluster. The MlrA enzyme opens the cyclic structure, and subsequently, linear toxin is ≥160 times less toxic. MlrB further degrades the linear MC.

Objective

The goal of this project is to reengineer and produce a shelf-stable MC degrading enzyme that can be used in the field to deactivate harmful toxins while cleanup personnel remediate MC harmful algal bloom (HAB) events in a safe environment.

Approach

The project team will develop a synthetically derived, bacterial-produced enzyme(s) capable of degrading MC. By producing a bicistronic MC-degrading clone, the MlrA enzyme—which linearizes the highly toxic cyclic MC, thus significantly reducing its toxicity—is coupled with the MlrB enzyme, which begins the natural degradation process by further cleaving the MC. The resultant clone expels the enzyme(s) and allows for high level production and eventual purification of the enzyme(s). The MlrAB will be purified and its effectiveness against MC in a variety of real-world matrices will be evaluated. The final product of this project is anticipated to be a shelf-stable, enzyme powder that can be safely administered to MC-impacted bloom areas to reduce toxicity to wildlife and cleanup crews.

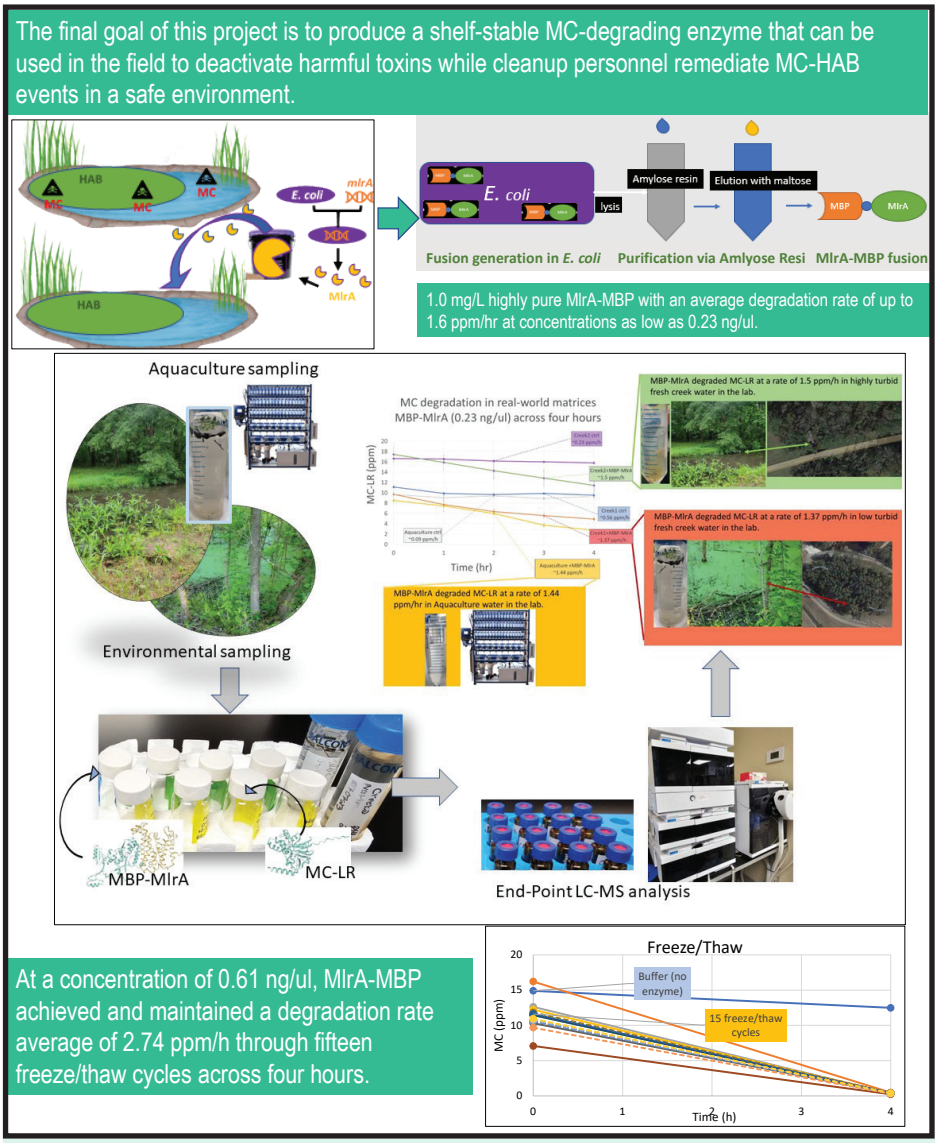


Figure 1. *Workflow diagram for project.*

Bacterial Remediation of Microcystin HAB Toxins

Lead: Carina M. Jung, PhD, ERDC, Carina.M.Jung@usace.army.mil
Co-Lead: Lyndsay Carrigee, PhD, ERDC, Lyndsay.A.Carrigee@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Major Milestones

Date	Milestone
Publications	**Tech Report:** Jung et al. In prep. "Bacterial Remediation of Microcystin-HAB Toxins." ERDC/EL TR.
Products	**US Patent** #17405012 "Recombinant proteins having enzyme activity against microcystin and methods of water remediation" (Submitted; in review)
Tech Transfer	**Industry Partner:** Possible transition to Allonia, LLC
Demonstration	Meso-/field-scale demo and cost/feasibility assessment for scale-up of MlrA application (Pending)

Partnership/Leveraging Opportunities

This work will leverage multiple collaborations and other work units. This project involves collaboration with multiple USACE district liaisons from Omaha, Jacksonville, and Buffalo District. In the future this project may be transferred to a partner, Allonia, LLC.

Value to USACE Mission

Once a bloom event occurs, detoxifying the water would be hugely beneficial to animal and human health and allow for the safety of HAB response team personnel.

Mitigation of Harmful Algal Bloom Toxins Using Deployable 3D-Printed Photocatalytic Devices

Lead: Alan J. Kennedy, ERDC, Alan.J.Kennedy@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

USACE maintains and improves inland and intra-coastal waterways, ports, and harbors. Threats from harmful algal blooms (HABs) to inland ecological resources and human health are increasing. Innovative solutions for HAB treatment (both cyanobacteria and thier toxins) are needed to mitigate blooms and decrease risks without introducing chemicals (e.g., copper algaecides, Tributyltin, and peroxides) that may have undesirable secondary effects.

Objective

Development of an effective treatment strategy for HABs in open and closed water systems that is customizable, reusable and avoids chemical additives was our goal. Photocatalytic TiO_2 nanoparticles are effective in the lab for breaking down toxins under UV-light by activating short-lived free radicals. However, TiO_2 cannot be field-deployed as free particles because they settle out of the photoactive zone and disperse into the environment. Here we designed, developed, and demonstrated 3D printable polymer composite containing TiO_2 photocatalyst as a sustainable HAB management technology.

Approach

Our research approach involved four interrelated tasks: (1) determine TiO_2 effectiveness on HABs, with microcystin from *Microcystis aeruginosa* being the target for treatment; (2) develop standard protocol to make and 3D print devices (Figure 1 and Figure 2); (3) perform tests to optimize effectiveness and reduce impacts to nontarget organisms; and (4) demonstrate technology effectiveness in a USACE-relevant mesocosm or field scale.

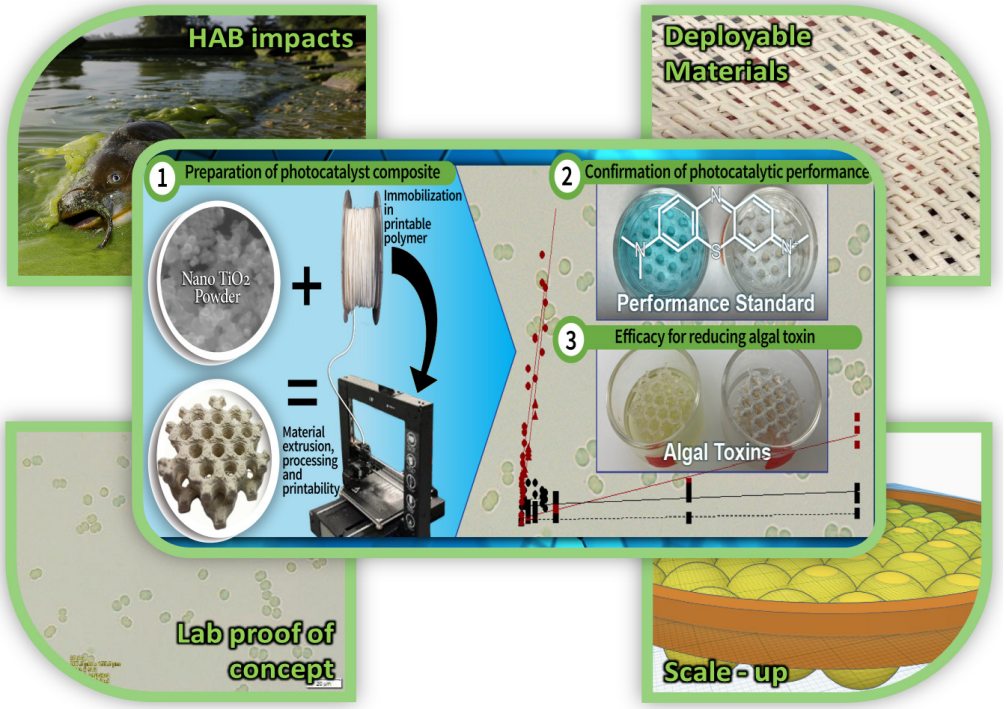


Figure 1. *Process for producing customizable, deployable, and retrievable material structures via 3D printing. https://doi.org/10.1016/j.cej.2022.140866*

Mitigation of Harmful Algal Bloom Toxins Using Deployable 3D-Printed Photocatalytic Devices

Lead: Alan J. Kennedy, ERDC, Alan.J.Kennedy@usace.army.mil

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Results

Lab results showed the photocatalytic performance of the 3D-printed TiO_2 was equivalent to that of free TiO_2 (Figure 3) and provided multiple advantages: (1) reduced nanoparticle toxicity to nontarget species; (2) retains reactive material in the water column for prolonged treatment; and (3) is retrievable and reusable. 3D-printed TiO_2 also degraded microcystin and was 12-times faster than UV light alone (Figure 4). Photocatalyst interactions with the polymer locally reduces pH, leading to enhanced microcystin adsorption to the photocatalyst and subsequent destruction. Further work will go toward faster treatment, technology scale up, and road mapping, including preliminary field demonstration.

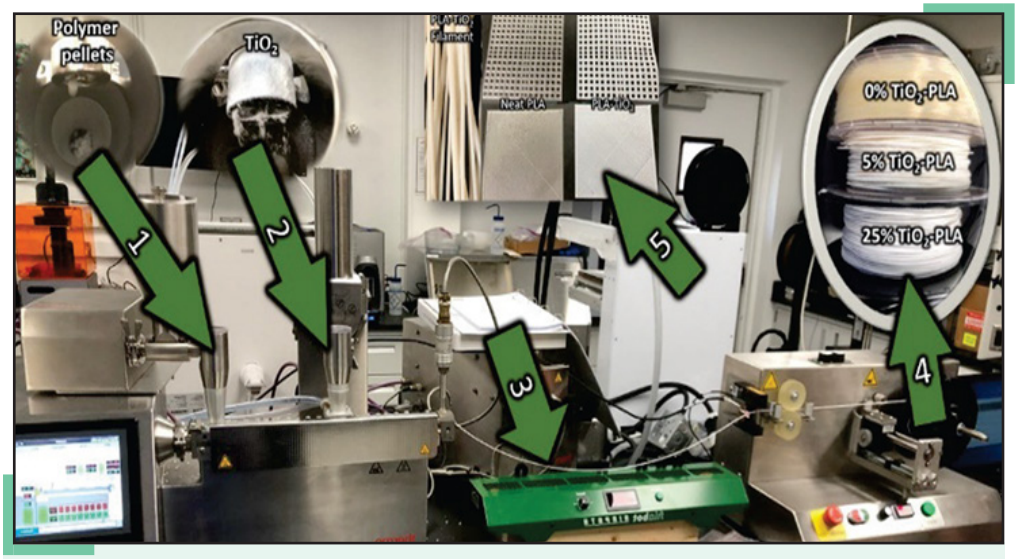


Figure 2. *Developed process to embed TiO_2 into filament that is 3D printable by inexpensive desktop printers. This process can be commercialized at a low cost. https://apps.dtic.mil/sti/citations/*

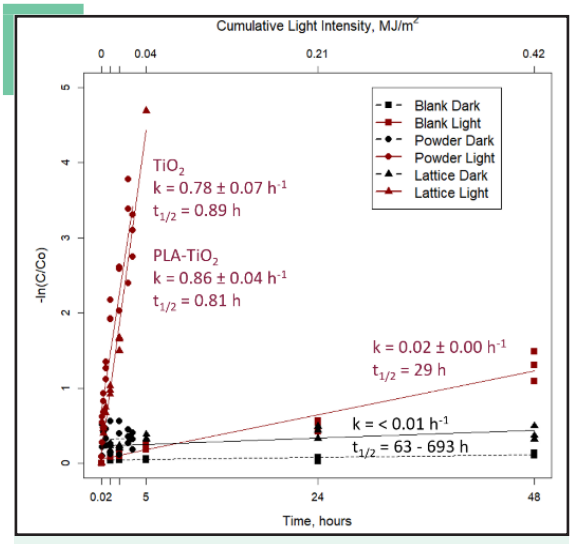


Figure 3: *Similar photocatalytic performance using methylene blue dye between free and 3D-printed photocatalyst. These data demonstrate successful performance of 3D-printed materials. https://doi.org/10.1016/j.cej.2022.140866.*

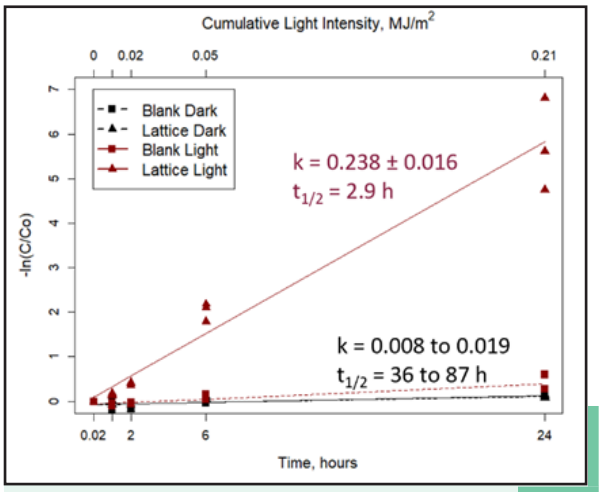


Figure 4: *3D-printed photocatalytic materials degrades algal toxins (microcystin) within environmentally relevant timeframes and environmental conditions. https://doi.org/10.1016/j.cej.2022.140866.*

Mitigation of Harmful Algal Bloom Toxins Using Deployable 3D-Printed Photocatalytic Devices

Lead: Alan J. Kennedy, ERDC, Alan.J.Kennedy@usace.army.mil

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Major Milestones

Deliverable	Description
Publications	**Tech Note:** Kennedy et al. 2022. *Sustainable Harmful Algal Bloom Mitgation by 3D Printed Photocatalytic Oxidation Devices (3D-PODs).* ERDC/TN ANSRP-22-1. Vicksburg, MS: US Army Engineer Research and Development Center. https://apps.dtic.mil/sti/citations/AD1166826. **Journal Article:** Kennedy et al. 2023. "Degradation of Microcystin Algal Toxin by 3D Printable Polymer Immobilized Photocatalyic TiO_2." *Chemical Engineering Journal* 455: 40866. https://doi.org/10.1016/j.cej.2022.140866. **Tech Report:** Kennedy et al. In prep. "Demo and Report: 3D printed, deployable/retrievable HABs control technology." ERDC/EL TR.
Products	**Software:** Eventually, will have downloadables for printing and can place online **Patents:** In progress; met with patent attorney in March 2023
Tech Transfer	Prototypes and digital library available to USACE districts for pilot use

Partnership/Leveraging Opportunities

This effort has synergy with other Aquatic Nuisance Species Research Program sponsored projects including Rapid Algae Flotation Techniques (RAFT) (PI: Clinton Cender) and Light-Based Mitigation Technology (LBMT) (PIs: Elizabeth Gao and Taylor Rycroft), with potential to float treat and UV treat HABs more effectively. A combination of the LBMT and 3D-printed TiO_2 technologies is proposed to harness strengths of both for maximal HAB treatment effectiveness. A 3D-printed flexible fabric mat could be used as a polishing step to reduce microcystin concentrations after LBMT inactivates the cells. The PIs have partners at USACE Jacksonville, Buffalo, Chicago, and Rock Island districts.

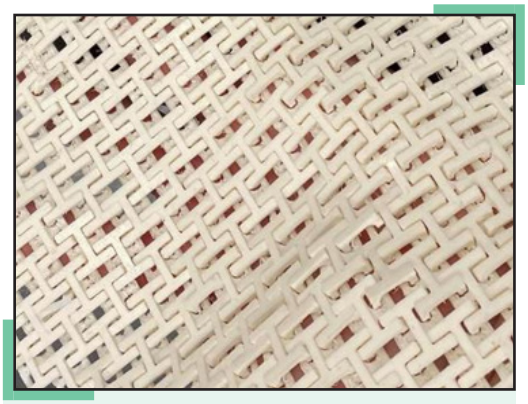


Figure 5: *Capability to 3D print flexible materials that can move with water.*

Value to USACE Mission

Optimization of deployable and retrievable technology available on-demand to combat HABs while avoiding legacy contamination (e.g., copper, peroxides, etc.). Provides USACE with a proactive, cost-effective, and reusable HABs solution while maintaining a sustainable mission.

Implications of Seasonal Anoxia and Sediment Resuspension on Nutrient Loading and HAB Initiation

Lead: Dr. Marissa Kneer, ERDC, Marissa.I.Kneer@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

This work unit addresses two tasks. First, project partners at Texas A&M University Galveston (TAMUG) require water quality analysis for a large-scale limnology study examining nutrient cycling in multiple Texas reservoirs in order to develop a comprehensive harmful algal bloom (HAB) risk model. Second, sediment resuspension and vertical mixing of nutrient-rich bottom-waters release nutrients that play a key role in HAB outbreak initiation, but internal nutrient loadings associated with sediments in reservoirs of the south central US are poorly understood. As a result, ERDC will conduct sediment nutrient experiments to inform develoment of the TAMUG HAB risk model.

Objective

The objective is to (1) complete water quality analysis for our TAMUG partners, and (2) determine the magnitude and stoichiometry of nutrient loading from sediments during seasonal water column mixing and resuspension events. Both data sets will be incorporated into a model useful for lake and watershed scale HABs management.

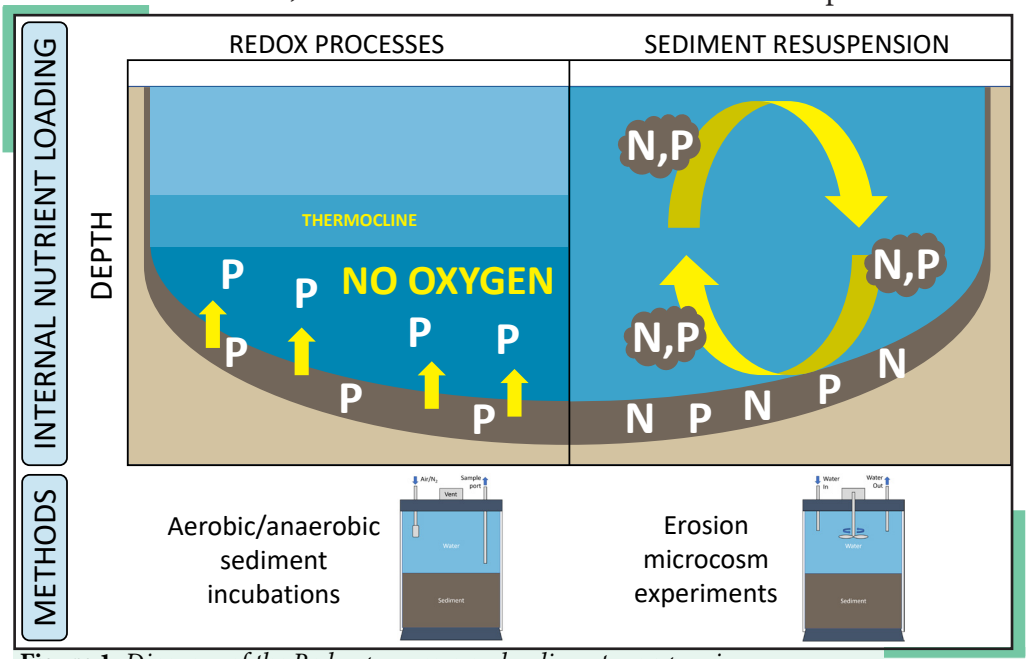


Figure 1. *Diagram of the Redox processes and sediment resuspension (note: P = phosphorous, N = nitrogen)*

Approach

(1) Characterize standard water quality parameters from several hundred seasonally stratified water quality samples collected by project partners at TAMUG; (2) evaluate sediment morphology, available nutrients, and relevant solid phases in eight Texas reservoirs; (3) quantify nutrient concentrations in surface water, bottom water, and sediment pore water; (4) determine magnitude of potential nutrient release from sediment to anoxic bottom waters using sediment incubations; and (5) investigate nutrient release from sediment due to sediment resuspension using an erosion microcosm system.

Results

Nutrient analysis has been completed on 560 water quality samples and data shared with TAMUG partners for continued development of our HAB risk model. Despite logistical challenges, sediment cores were collected from five reservoirs in Texas and incubation experiments are ongoing to determine the magnitude of nutrient loading during seasonal anoxia events (Figure 2). Following these incubation experiments, erosion microcosm experiments will begin to determine the contribution of particulate-phase nutrients to internal nutrient loading.

Implications of Seasonal Anoxia and Sediment Resuspension on Nutrient Loading and HAB Initiation

Lead: Dr. Marissa Kneer, ERDC, Marissa.I.Kneer@usace.army.mil

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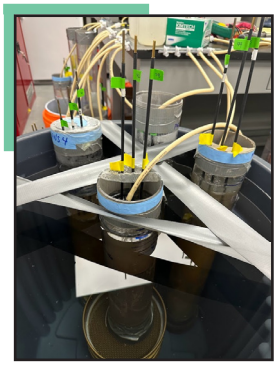




Figure 2. *(Left) Aerated sediment cores simulating the oxygenated lake bottom during the summer; and (right) intact sediment core collected using a vibra-corer for use in incubation and erosion microcosm experiments.*

Major Milestones

Delivered	Description
Publications	**Journal Article:** Kneer et al. In prep. "Impact of Sediment Characteristics and Seasonal Anoxia on Importance of Nutrient Loading in Warm Monomictic Reservoirs." *Journal of Lake and Reservoir Management.* **Journal Article:** Kieley et al. In prep. "Quantitative Model for HAB Prediction in Reservoirs of Southcentral TX." *Ecological Modeling.*
Tech Transfer	**Instruction Manual:** Kieley et al. In prep. "Quantitative Model for HAB Prediction in Reservoirs of Southcentral TX."
Products	**Quantitative Model** for HAB Prediction in Reservoirs of Southcentral TX.

Partnership/Leveraging Opportunities

This work is leveraging a collaboration wth research project partners from TAMUG (PI: Roelke). The collabortion will contribute to development of a comprehensive quantitative model to quantify HAB outbreak risk.

Value to USACE Mission

Characterization of sediments and quantification of sediment nutrient loading will improve lake-scale nutrient management strategies and contribute to watershed HAB risk model development.

Global Transcriptional Profiles Associated with Increasing Severity and Duration of a *Microcystis* Bloom

Lead: Lauren Krausfeldt, Nova Southeastern University

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Cyanobacterial harmful algal blooms (cyanoHABs) are a major concern in freshwater ecosystems around the world. CyanoHABs have significant negative consequences on the health of humans, animals, ecosystems, and the economy. There remains a need to develop novel approaches to predict, prevent, and disrupt the formation of cyanoHABs. Towards fulfilling this need, an understanding of the key physiological changes in cyanobacteria that lead to the formation and persistence of cyanoHABs is required.

