An evaluation of procedures for collection, transport and processing of particulate cyanobacterial samples

RFA 22051 Addendum#1

Dated: April 29, 2022

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This document has been prepared according to the United States Environmental Protection Agency publication *EPA Requirements for Quality Assurance Project Plans* dated March 2001 (QA/R-5).

A PROJECT MANAGEMENT

A1. Approval Sheet

James F. Haney, Project Manager
Emeritus Professor, University of New Hampshire — April 29, 2022 (Date)

Nancy Leland, M.S. Project Quality Assurance Officer
Affiliate Researcher, University of New Hampshire — April 29, 2022 (Date)

Jessica Iverson, Quality Assurance Officer
U.S EPA Region 1 — 5/2/22 (Date)

Adam Reilly, Project Officer
U.S EPA Region 1 — 5/2/22 (Date)

QAPP Update Log

Prepared/Revised By:	Date:	Revision No:	Summary of Changes:
Nancy Leland	4/29/2022	0.1	Collection of particulate cyanobacteria and size fractionation

A.2 Table of Contents

A.3 Distribution List

The following individuals must receive a copy of the approved QAPP in order to complete their role in this project.

Table 1. Distribution list and personnel responsibilities

Name	*Organizational Affiliation*	*Role*	*Contact Information (E-mail address)*
James Haney	UNH-CFB	Project Manager Contract Manager	jim.haney@unh.edu
Nancy Leland	UNH-CFB	Project Quality Assurance Officer	nancy.leland@unh.edu
Sheri Caseau	Martha's Vineyard Commission	Field Sampling Coordinator	caseau@mvcommission.org
Amanda McQuaid	UNH-CFB	Technical Advisor	amanda.mcQuaid@unh.edu
Adam Reilly	US EPA	Project Officer	reilly.adam @epa.gov
Jessica Iverson	US EPA	Quality Assurance Officer	iverson.jessica@epa.gov

A.4 Project Task Organization

James Haney (UNH-CFB) will serve as the Project manager and contract manager. The project manager will review, evaluate and approve the study design and sample site locations, coordinate with other monitoring efforts in the study areas, develop reporting deadlines, and verify completion of all tasks. As contract manager, Jim will authorize payments.

Nancy Leland (UNH-CFB) is the Project Quality Assurance Officer and is responsible for providing technical assistance for the preparation of field sampling and coordination of laboratory activities. The Quality Assurance Officer will be responsible for maintaining the QAPP and for ensuring that personnel have the most current approved version of the QAPP. Prior to conducting any sampling activities, the Quality Assurance Officer shall coordinate with the project team to ensure all mandatory QA protocols are understood. The duties include overseeing the collection and storage of samples, assisting in the implementation of field components, and managing all laboratory activities for the analysis of pigments and cyanotoxins. As contract manager, Nancy will monitor laboratory contract progress and maintain records.

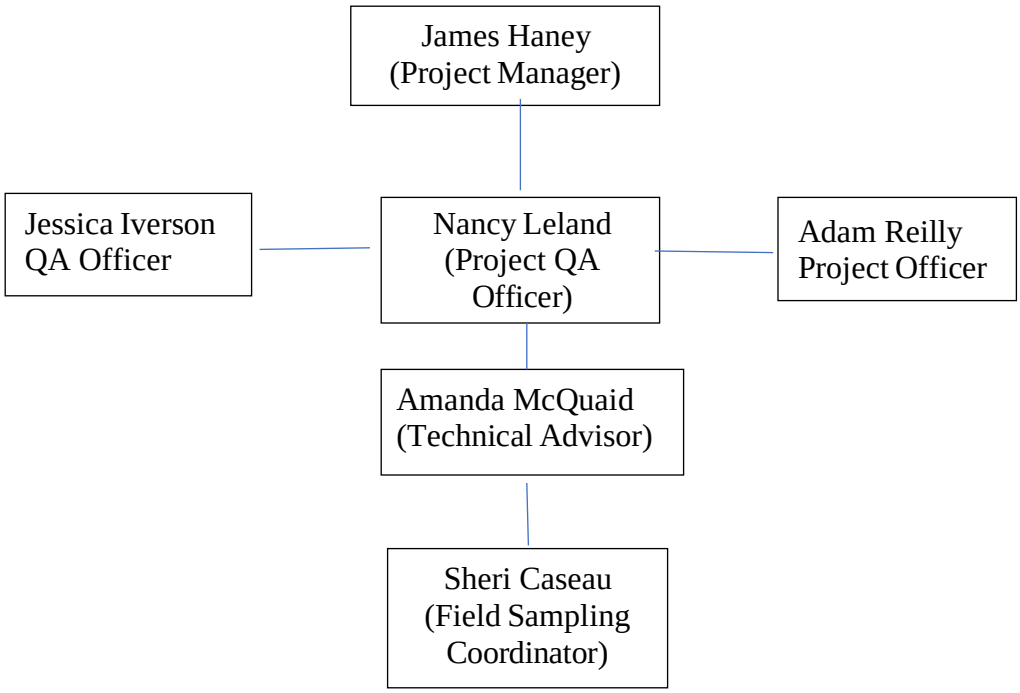
Sheri Caseau (MVC) will coordinate all field sampling efforts for this project. Duties include developing the schedule for the field team, maintaining adequate supplies and equipment, conducting the sampling, and ensuring proper sample preservation and shipment to appropriate laboratories. The Field Sampling Coordinator is responsible for keeping all field records.

