# A. Project Management

## A1. Title and Approval Sheet

**U.S. Environmental Protection Agency**

**Office of Research and Development**

**Center for Environmental Measurement and Modeling**

***Atlantic Coastal Environmental Sciences Division***

***Watershed and Estuarine Diagnostics Branch***

**Quality Assurance Project Plan**

**Title:** High resolution spatial and temporal dynamics of freshwater cyanobacteria HABs

**QA Category:**  A B

**ORD National Program Project/Task ID:** Click here to enter text.

**QAPP was Developed:**  Intramurally  Extramurally: Click here to enter text.

**QAPP Accessibility:** QAPPs will be made internally accessible via the [ORD QAPP intranet site](https://intranet.ord.epa.gov/quality-assurance/qapps?combine=&field_qapp_project_lead_value=&title=&field_lab_value=cemm&field_qapp_project_type_value=&field_division_value=) upon final approval *unless the following statement is selected*.

I do NOT want this QAPP internally shared and accessible on the ORD intranet site.

**Project Type(s) (check all that apply):**

Environmental Measurements  Environmental Technology  Decision Support Tool  Existing Data  Informatics Geospatial  Method Development  Model Application  Model Development

Software and Data Management Remote Sensing  Technical Assessment  Other

**­­­­­­­­­­­­­­­­­­­­­­­­­­**

**Approvals**

**Prepared by:**

Stephen Shivers

*Signature Date*

**Branch Chief:**

Betty Kreakie

*Signature Date*

**QA Manager:**

Joseph Livolsi

*Signature Date*

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| **QAPP Revision History** | | | |
| **QAPP ID Number** | **Prepared By** | **Date of Revision** | **Description of Change** |
|  |  |  |  |
|  |  |  |  |

## A3. Distribution List

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Unsure who to add here

## A4. Project Organization

|  |  |  |  |  |
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## A5. Problem Definition and Background

Cyanobacteria and other harmful algae have been established as a recurring and persistent threat to human health and the environment. These aquatic communities vary across and within regions depending on many site conditions. Therefore, providing information to local lake managers and recreational users of waterbodies is important in protecting human health from the threat of cyanobacterial blooms and high bacteria levels. To accomplish this, there is a need to understand the relationship between the occurrence of harmful algal blooms and E. coli and water quality parameters, such as dissolved oxygen, pH, conductivity, turbidity, and temperature. Generally, most traditional methods of collecting these data are often cost prohibitive for many agencies, especially state, local, and tribal agencies. However, the possibility exists to collect these data *in situ* with newer low-cost sensors if they can be shown to work as well as the more traditional methods. This proposal aims to evaluate low-cost sensors’ viability as alternative methods for generating this data. A comparison study is planned for co-locating low-cost sensors with traditional methods (*e.g.,* direct measures via sondes and grab samples analyzed in laboratories) at swim beaches in the Kansas City-metro area and in various locations in New England. Comparing results across broad regions with differing bloom characteristics, landscape legacies, and nutrient loads will provide more robust results and help test the performance of these low-cost sensors for many different lake types and settings. If successful, these sensors would provide a cost-effective solution for cyanobacteria bloom monitoring.

## A6. Project/Task Description

To evaluate the efficacy of these low-cost sensors, sensor platforms will be built using off the shelf sensors, Raspberry Pi boards as controllers, and mounted on a floating platform creating a CHEAP buoy. These CHEAP buoys will be deployed at several locations (TBD) throughout MO, KS, RI, and MA. Two CHEAP buoys will be co-located with two research buoys with YSI EXO2 sondes, while the others will be deployed in locations that will be regularly sampled with sondes. This design will allow for comparison of the CHEAP buoy sensors with more expensive research-grade sensors. Ideally, these will be deployed before blooms begin and remain in place through the end of the growing season.

