# 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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Jeffrey Hollister, ACESD

Betty Kreakie, Branch Chief WEDB, ACESD

Stephen Shivers, ACESD

## A4. Project Organization

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

Anthropogenic influences have contributed to eutrophication of water resources within the United States by introducing nitrogen and phosphorus to those waterbodies. Cyanobacteria are a component of the natural environment but can proliferate and form harmful algal blooms (HABs) under appropriate conditions (e.g. increasing temperature and nutrients). HABs are a particular area of interest for SSWR 4.3.1. Understanding these blooms is important because HABs have the potential to produce toxins, such as microcystin, and to reduce O2 levels upon collapse leading to hypoxia. These effects can negatively affect both human health and the environment. Many studies have been conducted on either spatial or temporal dynamics of HABs, but few have addressed both at high frequency. This project will investigate spatial and temporal dynamics of HABs using high frequency measurements and will address the following objectives/questions:

1. How are HAB dynamics changing over space and time?
   * Which algal indicators (i.e. chlorophyll a and phycocyanin) best describe these dynamics?
   * Does land use affect bloom dynamics?
2. Are toxins produced in the blooms?
   * Do toxins vary spatially and temporally?
   * How does toxin production relate to algal indicators?
3. What chemical and physical measurements (i.e. temperature and pH) best predict changes in HAB formation?
4. How does the cyanobacteria community change over space and time?

## A6. Project/Task Description

To investigate temporal dynamics of HABs, two buoys with sensor packages and weather stations will be deployed to two ponds (Mashapaug Pond and Shubael Pond). Ideally, these will be deployed before blooms begin and remain in place through the end of the growing season. To investigate spatial dynamics of HABs, a flow-through system (FLAMe – developed by University of Wisconsin) will analyze water samples while a boat navigates a predefined path in the ponds. Combining these two high frequency methods will allow for a comprehensive analysis of the spatial and temporal dynamics of HABs.

*Site Description*

Mashapaug Pond is intermediate in size (46.1 hectares), shallow (max depth = 5.2 m), and is the largest freshwater pond in the city of Providence, RI. The watershed drains 4.7 km2 of urban landscape. Mashapaug Pond receives flow from an unnamed tributary connected to Spectacle Pond, ground water, and storm water; the outflow provides flow to the Roger Williams Park pond complex. Mashapaug Pond has a long history of anthropogenic influences extending back to the 1600s. More recently, the Gorham Manufacturing Company operated between 1890 and 1986 adjacent to the pond and contributed to contamination within the pond. HABs occur frequently and persist from summer into the fall.

Shubael Pond is a small kettle pond (22.7 hectares) with a maximum depth of 12.2 m located in Barnstable, Massachusetts. Typical of a kettle pond, Shubael Pond lacks surface water connectivity with groundwater being the primary hydrologic connection. Shubael Pond, historically classified as oligotrophic, has experienced HABs in recent years resulting in pond closures to the public. Converting a block of houses northwest of the pond to improved septic systems, as part of the Nutrients Solution-Driven Research project, may reduce nitrogen inputs into Shubael Pond and reduce the occurrence of HABs. This project will provide supporting data to the Nutrients SDR project.

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| **2021** | | | | | **2022** | | | | **2023** | | | |
| **Q1** | | **Q2** | **Q3** | **Q4** | **Q1** | **Q2** | **Q3** | **Q4** | **Q1** | **Q2** | **Q3** | **Q4** |
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|  | **QAPP Preparation** | | | | | | | | | | | |
|  | **Data collection (buoys and FLAMe deployed)** | | | | | | | | | | | |
|  | **Sample analysis** | | | | | | | | | | | |
|  | **Data Analysis** | | | | | | | | | | | |
|  | **Report/manuscript 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 spatial and temporal dynamics of cyanobacterial blooms. 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

Stephen Shivers 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*

Data acquisition in the field using non-data logging sondes and 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 entered and transferred 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*