Amanda McQuaid (UNH-CFB) will provide an advisory role to provide technical assistance for the preparation and collection of field samples.

Organization chart and responsibilities

Figure 1 shows the organization of staff participating in the "Evaluation of collection, transport and processing of particulate cyanobacterial samples". The project team, responsible for the deliverable items, includes James Haney, Nancy Leland and Sheri Caseau. The parties responsible for playing an advisory role for the project include Amanda McQuaid.

Figure 1. Organizational chart



A.5 Problem Definition/Background

A comprehensive cyanobacteria monitoring program following the Quality Assurance Program Plan (CMC-QAPP) for the Cyanobacteria Monitoring Collaborative Program (EPA, 2017) was implemented in 2021 in collaboration with the Martha's Vineyard Commission (MVC), the Martha's Vineyard Shellfish Group (MVSG) and the Wampanoag Tribe of Gay Head (Tribe) to provide an island-wide assessment of cyanobacterial populations. A draft report has been submitted to the MVC documenting the preliminary results of this effort. The findings indicate that the existing CMC-QAPP (EPA, 2017) would be improved with modifications that address the collection, transport and processing of particulate cyanobacterial samples, in particular the use of size-fractionation for the collection of particulate cyanotoxins.

A.6 Project/Task Description

Because a protocol does not currently exist for the collection, transport and processing of particulate cyanobacterial samples, we are proposing a plan for a project verifying the use of these techniques in two aquatic systems within different salinities (Tashmoo Pond and Lagoon Pond). The proposed efforts will complement the collection protocols previously published (Leland et al, 2019) (EPA, 2017, Rev1. June 2021) and contained in the current report (Leland and Haney 2022).

Specifically, the information from this project will be used to address the following questions:

a. Are there benefits to collecting cyanobacterial samples as particulates as compared to the existing (water) method? Examples of benefits could include considerations related to salinity interference, cost savings for sample processing and minimization of data variability.
b. Does this method describe changes in cyanobacterial populations in response to nutrient inputs from stormwater discharges?
c. What information is necessary to evaluate the exposure potential to cyanotoxins from picocyanobacterial populations across a diverse range of aquatic habitats?
d. Can this technique be easily utilized by a broad range of end-users? Examples of this would include volunteer monitoring programs, such as the Wompanoag Tribe and other organizations such as the Martha's Vineyard Commission.

The procedures used for collecting and preparing the field samples will follow the U.S Environmental Protection Agency (2017) Quality Assurance Program Plan (QAPP) for the Cyanobacteria Monitoring Collaborative Program Rev 1, June 22, 2021 with modifications to Section 6.6 Stepwise procedures for sample collection, fractionation, preservation and analysis. Section 6.6 does not include the steps necessary for the collection of any particulate samples (WLW and <50µm) and/or the isolation of additional size fractions for collection as particulates (5µm) and/or filtrates (10µm, 5µm and 0.2µm). Samples (Table 2) will be collected at the proposed sampling sites (Figure 2) according to the project schedule (Table 3).

All water samples will be size fractionated into prelabeled sample containers following collection and appropriate serial filtration. Size fractions to be collected for particulate cyanotoxin analysis include 0.2µm – WLW, 0.2µm - 50µm, 0.2µm– 5.0µm, using 50µm nitex mesh, 10 µm nitex mesh, 5µm nitex mesh and 25mm glass-fiber filters (prefiltered, precombusted for 0.3-µm effective pore size), respectively. Size fractions to be collected as a filtrate includes <10µm, <5.0µm and <0.2µm (otherwise referred to as the dissolved sample). A volume of WLW will be used for each type of sample. The 0.2µm – WLW particulate sample will be collected by placing a recorded volume of WLW into a clean, plastic syringe fitted with a Swinnex filter holder passing through a prefiltered/precombusted 0.7 µm GFF 25mm filter until the filter has clogged. The filtered volume will be recorded. A final rinse of the particulate material collected on filters will be completed using 10mL of Milli-Q water and 10 mL of air prior to storage.