*Study Location(s):*Selected sampling locations in Kansas City, MO; Kansas City, KS; Rhode Island and Massachusetts

*Research Approach:*

1. Identify lakes in the Region 7 study area with swim beaches that may have high likelihood of blooms (relying heavily on the 10 years of local data in Region 7); ease of access and proximity to laboratories are key factors in site selection.
2. Identify lakes in New England for testing relationships between key parameters. Access to lakes, existing data, and proximity to ORD laboratory in Narragansett, RI are key factors in site selection.
3. Identify key parameters for study and determine responsible laboratories for conducting analyses.
4. Establish sampling frequency and determine which low-cost methods will be compared
5. Develop sampling strategy, analytical requests, shipping requirements; purchase sampling supplies and prepare project plan
6. Collect and analyze samples multiple times between Memorial Day and Labor Day (recreational season)
7. Analyze results and prepare reports

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| **2021** | | | | | **2022** | | | |
| **Q1** | | **Q2** | **Q3** | **Q4** | **Q1** | **Q2** | **Q3** | **Q4** |
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|  | **QAPP Preparation** | | | | | | | |
|  | **Data collection (buoys deployed)** | | | | | | | |
|  | **Sample analysis** | | | | | | | |
|  | **Data Analysis** | | | | | | | |
|  | **Report, manuscript, or presentation preparation and submission** | | | | | | | |

## A7. Quality Objectives and Criteria for Measurement Data

The overall quality objective for this project is to generate field data to evaluate the efficacy of low-cost sensors to identify bloom predictors. The quality objectives will be maintained by utilizing appropriate quality control measures in both the lab and the field. Regular calibration of field instruments (See Section B7 for schedule) should minimize error produced by the sondes (see Tables 1 and 2 for measurement range and resolution). Rigorous application of QA/QC policies in EPA SOPs (see Section B4 for analytical methods) will be applied during laboratory procedures to ensure data quality and minimize instrumentation or procedural error.

## A8. Special Training/Certifications

None of the field tasks require special training or certification. Standard training using analyzers at ACESD technicians or others who have expertise with the method. Training will be documented via the ORD competency forms.

## A9. Documents and Records

? will be responsible for maintaining and updating this QAPP. All field equipment calibrations will be logged in the field notebook upon completion. Any deviance that requires recalibration will also be noted. Dates and times of calibration standard creation for lab procedures will be noted in the lab notebook. Results from standard curves will also be added to the lab notebook.

*Field Tasks*

Site observations will be recorded in Rite in the Rain notebooks (see Section A.5 for description of data to be collected). Data will be transferred from buoy storage to EPA server storage, which is backed up regularly (see section B10 for details on data management). This study falls into QA Category B for basic environmental research and will follow records schedule 1035b, records will be held for twenty years after project is completed.

*Laboratory Tasks*

Data output from the Turner Designs Trilogy fluorometer (chlorophyll a analysis) will be transferred to a local laptop, converted to .csv format using R, and stored on EPA server storage (see section B10 for details on data management). All laboratory notes will be handwritten in an EPA approved laboratory notebook. This study falls into QA Category B for basic environmental research and will follow records schedule 1035b, records will be held for twenty years after project is completed.

# B. DATA GENERATION AND ACQUISITION

## B1. Experimental Design

CHEAP buoys will be placed within lakes before the bloom season begins (installed by May 31) to capture baseline data before bloom occurrence. These buoys will remain in place through the end of the bloom season (approximately November/December). A map with buoy coordinates will be added after deployment. Each buoy will have a suite of low-cost sensors (temperature, dissolved oxygen, pH, and turbidity) collecting physical data. Water samples will be collected once per month in triplicate for lab analyses (chlorophyll a, phycocyanin, and microcystin).

## B2. Sampling Methods

Physical parameters will be measured using sensors connected to a Raspberry Pi controller and mounted on a floating platform. The sensor package will be suspended just below the water surface. Temperature, dissolved oxygen, pH, and turbidity will be measured continuously. Data will be stored using onboard storage and will be retrieved periodically. We will explore methods for sending data via cell or satellite communications. A depth profile at 1m increments of physical parameters (temperature, conductivity, dissolved oxygen, and pH) will also be taken at the buoy location using a handheld multiparameter sonde at the time of water collection.