Approach

To address the mechanistic understanding of HABs caused by *Microcystis*, we performed in situ mesocosms on the Caloosahatchee River in south Florida during ongoing Microcystis bloom. We added daily additions of N in the forms of NH_4 and urea, a commonly used N fertilizer in agriculture. Measurements for temperature, phycocyanin (PC), chlorophyll a (chl a), conductivity, pH, fluorescent dissolved organic matter (fDOM), turbidity, and dissolved oxygen (DO) were collected daily at time point (T) T0, T24, T48, and T72, and additionally at T264, a time point chosen to represent a longer term impact. These measurements were done prior to daily nutrient addition using a YSI EXO multiparameter water quality sonde. Surface water samples at 0.05 m depth were collected monthly by the South Florida Water Management District (SFWMD) from Lake Okeechobee for DNA sequencing and subsequent comparison. We characterized the global transcriptional response of the Microcystis bloomto different nutrient additions that yielded differential bloom outcomes using a metatranscriptomic approach.

Results

By performing in situ mesocosms on the Caloosahatchee River in southwest Florida, a *Microcystis* bloom was successfully intensified with the addition of nitrogen. Metagenomics identified that *Microcystis panniformis* caused the bloom and was highly identical to *M. panniformis* identified in Lake Okeechobee, a major source of freshwater to the Caloosahatchee River. Metatranscriptomes characterized the global transcriptional response associated with increased bloom intensity upon treatment with ammonium and urea for 72 h, which primarily included upreg-

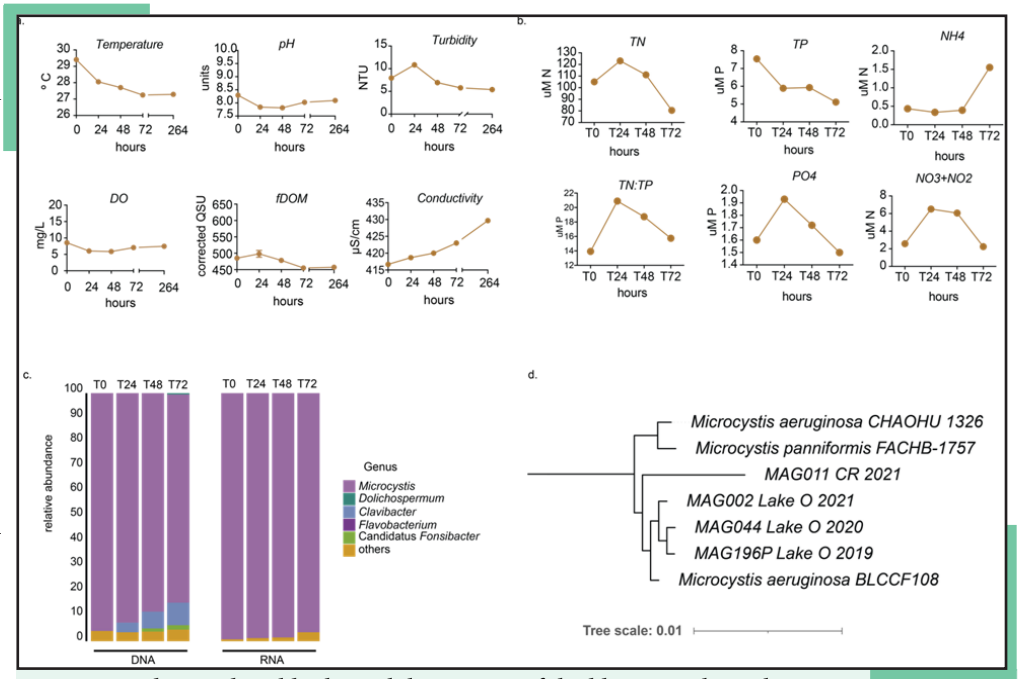


Figure 1. *Chemical and biological description of the bloom on the Caloosahatchee River (CR) in May 2021: (a) chemical data collected from the CR during the bloom on the CR using YSI; (b) nutrient chemistry in the CR during the bloom; (c) present and active microbial community composition from the bloom on the CR based on metagenomic (DNA) and metatranscriptome (RNA) reads, respectively; (d) phylogenetic tree showing relationships between Lake Okeechobee (O) and CR metagenome assembled genomes recovered from 2019-2021. MAGs on tree are labeled where and in what year they were recovered.*

Global Transcriptional Profiles Associated with Increasing Severity and Duration of a *Microcystis* Bloom

Lead: Lauren Krausfeldt, Nova Southeastern University

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

ulation photosynthesis machinery, phosphate transport, and carbon transport and metabolism. However, urea additions also caused upregulation of carbon storage, amino acid metabolism, microcystin biosynthesis, and several other processes associated with cell growth, and the bloom persisted several days longer in this treatment. Type II toxin-antitoxin systems, CRISPR-cas genes, and over 100 transposases were also upregulated indicating that phage defense and genome rearrangement were important in bloom proliferation. Importantly, these results show the stepwise response by Microcystis that leads to increased bloom intensity and duration.

Value to USACE Mission

This study, to our knowledge, is the first to successfully induce a Microcystis bloom using in situ mesocosms and characterize the global transcriptional response over several days. In doing this, the functions that are important in increasing severity and duration of a bloom experiencing N-limitation have been elucidated. This study also confirmed that phage defense and genome rearrangement are key mechanisms for the proliferation of Microcystis in nature. Finally, we demonstrate the potential importance of urea as N source in the Caloosahatchee River and elsewhere, as urea alone was able to support these outcomes. Since the use of urea as a N fertilizer is increasing globally, this demonstrates run-off containing urea could have severe implications on freshwater ecosystems.

Project Team

Lauren Krausfeldt[1*], Paisley Samuel[1], Robert P. Smith[1,2], Hidetoshi Urakawa[3], Barry H. Rosen[3], and Jose V. Lopez[3]

1. Department of Biological Sciences, Guy Harvey Oceanographic Center, Nova Southeastern University, Dania Beach, FL
2. Cell Therapy Institute, Kiran Patel College of Allopathic Medicine, Nova Southeastern University, Fort Lauderdale, FL
3. Department of Ecology and Environmental Studies, Florida Gulf Coast University, Fort Myers, FL

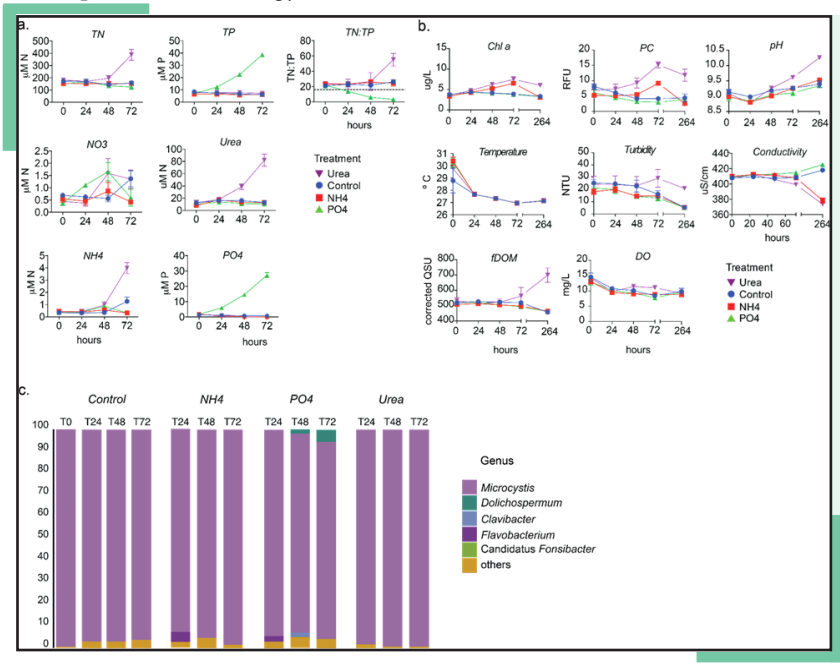


Figure 2. *Chemical and active microbial community in the mesocosms on the Caloosahatchee River: (a) nutrient concentrations in the mesocosm chambers at the surface at the start of the experiments (T0), after 24 h (T24), after 48 h (T48), and after 72 h (T72). Nutrients were added at increasing concentrations at T0, T24, and T48 and samples for nutrient concentrations were collected before nutrient dosing. Samples for dissolved nutrients were also collected after nutrient dosing and can be found in Figure S2; (b) chemical profiles in the mesocosm chambers at the surface at T0, T24, T48, T72, and one week later at T264. Chemical profiles were taken before nutrient dosing that occurred at T0, T24, and T48; (c) top 95% of the composition of the active microbial community in the control chambers at T0.*

Identification and Preventative Treatment of Overwintering Cyanobacterial Cells in Sediments

Lead: Dr. Andrew McQueen, ERDC, Andrew.D.McQueen@usace.army.mil

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Problem

Overwintering cyanobacterial cells in the sediment can contribute to harmful algal blooms (HABs), yet there are limited data on the identification and treatment of these "resting" cells.

Objective

The objective is to detect and treat overwintering cells in sediments to decrease seasonal severity of blooms.

Approach

First, conduct a literature review to determine detection methods, site selection criteria, and answer initial feasibility-related questions regarding algaecide applications to overwintering cells. Second, conduct bench-scale testing to evaluate analytical detection methods for overwintering cells and measure responses of cells to candidate algaecide exposures. Finally, evaluate performance of preventative algaecide applications at one or more field sites with known HAB history and confirmed overwintering cells in sediments (Figure 1).

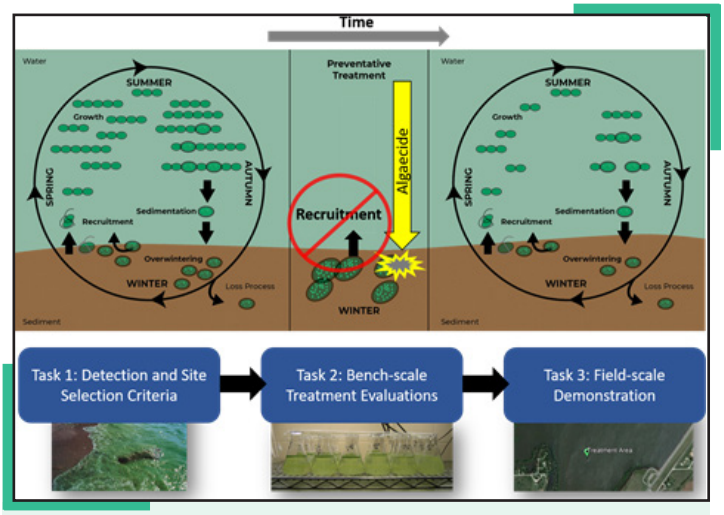


Figure 1. *Approach of identification and preventative treatment of overwintering cyanobacteria cells in sediment.*

The literature review demonstrated that there are multiple lines of evidence supporting algaecide efficacy for preventative treatments, which include (1) multiple field studies demonstrating scalability and efficacy of algaecides against benthic algae, (2) data suggesting similar sensitivities of overwintering and planktonic *Microcystis* cells to a peroxide algaecide (a commonly used active ingredient for algaecides in situ), and (3) a mesocosm study demonstrating a decrease in HAB severity following preventative treatments. This review informed data needed for identification and prioritization of potential treatment sites, metrics for measuring treatment success and potential efficacy of algaecides for preventative treatments of overwintering cells. These results provided bench-scale efficacy data for selected USEPA registered peroxide and copper-based algaecides (graphical abstract Figure 2), to inform future field scale demonstrations.

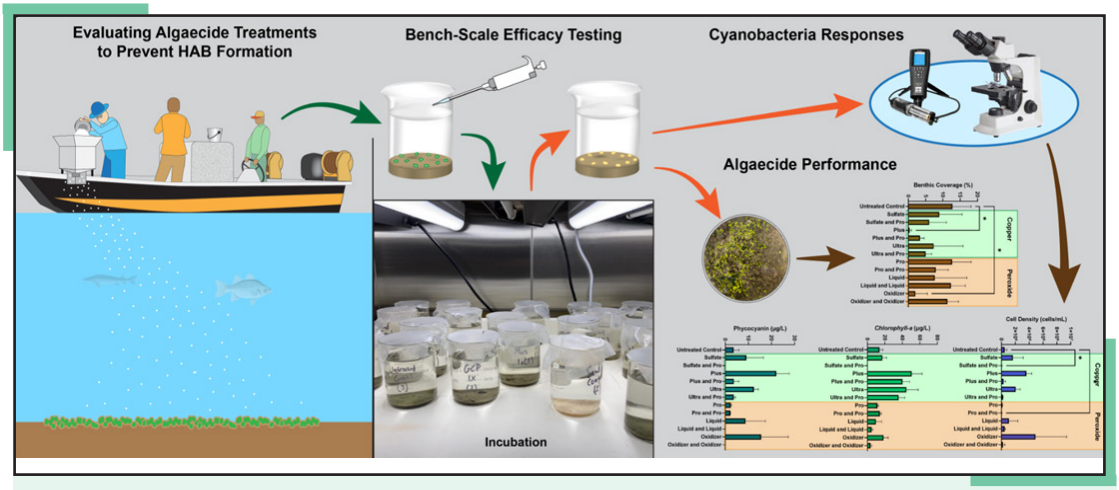


Figure 2. *Measure the efficacy of copper and peroxide-based algaecides for preventative treatment of overwintering cyanobacteria.*

Identification and Preventative Treatment of Overwintering Cyanobacterial Cells in Sediments

Lead: Dr. Andrew McQueen, ERDC, Andrew.D.McQueen@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

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Results

Bench-scale treatment efficacy data indicated that peroxide-based algaecides were effective at decreasing the growth potential of overwintering cells. Cell density and phycocyanin concentrations were useful response measurements (Figure 3).

Task 3 field demonstration was conducted in FY23. Early trials in an 80-acre pond in Kansas indicated that, following the preventative treatments, average cell densities of HAB-forming cyanobacteria were consistently lower in the water column of the treatment zone as compared to the untreated zone for the post-treatment sampling events in May through October (Figure 4).

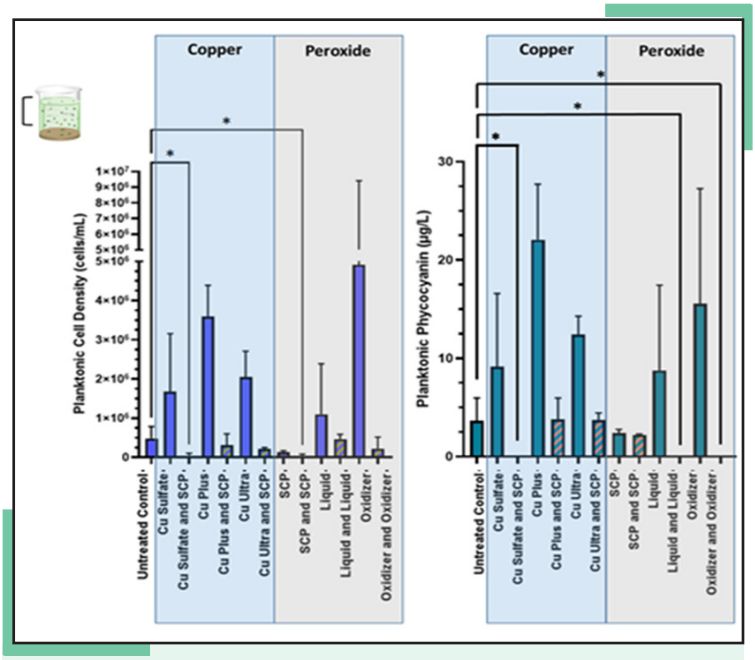


Figure 3. *The graph shows Planktonic responses of cyanobacteria from sediments containing overwintering cells following algaecide exposure and a 14-day incubation.*

Partnership/Leveraging Opportunities

This work is leveraging multiple collaborations and other work units including the Kansas Department of Health and Environment (KDHE), USACE Tulsa District (SWT), and USACE Kansas City District (NWK). Successfully collaborated with KDHE and USACE-SWT on a field-scale demonstration in Kansas leveraging funds from KDHE. Additional collaboration is planned in FY23.

Value to USACE Mission

Early detection and preventive treatment of overwintering cells can provide substantial value to operations by potentially decreasing severity of bloom events, delaying seasonal onset of blooms, minimizing ecological and human health risks, and minimizing costs of mitigation.



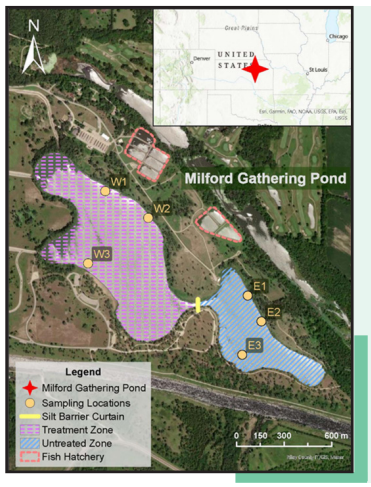


Figure 4. *Initial field-scale demonstrations (Milford Gathering Pond, Kansas) treating overwintering cells in sediments indicate decreased severity of HAB. The map depicts the treated and untreated (control) areas of the lake.*

Identification and Preventative Treatment of Overwintering Cyanobacterial Cells in Sediments

Lead: Dr. Andrew McQueen, ERDC, Andrew.D.McQueen@usace.army.mil

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Major Milestones

Deliverables	Description
Publications	**Tech Report:** Calomeni et al. 2022. I*dentificatoin and Preventative Treatment of Overwintering Cyanobacteria in Sediments: A Literature Review.* ERDC TR-22-10. Vicksburg, MS: US Army Engineer Research and Development Center. http://dx.doi.org/10.21079/11681/45063. **Journal article:** Calomeni et al. 2023. "Approach for Identification and Prioritization of Sites for Preventative Management of HABs by Targeting Overwintering Cyanobacteria in Sediments." *Journal of the Aquatic Plant Management Society* 61:30–41. https://apms.org/journal/. **Journal article:** Calomeni et al. 2023. In review. "Bench-Scale Evaluation of Algaecides for the Preventative Treatment of Overwintering Cyanobacteria in Sediments from a HAB Impacted Pond." *Ecotoxicology and Environmental Safety* **Journal article:** Calomeni et al. 2023. In review. "Overwintering Cyanobacteria: Contribution to HABs and Implicatons for Preventative Management in Freshwater Resources of the US." *Journal of Applied Ecology*. **Journal article:** Kinley-Baird et al. 2023. In review. "Evaluation of Preventative Algaecide Treatments for Overwintering Cyanobacteria in Sediments of a Central USA Lake." **Tech report:** McQueen et al. In prep. "A Management Plan for the Monitoring and Preventative Treatment of Overwintering Cyanobacteria." ERDC TR. **Articles:** • **Seiter-Moser et al. 2021.** "Creating Scalable Solutions for HABs." *The Military Engineer.* https://samenews.org/creating-scalable-solutions-for-harmful-algae-blooms/. • **Kuzmitski. 2022.** ERDC Builds Robust Research Portfolio of HAB Solutions for USACE, Nation. *The Corps Environment.* https://media-cdn.dvidshub.net/pubs/pdf_62208.pdf.
Demonstrations	**110-acre field-scale Peroxide Algaecide Treatment Efficacy Demonstration** on a Historically HAB impacted waterbody. Milford Gathering Pond, Kansas. April 2022 **Field-scale Peroxide Algaecide Treatment Efficacy Demonstration** on a Historically HAB impacted waterbody. Big11 Pond, Kansas. March 2023

Evaluation of Cavitation as Potential HAB Management Technology

Lead: Victor Medina, Formerly ERDC Environmental Laboratory

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

USACE maintains waterways, reservoirs, and recreational areas that can all be impacted by harmful algal blooms (HABs). Scalable technologies are needed that can minimize HAB severity and duration and thereby reduce HAB impacts to USACE.

Objective

The objective of this project is to explore cavitation as potential HAB management strategy using a phased laboratory to field approach.

Approach

Cavitation releases microbubbles that collapse violently, creating a pressure change that may be effective in destroying cyanobacteria. The bubble collapse creates reactive oxygen species such as superoxide and hydroxyl radical, which have potential to degrade cyanotoxins. Previous studies have shown a reduction of *Microcystis aeruginosa* as measured by chlorophyll and reduction of the toxin Microcystin LR following exposure to cavitation bubbles. Cavitation is a potentially flexible technology in that could be deployed in a number of ways to address cyanobacteria HABs: as a single point; as an array or net to create a barrier; as a fixed system; or as transportable units. It could also potentially be deployed as a towed system, moving across a waterbody while providing treatment. This study featured a laboratory test where cyanobacteria were exposed to a cavitation apparatus.

Results

The laboratory tests involved passing cyanobacteria-containing water through a needle valve tuned to produce cavitation bubbles. A decrease in chlorophyll was measured as the water circulated through the valve repeatedly. However, this experimental design prevented assessment of cavitation bubble exposure versus effect of the needle valve itself on the cyanobacteria. Accordingly, a "no go" decision was made and this approach did not proceed to larger scale or field trials.

Value to USACE Mission

Scaleable technologies that are capable of HAB prevention and management are needed to reduce HAB severity and impact frequency to our nation's freshwater resources. Although this project reached a "no go" decision due to experimental design challenges, cavitation and other technologies that produce reactive species that can neutralize cyanobacteria and their toxins have great potential for further development.



Figure 1. *Equipment used during the experiment.*

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

Lead: Dr. Martin Page, ERDC, Martin.A.Page@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Removing harmful algae from waterbodies is not scalable due to logistical challenges associated with managing the large volumes of potentially toxic algal biomass.

Objective

Develop a scalable, rapidly-deployable system that removes harmful algae from waterbodies and efficiently manages the resulting biomass.

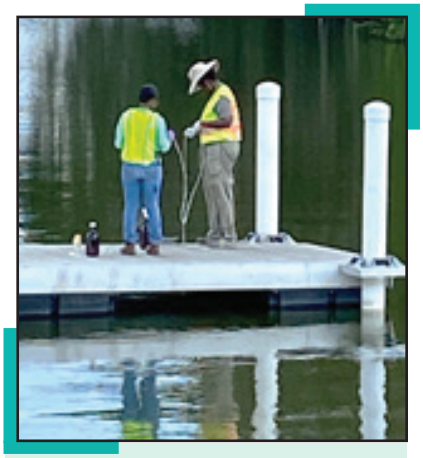


Figure 1. *Researchers in the field.*

Approach

HABITATS integrates high-throughput, energy-efficient technologies for interception, treatment, and transformation of HABs. It employs resource recovery to increase physical and economic scalability of HAB removal from large waterbodies. The removed biomass is converted into fuel and fertilizer feedstocks, resulting in a more sustainable solution that can generate more energy than it uses. The system destroys cyanotoxins, both in the treated water and in the removed biomass, while removing intracellular nutrients from the water, decreasing total phosphorus and nitrogen levels in the processed water by up to 93% and 55%, respectively. A rapidly deployable system has been developed that is now undergoing optimization and validation in field settings.



Figure 2. *Researchers in the lab.*

Major Milestones

Date	Milestone
FY21, Q4	Development of technology transfer documentation, updating scalability and economic impact models, and technical publication.
FY21, Q4	Field assessments of in situ microflotation to increase economic scalability of HABITATS (initial assessment completed; redesign and optimization ongoing).
FY21, Q4	Field demonstration of onshore treatment system in Florida, including on-site fuel generation (completed; system optimization ongoing).
FY22, Q3	Assembly and installation of a deployable onshore system that can treat 2 million gallons per day (10-fold higher treatment capacity than previously-tested systems, validation ongoing).
FY22, Q3	Awarded contract for the world's largest deployable hydrothermal liquefaction system for transforming algal biomass into fuel (completed).
FY23, Q3	Assemble final deployable prototype and establish HAB mitigation test bed at Lewisville Aquatic Research Facility.
FY23, Q4	Complete prototype optimization testing.
FY24, Q2	Complete training videos and manuals to support technology transfer.