The Astoria Pacific autoanalyzer (analytical chemistry) and microplate reader (ELISA) will output data in spreadsheet form. (See Section A.5 for description of data to be collected). These spreadsheets will be inspected for potential problems before being stored on EPA server storage. Data output from the Turner Designs Trilogy fluorometer 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

Two buoys will be placed at the deepest point within Mashapaug and Shubael ponds 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 multiparameter sonde collecting physical and biological data (temperature, pH, dissolved oxygen, conductivity, turbidity, chlorophyll a and phycocyanin), as well as a nitrate sensor, collecting data continuously (15 minute intervals). Water samples will be collected once to two times per month (depending on local pond conditions) in triplicate for lab analyses (zooplankton and phytoplankton identification, chlorophyll a, phycocyanin, microcystin, TN, TP, NO3, PO4, and NH4). Concurrent with water sample collection, the FLAMe system will follow a predetermined path and collect continuous spatial data covering the entire pond using the same sensors that are on the buoys.

## B2. Sampling Methods

Physical and biological parameters will be measured using a YSI EXO2 multiparameter sonde. This sonde will be suspended approximately 0.75 m below the water surface. Temperature, conductivity, dissolved oxygen, pH, turbidity, chlorophyll a, and phycocyanin will be measured continuously with the YSI EXO 2 multiparameter sonde. Data will be stored by a data logger 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 YSI ProDSS multiparameter sonde.

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.

Zooplankton collection will follow the procedure of the National Lake Assessment (EPA 841-B-11-003). Two plankton nets (150 µm and 50 µm mesh size) will be towed vertically through the water column at a steady rate (0.3 m/s or 16.7 sec for each tow) at the index site within each lake. Using two different net sizes will allow for collection of different size fractions of zooplankton. A single 5 m tow will be used for each net size (3 m at Mashapaug). After towing, zooplankton will be rinsed into a bucket using a squirt bottle filled with DI water, narcotized using CO2 tablets and transferred to labeled storage containers containing 70% ethanol.

## 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*, phycocyanin, NO3, PO4, and NH4 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.

Nutrient analysis: The water that was filtered will be used for nutrient analysis (NO3, PO4, and NH4) and will be distributed to 20 mL scintillation vials for storage (below 0 °C) until analysis. Unfiltered water for nutrient analysis (TN and TP) will also be distributed to 20 mL scintillation vials for storage (below 0 °C) until analysis, which will occur within 48 hours of digestion.

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.

Zooplankton samples will be stored in pre-labeled containers and stored in 70% ethanol until analysis.

Phytoplankton samples will be fixed on microscope slides as permanent mounts and identified during the off season (SOP in development).

## 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.

Samples will be digested before TN/TP analysis. This digestion will follow ACESD SOP, which is currently in development.

Nutrient (TN, TP, NO3, PO4, and NH4) determination will use segmented flow analysis performed on an Astoria-Pacific Micro-Segmented Flow Autoanalyzer. The following EPA standard methods will be used as guidance:

• Method 350.1 Determination of Ammonia Nitrogen

• Method 353.2 Determination of Nitrate-Nitrite

• Method 365.1 Determination of Phosphorus

These methods have been modified for use by the ACESD laboratory and the ACESD SOP describes a modified procedure (J-ACESD-EMRB-SOP-3076-1, Nutrient Analysis by the Astoria-Pacific Astoria2 Micro-Segmented Flow Autoanalyzer).

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 DO, turbidity, conductivity, and pH sensors will be calibrated weekly with known standards and checked before each sampling trip. The AlgaeTorch is factory calibrated

every two years per manufacturer recommendation and is maintained by Anne Kuhn. Manufacturer calibration is verified by using a calibration test cylinder. Accuracy of the AlgaeTorch will also be verified by correlating sonde output vs. chlorophyll a measured by fluorometry. 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

Mashapaug Pond has been monitored by the University of Rhode Island’s Watershed Watch monitoring program and Shubael Pond has been monitored by the Town of Barnstable in recent years. Data from both programs will be used as historical context and to aid in data interpretation.

## 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 (Shivers 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 |  |