Secchi transparency will be measured using a Secchi disk. The Secchi disk will be lowered on the shaded side of the boat until it disappears and the depth will be recorded in the field notebook (EPA 841-B-11-003). The disk will be lowered 0.5 m below where the disk is no longer visible, raised slowly until it reappears, and the disappearance and reappearance depths will be recorded in the field notebook. To minimize error, the same person will take Secchi disk depth readings at each site.

Water samples will be collected in triplicate from the surface in acid-washed 1 L amber bottles. The bottle shall be triple rinsed with lake water before collecting the final sample. Bottles will be placed in a cooler on ice until returning to the lab.

## B3. Sample Handling and Chain of Custody

Field collection bottles will be clearly labeled at the time of collection with the date and site name. Bottles will be stored in a cooler on ice until returning to the lab to prevent sample degradation caused by heat and/or light. All samples will be filtered and/or stored within 24 hours of collection. Chlorophyll *a* and phycocyanin samples will be filtered using pre-ashed GF/F (0.7 µm) filters.

Chlorophyll *a:* Samples will be filtered under reduced light conditions (no direct sunlight). 400 mL of water will be filtered onto filters for chlorophyll *a* analysis. If chlorophyll *a* concentrations are high causing reduced filtration rates, smaller volumes of water may be filtered. The volume of water that was filtered will be written on the foil packet along with the site name and collection date. Filters will be wrapped in foil and stored frozen (below 0 °C) until extraction. To start extraction, filters will be placed in 15 mL polystyrene tubes (prefilled with 90% acetone). The tubes will be stored in the freezer for a minimum of 12 hours before analysis.

Phycocyanin: An additional 400 mL of water will be filtered onto filters for phycocyanin analysis using the same method as was described for chlorophyll a. Filters will be wrapped in foil and stored in the freezer until extraction. The volume of water that was filtered will be written on the foil packet along with the site name and collection date. Filters will be wrapped in foil and stored frozen (below 0 °C) until extraction.

Cyanotoxin: Unfiltered water for cyanotoxin analysis will be distributed into 20 mL glass scintillation vials, frozen (below 0 °C), and held until processing.

Chlorophyll a and phycocyanin will be extracted and analyzed within 60 days of collection.

## B4. Analytical Methods

Chlorophyll a determination will use fluorometric analysis. A known quantity of water will be filtered through 47 mm GF/F filters. Frozen filters will be placed in 15 mL polystyrene tubes (containing 10 mL of 90% acetone) and sonicated in a sonicating water bath for 20 minutes. Determination will proceed following the ACESD SOP for non-acid determination of chlorophyll a using a Turner Designs Trilogy fluorometer (J-ACESD-MAB-SOP-1425-0, Non-Acid Determination of Chlorophyll a Using a Turner Designs Trilogy Fluorometer).

Phycocyanin determination will use fluorometric analysis. A known quantity of water will be filtered through 47 mm GF/F filters. Frozen filters will be placed in 30 mL centrifuge tubes containing 20 mL of 50 mM phosphate buffer and sonicated in a sonicating water bath for 15 minutes under reduced light. The samples will be refrigerated for 2 hours then placed in a dark storage cabinet to warm to room temperature (total extract time of 3 hours). The samples will be analyzed for phycocyanin using a fluorometer fitted with a phycocyanin module (Orange) based on Kasinak et al 2015 and will follow J-ACESD-MAB-SOP-3949-0, Determination of Phycocyanin Using a Turner Designs Trilogy Fluorometer.

Cyanotoxin determination will use enzyme-linked immunosorbent assays (ELISA). Unfiltered water samples in 20 mL glass scintillation vials will undergo a freeze thaw cycle three times. After the third cycle, water will be filtered using a 25mm glass fiber syringe filter (1.2 μm) and transferred to a new glass scintillation vial. The assays will proceed according to kit manufacturer instructions and EPA guidelines (EPA Method 546 and EPA 841-B-11-004).

## B5. Quality Control

Field blanks will be filtered for all fluorometric analyses. QC checks, such as spikes and duplicates, are integral to ensuring data integrity and will be used whenever possible. QC checks are method dependent and are discussed in detail in the methods listed in the appendix.