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

Lead: Dr. Martin Page, ERDC, Martin.A.Page@usace.army.mil

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Partnership/Leveraging Opportunities

Ongoing field studies within USACE's Aquatic Nuisance Species Research Program have included collaboration with academic, industrial, and government partners, such as the University of Illinois, AECOM, Ecologix, and Elastec, and the Department of Energy's Pacific Northwest National Laboratory. Field studies have been coordinated with state regulatory agencies, including the Florida Department of Environmental Protection, the New York Department of Environmental Conservation, and the Ohio Department of Natural Resources. Regional USACE field support has included members from Jacksonville, New England, and Buffalo Districts.



Figure 3. *Representatives from AECOM and Genefuel discuss hydrothermal liquefaction with University of Illinois partners at the ERDC HABITATS field demonstration in Florida (2022).*

Value to USACE Mission

HABITATS can be deployed at key locations, such as spillways and canals, to reduce transport of algae, intracellular nutrients, and potential toxins through critical waterways, reducing environmental impacts on downstream waterbodies and communities.



Figure 4. *Various images taken during research field deployment.*

Efficacy of Algaecides and Novel Ozone Nanobubble Technology on Prevention and Management of HABs

Lead: Heather Raymond, The Ohio State University

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Lab and field trials have demonstrated the efficacy of algaecides on cyanobacteria control at warmer temperatures, but data on treatment potential at lower temperatures is limited. There are increasing reports of cyanotoxin producing harmful algal blooms (HABs) in temperate regions under cooler conditions. Faced with limited options, water managers may apply algaecides that could have little to no benefit, but high cost. Having a better understanding of temperature limitations for different algaecide formulations could provide a cost savings and increase effectiveness.

Nanobubble ozone treatment (NBOT) is an emerging alternative HAB prevention and control strategy with potential effectiveness at lower temperatures. By incorporating ozone into nanobubbles, ozone may persist for longer periods, diffuse slower, increase hydroxyl radical production, and increase reaction times with contaminants. Preliminary studies in Florida and Ohio have yielded promising results, but the modes of action for cyanobacteria control have not been fully elucidated and it is unclear how changing environmental conditions impact treatment and dose requirements. How this treatment potentially impacts nutrient cycling is also poorly understood. Controlled studies are limited and full-scale sustained lake treatments have not been conducted. A lack of data on potential impacts to nontarget organisms has created barriers for approval of larger scale treatment trials.

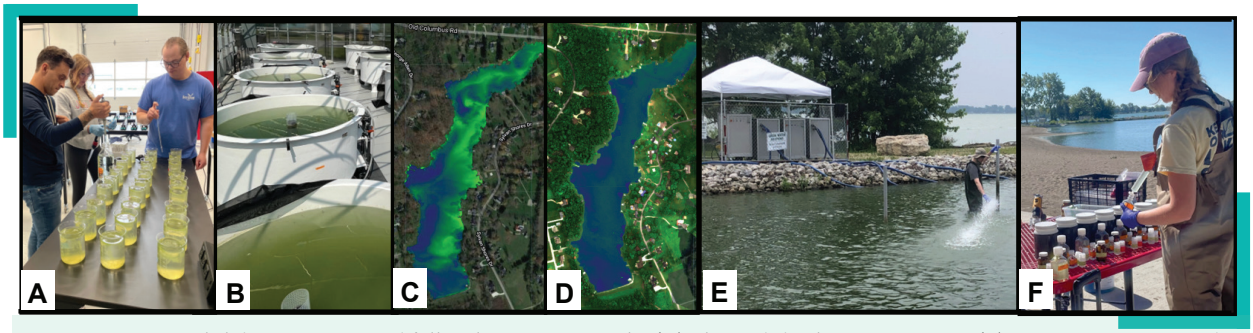


Figure 1. *Example lab, mesocosm, and full scale treatment trials: (A) algaecide beaker experiments; (B) mesocosm NBOT and algaecide comparison; (C) NBOT lake treatment trial, pretreatment; (D) NBOT late treatment trial, posttreatment; and (E and F) NBOT state park beach treatment trial.*

Objective

This project aims to evaluate effectiveness and potential impact to nontarget organisms of NBOT technology for cyanobacteria control and nutrient abatement. NBOT treatments are also being compared to traditional registered algaecides (multiple copper and hydrogen peroxide based formulations) and efficacy of both treatments at lower temperatures and on cold-tolerant cyanobacteria is being evaluated. Experiments have been conducted in the lab, in controlled mesocosms, and in full-scale field trials. Overall, this project will provide scientifically defensible data that water managers need to make informed decisions related to algaecide use and alternative NBOT treatments.

Approach

NBOT Lab Experiments. Controlled experiments aimed to quantify bubble size distribution, density, lifetime, and zeta potential, and the aqueous ozone and hydroxyl radical produced in solution during and following NBOT treatment. Production of high densities of nanobubbles was confirmed using a nanosight, but microbubbles were also produced in the bench scale unit. The total hydroxyl radical production from nanobubble collapse alone (no ozone addition) was found to be minimal (less than 5 µg/L over 100 days) and insufficient for the oxidation demand for most treatment needs. These results indicate that nanobubble technology without ozone gas claiming to work through the action of hydroxyl radical production is not a suitable treatment method. This finding is important, since several companies currently market nanobubble-only treatment technologies. In NBOTs, the majority of ozone is released to aqueous phase within the unit, less than 10% persists within nanobubbles, and a small fraction is converted to hydroxyl radical. Year 3 NBOT lab experiments will aim to quantify hydrogen peroxide production, investigate effects of NBOT treatment on nutrient cycling and disolved organic material (DOM), and evaluate ozone dosing requirements based on variable water quality parameters.

Efficacy of Algaecides and Novel Ozone Nanobubble Technology on Prevention and Management of HABs

Lead: Heather Raymond, The Ohio State University

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Approach (cont.)

Algaecide Beaker Experiments. Low-temperature (10°C, 15°C, and 20°C) replicated algaecide experiments using 5–6 copper and peroxide-based algaecides at three doses were conducted on a spring and fall Grand Lake Saint Marys–sourced *Planktothrix*-dominant bloom and on a higher-temperature (17°C, 22°C, and 27°C) late-summer Lake Erie–sourced *Microcystis* and *Dolichospermum* bloom. The algaecides did not perform well in terms of cyanobacteria reduction in cooler temperatures, yet intercellular toxins were released, which could have implications for drinking water supplies. There were differences in algaecide response between trials, with reduced effectiveness in the spring versus fall collected *Planktothrix* bloom. The warmer Lake Erie–sourced bloom responded best to treatment. Metatranscriptomic analysis is in process.

Mesocosm Trials. A mesocosm trial was conducted at Stone Laboratory using Lake Erie–sourced water. Copper and hydrogen peroxide based algaecides were compared to two NBOT ozone doses in a replicated study. The algaecides, applied following recommended label doses, had severe detrimental effects on the zooplankton community while the lower dose NBOT treatment had a positive impact on zooplankton community (increased abundance) and the higher dose NBOT treatment only impacted *Bosmina* populations. Overall, the NBOT treatments had less negative impact to nontarget organisms as compared to algaecides. A second mesocosm trial focused on impact of DOM on NBOT dosing requirements will occur in 2023. Cell staining and metatranscriptomic analyses are being employed to better elucidate mode of action for cyanobacteria control.

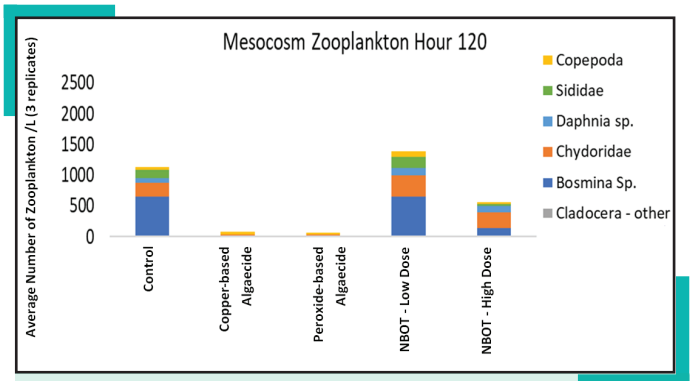


Figure 2. *Graph of results.*

Full-Scale NBOT Trials. Multimonth NBOT trials were conducted at Lake Sylvan and Grand Lake Saint Marys West Beach, and a shorter term stream trial took place at the Saint Marys River. At Lake Sylvan following increased NBOT dose, cyanobacteria concentrations decreased, the community shifted, and recreational advisories were avoided. At a neighboring no-treatment lake with similar bloom, cyanobacteria concentrations increased during the same period and advisories were necessary. Cyanobacteria and toxin concentrations were higher during the Grand Lake Saint Marys beach NBOT trial and treatment effects were localized. Recreational advisories at the treatment beach were reduced compared to neighboring beaches. Repeat beach treatment trials are planned for summer 2023 and additional sampling will occur to better delineate treatment effect and spatial extent from treatment units. A short-term stream trial was conducted in November 2022, and declines in cyanobacteria and toxin concentrations were observed post treatment. An additional stream trial is planned for 2023. How changes in DOM resulting from NBOT treatment affect the phytoplankton community is also being evaluated.

Partnership/Leveraging Opportunities

The Ohio State University: Eugene Braig, Dr. Justin Chaffin, Dr. Rachel Gabor, Dr. Linda Weavers, Dr. Chris Strasbaugh, Dr. Sami Khanal, Dr. Billy Fagan, Dr. Sam Miller, Haley Kuhn, Josh Fuchs, Jack Deptola, Elyse Bonner, Shannon Thayer, Vinny Anderson, Mark Tischer, Haleigh Fernandez, Autumn Taylor, Keara Stanislawczyk, Ian Crumrine, Holly Stanley, Katie Gaffney, Anne Gerhart, Megan Greige, Matt Romanko, Neha Joshi, and Mark Bola. University of Florida: Dr. H. Dail Laughinghouse III, Dr. David Berthold, Dr. Forrest Lefler, Maximiliano Barbosa, and Jing Hu.

Additional Collaborators: Dr. Peter Moeller (NOAA), Dr. Mike Elovitz (USEPA), Dr. Heath Mash (USEPA), Dr. Stephen Jacquemin (Wright State University), Ohio Department of Natural Resources, Ohio EPA, Sylvan Lake Association, Grand Lake Saint Marys Restoration Commission, and Green Water Solutions LLC.

CyanoHAB Nowcast/Forecast for Lake Okeechobee

Lead: Rick Stumpf, NOAA National Centers for Coastal Ocean Science, richard.stumpf@noaa.gov

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Lake Okeechobee is critical to the USACE Jacksonville District's (SAJ) Central and South Florida Project, which provides flood control, acts as a public water supply, prevents saltwater intrusion, protects fish and wildlife, and supports agriculture. In recent years, large toxic cyanobacteria blooms have complicated lake management. Effective and timely lake management decision-making relies on easy-to-use tools that quickly communicate information about bloom location, movement, and intensity.

Objective

NOAA will provide cyanobacteria predictive models for Lake Okeechobee for use in lake management. Satellite data will be used to identify bloom intensity, extent, and location to enhance monitoring and provide forecasts. Nowcasts (1–5 days) will show likely bloom movement, while long-term forecasts (approximately 1 month) will provide guidance on the likely timing and development of more intense blooms. The models will be easy to run and allow SAJ to apply management strategies that reduce the impacts of blooms on the lake and receiving waters.

Approach

Satellite data will be used to provide detailed information on bloom location for a 20+ year time series and be used for routine monitoring and developing forecasts. The satellite data will be used to initialize a model to forecast bloom location for several days forward. Satellite data will be combined with environmental and water sample data to identify conditions that favor bloom intensification, and lead to more, or less, intense blooms. The resulting models will be designed for easy operation, with the intended long-term implementation at SAJ. A consistent satellite data set will allow for future observations to be added to the time series.

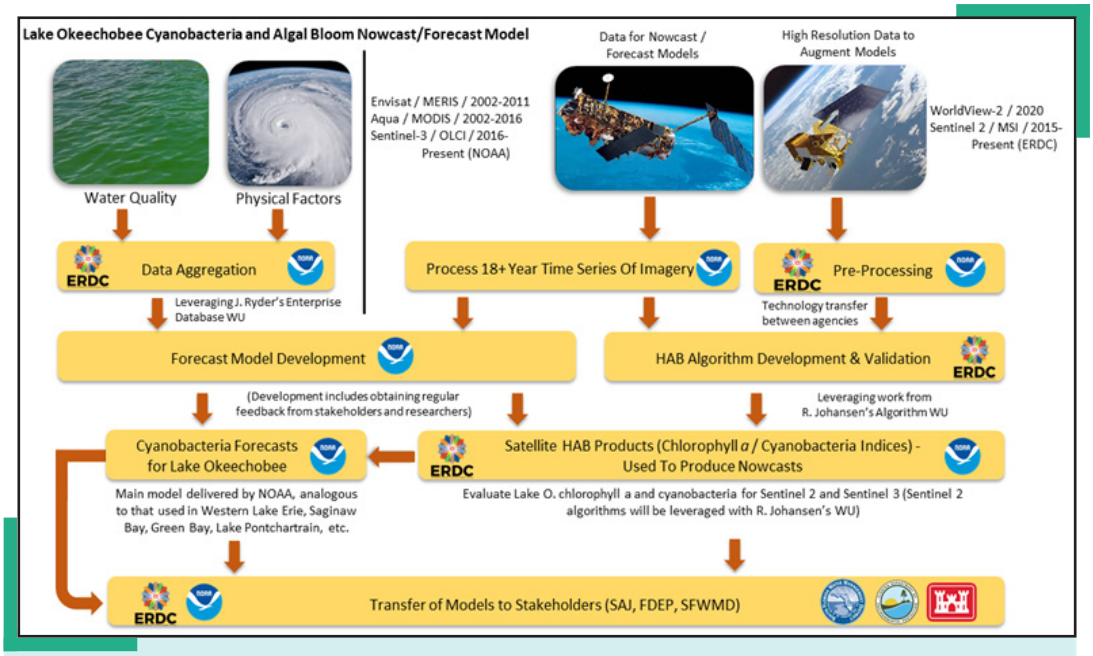


Figure 1. *Project concept diagram.*

CyanoHAB Nowcast/Forecast for Lake Okeechobee

Lead: Rick Stumpf, NOAA National Centers for Coastal Ocean Science, richard.stumpf@noaa.gov

USACE Harmful Algal Bloom Research & Development Initiative

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Major Milestones

Deliverables	Description
Publications	**Journal article:** Mishra, S., R. P. Stumpf, and A. Meredith. 2023. "Constructing a Consistent and Continuous Cyanobacteria Bloom Monitoring Product from Multi-Mission Ocean Color Instruments." *Remote Sensing* 15 (22): 5291. https://doi.org/10.3390/rs15225291. **Literature review** of key environmental forcng factors that drive cyanobacterial blooms in Lake Okeechobee **Development and validation** of the cyanobacteria nowcast and forecast models
Technology Development	**Intercomparison and evaluation** of satellite algorithms for cyanobacteria and chla **Nowcast model (1–5 days)** based on satellite imagery, transferable to SAJ **Forecast model** (for weeks to seasonal), transferable to SAJ
Other	Identification of Lake Okeechobee data and existing models relevant to stakeholder priorities Demonstration and training of models with stakeholder feedback

Partnership/Leveraging Opportunities

This project establishes a partnership with ERDC researchers for applying satellite technology to cyanoHAB monitoring. In addition, the project utilizes the South Florida Water Management District (SFWMD) Lake Okeechobee Ecosystem Model to create a forecast tool, which likewise uses their DBHydro database for retrospective and real-time data. While SAJ and SFWMD are key partners, they are also end-users of the products. National Centers for Coastal Ocean Science resources have provided the satellite data processing system and includes a partnership with the University of Florida to understand the environmental factors.

Value to USACE Mission

The models will provide USACE resource managers and regional partners with a critical new decision support tool for cyanoHAB monitoring, providing the capacity to track bloom development, timing, size, and distribution in Lake Okeechobee. Not only will it be a nationally relevant tool adaptable to reservoirs across USACE but also will accommodate a broad range of end-users with limited modeling experience. Furthermore, the partnership helps build interagency capabilities, joining unique scientific approaches and experience, for developing value-added products that increase efficiency and decision-making in routine cyanoHAB monitoring programs.

UAS Survey to Support EPA Region 7 HAB Monitoring

Lead: Molly Reif, ERDC, molly.k.reif@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Harmful algal bloom (HAB) monitoring continues to be challenging for many agencies and it is unclear how new technology, such as unmanned aircraft systems (UAS), can assist with monitoring and better understanding of hyperspectral image capabilities for detecting HABs.

Objective

Assist EPA Region 7 with ongoing HAB research and development by evaluating new UAS technology, including hyperspectral imagery for identifying spectral signatures of cyanobacteria genera and species.

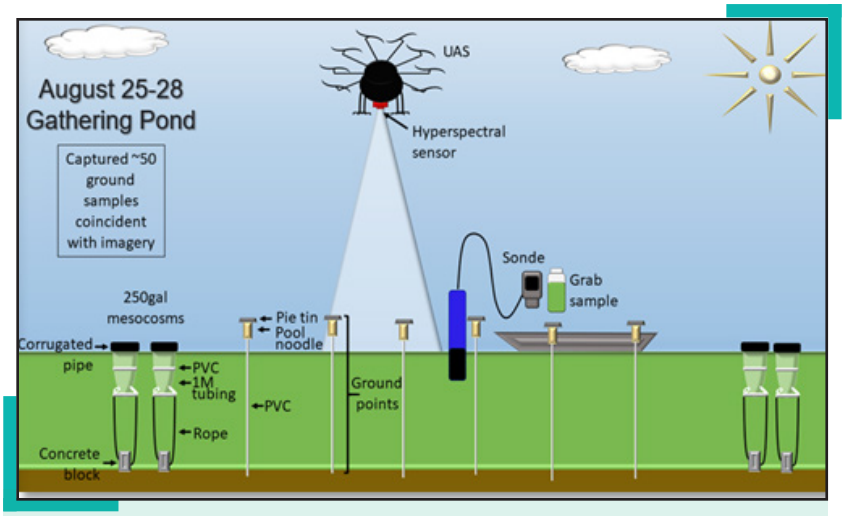


Figure 1. *Conceptual diagram.*

Approach

Conducted a two-part experiment to evaluate hyperspectral imagery by (1) performing laboratory hyperspectral imaging analysis to better understand and identify relevant spectral bands specific to the cyanobacteria genera and species present in both laboratory monocultures (cyanobacteria and eukaryotic algae) and field samples from Milford Lake, Kansas and (2) conducting a coordinated field survey with simultaneous collection of in situ samples (physical water samples and sonde measurements) and UAS hyperspectral imagery over a small target area in Milford Lake, Kansas.

Major Milestones

Deliverable	Description
Publications	**Journal Article:** Pokrzywinski, K., R. Johansen, M. Reif, S. Bourne, S. Hammond, and B. Fernando. 2022. "Remote Sensing of the Cyanobacteria Life Cycle: A Mesocosm Temporal Assessment of a *Microcystis* sp. Bloom Using Coincident Unmanned Aircraft System (UAS) Hyperspectral Imagery and Ground Sampling Efforts." *Harmful Algae* 117:102268. https://doiorg/10.1016/j.hal.2022.102268. **Journal Article:** Pokrzywinski et al. In prep. "Hyperspectral Remote Sensing to Identify Spectral Signatures of Cyanobacteria Genera and Species."
Documents	**Internal Field Plan:** Reif et al. 2021. Field sampling plan and overall data collection & coordination plan for the Gathering Pond, Kansas (ERDC, EPA, NOAA, University of Florida, and USACE Kansas City District [NWK]).
Products	**Spectral Library (Reference) Database:** Link to federal partners (anticipated)

UAS Survey to Support EPA Region 7 HAB Monitoring

Lead: Molly Reif, ERDC, molly.k.reif@usace.army.mil

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Partnership/Leveraging Opportunities

ERDC's Environmental Laboratory has a variety of current and ongoing research and development efforts to evaluate the efficacy of UAS and other remote-sensing technologies for improved monitoring of HABs. Specifically, this work leverages multiple agency resources to evaluate UAS technology. It will provide a critical step in examining the technology for species identification and, potentially, to better understand the relationship between toxin production and species-specific spectral characteristics that can be used for monitoring. As such, coordination with other agencies is likewise ongoing to advance the state of the science for maximizing benefits to improve HAB response.

Value to USACE Mission

This work leverages support from EPA Region 7, including funding and field sampling; assistance from NWK for boat support; support to NOAA for field sampling, sample processing, and spectral analysis; and support to the University of Florida for field sampling and sample processing/analysis.



Figure 2. *Researchers in the field collecting samples.*

Remote-Sensing Software Tools for HAB Monitoring, Tool Refinement, and Improved Technical Transfer

Lead: Molly Reif, ERDC, molly.k.reif@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Supporting documentation, technical training, and marketing is needed for USACE water quality managers to better utilize new remote-sensing software tools to assist with water quality monitoring.

Objective

To better facilitate technical transfer of newly developed remote-sensing software tools for HAB monitoring, as well as add functionality to the HAB Explorer web app viewer.

Approach

This work has the following goals: (1) update and add functionality to the HAB Explorer web app viewer in partnership with Esri and subsequently make the web app publicly available, and (2) produce supporting documentation, technical training, and marketing to better facilitate technical transfer (e.g., tool summary document and presentations, quick-guide tool matrix, tool maintenance updates, finalized publication of ArcGIS Pro user-guide technical report, and recorded/in-person technical tool demonstrations).

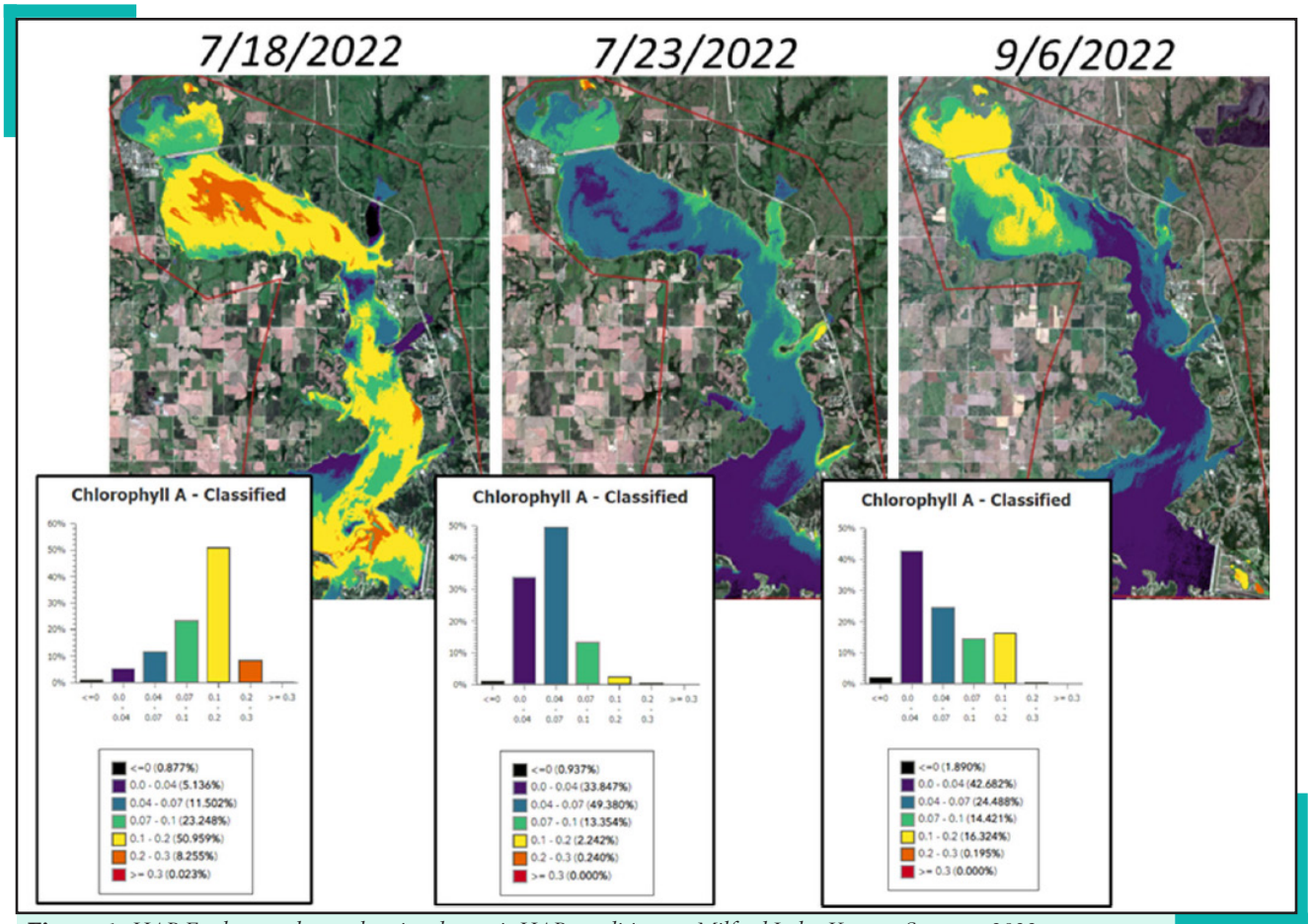


Figure 1. *HAB Explorer web app showing dynamic HAB conditions at Milford Lake, Kansas, Summer 2022.*

Remote-Sensing Software Tools for HAB Monitoring, Tool Refinement, and Improved Technical Transfer

Lead: Molly Reif, ERDC, molly.k.reif@usace.army.mil

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Major Milestones

Deliverable	Description
Publications	**Tech Report:** Saltus, C. L., M. K. Reif, and R. A. Johansen. 2022. *Waterquality for ArcGIS Pro Toolbox: User's Guide*. ERDC/EL SR-22-6. Vicksburg, MS: US Army Engineer Research and Development Center. http://dx.doi.org/10.21079/11681/45362.
Documents	**Brochure:** Engineer Research and Development Center. 2022. "Remote Sensing Tools to Assist USACE Water Quality Monitoring." In *Civil Works R&D Value to the Nation: 2022 Edition*. ERDC B-22-2. http://dx.doi.org/10.21079/11681/43465. **Brochure:** Engineer Research and Development Center. 2023. "Remote Sensing Tools for Water Quality and HAB Monitoring." In *Civil Works R&D Value to the Nation: 2023 Edition*. ERDC B-23-1. http://dx.doi.org/10.21079/11681/46532. **News Article:** Kuzmitski, H. 2023. "Remote Sensing Gives USACE an Edge at Detecting Harmful Algal Blooms." USACE Headquarters Website. https://www.usace.army.mil/Media/News/NewsSearch/Article/3277031/remote-sensing-gives-usace-an-edge-at-detecting-harmful-algal-blooms/.
Products	**Software Tool:** ArcGIS Pro Waterquality Toolbox v1.1 Update. http://dx.doi.org/10.21079/11681/42240. **Software Tool:** HAB Explorer Phase 2 Update. https://arcportal-ucop-corps.usace.army.mil/hab/.
Tech Transfer	**Virtual training** to USACE water managers, re: ArcGIS Pro for waterquality and HAB Explorer. Webinar, February 2022. https://corpslakes.erdc.dren.mil/employees/invasive/exchange.cfm?Option=Webinar&Type=Past&CoP=nrm&Id=660&ICS=No. **Software Demo** to USACE water managers, re: software tool series. WQ Workshop, February 2023.

Partnership/Leveraging Opportunities

In FY22, funding was received from the Great Lakes and Ohio River Division initiate technical transfer ($22,000).

Value to USACE Mission

The software tools will provide improved capabilities for HAB monitoring and management (proactive versus reactive) and can help reduce costs through minimizing and prioritizing field sampling efforts. In addition, the tools have widespread applicability to USACE projects, and they transform and leverage years of USACE-funded remote-sensing investigative analyses into tools.

Factors Critical to Long-Term Lake and Reservoir Management: Relationships Between Land Use, Nutrient Loading, Inflows, HABs, and Anoxia

Lead: Dr. Daniel Roelke, Texas A&M University Galveston
Co-PI: Dr. Jessica Labonte, Texas A&M University Galveston

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Lakes in the southcentral US are warm-monomictic systems, many of which have become eutrophic or hypereutrophic, raising concerns about harmful algal blooms (HABs) and hypoxia. HABs associated with prymnesiophytes and cyanobacteria have been recurrently problematic and the frequency, magnitude and duration of HABs are likely to increase with climate change. We seek to better understand the drivers of inland HABs and hypoxia in the region, with a particular focus on nitrogen loading. Recent advancements in understanding have shown a selection for prymnesiophytes and some toxic cyanobacteria at high nitrogen-to-phosphorus ratio (N:P). We are sampling 20 reservoirs and their tributaries, spanning a pronounced east–west annual precipitation gradient across Texas. Samplings occur in the spring and summer each year, and measured parameters include chlorophyll-a, microcystins, inorganic and total nutrients, microbial community composition (using metagenomics), microbial functions (using metatranscriptomics), and several water quality parameters. Inflows and nutrient loadings are being determined using the Hydrologic and Water Quality System (HAWQS) calibrated with USGS gauge data, as well as tributary nutrient samples. Principal component analysis of data collected thus far reveal a positive relationship between NO_3/NO_2 and total microcystins, though this trend was heavily driven by an ongoing bloom in one lake. Excluding this lake from analysis, a strong relationship still exists between NO_3/NO_2 and total microcystins in the bottom waters of shallow stations (6 m, standardized sampling), demonstrating the persistence of these toxins even when mixed to aphotic depths of the water column. Our ongoing work explores whether microcystins are widespread in tributaries to these lakes, as is the case in other regions of the country. We are comparing tributary microcystins to in-lake microcystins to assess the scale of cyanotoxin loading, and comparing land-use practices in the watersheds of these lakes with a focus on agricultural, industrial, and municipal practices.

Objective

HABs and anoxia events are an ongoing problem in lakes and reservoirs of the southcentral US, resulting in economic losses in the tens of millions of dollars to regional economies. Managing land-use and nutrient loadings in these watersheds, as well as managing in-lake chemical cycles, vertical mixing, and hydrology, to prevent HAB incidence and curb anoxia events is likely far less costly. But development of such management schemes requires a better understanding of the form and functioning of these systems and their sensitivity to environmental factors. That essential knowledge will be discovered in this research. Our objective is to advance understanding of how early season nutrient loading magnitude and stoichiometry, and hydraulic residence time in lakes and reservoirs with varied surrounding land-use practices influences HABs, anoxia, and microbial functioning.

Approach

We are collecting tributary inflow records, measuring nutrient concentrations, and compiling reservoir volume and discharge data for 20 lakes and reservoirs spanning an east–west precipitation gradient across the southcentral US, which enables estimation of early season nutrient loadings and hydraulic residence times. From these lakes and reservoirs, we are determining in the early season and late summer an estimation of internal loading through sediment resuspension and vertical mixing of nutrient-rich, bottom-waters; total plankton community composition using metagenomics; a suite of water quality parameters that include temperature, conductivity, pH, turbidity, oxygen reduction potential, and dissolved oxygen, and also cyanotoxins using immunoassays; estimation of important ecosystem functions using transcriptomics; and categorizing land-use practices in areas surrounding each of the lakes and reservoirs. We will quantify

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Approach (cont.)

relationships between land-use practices, nutrient loading, hydraulic residence time, microbial and bacteriophage composition, HAB species prevalence and microbial functioning, and create quantitative models useful for management decisions accompanied by a user's manual so that nontechnical personnel can apply the models.

Assessment

With the exception of the sediment coring, field sampling and collection have gone well. The only times a station was notsampled was because it was inaccessible due to low water levels in the target lake, which prevented launching of the boat. When this occurred, samples were taken by wading into the lake from the shoreline. Collection of sediment cores for laboratory experiments has proved challenging. The coring methods employed thus far have not been effective at collecting cores, given the sediment types of these lakes and the depth of the water column over which the corers are deployed.

With the exception of the sediment coring experiments and sequenced sample processing, all samples have been processed and data analyzed. Analysis of this data, thus far, shows support for the primary hypothesis that a relationship between phycotoxins and nitrogen should exist. Sediment core experiments have not been conducted yet because of the difficulties encountered collecting cores. Regarding the processing of sequenced samples, this occurred because of personnel issues we encountered that led to the departure of the postdoctoral researcher on the project. This resulted in all DNA extractions having to be redone, among other issues. We are in the search process for hiring another postdoctoral researcher.

The watershed modeling using HAWQS is underway. A comparative analysis in all stream segments with USGS flow gauges is being performed to calibrate the HAWQS model. Once completed, we will be able to use HAWQS to determine the total inflows into the lakes being studied.

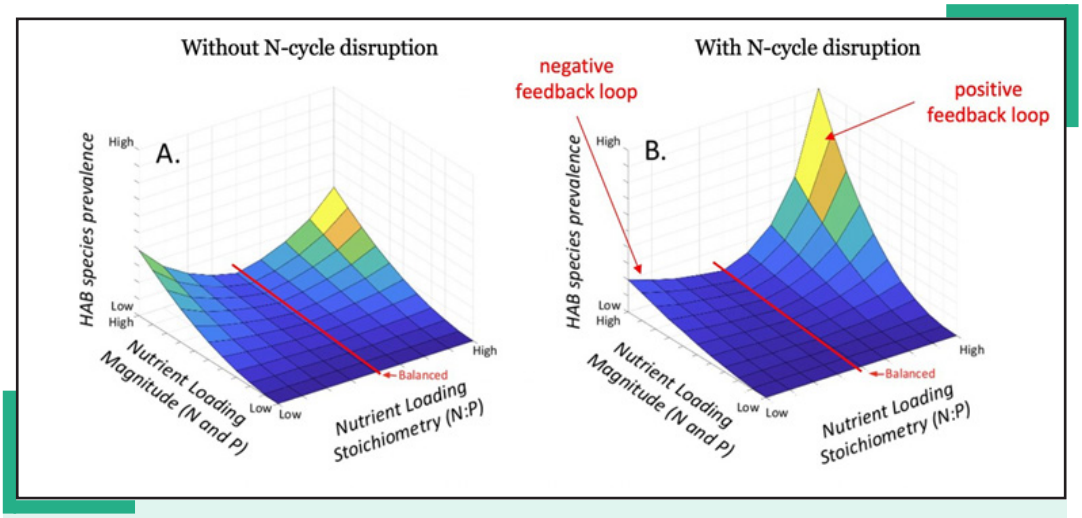


Figure 1. *Hypothesized conceptual model shows a relationship between nutrient loading magnitude and stoichiometry (in regards to N:P) and the prevalence of HAB species (here, nutrient loading is the combined external and internal loading): (A) without and (B) with N-cycle disruption.*

Factors Critical to Long-Term Lake and Reservoir Management: Relationships Between Land Use, Nutrient Loading, Inflows, HABs, and Anoxia

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Major Milestones

Deliverable	Description
Publications	**Tech Report:** Roelke and Labonté. 2023. "Factors Critical to Long-Term Lake and Reservoir Management: Relationships between Land-Use, Nutrient Loading, Inflows, HABs and Anoxia." Submitted Cumulative Quarter Report. **Journal Article:** Kieley et al. In prep. "Microcystins Correlate with Nitrate and Nitrite in Warm-Monomictic Lakes of South-Central USA and May Disrupt Denitrification." *Science of the Total Environment*. **Journal Article:** Roelke et al. Anticipated. "Biogeography of Toxic Cyanobacteria and Their Phages across Twenty Warm-Monomictic Lakes in Texas." **Journal Article:** Klobusnik et al. Anticipated. "Viral Distribution and Lifestyles across a Range of Warm Monomictic Lakes." **Journal Article:** Cagle et al. "Modeling Cyanobacteria Bloom Formation and Toxin Production."
Products	**Bioinformatic pipeline** to analyze and compare metagenomic and metatranscriptomic data (from quality control, assembly, gene calling, and annotations to genome assembly, phylogenomics, differential expression, and statistical analyses); initial pipeline was developed using Buffalo Springs Lake data sets. **GUI-driven computational model** to show relationships between alter inflows, nutrient loadings, and user selected parameters such as HAB species, chlorophyll-a, dissolved oxygen, etc.
Tech Transfer & Demonstrations	GUI-driven computational model demonstration (anticipated).

Evaluation of Historic Water Quality Information and HAB Events: Technical Guidance for Routine Monitoring Programs

Lead: Dr. Jodi L. Ryder, ERDC, Jodi.L.Ryder@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

The occurrence and geographic distribution of cyanobacterial harmful algal bloom (HAB) events is increasing. USACE districts need efficient data informatics to evaluate water quality in the context of historical observations to understand trends and disturbances in the systems they monitor.

Objective

To identify high value water quality and phytoplankton parameters and maximize the information contained in the Enterprise Water Quality Database (EWQD) for understanding the occurrence of HABs at USACE projects through standardized analysis accessible to any USACE district.

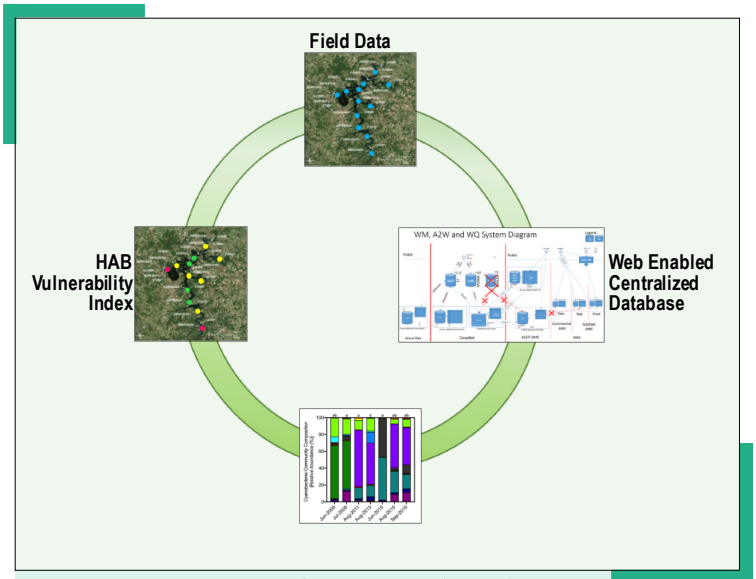


Figure 1. *Project concept for water quality informatics.*

Approach

This project adds on-demand analysis capability to the EWQD (under development) to deploy standardized workflows and a HAB vulnerability index. The EWQD is being created to compile and archive water quality data collected at USACE projects in a discoverable format. Additional functionality for assessing water quality with relevance for HABs is being created through search functions, data visualizations, derived variable and index calculations, graphing functions, built-in statistical analysis, and standardized data extraction tools. The tools under development are deployed with the web services that provide access to the database so they are available for any USACE water quality observation site stored in the database. District enrollment is ongoing.

Results

Several new web service functionalities were added to the EWQD. In a dashboard view, users can access recorded water quality data by parameter and instantly see where the most recent value falls in the long-term data set using a box and whisker plot. Heat maps show when samples have been collected in a graphical format. Single parameter and composite eutrophication indices are calculated and plotted by date to examine long-term trends on lake health. Biological samples can now be stored in the database and recalled for plotting in multiple methods to examine sample biodiversity.

Trends at Barkley

Sample Year	Parameter	Units	Lowest Minimum	Highest Minimum	Lowest 25th	Highest 25th	Lowest Median	Highest Median	Lowest 75th
2018	CHLRPHYL B UG/L	ug/L	0.1	1.06	0.11	1.29	0.495	1.565	0.97
2018	CHLRPHYL C UG/L	ug/L	2.03	3.27	2.13	3.44	2.535	4.705	3.73
2022	WATER TEMP CENT		7	7	7	7	7	7	7
2022	TRANSP SECCHI METERS		7	7	7	7	7	7	7
2022	DO SATUR PERCENT	%	80	100	80	100	80	100	80
2022	DO SATUR PERCENT		81	81	81	81	81	81	81

Figure 2. *An example of the on-demand analysis tools being deployed in the web service access to the Enterprise Water Quality Database to facilitate interpretation of routine water quality data. This figure shows one exportable report available in the system.*

Evaluation of Historic Water Quality Information and HAB Events: Technical Guidance for Routine Monitoring Programs

Lead: Dr. Jodi L. Ryder, ERDC, Jodi.L.Ryder@usace.army.mil

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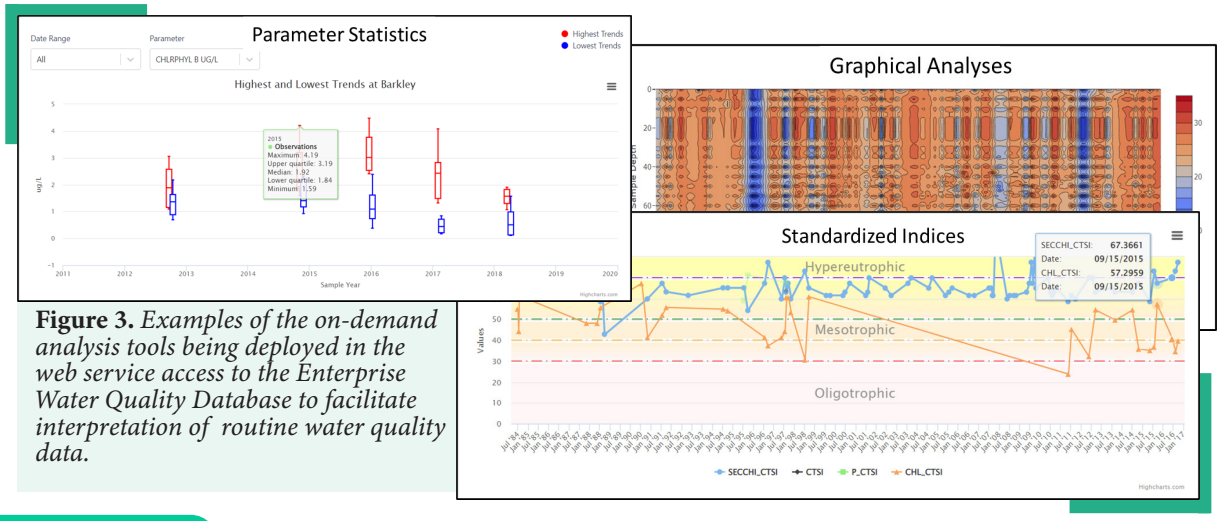


Figure 3. *Examples of the on-demand analysis tools being deployed in the web service access to the Enterprise Water Quality Database to facilitate interpretation of routine water quality data.*

Major Milestones

Deliverable	Description
Tech Transfer	**Technology:** Minable HAB database **Technology:** HAB Vulnerability Index algorithim and method **Technology:** Prototype map interface for the visualization of HAB Vulnerability Index (in progress)
Publications	**Technical Note:** "HAB Vulnerability Index Using Routine Water Quality Data" (in progress) **Technical Note:** "User Guidance for HAB Relevant Data in the USACE Enterprise Water Quality Database" (in progress) **Technical Report:** "Use of the USACE Enterprise Water Quality Database to Visualize HAB Vulnerability" (in progress)
Documents	**Conference Submission: "**HAB Vulnerability Index Case Study" (in progress)

Partnership/Leveraging Opportunities

This work leverages the "Enterprise Water Quality Database" development effort and is leveraged by "Operational Strategies for HABs Management in Inland Reservoirs and Universal Algorithm Development for the Detection of HABs and Water Quality Parameters Using Satellite Remote Sensing" project.

Value to USACE Mission

The project aims to maximize the value of routine water quality data by enabling rapid processing of historic and newly input data in the Enterprise Water Quality Database.

Learn about other EL research areas, including
Aquatic Nuisance Species
Aquatic Plant Control
Ecosystem Management and Restoration

Find ERDC on the web at:

Operational Strategies for HAB Management in Inland Reservoirs

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Lead: Dr. Jodi L. Ryder, ERDC, Jodi.L.Ryder@usace.army.mil

Problem

Given the dynamic interplay between flow, water quality, and harmful algal blooms (HABs) it is difficult to anticipate the impacts of operational decisions on water quality at inland reservoirs.

Objective

To develop a model that leverages existing and ongoing routine water quality monitoring for nutrient water quality, chlorophyll-a, and other indicators of algae activity in a process-informed modeling framework to make short-term predictions of water quality responses to operational and flow perturbations.

Approach

Historically, water quality monitoring focused on geochemical parameters, nutrients, and known contaminants and was routinely performed at intervals to evaluate if water quality was declining over the long term. Water quality models were also structured toward answering questions about how small changes would affect a reservoir system in the long term. The hydrodynamic and water quality information used for these models tended to focus on average values or specific periods of intense study. However, HABs are typically short-lived periods of rapid water quality change. There can be many causes (both chemical and physical) so there is no singular best action to mitigate a bloom. The first component of this project, completed in 2020–2022, was to examine which water quality and hydrodynamic parameters were most indicative of blooms at several lakes where high-frequency monitoring of water temperature, chlorophyll-a, and phycocyanin, proxies for algae and cyano-HABs, were recorded. The second component is to develop models for short-term prediction of bloom likelihood that incorporate hydrodynamic conditions so that the models can be used to evaluate the potential effects of operational flow changes at reservoirs.

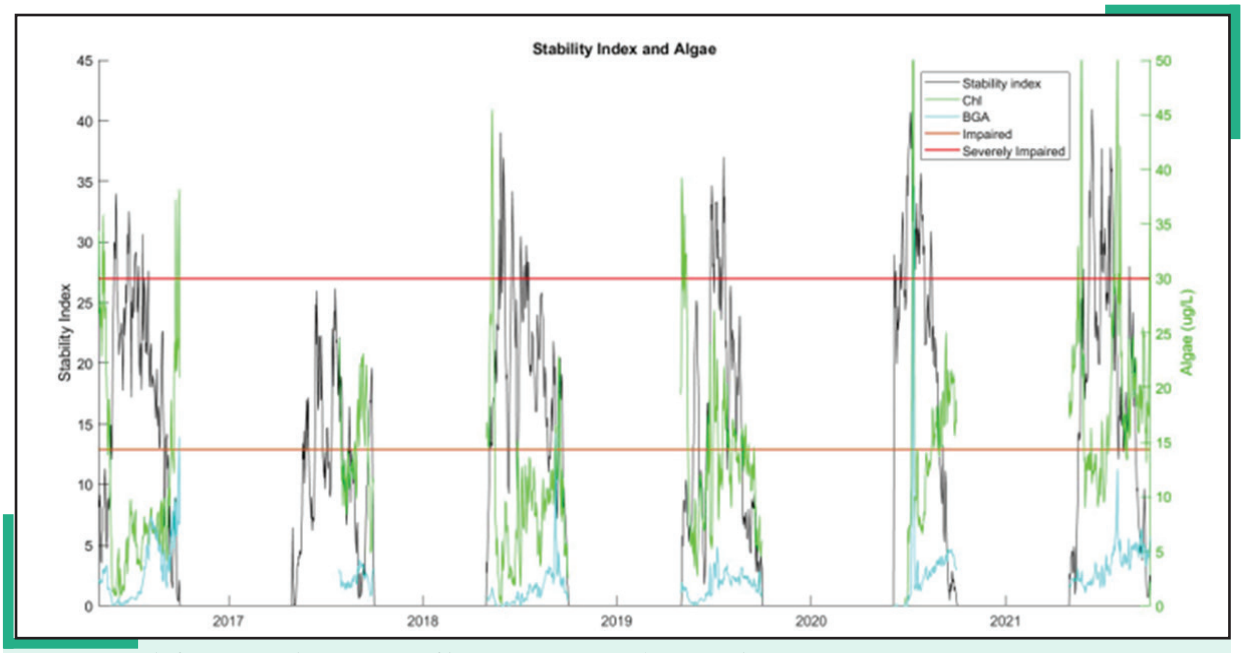


Figure 1. *High frequency observations of key parameters indicating algae presence.*

Operational Strategies for HAB Management in Inland Reservoirs

Lead: Dr. Jodi L. Ryder, ERDC, Jodi.L.Ryder@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative



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Approach (cont.)