## B6/B7. Instrument/Equipment Calibration, Testing, Inspection, Maintenance

All analytical equipment (Astoria-Pacific segmented flow autoanalyzer, fluorometer, and microplate reader), sondes, pipettes, and balances are maintained in accordance with manufacturer standards by ACESD. The Secchi disk will be inspected for proper rope attachment before each use.

Instrument calibration is critical for ensuring data quality and will be performed frequently. The handheld sondes will be calibrated before each sampling trip. Fluorometer accuracy will be assessed before each sample run using a secondary solid calibration standard. Other analytical equipment will be calibrated before each sample run by the designated operator at ACESD.

## B8. Inspection/Acceptance of Supplies and Consumables

All research team members are responsible for ensuring all necessary supplies and consumables (i.e. pH buffers and conductivity standards) are available when needed.

## B9. Non-direct Measurements

Will be updated once lakes are chosen.

## B10. Data Management

All field data will be recorded on Rite-In-The-Rain paper to prevent reduced legibility from contact with water. All handwritten data will be transferred approximately weekly to the database. Data from analytical equipment will be transferred to the database shortly after procedure completion. Data for this task is to be under version control (e.g. via git) and will be stored both locally on team members OneDrive and remotely on GitHub. Access to the database will be available for all project collaborators via GitHub.

As this project will combine data from multiple field sensors, lab instruments, and hand written notes, great care will need to be taken in merging the data into an analytical dataset. The dataset itself can be fairly simply constructed as a flat .csv file. Raw data from laboratory instruments (immediately following procedure completion) and files from field sensors with data loggers (weekly) will be downloaded as raw files into the version controlled repository. Manual data entry will be conducted via an Excel front-end with initial quality control measures applied to those fields (e.g. throwing an error if water temp is not between 0-100 degrees Celsius). Data aggregation for all sources will be scripted and automated as much as is feasible.

Code for this project will be developed following standard best practices which include full documentation, code review, and use of a version control system (i.e. git). Collaboration on code development will be facilitated via GitHub.

R will be the primary analytical language; however we will explore others (e.g. python , javascript, c++, etc.) as required. The computational work for this project relies on open source software, and versions of most open source software packages change often. Thus, specifying these *a priori* is not recommended as versions will change. To ensure reproducibility of our work we will include specifications of software and operating system details (e.g. versions of R, packages, and operating system) for all research products such that others can recreate the computational environment used for our analyses.

Lastly, all code, data, and documents will be managed as a research compendium (e.g. Marwick et al. 2018, <https://doi.org/10.1080/00031305.2017.1375986>). The compendium will be available via GitHub, archived on Zenodo, and will follow standard for research compendia written in the R language. A final README file will outline the file and directory structure and will be completed upon completion of the project.

# C. ASSESSMENTS AND OVERSIGHT

## C1. Assessments and Response Actions

Describe any audits or assessments that will be done during the project. Will readiness reviews be done prior to sample collection or analysis? Will proficiency testing take place? Do field activities need to be audited after training? Describe corrective action procedures should audits reveal a deficiency (e.g., retraining of lab technicians).

If no additional audits are needed, simply defer to the CEMM QA audit program. The QA manager assigned to this project may determine that a project-specific audit is needed depending on the visibility of the project and may add this info to the QAPP when reviewing it.

The project co-leads (Henderson and Hollister) will be responsible for overall oversight of the project. They will also initiate action in response to QA/QC issues. This research project falls into QA Category B. Assessments are not required but may occur at the discretion of management and/or QA staff, in which case they will be discussed, scheduled, and conducted at the convenience of QA manager and the project staff.

## C2. Reports to Management

Describe the way management will be kept informed regarding the progress of the project including any assessment activities. Identify the type of progress reports that might be written, the frequency, and who reports will be delivered. Specify who is responsible for preparing and distributing the reports.

Annual reports will be provided to management, if requested, as a measure of accountability and a barometer of project success.