	Hypolimnetic Withdrawals	Horizontal Flushing	Pulsed Inflow	Mixing
Management Strategy	Removal of hypolimnetic layer	Removal of surface layer	Upstream water used to break up downstream blooms	Use of artificial mixing to entrain cyanobacteria
Works By	Reducing nutrients	Direct biomass removal	Dilution of cells and altering environmental conditions	Light limitation
Stratification	Retains stability	Retains stability	Breaks up	Breaks up
Nutrients	Decreases legacy nutrients released from sediments	Decreases surface nutrients	Alters nutrient distribution	Alters nutrient distribution
Temperature	Temperature increase in hypolimnetic layer	Hypolimnetic temperature retained, surface temperature reduced	Decrease surface temperature, more uniform profile	More uniform temperature profile
Outflow	Cold, oxygen depleted, nutrient rich	Warm, oxygen rich, eutrophic	N/A	N/A

Major Milestones

Date	Milestone
FY21, Q4	Development of literature-based HAB-specific processes and parameters (complete)
FY22, Q4	**Journal Article:** "The Manipulation of Limnological Parameters to Impact HAB Events" (in review)
FY23, Q3	**Technical Report:** "Water Quality Trends and Observed Impacts of Reservoir Operations" (in progress)
FY23, Q2	**Technical Note:** Model Design report (in progress)
FY23, Q3	**Technical Report:** Model Dem/Val report (in progress)
FY23, Q3	Model release (in progress)
FY23, Q3	**Technical Report:** "Use of the USACE Enterprise Water Quality Database to Visualize HAB Vulnerability" (in progress)

Operational Strategies for HAB Management in Inland Reservoirs

Lead: Dr. Jodi L. Ryder, ERDC, Jodi.L.Ryder@usace.army.mil

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Partnership/Leveraging Opportunities

This work will leverage ongoing field studies within USACE's Aquatic Nuisance Species Research Program, such as HABITATS, which includes collaborations with New York State Department of Environmental Conservation and Florida Department of Environmental Protection. In addition to government collaborations, commercial products are being evaluated, modified, and optimized in the RAFT study.

Value to USACE Mission

This product enables the predictive use of modeling tools to test reservoir operational strategies to maintain regulatory and stakeholder requirements with respect to multiple water quality and hydrodynamic parameters. The capability to change reservoir conditions to prevent or reduce bloom impact would represent a cost effective mitigation strategy. However, because hydrodynamic conditions can both positively and negatively impact water quality, it is important to be able to test changes before enacting them.

Development and Validation of Early Detection and Sustained Treatment Methods to Control HABs in Inland Source Waters in Ohio

Lead: Young Seo, University of Toledo, Youngwoo.Seo@UToledo.edu
Co PIs: Thomas Bridgeman, Dae-Wook Kang, and Yakov Lapitsky, University of Toledo

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Many freshwater systems in Ohio have suffered from chronic cyanobacterial harmful algal blooms (cHABs), which compromise the quality of water for consumption, aquaculture, and recreation. There are significant research needs to develop field-applicable rapid detection and monitoring methods in conjunction with sustained treatment technologies for HAB-associated risk management.

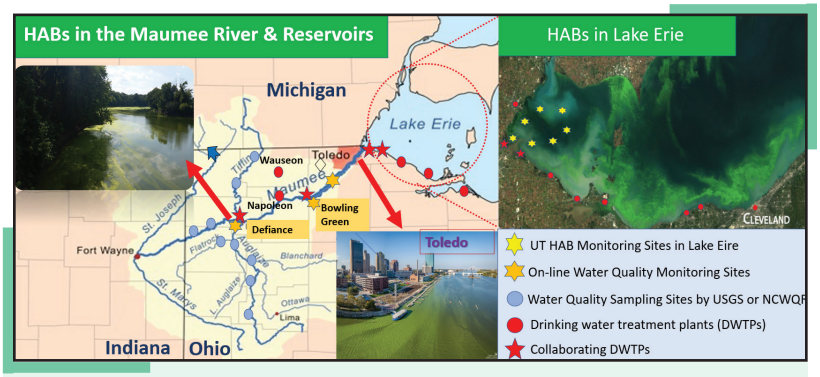


Figure 1. *cHAB in inland source waters in Ohio.*

Objective

Provide detection methods to better assess bloom dynamics in near real-time, over the long term, and in response to HAB treatment. Provide data-driven guidance for the optimal application of treatment methods in HAB-impacted inland waters.

Approach

Develop cost-effective, field-applicable technologies to mitigate HAB risks via (1) improved fluorometric sensors and identification of novel early-warning biomarkers that rapidly monitor the state of HABs, (2) nature-inspired biological and chemical treatment methods for the removal of cyanobacteria and their toxins (e.g., sustained algaecide release and/or biological treatment), and (3) the integration of these developed methods with currently applied HAB management processes.

Results

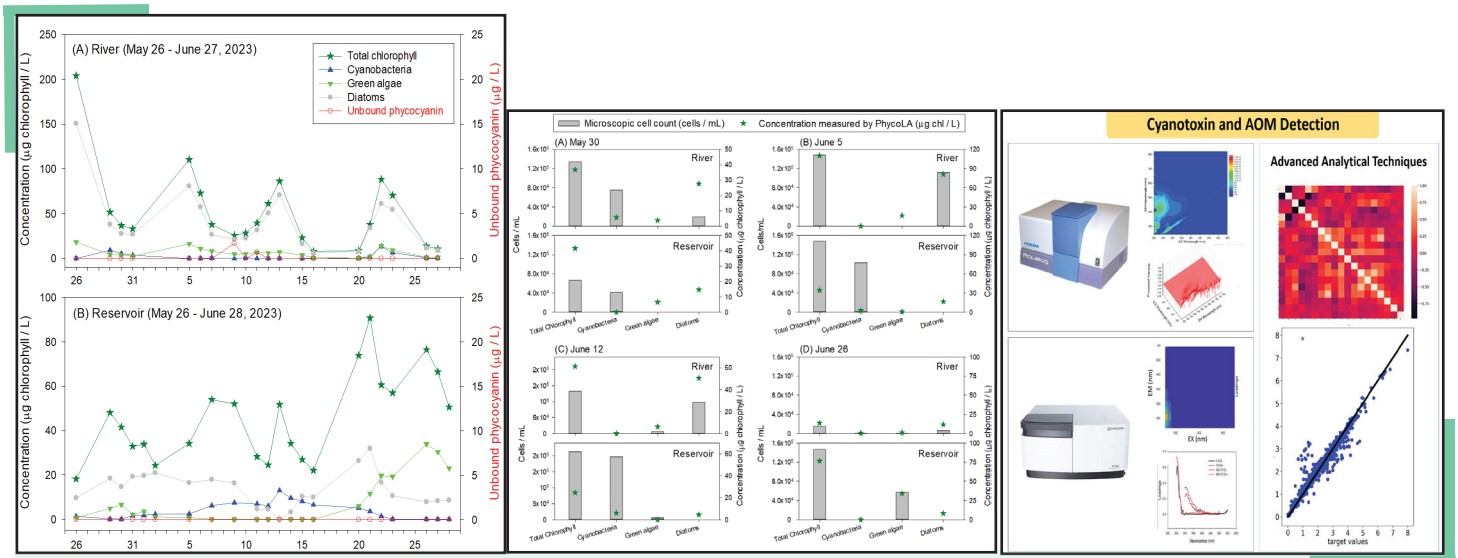


Figure 2. *Examples of the performance of fluorometric monitoring systems for cHABs and their toxins (further data collection is ongoing).*

Development and Validation of Early Detection and Sustained Treatment Methods to Control HABs in Inland Source Waters in Ohio

Lead: Young Seo, Univiversity of Toledo, Youngwoo.Seo@UToledo.edu
Co PIs: Thomas Bridgeman, Dae-Wook Kang, and Yakov Lapitsky, University of Toledo

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Results (cont.)

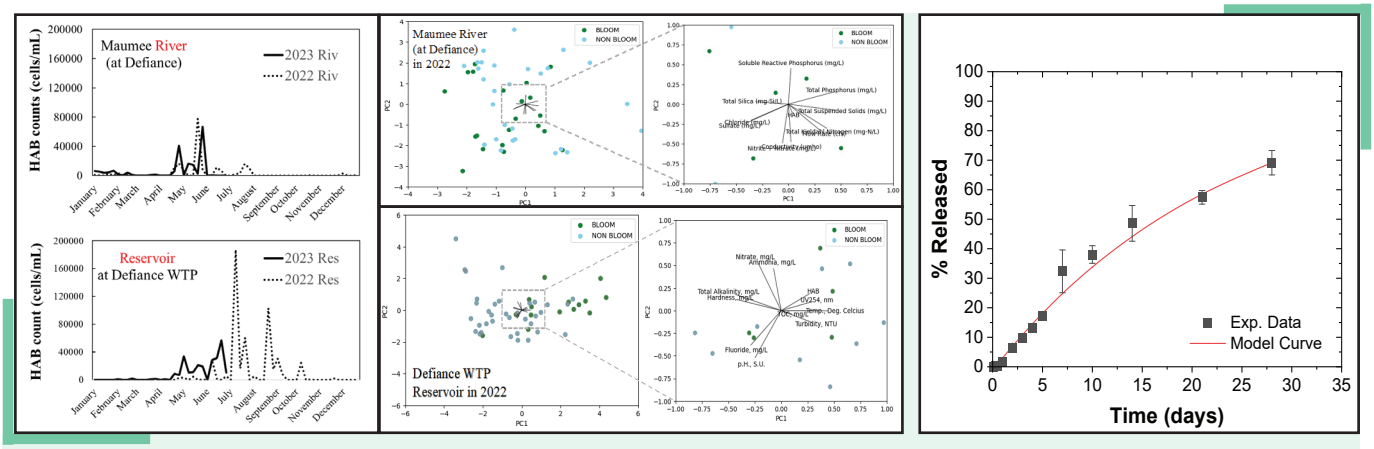


Figure 3. Left, changes in HAB counts and, middle, PCA plots of HAB counts and environmental factors at Maumee River and a Reservoir (Defiance, Ohio). Right, partial algaecide release profile from a scaled-down algaecide-releasing buoy (further data collection is ongoing).

Major Milestones

Deliverable	Description
Publications	**Tech Report:** Seo et al. In preparation. "Development and Validation of Early Detection and Sustained Treatment Methods to Control HABs in Inland Source Waters in Ohio." **Journal Publications:** • "Application of Machine Learning to Omics-Based Data: A Review of Recent Advances in Understanding of Microbial Dynamics in Natural and Engineered Systems" (under review) • "The Use of Fluorometry to Guide the Tracking and Treatment of Algal Blooms in Inland Reservoirs" (in preparation) • "Long-Term and Seasonal Trend of Phytoplankton Dynamics in Maumee River and a Reservoir" (in preparation) • "Spatial Dynamics of Microbial Consortia and Harmful Algal Blooms in Maumee River" (in preparation) • "Reservoir Management Practice that Affects Microbiome Dynamics and Water Quality" (in preparation) • "Algaecide-Releasing Buoys for Sustained Harmful Algal Bloom Control" (in preparation) • "Application of Machine Learning to Detect Algal Organic Matter and Cyanotoxins" (in preparation) • "Biological Treatment of Nutrients and Cyanotoxins" (in preparation)

Development and Validation of Early Detection and Sustained Treatment Methods to Control HABs in Inland Source Waters in Ohio

Lead: Young Seo, Univ. of Toledo, Youngwoo.Seo@UToledo.edu
Co PIs: Thomas Bridgeman, Dae-Wook Kang, Yakov Lapitsky, Univ. of Toledo

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Major Milestones (cont.)

Deliverable	Description
Products	**Data and Code Repository**
Demonstrations	Demonstration and training in the use of PhycoLA algal fluormeter at Defiance Drinking Water Treatment Plant (DWTP), May 2023 Algaecide treatment to control HAB and other phytoplanktons. Defiance Reservoir, Ohio. June–August 2023 Mesocosm and macrocosm (field) testing/demonstrations of the algaecide releasing buoys at the Bowling Green and Defiance DWTP Reservoirs (in Northwest Ohio) beginning 2024
Marketing Video	University of Toledo. 2021. "Safeguarding Water." https://www.youtube.com/watch?v=pZexjP-W7JGs&t=1s
Tech Development	Kober, U. A., Y. Seo, and Y. Lapitsky. In preparation. "Algaecide-Releasing Buoys for Sustained Harmful Algal Bloom Control (An Invention Disclosure)"

Partnership/Leveraging Opportunities

This work leverages multiple collaborations with municipalities in Ohio (Cities of Toledo, Bowling Green, Defiance, and Waseon), industry partners (bbE Moldaenke, SePRO, and Horiba), and the US Environmental Protection Agency.

Value to USACE Mission

The outcomes of the successful project will lead to the development of advanced detection methods to better assess bloom dynamics in near real-time, over the long term, and in response to HAB treatment. They also provide data-driven guidance for optimal application of treatment methods in HAB-impacted inland source waters.



Figure 4. *Images taking during research in the laboratory and during field test.*

Active Early Detection and Diagnosis of HABs with Scalable Biological Treatment Strategies

Lead: Young Seo, University of Toledo, Youngwoo.Seo@UToledo.edu
Co-PIs: Thomas Bridgeman, Dae-Wook Kang, University of Toledo

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Early warning of cyanobacterial bloom occurrence and bloom condition is necessary to respond to harmful algal bloom (HAB) occurrences with appropriately targeted water treatment methods.

Objective

Advance molecularly informed, near real-time HAB diagnostics to support improved HAB monitoring and to optimize HAB treatment processes.

Approach

Develop cost-effective, field applicable technologies to mitigate HAB risks via (1) improved fluorometric sensors and identification of novel early warning biomarkers that monitor—in near real-time—the state (and treatment response) of HABs, (2) nature-inspired biological treatment methods for the removal of cyanobacteria and their toxins (e.g., cyanophages, environmentally friendly algaecides, and/or biofilters), and (3) the integration of these developed methods with currently applied HAB management processes.

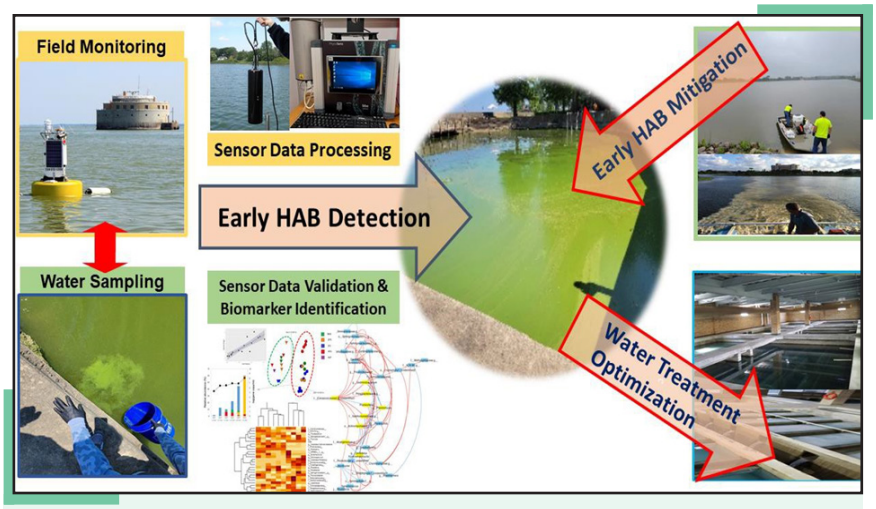


Figure 1. *Schematic diagram for the project goal.*

Results

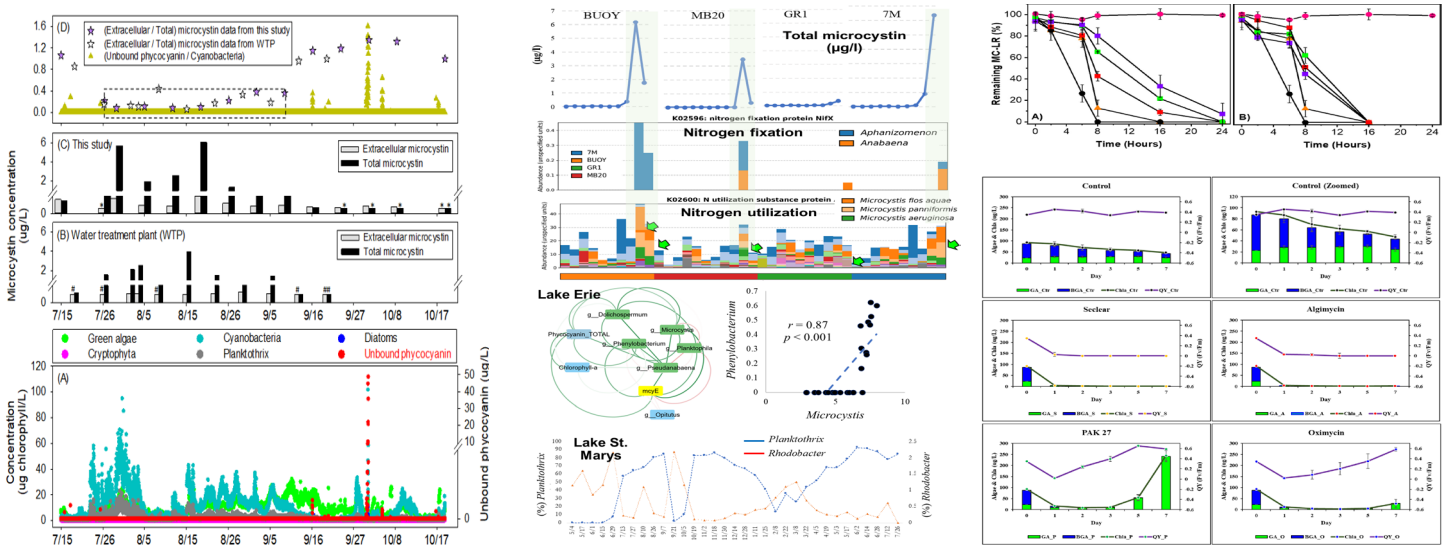


Figure 2. *Sample data displaying obtained outcomes of each objective: (left) unbound phycocyanin serves as an early indicator of HAB cell lysis and release of toxins into the environment, (middle) nitrogen metabolism plays a significant role on HABs both in Lake Erie and Lake St. Marys, and (right) biological and chemical treatments show their potentials to control cyanobacteria and their toxins.*

Active Early Detection and Diagnosis of HABs with Scalable Biological Treatment Strategies

Lead: Young Seo, University of Toledo, Youngwoo.Seo@UToledo.edu
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Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Results (cont.)

Sampling was consistently conducted biweekly in both Lake Erie and Grand Lake St. Marys, Ohio. In Lake Erie, multiple additional sampling locations, including a low service water pump station, were included to improve the capture of microbial activity before, during, and after cyanoHABs. The study monitored physicochemical and pigments parameters to gain insights into cyanoHABs, and the levels of toxins were also measured using liquid chromatography with tandem mass spectrometry. To investigate microbial dynamics of cyanobacterial species and their neighboring community, amplicon sequencing (16S, 18S, and fungal internal transcribed spacer) was employed. The functional roles on cyanoHABs were investigated through metagenomic sequencing, targeting bacterial and viral DNA. Multiomic data were then aligned with qantitative polymerase chain reaction data (targeting cyanobacterial 16S, mcyE, and mlrA genes) and water parameter data to validate potential biomarkers for early warning of cyanoHABs. Additionally, a data-driven machine learning approach, combining sequencing and water physicochemical data, was considered to efficiently integrate rich multiomic and physicochemical data for the identification of early warning biomarkers.

Both lab and field tests (mesocosm tests at Lake Erie and full-scale field tests at a reservoir in Bowling Green, Ohio) were conducted for selected commercial algaecides. The influence of commercial algaecide treatment on cyanobacteria inactivation and the release of algal organic matter and toxins, as well as on microbial community responses were monitored. Additionally, the team explored new methods of controlling cyanobacteria by targeting carbon dioxide fixation and evaluating the efficacy of cyanophage treatment. They found that a combination of cyanophages and low-dose algaecide treatment was effective in controlling cyanobacteria blooms and that the lytic activity of cyanophages was enhanced with low-dose peroxide treatment. The team also constructed and tested lab-scale biofilters using cyanotoxin-degrading bacteria for removing microcystin-LR, which were found to be effective in removing microcystin-LR with reduced lag phage time for toxin biodegradation. Research findings of the project have been disseminated through peer-reviewed journal articles and presentations at national and international conferences. Based on the results of the first two years of the project, the team has published two peer-reviewed journal articles and presented their findings at 15 research conferences. The team also has one manuscript currently under review, and is preparing seven more manuscripts for submission.



Figure 2. *Algecide tests at a lake in Bowling Green, Ohio.*

Active Early Detection and Diagnosis of HABs with Scalable Biological Treatment Strategies

Lead: Young Seo, University of Toledo, Youngwoo.Seo@UToledo.edu
Co-PIs: Thomas Bridgeman, Dae-Wook Kang, University of Toledo

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Major Milestones

Deliverable	Description
Publications	**Journal Articles:** Baranwal, P., D.-W. Kang, and Y. Seo. 2022. "Impact of Algal Organic Matter and Humic Substances on Microcystin-LR Removal and Their Biotransformation during Biodegradation." *Science of the Total Environment* 852:157993. https://doi.org/10.1016/j.scitotenv.2022.157993. Jeon, Y. P. Baranwal, L. Li, K. Piezer, and Y. Seo. 2022. "Review: Current Understanding on Biological Filtration for the Removal of Microcystins." *Chemosphere* 313:137160. https://doi.org/10.1016/j.chemosphere.2022.137160. In review. "Field and Laboratory Studies of Fluorescence-Based Early Warning Systems for Tracking Cyanobacterial Cell Lysis and Microcystins Release." *Water Research*. In review. "Inhibition of CO_2 Fixation in the Microcystis aeruginosa as a Potential Target for the Control of Freshwater Cyanobacterial Harmful Algal Blooms." *Environmental Science & Technology*. In prep. "Assessing Release of Intracellular Organic Matter from Cyanobacteria Following Application of Commercial Algaecides." *Journal of Hazardous Materials*. In prep. "Synergistic Impact of Combined Application of Cyanophage and Algaecide against Bloom Forming Cyanobacteria." *Harmful Algae*. In prep. "Metagenomic Insights into the Impact of Commercial Algaecides on Harmful phy-Toplankton and Microbial Community Responses: Mesocosm Study." *Water Research*. In prep. "Synergistic Role of Endogenous Multispecies Biofilms in Biological filters for Cyanotoxin Removal." *Water Research*. In prep. "An Insight into Microbial Community Dynamics and Interactions of the Persistent Planktothrix-Dominated Bloom in Grand Lake St. Marys." *Harmful Algae*. In prep. "A Metagenomic Approach to look into Lake Erie Cyanobacterial Harmful Algal Bloom, Quest for an Early Warning Biomarkers." *Water Research*.
Products	**Invention Disclosure.** Target specific inhibition and control of cyanobacteria and green algae. May 2023
Tech Transfer and Demonstrations	**Sensor system deployment and real time monitoring of water.** City of Toledo water treatment plant operators. July–October 2022 **Sensor system deployment and training.** City of Defiance water treatment plant operators. May 2023

Cyanobacteria Assessment Network: Pilot Study with Sentinel-2–derived Chlorophyll Data (CyAN-S2)

USEPA, NOAA, NASA, USGS, and USACE—An Interagency Collaboration

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Problem

Harmful algal blooms (HABs) are a global problem impacting the health and safety of both inland and coastal waters. Excessive algal biomass and toxin production negatively impact water uses such as recreation, consumption, livestock, fisheries, and irrigation. Algal, both phytoplankton and cyanobacterial, biomass can result in surface scums, taste and odor issues, hypoxia, reduced aquatic diversity, and negative socioeconomic impacts. Despite ecosystem, economic, and public health concerns, toxins are infrequently assessed due to the cost, required expertise, and time necessary for proper analysis. Moreover, measuring indicators of algal biomass, such as chlorophyll-a, can be time and cost intensive. State and federal agencies are faced with the challenge of operating, maintaining, and monitoring hundreds to thousands of water bodies, covering large geographic areas with limited water quality staff.

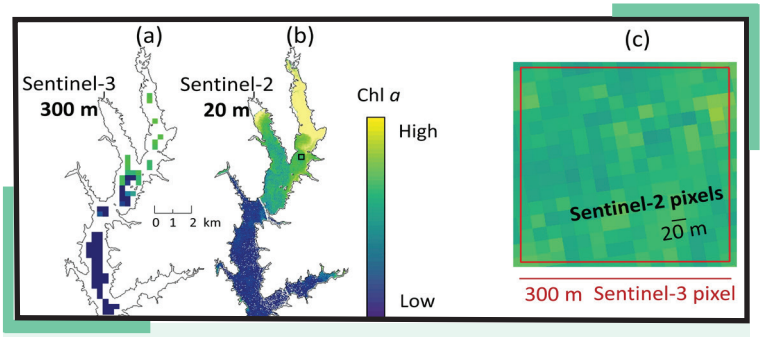


Figure 1. *Comparison of the spatial coverage from the (a) original CyAN project Sentinel-3 300 m pixel resolution, and (b) Sentinel-2 20 m pixel resolution in the northern section of Jordan Lake, North Carolina; (c) an example of a single Sentinel-2 finer resolution pixels.*

Objective

The US Army Corps of Engineers (USACE) manages more than 400 lakes and reservoirs that would benefit from monitoring chlorophyll-a with Sentinel-2 (S2) satellite imagery. This pilot project seeks to develop a national chlorophyll-a product capable of 10–60 m spatial resolution (Figure 1), which would enable satellite HAB monitoring for more than 270,000 lakes and reservoirs.

Approach

This one-year USACE-sponsored pilot project will build initial CyAN-S2 infrastructure and demonstrate its potential using HAB data sets in Florida, Ohio, and Oregon. Key steps include: (1) in situ and S2 data assimilation; (2) develop new validation approaches specific to higher-resolution S2 data; (3) explore initial S2 spatial and temporal compositing; and (4) produce a pilot study report. This pilot study will encompass 29 USACE reservoirs, including Harsha Lake in Ohio; 7 USACE reservoirs in Florida, including Lake Okeechobee and the Lake Watch program; and 18 USACE reservoirs in Oregon, including Detroit Lake. Cross validation of Sentinel-3 (S3) and S2 approaches will be accomplished using data from 212 lakes that are resolvable by both S3 and S2, which include 23 USACE reservoirs. This one-year pilot is an interagency collaboration, with USACE leading algorithm evaluation and aggregation of in situ data; USEPA leading development of a scoring rubric to be used during quality control review and validation; USGS leading harmonization and provision of in situ chlorophyll-a observations and aquatic reflectance data sets; NASA leading strategic approaches to satellite image composites and data delivery; and NOAA leading the design methods and metrics for establishing quality control of the satellite data and temporal comparisons. The NOAA National Environmental Satellite, Data, and Information Service (NEDIS) has agreed to provide in-kind support for hosting the S2 national chlorophyll product both during the pilot phase and long-term.

Milestones

CyAN-S2 Pilot Study Report.

Value to USACE Mission

Monitoring of smaller lakes and reservoirs is needed to proactively protect human, animal, and environmental health and economic prosperity and support the 2014 Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA), Clean Water Act, and Safe Drinking Water Act. Building a monitoring capability with S2 will provide timely data for >400 USACE-managed lakes and reservoirs and >270,000 lakes and reservoirs with a potential annual avoided cost of $42M/year.

Profiling Microbial Community Composition and Functions in the Lake Okeechobee Watershed

Lead: Viviana Mazzei, USGS, vmazzei@contractor.usgs.gov

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Lake Okeechobee (Lake O) is a model ecosystem to study the patterns and processes of phytoplankton assembly in large, shallow subtropical lakes with coastal connectivity. Novel anthropogenic environmental gradients created by surrounding land use types, natural and managed hydrologic processes, and climate change create spatial and temporal heterogeneity that are likely driving phytoplankton assemblage structure and HAB dynamics within the Lake O system.

Objective

(1) Describe spatial and temporal patterns and drivers of phytoplankton community structure, HAB events, and toxin-production in the Lake O system, and (2) experimentally test the effects of NO_3, NH_4 and PO_4 enrichment on short term phytoplankton dynamics and toxin production.

Approach

Monthly water samples were collected at 21 locations for quantitative phytoplankton taxonomic analysis and measurement of cyanotoxins and cyanotoxin biosynthesis gene concentrations from March 2019 to October 2021 (32 months). During this period, six independent in situ nutrient amendment mesocosm experiments were conducted at different times of the year at the Franklin Lock and Dam on the Caloosahatchee River, which drains Lake O to the west into the Gulf of Mexico.

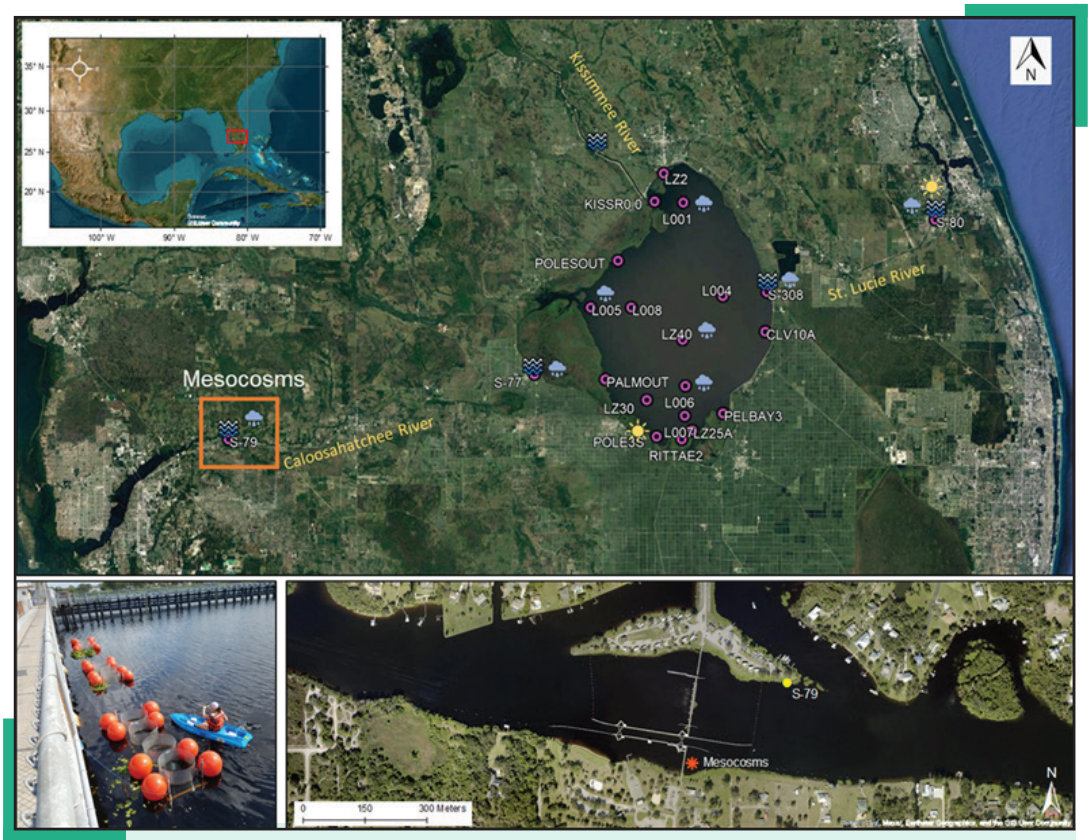


Figure 1. *Location of the 21 sampling locations. Stacked wavy lines indicate locations of lock and dam structures controlling lake inflows and outflows. Cloud icons indicate sites where precipitation data are routinely collected. Sun icons indicate where total solar radiation data were collected. The location of the mesocosm experiments relative to the other sampling stations is marked by the box. The bottom panel shows a close up of the Franklin Lock and Dam site of the mesocosm experiments and a photo of the experimental structure and design.*

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Major Milestones

Deliverable	Description
ScienceBase Data Release	**Mazzei, V.,** and K. Sullivan. 2022. *Caloosahatchee River Nutrient Enrichment Mesocosms: Phytoplankton Taxonomic Quantification September 2019, June 2020, September 2020, February 2021.* US Geological Survey data release. https://doi.org/10.5066/P99ELCEC. **Mazzei, V.,** and K. Sullivan. 2023. *Caloosahatchee River Nutrient Enrichment Mesocosms: USGS Phytoplankton Taxonomic Quantification February 2021, May 2021, July 2021*. US Geological Survey data release. https://doi.org/10.5066/P96LONDP. **Mazzei, V.,** and K. Sullivan. 2022. *Monthly Phytoplankton Taxonomic Quantification in Lake Okeechobee and the Okeechobee Waterway, Florida, USA, 2019–2021.* US Geological Survey data release. https://doi.org/10.5066/P9QOJ4JZ. Karwacki, E. E., and **V. Mazzei**. 2022. *Quantitative Polymerase Chain Reaction (qPCR) Cyanotoxin Data for Eight Mesocosm Experiments in the Caloosahatchee River, Florida from May 2019 through August 2021.* US Geological Survey data release. https://doi.org/10.5066/P9D52SZ1. Karwacki, E. E., and **V. Mazzei**. 2022. *Quantitative Polymerase Chain Reaction (qPCR) Cyanotoxin Data for 21 Sites on Lake Okeechobee and the Okeechobee Waterway, Florida, USA Collected Monthly from March 2019 through October 2021.* US Geological Survey data release. https://doi.org/10.5066/P9CFALMR.
Journal Article	**Mazzei, V.,** E. E. Karwacki, K. A. Loftin, J. V. Lopez, L. E. Krausfedlt, B. H. Rosen, and H. Urakawa. In review. "Phytoplankton Responses to Experimental Nitrogen and Phosphorus Loading in a Eutrophic and Turbid Subtropical River." *Ecological Applications*. **Mazzei, V.**, K. Sullivan, and K. A. Loftin. In review. "Phytoplankton Assemblage Structure, Drivers, and Thresholds in the Lake Okeechobee System, Florida, USA with an Emphasis on Cyanobacterial HAB Taxa." *Limnology & Oceanography*.

Partnership/Leveraging Opportunities

The USGS Environmental Health Toxic Substance Hydrology Program provided additional funding support for this project. The South Florida Water Management District collected the monthly field samples in tandem with their routine water quality monitoring samples, allowing their water quality data to be leveraged for this project.

Value to USACE Mission

Understanding cyanobacteria community composition and how it varies as a function of nutrient concentrations and over time in Lake Okeechobee provides new insight into the cyanoHABs that plague Lake Okeechobee. This fundamental knowledge has the potential to inform novel prevention and management strategies in Lake Okeechobee and cyanoHAB research in freshwaters across the nation.

Flocculation of Freshwater Microalgae Using Naturally Derived Biomolecules

Lead: Dr. Catherine C. Thomas, ERDC, Catherine.C.Thomas@usace.army.mil

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Harmful algal blooms (HABs) have increased in frequency and distribution, impacting communities throughout the US Chemical based treatments for HABs can not only introduce harsh chemicals into the environment, but also prompt the release of toxins from damaged or dying cyanobacteria cells. Thus, alternative treatments using natural materials to bind and precipitate microalgae is of great interest.

Objective

The objectives of this work were to (1) assess the binding efficiency/affinity of naturally derived compounds including chitosan, bio-based cationic compounds, and various types of modified clays (or any combination of chitosan, starches, or clays) to two cyanobacteria species (*Microcystis aeruginosa* and *Anabaena* spp.) typically found in freshwater systems,and (2) determine the stability of algal flocs over time under static and turbulent conditions.

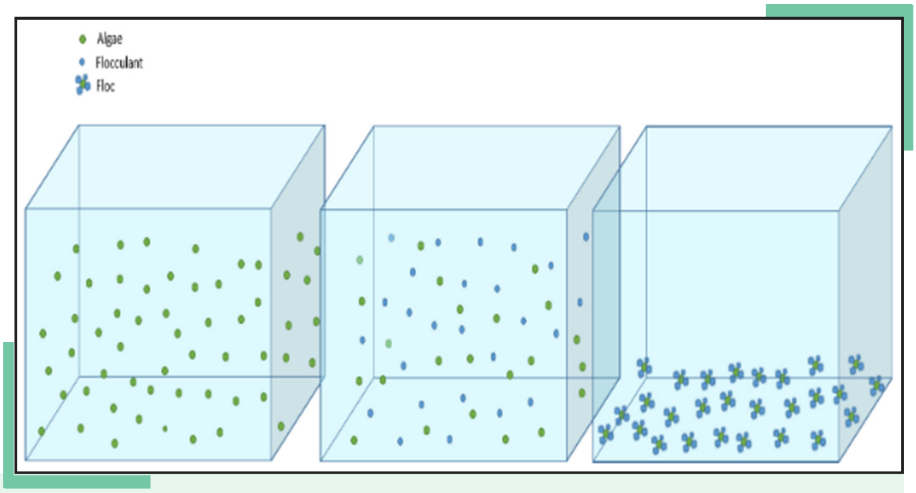


Figure 1. *Schematic illustration of the anticipated behavior.*

Approach

We evaluated the binding affinity of the test amendments to cyanobacteria species and determined optimal dosing amounts using experiments performed under conditions of varying pH and dissolved oxygen (DO) concentrations. Flocculation and precipitation kinetics were also determined under static and mixing conditions. The highest performing amendment and/or application ratios were down-selected for further testing of mixed cyanobacteria cultures in the presence of sediment at mesocosm scale in a wave simulation tank. Commercially available polymers used in experimentation, BioFlow and GeoFlow, were composed of chitosan acetate and a proprietary blend of bentonite, zeolite, and calcium carbonate, respectively. Flocculation and precipitation tests were performed in the presence of total suspended solid (TSS) sediment concentrations up to 220 mg/L, mimicking high turbidity water.



Figure 2. *Precipitated algae flocs formed from (left to right) BioFlow + chitosan flakes; BioFlow + GeoFlow; chitosan powder + modified bentonite clay; and chitosan flakes + modified bentonite clay.*

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Results

Evaluations indicated that two amendment combinations were most effective in binding and precipitating cyanobacteria: (1) BioFlow (a commercially available form of chitosan acetate) + GeoFlow (a proprietary blend of bentonite, zeolite, and calcium carbonate) and (2) Bio-Flow + chitosan flakes (1.6–2.5 mm). Regarding floc stability, flocs formed by the BioFlow + chitosan flake combo were unaffected by fluctuating pH (4–10) and DO concentrations. The BioFlow + GeoFlow combo, however, yielded pH-dependent floc; increasingly acidic conditions (less than pH 5.5) caused the resuspension of flocs back into solution. Flocculation and precipitation tests showed that increasing TSS concentrations improved precipitation rates, likely due to the formation of larger, heavier particles. However, in wave tank simulations, flocculation and precipitation of algae was less effective due to low intensity mixing induced by wave motion. Although the chitosan acetate + chitosan flakes combo demonstrated efficacy to induce sedimentation of algal flocs in the presence of suspended sediment, wave simulations suggest that open water application of the materials is not recommended.



Figure 4. *Algae flocs in wave tank formed by BioFlow + chitosan flakes.*

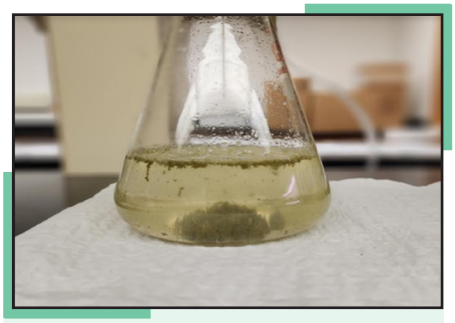


Figure 3. *Algae floc formed by Bio-Flow + chitosan flakes in the presence of suspended sediment at the highest tested TSS concentration (220 mg/L).*

Major Milestones

Deliverable	Description
Publication	**Tech Report.** Thomas et al. In prep. "Flocculation and Precipitation of Freshwater Microalgae using Bio-Based Polymers." ERDC TR.

Partnership/Leveraging Opportunities

This work leveraged that of "Research on Algae Flotation Techniques (RAFT)" (PI: Cender), another USACE Aquatic Nuisance Species Research Program project, in which flocculants were investigated for ability to achieve algae flotation in open water applications.

Value to USACE Mission

Results from our research provide valuable data for practical HAB management solutions involving the application of environmentally benign polymers in low-concentration HAB-impacted waters. Our approach provides a method to disrupt the formation of large algal colonies and thus prevents the spread of algae in a USACE managed lake or reservoir.

Ultrasound as a Source Water Reservoir Management Strategy to Control Cyanobacteria Blooms

Lead: Linda Weavers, PhD, PE, The Ohio State University

USACE Harmful Algal Bloom Research & Development Initiative

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Problem

Ultrasound is a physical reservoir management strategy that may be an important bloom prevention and mitigation strategy with over 10,000 installations worldwide. From studies primarily in a lab setting at high power, ultrasound has been shown to collapse gas vesicles in cells using buoyancy as an evaluation tool for gas vesicle collapse, break filamentous cyanobacteria, inhibit growth, reduce cell concentrations, inactivate cells, reduce photosynthetic activity, and increase sedimentation rates. Information on effects at low power used in reservoir management are limited. Compared to other physical strategies and use of algaecides, ultrasound has the advantage of low energy, no chemical addition, and the possibility that cell lysis and release of toxin does not occur.

Presently, information on its effective use is mixed and rather limited. In particular, ultrasonic devices are installed to cover an entire reservoir and remain on for an entire season. Due to this mode of use, there are not paired control experiments to conclusively determine the mechanism by which ultrasound is working. Prior to this project, we investigated a mechanism cited by ultrasound vendors, collapse of gas vesicles, and did not find evidence that this mechanism was active.

Objective

This project aims to explore mechanisms that may be active in mitigating HABs to provide targeted guidance on deployment of these systems. We use smart deployment of ultrasound or combining ultrasound with an emerging algaecide, namely hydrogen peroxide (H_2O_2), which is anticipated to significantly improve its efficacy while decreasing algaecide use. Elucidating how ultrasound may affect cyanobacteria is important to resolve why it appears to be effective in some cases and not in other cases. This knowledge related to the use of ultrasound in reservoirs will allow for more informed decision-making related to use of this emerging technology allowing for smart deployment.

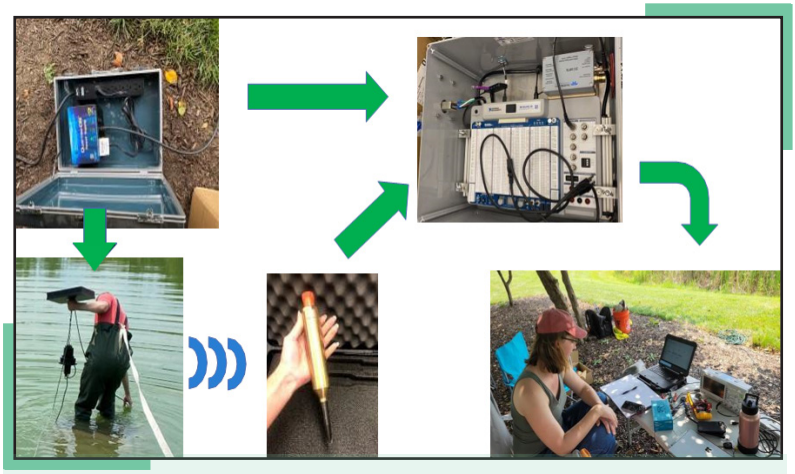


Figure 1. *Components for measuring ultrasound in lab and field deployments.*

Approach

Year 2 effort occurred in the laboratory and in the field. One aspect of lab work focused on whether sonoporation and/or endocytosis may be occurring. We modified methods using cell stains and flow cytometry to determine if cell permeance is higher in the presence of ultrasound. Additionally, in the laboratory, work has begun to explore the combined effect of ultrasound and hydrogen peroxide to determine if ultrasound can reduce the amount of algaecide required for treatment.

Moreover, in Year 2, we measured ultrasound in the field in a reservoir and in a lab tank with a hydrophone, National Instruments ELVIS board, and Lab View computer program (Figure 1). We have successfully monitored the sound field in a field deployed ultrasonic unit and collected water quality information with an EXO Sonde in two different reservoirs and on different days at different distances from the ultrasonic source.

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Year 2 Results

To assess the potential for sonoporation to occur and be a mechanism affecting cyanobacteria exposed to field deployed ultrasound, we developed a method for testing this mechanism. Due to the natural fluorescence from phycocyanin and chlorophyll a, interfering with common stains used, we tested five stain combinations before finding suitable combination (SYTOX Green and DAPI). We confirmed that under ultrasound conditions in which human cells undergo sonoporation, *Microcytis* sp. also undergoes sonoporation. Sonoporation experiments over a 10 min time period revealed that sonoporation occurs rapidly with 70% of the cells stained with the impermeant stain in 5 min. Using a hydrophone, we confirmed that this condition is above the cavitation threshold, not consistent with field conditions. Next steps will be to perform experiments at conditions consistent with field ultrasound conditions to determine if sonoporation is occurring in the field deployed systems.

Additional container studies are being conducted in the presence of hydrogen peroxide, with and without ultrasound. We investigated the treatment of 105 and 106 *Microcystis* cells/mL with 2 and 4 mg/L hydrogen peroxide. At 105 cells/mL, degradation of hydrogen peroxide occurred over 24 hr. Chlorophyll a increased in the no treatment and 2 mg/L hydrogen peroxide condition. Chlorophyll a remained stable in the 4 mg/L condition over the 3-day experiment. At the higher cell concentration of 106 cells/mL, hydrogen peroxide degraded over 24 hr. Chlorophyll a decreased over 24 hr in all conditions and then rebounded. Enumerations of the 106 cells/mL condition indicated an increase in cells in the control, decreases in the 2 mg/L, and stable cell numbers in the 4 mg/L hydrogen peroxide condition. This work will be repeated prior to experiments in the presence of ultrasound.

In Year 2, hydrophone measurements of ultrasound emanating from the field device was conducted in a small tank in the laboratory to determine if there was directionality of the sound from the device and to determine the stability of measurements with time. This work confirmed there is no directionality of the sound. We also determined that soaking the hydrophone and using an uninterruptible power supply improved the reproducibility of our measurements. In addition, the cycling of the ultrasound is such that we can reduce the time of our measurement capture while not biasing results, thereby improving the efficiency of our measurements.

Hydrophone and water quality measurements were conducted at two reservoirs at the City of Wilmington, Ohio. The reservoir is generally well mixed over the depth we monitored based on temperature and water quality profiles with depth. In addition, no obvious water quality trends were observed at different distance from the ultrasonic unit. Hydrophone measurements indicated an exponential decrease in the ultrasonic signal with distance from the unit. Values measured are well below the cavitation threshold, suggesting a low likelihood for cell lysis and release of cyanotoxins from cyanobacteria present in the reservoirs. Work will continue in Year 3 in reservoirs measuring field ultrasound and water quality. We have two new water utility partners that have recently installed ultrasonic units in reservoirs. We are developing a comprehensive field study in the reservoir nearest Columbus, Ohio, that has multiple similar reservoirs. Their reservoirs will allow us to monitor untreated and treated reservoirs for water quality to observe differences.