# D. DATA VALIDATION AND USABILITY

## D1/D2. Data Review, Verification, and Validation/Verification and Validation Methods

Describe how the data will be reviewed for completeness (including sample metadata), accuracy (as with transcription or transformation errors), and conformance to method specifications. Describe how you will reject or accept data. List any data qualifiers that will be reported with the data. Data validation should include an assessment of the data and its quality relative to the end use. Describe data verification and validation methods, including software to be used in verification or validation.

All data produced by analytical equipment will be reviewed for issues upon output. All handwritten data will be inspected and reviewed for issues created when transferring from notebook to database.

The inclusion of spikes and duplicates during analyte determination will validate data quality. All analytical output will be reviewed to ensure that QC checks are within the tolerances established in the corresponding methodologies. All manually entered data will be inspected for potential problems (e.g. transpositions).

## D3. Analysis and Reconciliation with User Requirements

Any analytical output that exceeds method tolerances will be rerun on a batch scale and reviewed again upon completion. Any errors found in manually entered data will be verified against the original handwritten data logs and corrected as needed.

Describe what types of statistical analyses may be applied. State if a statistician was consulted. (Planning for the types of statistical analyses helps inform the experimental design.)

Direct from [EPA QA/G-5](https://www.epa.gov/sites/production/files/2015-06/documents/g5-final.pdf): *This element is to describe how you will evaluate the*

*validated data to see if it answers the original questions asked, i.e., the measurement quality objectives or data quality objectives.* *Describe how data will be presented, e.g., tables or charts, to illustrate trends, relationships, and anomalies. Discuss how limitations on the use of the data will be handled and reported to the decision makers.*

# E. References

Kasinak, J-M, B. Holt, M. Chislock, and A. Wilson. 2015. Benchtop Fluorometry of Phycocyanin as a Rapid Approach for Estimating Cyanobacterial Biovolume. Journal of Plankton Research 37: 248-257.

USEPA. 1993. Method 350.1 Determination of Ammonia Nitrogen by Semi-Automated Colorimetry. U.S. Environmental Protection Agency, Cincinnati, OH.

USEPA. 1993. Method 353.2 Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry. U.S. Environmental Protection Agency, Cincinnati, OH.

USEPA. 1993. Method 365.1 Determination of Phosphorus by Semi-Automated Colorimetry. U.S. Environmental Protection Agency, Cincinnati, OH.

USEPA. 2011. 2012 National Lakes Assessment, Field Operations Manual. EPA 841-B-11-003. U.S. Environmental Protection Agency, Washington, D.C.

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USEPA. 2013. Nutrient Analysis by the Astoria-Pacific Astoria2 Micro-Segmented Flow Autoanalyzer. Atlantic Ecology Division, Narragansett, RI.

USEPA. 2016. Method 546 Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay. U.S. Environmental Protection Agency, Cincinnati, OH.

USEPA. 2016. Non-Acid Determination of Chlorophyll *a* Using a Turner Designs Trilogy Fluorometer. Atlantic Ecology Division, Narragansett, RI.

**Table 1: YSI EXO 2 sonde probe specifications**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Range | Accuracy | Resolution |
| Temperature | -5-50 °C | ± 0.2 °C | 0.001 °C |
| Dissolved oxygen | 0-50 mg/L | (0-20 mg/L: ± 0.1 mg/L or 1.0% of reading; 20-50 mg/L: ± 5% of reading ) | 0.01 mg/L |
| pH | 0-14 | ± 0.2 | 0.01 |
| Salinity | 0-70 ppt | ± ( 0.2 ppt or 2.0% of reading) | 0.01 ppt |
| Conductivity | 0-100 mS/cm | ± (1.0 % of reading or 0.002 mS/cm) | 0.001 mS/cm |
| Chlorophyll a | 0-100 rfu; 0-400 µg/L | Linearity: r2≥0.999 for Rhodamine WT | 0.01 rfu; 0.01 µg/L |
| Phycocyanin | 0-100 rfu; 0-100 µg/L | Linearity: r2≥0.999 for Rhodamine WT | 0.01 rfu; 0.01 µg/L |
| Turbidity | 0-4000 FNU | 0.3 FNU or ± 2% of reading |  |