Ultrasound as a Source Water Reservoir Management Strategy to Control Cyanobacteria Blooms

Lead: Linda Weavers, PhD, PE. The Ohio State University

USACE Harmful Algal Bloom Research & Development Initiative

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Year 2 Results

Deliverable	Description
Presentations	L. K. Weavers. 2022. "Evaluation of Nanobubble Ozone Technology (NBOT) and Ultrasound for Cyanobacterial Harmful Algal Bloom Control." Department of Civil and Environmental Engineering, Case Western Reserve University, June 15. L. K. Weavers. 2022. "Evaluation of Ultrasound for Cyanobacterial Harmful Algal Bloom Control." *OTCO Reservoir Management Workshop Webinar*, July 12. E. Bonner, E. Crafton-Nelson, Z. Bohrerova, and L. K. Weavers. 2022. "Evaluating Hydrogen Peroxide (H_2O_2) Induced Suppression of *Microcystis aeruginosa* (*M. aeruginosa*)." Abstract. Water Management Association of Ohio, November 8–9. M. Tischer, C. R. Weaver, C.-M. Cheng, E. Crafton, Z. Bohrerova, S. Thayer, E. Bonner, R. Vehar, and L. K. Weavers. 2022. "Field Measurement of Ultrasound and Water Quality Parameters to Assess Its Effectiveness as a Source Water Reservoir Management Strategy to Control Cyanobacteria Blooms." Abstract. Water Management Association of Ohio, November 8–9. S. Gill, M. Tischer, S. Thayer, E. Bonner, R. Vehar, C.-M. Cheng, L. K. Weavers, Z. Bohrerova, C. R. Weaver, and E. Crafton. 2022. "Field and Bench-Scale Analysis of Mechanism for Ultrasonic Control of Harmful Algal Blooms." Abstract. Water Management Association of Ohio, November 8–9. M.A. Tischer, C. R. Weaver, E. Crafton, C.-M. Cheng, R. Vehar, S. Gill, T. Belleville, and L. K. Weavers. 2023. "Ultrasound as a Source Water Reservoir Management Strategy to Control Cyanobacteria Blooms." Abstract. *Association of Environmental Engineering and Science Professors (AEESP) Biennial Conference*, Boston, MA, June 20–22. E. N. Bonner, E. Crafton, Z. Bohrerova, S. Thayer, M. Tischer, C.R. Weaver, C.-M. Cheng, L. Nemec, and L. K. Weavers. 2023. "Evaluating Ultrasound and Hydrogen Peroxide (H2O2) Induced Suppression of Cyanobacteria." Poster abstract. *2023 One Water Technical Conference*, Columbus, OH, August. M. Tischer, C. R. Weaver, E. Crafton, C.-M. Cheng, R. Vehar, S. Gill, T. Belleville, and L. K. Weavers. 2023. "Ultrasound as a Source Water Reservoir Management Strategy to Control Cyanobacteria Blooms." Oral abstract. *2023 One Water Technical Conference*, Columbus, OH, August.
Publications	E. N. Bonner, E. Crafton, Z. Bohrerova, S. Thayer, M. Tischer, C.R. Weaver, and L. K. Weavers. In prep. "Evaluation of Cyanobacteria Suppression by Hydrogen Peroxide and Ultrasound." *AWWA: Water Science*. M. A. Tischer, C. R. Weaver, E. Crafton, K. Murphy, R. Vehar, C.-M. Cheng, L. K. Weavers. In prep. "Field Use of Ultrasound for Management of Algal Blooms: Evaluation of Sound Pressure and Water Quality. *Harmful Algae*.
Theses	Shannon Thayer. 2023. "Investigating Sonoporation in Cyanobacteria Exposed to Ultrasound." MS Thesis. Elyse Bonner. 2023. "Evaluation of Cyanobacteria Suppression by Hydrogen Peroxide and Ultrasound." MS Thesis. Mark Tischer. In prep. "Field Use of Ultrasound for Management of Algal Blooms: Evaluation of Sound Pressure and Water Quality."

Determining Environmental Triggers of HAB and Toxin Production for the Purpose of HAB Prediction, Detection, and Management (HAB-PDM) in Flowing Waters

Lead: Dr. Mindy Armstead, Department of Natural Resources and the Environment, Marshall University, Huntington, West Virginia

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Harmful algal blooms (HABs) in riverine ecosystems are increasing in severity and frequency. Understanding what has caused this increase is complicated due to the complex hydrogeology, water quality, and biology associated with riverine ecosystems. The additional variability associated with managed river ecosystems further complicates our knowledge of algal community dynamics in such systems. While the general conditions that support bloom formation (i.e. low flow, high temperatures, and nutrient abundance) are understood, the interaction of these factors with the complex hydrology of managed riverine ecosystems and the algal community dynamics in these systems have not been well characterized, leading to data gaps for bloom prediction and management. Specific predictions of bloom timing and location and an ability to thwart bloom development, predict the extent of toxin production, reduce bloom duration, or effectively remediate blooms are all dependent on understanding biotic and abiotic conditions in these complex and poorly described ecosystems.

Objective

There is a definitive need to understand algal community dynamics and HAB development in managed river ecosystems. This research addresses data gaps identified in understanding of hydrology and ecology in these systems with application of the knowledge for bloom prediction and management. Specific objectives of the research are as follows: (1) detect: identify HAB sources, ecological triggers, and regulators of cell growth/toxin production; (2) predict: develop hydrodynamic, hydrologic, and machine learning models to simulate the role of hydrology, nutrients, and light attenuation on HAB growth; and (3) Manage: Investigate how riverine management affects HAB formation.

Approach

Our collaboration takes a broad approach to the diverse objectives aiming to fill data gaps by identifying HAB sources, identifying HAB triggers and cell growth/toxin production mediators, investigating the role of managed river hydrology in bloom formation, and preparing for the scalability and transferability of methods and tools developed. Field efforts in tributaries and the mainstem of the Ohio River investigate algal sources and community dynamics in the managed river ecosystem. Simultaneous laboratory efforts investigate cell growth and HAB triggers to support the field investigations and inform predictive models. Congruent hydrologic and hydrodynamic modeling efforts, along with statistical evaluations and machine learning efforts, test multiple approaches to bloom prediction and management. Project outcomes will include protocols/guidance for data collection for HAB monitoring and predictions, and a predictive tool for HABs in a managed river ecosystem.



Figure 1. *The Ohio River.*

Determining Environmental Triggers of HAB and Toxin Production for the Purpose of HAB Prediction, Detection, and Management (HAB-PDM) in Flowing Waters

Lead: Dr. Mindy Armstead, Department of Natural Resources and the Environment, Marshall University, Huntington, West Virginia

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Major Milestones

Date	Milestone
FY24, Q1	Contribution of landscape/basin scale hydrologic and water quality loadings to HABs in the Greenup Pool of the Ohio River. Technical report and/or white paper, input to hydrodynamic model.
FY24, Q2	Use of machine learning models to determine critical ecological parameters contributing to HAB formation. Technical report and/or white paper, input to hydrodynamic model.
FY24, Q4	Field study and analysis informing the contribution of tributaries to mainstem HAB formation, as well as filling data gaps on managed river ecosystem algal community dynamics and providing input for hydrodynamic, hydrologic, and machine learning models. Technical report and/or white paper.
FY24, Q4	Laboratory determination of environmental triggers/thresholds for algal growth and toxin production. Technical report and/or white paper.
FY24, Q4	Summary of tributary influences on mainstem HAB conditions to support mainstem modeling efforts. Technical report and/or white paper, input to hydrodynamic model.
FY25, Q3	Hydrodynamic model and scenario analysis for predicting/managing HABs in the Greenup Pool of the Ohio River. Technical report and/or white paper.
FY25, Q3	**Technical Report:** Guidance for data collection for optimal HAB detection.
FY25, Q3	**Technical Report:** Guidance for data collection to inform HAB predictive tool development.

Partnership/Leveraging Opportunities

This work will leverage multiple collaborations and other work units. Additionally, this effort utilizes the cross disciplinary expertise of the collaborators, representing biological, toxicological, and engineering disciplines from five universities. Our modeling and machine learning efforts will leverage available data from academic, public, and private sector sources to maximize the use of available data.

Value to USACE Mission

This research will alleviate disruption of aquatic uses due to adverse impacts from HABs, which are notable at USACE projects authorized for transportation, aquatic recreation, and water supply. Our efforts will increase the knowledge of HAB formation requirements and improve risk assessment and management of HABs in ecosystems with USACE management and oversight by providing methods for the monitoring, prediction, and management of HAB development, as well as a framework scalable and transferable to other managed river ecosystems.

Coupling Lake, Estuarine, and Watershed Models to Elucidate Role of Freshwater Discharges in Driving HAB Severity, Location, and Timing

Lead: David Kaplan, University of Florida

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Multiobjective management of Lake Okeechobee (Lake O) has been a "wicked problem" for USACE for nearly 100 years. The need to maintain in-lake storage for flood protection while meeting water quantity and quality requirements for flows to the Everglades has led to frequent discharges to the Caloosahatchee and St. Lucie Estuaries. While Lake O discharges have been associated with both freshwater and marine algal blooms, connections among lake management, watershed-derived nutrients, phytoplankton dynamics, and coastal hydrodynamics have not yet been fully resolved.

Objective

This project seeks to develop improved data- and model-driven guidance for Lake O releases and Caloosahatchee watershed management via four linked objectives: (1) adapting and coupling Lake O, Caloosahatchee watershed, and estuarine hydrodynamic models; (2) augmenting existing water quality data with real-time, monthly, and event-driven sampling; (3) quantifying estuarine residence times, nutrient concentrations, and phytoplankton communities in relation to environmental drivers; and (4) applying model and data synthesis to identify optimal conditions for Lake O discharge.

Approach

This project integrates multiple modeling, data collection, and analysis approaches. Modeling approaches include two Lake O models to simulate the effects of watershed loading and discharge decision-making on lake outflows and nutrient/algae loads; process- and machine learning-based models of Caloosahatchee River watershed flows and nutrient loads; and a coastal hydrodynamic model to understand how river discharge (including Lake O releases) affects estuarine residence times and water quality. Water quality monitoring and analyses include installation of real-time sensors and two years of discrete monthly sampling to

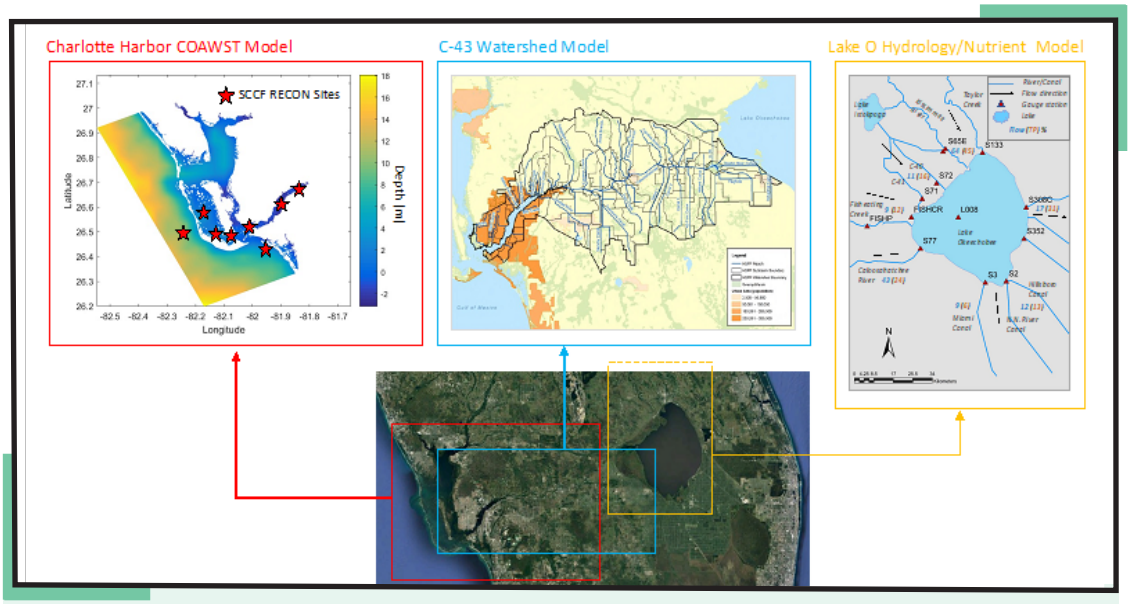


Figure 1. *To better understand the role freshwater discharge plays in HAB formation, researchers are combining data and models spanning from Lake Okeechobee to the Caloosahatchee River Estuary. The effort utilizes lake, watershed, and coastal hydrodynamic modeling to assess how lake operation decisions affect estuarine circulation, residence time, and water quality, including particle tracking within the Caloosahatchee River.*

Coupling Lake, Estuarine, and Watershed Models to Elucidate Role of Freshwater Discharges in Driving HAB Severity, Location, and Timing

Lead: David Kaplan, University of Florida

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Approach (cont.)

characterize phytoplankton communities and track nutrient sources. Statistical analyses include trend and change analysis for Lake O discharges, development of concentration-discharge relationships for the lake and watershed, and predictive models for phytoplankton taxa as a function of environmental drivers.

Major Milestones

Deliverable	Description
Journal Articles	Hewageegana, V. H., M. Olabarrieta, and J. M. Gonzalez-Ondina. 2023. "Main Physical Processes Affecting the Residence Times of a Micro-Tidal Estuary." *Journal of Marine Science and Engineering* 11 (7): 1333. https://doi.org/10.3390/jmse11071333. Phlips, E. J., S. Badylak, A. L. Mathews, E. C Milbrandt, L. R. Montefiore, E. S. Morrison, N. Nelson, and B. Stelling. 2023. "Algal Blooms in a River-Dominated Estuary and Nearshore Region of Florida, USA: The Influence of Regulated Discharges from Water Control Structures on Hydrologic and Nutrient Conditions." *Hydrobiologia* 850:4385–4411. https://doi.org/10.1007/s10750-022-05135-w. Shi, L., C. Ortals, A. Valle-Levinson, and M. Olabarrieta. 2023. "Influence of River Discharge on Tidal and Subtidal Flows in a Microtidal Estuary: Implication on Velocity Asymmetries." *Advances in Water Resources* 177:104446. https://doi.org/10.1016/j.advwatres.2023.104446. Tarabih, O. M., T. D. Dang, R. Paudel, and M. E. Arias. 2023. "Lake Operation Optimization of Nutrient Exports: Application of Phosphorus Control in the Largest Subtropical Lake in the United States." *Environmental Modelling & Software* 160:105603. https://doi.org/10.1016/j.envsoft.2022.105603. Montefiore, L. R., D. Kaplan, E. J. Phlips, E. C. Milbrandt, M. Arias, E. Morrison, and N. G. Nelson. In review. "Relative Contributions of Reservoir Releases and Basin Inputs on Downstream Water Quality in a Highly Engineered Watershed." *Water Resources Research.* Orozco-Lopez, E., and D. Kaplan. In review. "Interpretable Transformer Neural Network Prediction of Diverse Environmental Time Series Using Weather Forecasts." *Water Resources Research.*
Tech Reports	Kaplan, D., M. Arias, E. Milbrandt, E. Morrison, N. Nelson, M. Olabarrieta, E. Phlips, et al. 2022. *Year 1 Annual Report (October 1, 2021 – September 30, 2022): Coupling Lake, Watershed, and Estuarine Models to Better Understand the Role of Engineered Freshwater Discharges in Driving the Severity, Location, and Timing of Harmful Algal Blooms.* Technical report to the US Army Corps of Engineers Engineering Research Development Center, Aquatic Nuisance Species Research Program, Gainesville, FL. Olson, L. 2023. "Assessing Annual Changes in Water Quality in the Caloosahatchee River and Estuary." Undergraduate thesis, University of Florida.

Coupling Lake, Estuarine, and Watershed Models to Elucidate Role of Freshwater Discharges in Driving HAB Severity, Location, and Timing

David Kaplan, University of Florida

USACE Harmful Algal Bloom Research & Development Initiative

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Major Milestones (cont.)

Deliverable	Description
Products	One-page "Fact Sheets" summarizing the key findings and take-home messages from each of the journal publications listed above are currently in development.

Partnership/Leveraging Opportunities

Project outcomes are being leveraged by the new project "Integrating Modeling Tools and Observations for Prediction and Management of Harmful Algal Blooms in the St. Lucie Estuary and Watershed." End-user workshops and scenario development efforts are being coordinated among the two projects. A kickoff meeting was held in February 2023 and included the project team and representatives from the Florida Department of Environmental Protection, US Army Corps of Engineers Jacksonville District, and the South Florida Water Management District (which is also a direct project partner).

Value to USACE Mission

Understanding how lake, estuarine, and watershed operations influence the severity, location, and timing of HABs in and around Lake Okeechobee would give valuable insight for project managers that can guide more stratetgic operational decisions. This fundamental knowledge has the potential to inform novel prevention and management strategies in Lake Okeechobee and cyanoHAB research in freshwaters across the nation.

Appendix 2. HAB R&D Community Engagement

USACE Interagency Freshwater Harmful Algal Bloom (HAB) Research & Development (R&D) Workshops and USACE HAB Listening Sessions

The ERDC Environmental Laboratory (EL) Civil Works Technical Director's Office hosted three USACE Interagency Freshwater HAB R&D Workshops and three USACE HAB R&D Listening Sessions between 2021 and 2023. Objectives of the USACE Freshwater HAB R&D Workshops were to share USACE HAB R&D priorities and current research with interested federal, state, and university partners; to listen and learn about our partner's HAB R&D priorities and current research; and to solicit input from our partners regarding HAB R&D needs moving forward. Invited presentations were given by multiple federal and state agency partners (National Oceanic and Atmospheric Administration, US Environmental Protection Agency, US Geological Survey, US Bureau of Reclamation, US Food and Drug Administration, US Department of Agriculture Natural Resources Conservation Service, US Department of Agriculture Agricultural Research Service, and Centers for Disease Control and Prevention; Ohio, New York, and Florida) and by multiple universities. The objective of the USACE HAB R&D Listening Sessions was to gather feedback from USACE staff that deal directly with HABs in the field on (1) priorities, challenges, and ideas pertaining to HAB prevention, detection, and management/treatment; (2) barriers to the use of existing HAB technologies (e.g., lack of performance or cost data); and (3) HAB technologies that have been used but failed or were ineffective. Invited presentations were given by USACE districts each year, which both informed USACE HAB R&D needs and promoted information exchange and collaboration amongst USACE staff. Indeed, workshops and listening sessions have been invaluable to developing and delivering the USACE Freshwater HAB R&D Initiative—and were central in identifying remaining HAB R&D gaps described in the *Moving Forward Together* section of this report. Highlights from these community engagements are summarized below. Agendas from each community engagement workshop and listening session are included in Figures 1–6 below.

Data gaps identified included (1) understanding the role of algal seeding, seed thresholds, and treatment options for HABs; (2) harnessing molecular tools and other environmental parameters to elucidate HAB drivers and dynamics; (3) the need for a database containing repository of HAB prevention, detection, and management technology performance data; (4) the critical challenge of predicting HAB onset and toxin formation; and (5) benthic HABs as an emerging concern because they are more likely to be anatoxin producers and will require different monitoring and management approaches compared to planktonic HABs.

Opportunities identified included (1) agencies working together on joint projects to leverage authorities to accelerate HAB R&D; (2) transferring HAB knowledge and progress made for marine systems to freshwater systems; (3) capitalizing on remote sensing progress by combining autonomous vehicle and satellite surveillance for improved HAB monitoring; and (4) the Great Lakes and South Florida were identified as top locations for interagency collaboration.

USACE needs identified included (1) user-friendly models for HAB prediction; (2) rapid and reliable toxin detection to support water manager decision-making and communication with the public; (3) ecosystem- and watershed-scale HAB management technologies; (4) approaches for riverine HAB management; (5) HAB policy; and (6) streamlining and standardizing HAB practices for improved communication, data sharing, and technology transfer.

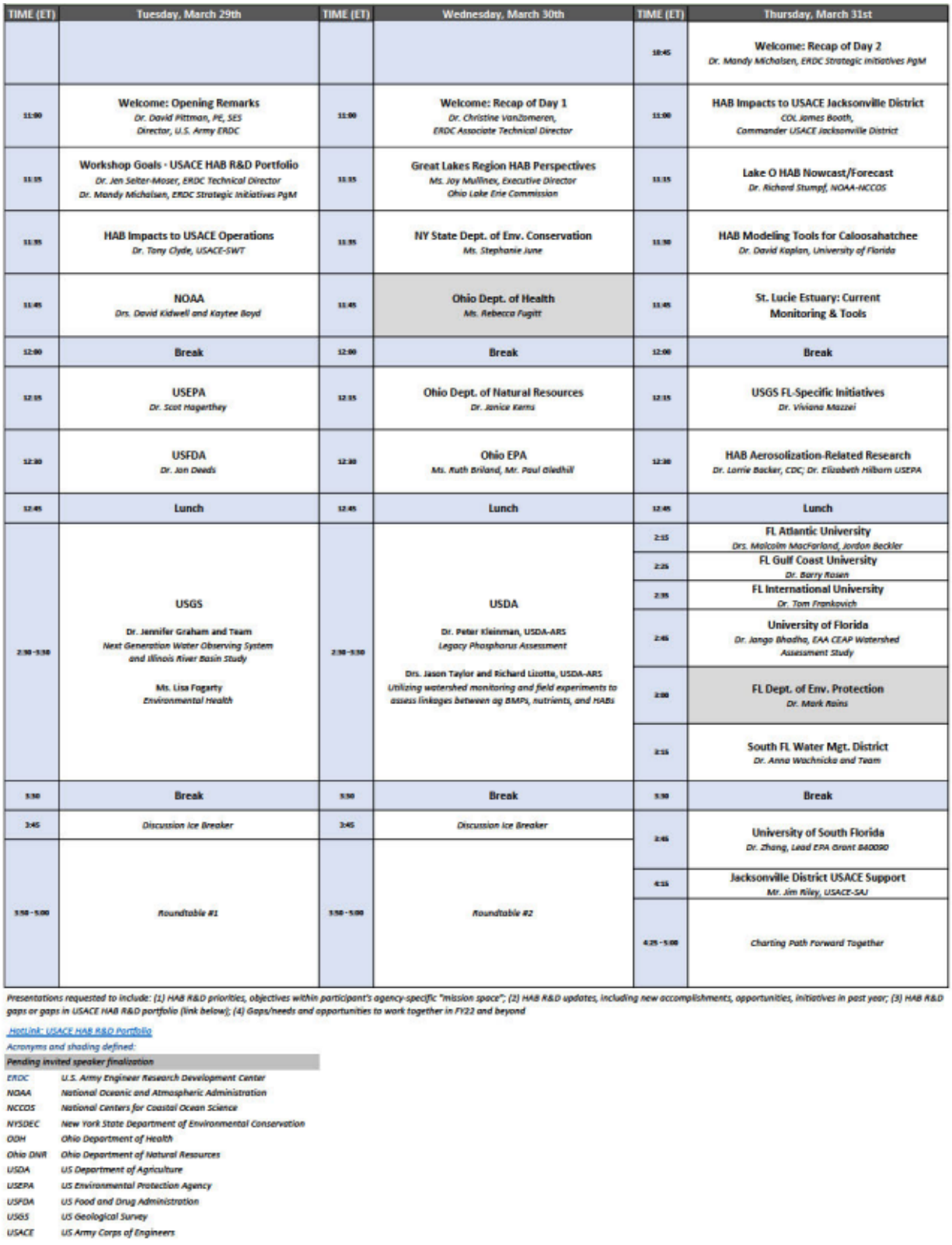
TIME (CT)	Tuesday, May 4th	TIME (CT)	Wednesday, May 5th	TIME (CT)	Thursday, May 6th
1:00	**Welcome** *Dr. Ty Wamsley, ERDC* USACE Civil Works Area Business Lead Director, Coastal and Hydraulics Laboratory	1:00	**Welcome** *Dr. Christine VanZomeren, ERDC*	1:00	**Welcome** *Mr. Mike Greer, ERDC*
1:15	**Workshop Goals and Objectives** *Dr. Jen Seiter-Moser & Team, ERDC* *Acting Technical Director*	1:15	**USACE HAB R&D Portfolio Overview** *Dr. Mandy Michalsen, ERDC*	1:15	**USACE Field HAB R&D Listening Session Debriefing** *Ms. Heather Theel, ERDC*
1:30	**Great Lakes Region** *Ms. Joy Mullinex, Executive Director* *Ohio Lake Erie Commission*	1:30	**USEPA** *Dr. Brenda Rashleigh*	1:30	**Ohio DNR** *Dr. Janice Kerns*
1:45	**HAB Impacts to USACE Operations** *Dr. Tony Clyde, USACE-SWT*	1:45	**ODH** *Ms. Rebecca Fugitt*	1:45	**Ohio EPA** *Ms. Ruth Briland, Mr. Paul Gledhill*
2:00	**USGS** *Drs. Jennifer Graham and Lisa Fogart*	2:00	**USFDA** *Dr. Jan Deeds*	2:00	**FL** *Mr. Jim Riley, USACE-SAJ*
2:15	**USDA** *Drs. Lisa Durlancik and Teferi Tsegaye*	2:15	**USBR** *Dr. Jacque Keele*	2:15	**USDA** *Drs. Lisa Durlancik*
2:30	**NOAA** *Drs. David Kidwell and Kaytee Boyd*	2:30	**NYSDEC** *Dr. Alexander Smith, Ms. Stephanie June, Ms. Karen Stainbrook*	2:30	**Break**
2:45	**Break**	2:50	**Break**	2:45	
3:00	*Discussion Ice Breaker*	3:00	*Discussion Ice Breaker*	3:00	*Discussion Ice Breaker*
3:05 - 4:00	**Roundtable Discussion Topic #1:** Current obstacles to HAB R&D besides funding	3:05 - 4:00	**Roundtable Discussion Topic #2:** What R&D areas are showing the most promise, and how can they be accelerated?	3:05 - 4:00	**Roundtable Discussion Topic #3:** Do you have ongoing research that would benefit from additional scientific and engineering expertise?

Overviews requested to include: (1) HAB R&D Goals/Objectives within participant's agency -specific Mission Areas; (2) HAB Research Trends and Topics; (3) HAB R&D Technologies Lessons Learned; (4) Funding/Leveraging Opportunities and Constraints

Acronyms:

NOAA	*National Oceanic and Atmospheric Administration*
NYSDEC	*New York State Department of Environmental Conservation*
ODH	*Ohio Department of Health*
Ohio DNR	*Ohio Department of Natural Resources*
USBR	*US Bureau of Reclamation*
USDA	*US Department of Agriculture*
USEPA	*US Environmental Protection Agency*
USFDA	*US Food and Drug Administration*
USGS	*US Geological Survey*
USACE	*US Army Corps of Engineers*
SFWMD	*South Florida Water Management District*
FDEP	*Florida Department of Environmental Protection*

Figure 1. Agenda from the Interagency Freshwater HAB R&D Workshop, hosted virtually via WebEx and in Vicksburg, Mississippi, May 4–6, 2021.



TIME (ET)	Tuesday, March 29th	TIME (ET)	Wednesday, March 30th	TIME (ET)	Thursday, March 31st
				10:45	**Welcome: Recap of Day 2** *Dr. Mandy Michalsen, ERDC Strategic Initiatives PgM*
11:00	**Welcome: Opening Remarks** *Dr. David Pittman, PE, SES* *Director, U.S. Army ERDC*	11:00	**Welcome: Recap of Day 1** *Dr. Christine VanZomeren,* *ERDC Associate Technical Director*	11:00	**HAB Impacts to USACE Jacksonville District** *COL James Booth,* *Commander USACE Jacksonville District*
11:15	**Workshop Goals - USACE HAB R&D Portfolio** *Dr. Jen Selter-Moser, ERDC Technical Director* *Dr. Mandy Michalsen, ERDC Strategic Initiatives PgM*	11:15	**Great Lakes Region HAB Perspectives** *Ms. Joy Mullinex, Executive Director* *Ohio Lake Erie Commission*	11:15	**Lake O HAB Nowcast/Forecast** *Dr. Richard Stumpf, NOAA-NCCOS*
11:35	**HAB Impacts to USACE Operations** *Dr. Tony Clyde, USACE-SWT*	11:35	**NY State Dept. of Env. Conservation** *Ms. Stephanie June*	11:30	**HAB Modeling Tools for Caloosahatchee** *Dr. David Kaplan, University of Florida*
11:45	**NOAA** *Drs. David Kidwell and Kaytee Boyd*	11:45	**Ohio Dept. of Health** *Ms. Rebecca Fugitt*	11:45	**St. Lucie Estuary: Current Monitoring & Tools**
12:00	**Break**	12:00	**Break**	12:00	**Break**
12:15	**USEPA** *Dr. Scot Hagerthey*	12:15	**Ohio Dept. of Natural Resources** *Dr. Janice Kerns*	12:15	**USGS FL-Specific Initiatives** *Dr. Viviana Mazzei*
12:30	**USFDA** *Dr. Jon Deeds*	12:30	**Ohio EPA** *Ms. Ruth Briland, Mr. Paul Gledhill*	12:30	**HAB Aerosolization-Related Research** *Dr. Lorrie Backer, CDC; Dr. Elizabeth Hilborn USEPA*
12:45	**Lunch**	12:45	**Lunch**	12:45	**Lunch**
2:30 - 3:30	**USGS** **Dr. Jennifer Graham and Team** *Next Generation Water Observing System and Illinois River Basin Study* **Ms. Lisa Fogarty** *Environmental Health*	2:30 - 3:30	**USDA** **Dr. Peter Kleinman, USDA-ARS** *Legacy Phosphorus Assessment* **Drs. Jason Taylor and Richard Lizotte, USDA-ARS** *Utilizing watershed monitoring and field experiments to assess linkages between ag BMPs, nutrients, and HABs*	2:15	**FL Atlantic University** *Drs. Malcolm MacFarland, Jordon Beckler*
				2:25	**FL Gulf Coast University** *Dr. Barry Rosen*
				2:35	**FL International University** *Dr. Tom Frankovich*
				2:45	**University of Florida** *Dr. Jango Bhadha, EAA CEAP Watershed Assessment Study*
				3:00	**FL Dept. of Env. Protection** *Dr. Mark Rains*
				3:15	**South FL Water Mgt. District** *Dr. Anna Wachnicka and Team*
3:30	**Break**	3:30	**Break**	3:30	**Break**
3:45	*Discussion Ice Breaker*	3:45	*Discussion Ice Breaker*	3:45	**University of South Florida** *Dr. Zhang, Lead EPA Grant 840090*
				4:15	**Jacksonville District USACE Support** *Mr. Jim Riley, USACE-SAJ*
3:50 - 5:00	*Roundtable #1*	3:50 - 5:00	*Roundtable #2*	4:25 - 5:00	*Charting Path Forward Together*

Presentations requested to include: (1) HAB R&D priorities, objectives within participant's agency-specific "mission space"; (2) HAB R&D updates, including new accomplishments, opportunities, initiatives in past year; (3) HAB R&D gaps or gaps in USACE HAB R&D portfolio (link below); (4) Gaps/needs and opportunities to work together in FY22 and beyond

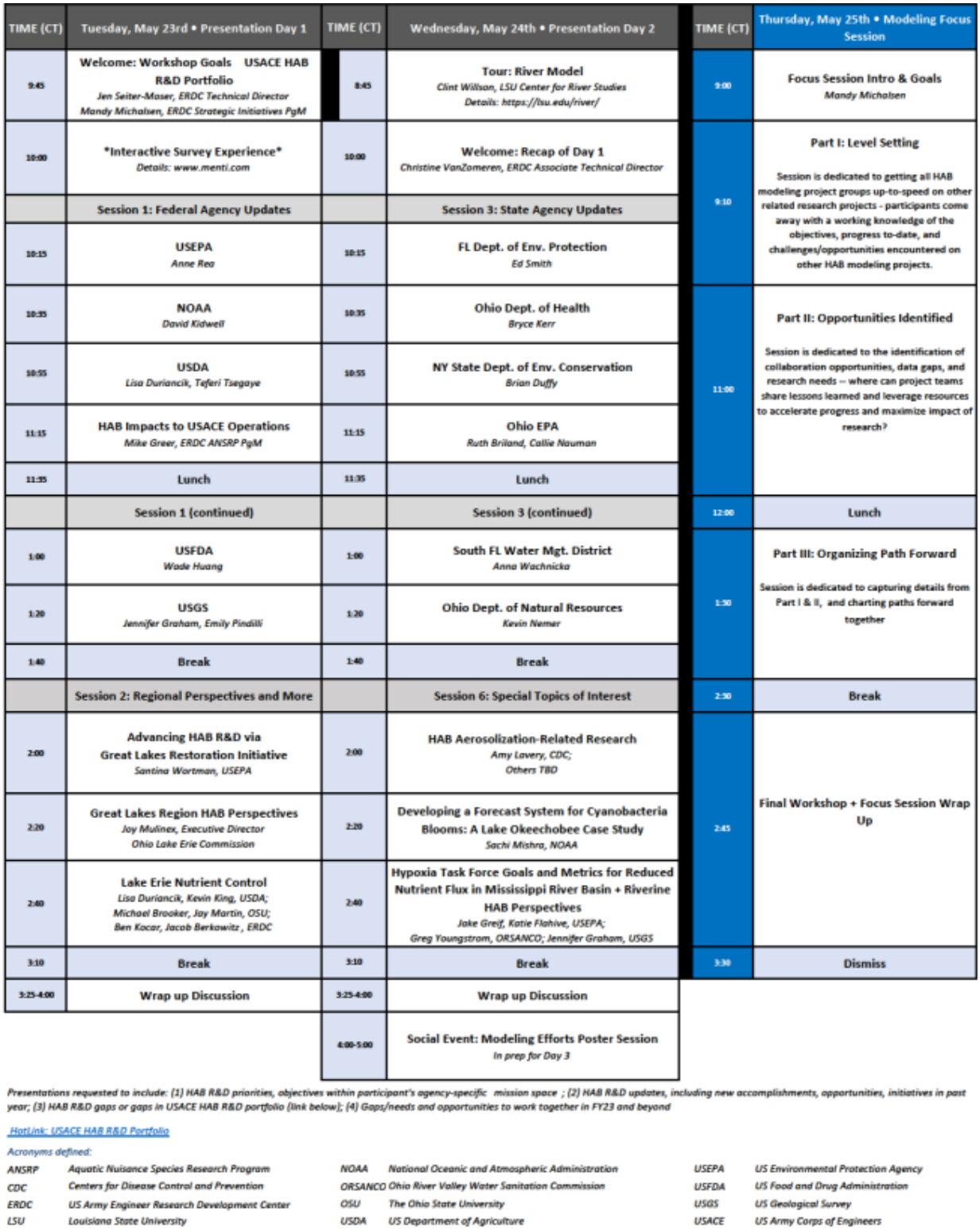
Hotlink: USACE HAB R&D Portfolio

Acronyms and shading defined:

Pending invited speaker finalization

ERDC	*U.S. Army Engineer Research Development Center*
NOAA	*National Oceanic and Atmospheric Administration*
NCCOS	*National Centers for Coastal Ocean Science*
NYSDEC	*New York State Department of Environmental Conservation*
ODH	*Ohio Department of Health*
Ohio DNR	*Ohio Department of Natural Resources*
USDA	*US Department of Agriculture*
USEPA	*US Environmental Protection Agency*
USFDA	*US Food and Drug Administration*
USGS	*US Geological Survey*
USACE	*US Army Corps of Engineers*

Figure 2. Agenda from the Interagency Freshwater HAB R&D Workshop, hosted virtually via WebEx and in Clewiston, Florida, March 29–31, 2022.



TIME (CT)	Tuesday, May 23rd • Presentation Day 1
9:45	**Welcome: Workshop Goals USACE HAB R&D Portfolio** *Jen Seiter-Moser, ERDC Technical Director* *Mandy Michalsen, ERDC Strategic Initiatives PgM*
10:00	***Interactive Survey Experience*** *Details: www.menti.com*
	Session 1: Federal Agency Updates
10:15	**USEPA** *Anne Rea*
10:35	**NOAA** *David Kidwell*
10:55	**USDA** *Lisa Duriancik, Teferi Tsegaye*
11:15	**HAB Impacts to USACE Operations** *Mike Greer, ERDC ANSRP PgM*
11:55	**Lunch**
	Session 1 (continued)
1:00	**USFDA** *Wade Huang*
1:20	**USGS** *Jennifer Graham, Emily Pindilli*
1:40	**Break**
	Session 2: Regional Perspectives and More
2:00	**Advancing HAB R&D via Great Lakes Restoration Initiative** *Santina Wortman, USEPA*
2:20	**Great Lakes Region HAB Perspectives** *Joy Mulinex, Executive Director* *Ohio Lake Erie Commission*
2:40	**Lake Erie Nutrient Control** *Lisa Duriancik, Kevin King, USDA;* *Michael Brooker, Jay Martin, OSU;* *Ben Kocar, Jacob Berkowitz, ERDC*
3:10	**Break**
3:25-4:00	**Wrap up Discussion**

TIME (CT)	Wednesday, May 24th • Presentation Day 2
8:45	**Tour: River Model** *Clint Willson, LSU Center for River Studies* *Details: https://lsu.edu/river/*
10:00	**Welcome: Recap of Day 1** *Christine VanZomeren, ERDC Associate Technical Director*
	Session 3: State Agency Updates
10:15	**FL Dept. of Env. Protection** *Ed Smith*
10:35	**Ohio Dept. of Health** *Bryce Kerr*
10:55	**NY State Dept. of Env. Conservation** *Brian Duffy*
11:15	**Ohio EPA** *Ruth Briland, Callie Nauman*
11:35	**Lunch**
	Session 3 (continued)
1:00	**South FL Water Mgt. District** *Anna Wachnicka*
1:20	**Ohio Dept. of Natural Resources** *Kevin Nemer*
1:40	**Break**
	Session 6: Special Topics of Interest
2:00	**HAB Aerosolization-Related Research** *Amy Lavery, CDC;* *Others TBD*
2:20	**Developing a Forecast System for Cyanobacteria Blooms: A Lake Okeechobee Case Study** *Sachi Mishra, NOAA*
2:40	**Hypoxia Task Force Goals and Metrics for Reduced Nutrient Flux in Mississippi River Basin + Riverine HAB Perspectives** *Jake Greif, Katie Flahive, USEPA;* *Greg Youngstrom, ORSANCO; Jennifer Graham, USGS*
3:10	**Break**
3:25-4:00	**Wrap up Discussion**
4:00-5:00	**Social Event: Modeling Efforts Poster Session** *In prep for Day 3*

TIME (CT)	Thursday, May 25th • Modeling Focus Session
9:00	**Focus Session Intro & Goals** *Mandy Michalsen*
9:10	**Part I: Level Setting** Session is dedicated to getting all HAB modeling project groups up-to-speed on other related research projects - participants come away with a working knowledge of the objectives, progress to-date, and challenges/opportunities encountered on other HAB modeling projects.
11:00	**Part II: Opportunities Identified** Session is dedicated to the identification of collaboration opportunities, data gaps, and research needs -- where can project teams share lessons learned and leverage resources to accelerate progress and maximize impact of research?
12:00	**Lunch**
1:30	**Part III: Organizing Path Forward** Session is dedicated to capturing details from Part I & II, and charting paths forward together
2:30	**Break**
2:45	**Final Workshop + Focus Session Wrap Up**
3:30	**Dismiss**

Presentations requested to include: (1) HAB R&D priorities, objectives within participant's agency-specific mission space ; (2) HAB R&D updates, including new accomplishments, opportunities, initiatives in past year; (3) HAB R&D gaps or gaps in USACE HAB R&D portfolio (link below); (4) Gaps/needs and opportunities to work together in FY23 and beyond

HotLink: USACE HAB R&D Portfolio

Acronyms defined:

ANSRP	Aquatic Nuisance Species Research Program	NOAA	National Oceanic and Atmospheric Administration	USEPA	US Environmental Protection Agency
CDC	Centers for Disease Control and Prevention	ORSANCO	Ohio River Valley Water Sanitation Commission	USFDA	US Food and Drug Administration
ERDC	US Army Engineer Research Development Center	OSU	The Ohio State University	USGS	US Geological Survey
LSU	Louisiana State University	USDA	US Department of Agriculture	USACE	US Army Corps of Engineers

Figure 3. Agenda from the 3rd Interagency Freshwater HAB R&D Workshop, hosted virtually via WebEx and in Baton Rouge, Louisiana, May 23–25, 2022.

TIME (CT)	Monday, April 19th
12:00	**Welcome/Opening Remarks** HQUSACE
12:05	**Meeting Goals and Objectives** Dr. Jennifer Seiter-Moser, ERDC ENV CW Acting Technical Director
12:10	**Overview of HAB Impacts to USACE Operations** Dr. Tony Clyde, SWT
12:25	**USACE HAB R&D Portfolio Overview** Dr. Mandy Michalsen, ERDC HAB Program Coordinator
12:40	**WRDA 2020 Update** Ms. Mindy Simmons, HQUSACE Senior Policy Advisor
12:50	**Q/A**
13:00	**USACE HAB Field R&D Perspectives** SAJ - Jim Riley
13:10	LRD Thad Tuggle (LRH)
13:20	LRD Zach Wolf (LRH)
13:30	LRD Rose Reilly (LRP)
13:40	NAD Alicia Palmer/Greg Wacik (NAB)
14:00	**BREAK**
14:15	MVD (Upper 3 Districts) Nicole Manasco (MVR)
14:25	SPD Justin Reale (SPA) / Erich Emery (LRD)
14:35	NWD Tina Lundell (NWP)
14:45	NWD Marvin Boyer (NWK)
14:55	NWD Eric Zimdars (NWS)/ Tony Clyde (SWT)
15:05	SWD Tony Clyde (SWT)
15:15 - 16:00	**Discussion/Q&A** Facilitated by: Mandy Michalsen (ERDC) and Mike Greer (LRB)

Acronyms: HQUSACE, Headquarters U.S. Army Corps of Engineers; ERDC ENV CW, U.S. Army Engineer Research Development Center, Office of the Technical Director, Civil Works Environmental Engineering and Sciences; SWT, Tulsa District USACE;SAJ, Jacksonville District USACE; LRD, Great Lakes and Ohio River Division USACE; LRH, Huntington District USACE; LRP, Pittsburgh District USACE; MVD, Mississippi Valley Division; SPD, South Pacific Division USACE; SPA, Albuquerque District USACE; NWP, Portland District USACE; NWD, Northwest Division USACE; NWK, Kansas City District USACE; NWS, Seattle District USACE; LRB, Buffalo District USACE

Figure 4. Agenda from the USACE HAB R&D Listening Session, hosted virtually via WebEx April 19, 2021.

TIME (CT)	Monday, March 21st
12:00	**Welcome/Opening Remarks: Meeting Goals and Objectives** Dr. Jennifer Seiter-Moser, ERDC ENV CW Technical Director
12:05	**Overview of HAB Impacts to USACE Operations** Dr. Tony Clyde, SWT
12:10	**USACE HAB R&D Updates** Dr. Mandy Michalsen, ERDC HAB Program Coordinator
12:25	**WRDA 2020 Update** Speaker TBD
12:40	**USACE HAB Field R&D Perspectives: Updates from 2021 HAB Season** SAJ - Jim Riley
12:50	LRD Thad Tuggle (LRH)
13:00	LRD Zach Wolf (LRL)
13:10	LRD Carl Nim (LRP)
13:20	NAD Alicia Palmer (NAB)/Greg Wacik (NAP)
13:30	**BREAK**
13:40	MVD (Upper 3 Districts) Nicole Manasco (MVR)
14:00	SPD Justin Reale (SPA)
14:15	NWD Sarah Burnet (NWP)
14:25	NWD Marvin Boyer (NWK)
14:35	NWD Eric Zimdars (NWS)
14:45	SWD Tony Clyde (SWT)
14:55	**Discussion/Q&A** Facilitated by: Tony Clyde (SWT), Erich Emery (LRD), and Mandy Michalsen (ERDC)

Acronyms: HQUSACE, Headquarters U.S. Army Corps of Engineers; ERDC ENV CW, U.S. Army Engineer Research Development Center, Office of the Technical Director, Civil Works Environmental Engineering and Sciences; SWT, Tulsa District USACE;SAJ, Jacksonville District USACE; LRD, Great Lakes and Ohio River Division USACE; LRH, Huntington District USACE; LRP, Pittsburgh District USACE; MVD, Mississippi Valley Division; SPD, South Pacific Division USACE; SPA, Albuquerque District USACE; NWP, Portland District USACE; NWD, Northwest Division USACE; NWK, Kansas City District USACE; NWS, Seattle District USACE; LRB, Buffalo District USACE

Figure 5. Agenda from the USACE HAB R&D Listening Session, hosted virtually via WebEx March 21, 2022.

TIME (CT)	Monday, February 27th
12:00	**Welcome/Opening Remarks: Meeting Goals and Objectives** Dr. Jennifer Seiter-Moser, ERDC ENV CW Technical Director
12:05	**USACE and HABs – a National Perspective** USACE Policy (Erich Emery), Great Lakes (Jim Luke), and Florida Engagements (Matthew Parrish)
12:20	**USACE HAB R&D, WRDA 2022 Updates** Dr. Mandy Michalsen, ERDC Lead PgrM for HABs
12:30	**USACE HAB Field R&D Perspectives: Updates from 2022 HAB Season** SPD Justin Reale (SPA)
12:40	LRD Thad Tuggle (LRH)
12:50	LRD Kristin Berger (LRL)
13:00	**BREAK**
13:15	LRD Andi Fitzgibbon (LRP)
13:25	NAD Brianna Treichler (NAP)
13:35	MVD (Upper 3 Districts) Nicole Manasco (MVR)
13:50	SAD Jim Riley (SAJ)
14:00	NWD Sarah Burnet (NWP)
14:10	NWD Marvin Boyer (NWK)
14:20	NWD Eric Zimdars (NWS)
14:30	SWD Chris Gilliland (SWT)

Acronyms: HQUSACE, Headquarters U.S. Army Corps of Engineers; ERDC ENV CW, U.S. Army Engineer Research Development Center, Office of the Technical Director, Civil Works Environmental Engineering and Sciences; SWT, Tulsa District USACE;SAJ, Jacksonville District USACE; LRD, Great Lakes and Ohio River Division USACE; LRH, Huntington District USACE; LRP, Pittsburgh District USACE; MVD, Mississippi Valley Division; SPD, South Pacific Division USACE; SPA, Albuquerque District USACE; NWP, Portland District USACE; NWD, Northwest Division USACE; NWK, Kansas City District USACE; NWS, Seattle District USACE; LRB, Buffalo District USACE

Figure 6. Agenda from the 3rd USACE HAB R&D Listening Session, hosted virtually via WebEx February 27, 2023.

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14. ABSTRACT

Harmful Algal Blooms (HABs) represent a significant and costly threat to our nation's economy and natural resources. This report outlines the US Army Corps of Engineers, Engineer Research and Development Center's (USACE-ERDC's) approach to deliver scalable technologies for prevention, early detection, and management of HABs to reduce HAB event frequency, severity, and duration.

15. SUBJECT TERMS

Algal blooms, Cyanobacterial blooms, Engineer Research and Development Center (U.S.)--Research, Environmental management, Technology--Development, Water quality

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