The <50µm filtrate will be collected using gravity filtration by passing a recorded volume of WLW through a 50µm ring net. The 0.2µm – 50µm particulate sample will be collected

by placing a volume of <50µm filtrate into a clean, plastic syringe fitted with a Swinnex filter holder passing through a prefiltered/precombusted 0.7 µm GFF 25mm filter until the filter has clogged. The filtered volume will be recorded. A final rinse of the particulate material collected on filters will be completed using 10mL of Milli-Q water and 10 mL of air prior to storage. The <10µm filtrate will be collected using gravity filtration by passing a recorded volume of <50µm filtrate through a 10µm ring net. The <5.0µm filtrate will be collected by placing a recorded volume of <10µm filtrate into a clean, plastic syringe fitted with a Swinnex filter holder passing through a 5µm nylon mesh filter. The 0.2µm – 5.0µm particulate sample will be collected by placing a volume of <5µm filtrate into a clean, plastic syringe fitted with a Swinnex filter holder passing through a 0.7 µm GFF 25mm filter until the filter has clogged. The filtered volume will be recorded. A final rinse of the particulate material collected on filters will be completed using 10mls of Milli-Q water and 10 mL of air prior to storage. The Swinnex filter holders (with filters intact) will be placed in Whirl-Pak bags and placed on ice and/or immediately frozen. Sample processing for fluorometric analysis will be conducted by removing each filter from the filter holders, placing in prelabeled microcentrifuge vials and combining with 1.5 mls of Milli-Q water. The sample will be vortexed for 3 minutes, centrifuged for 10 minutes at 10,000 rpm, supernatant removed with a pipette and transferred to a cuvette for fluorometric analysis. Following fluorometric analysis the sample will be returned to the microcentrifuge tube for two additional freeze-thaw-vortex cycles to complete the extraction procedure for cyanotoxin analysis.

Table 2. Proposed project parameters

Parameters	**Analytes**
Water Chemistry	Phycocyanin-particulate and water (extracted), Phycoerythrin-particulate and water (extracted), Microcystin LR-particulate and water, other cyanotoxins-particulate and water
Biological	Zooplankton, Cyanobacteria composition

Table 3. Project Schedule

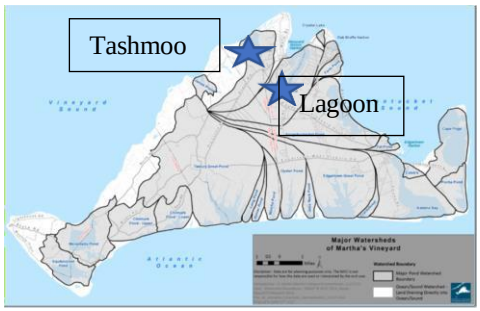
Pre-Implementation

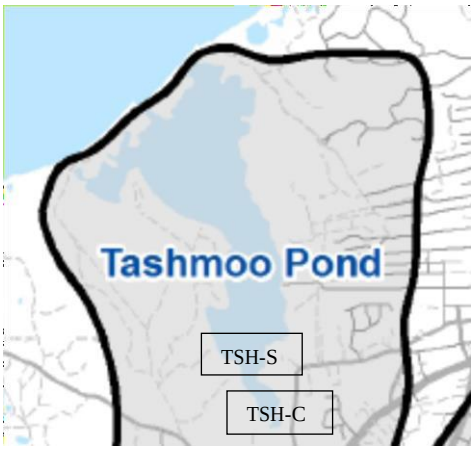
Task	1/1	2/1	3/1	4/1	5/1	6/1	7/1	8/1	9/1	10/1	11/1	12/1	1/1	2/1	3/1	4/1	5/1	6/1	7/1	8/1	9/1
Sample Site ~~Selection~~																					
Field Collection																					
Samples to lab																					
Lab analysis																					
Data entry																					
Data analysis																					

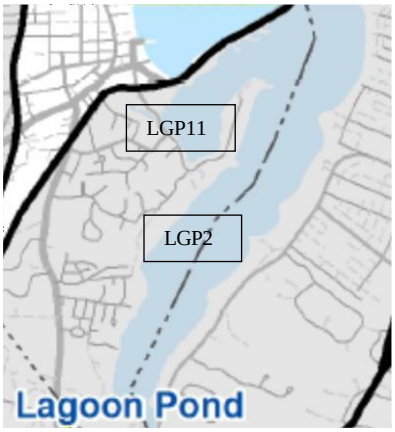
Post-implementation

Task	1/1	2/1	3/1	4/1	5/1	6/1	7/1	8/1	9/1	10/1	11/1	12/1	1/1	2/1	3/1	4/1	5/1	6/1	7/1	8/1	9/1
Installation SCM																					
Field Collection																					
Samples to lab																					
Lab analysis																					
Data entry																					

Figure 2. Proposed sampling sites







A.7 Quality Objectives and Criteria

Data quality requirements including criteria for accuracy and precision for discrete and in situ water chemistry parameters are listed in Tables 4, and for biological parameters in Table 5. The application of the requirements in these tables are described below as well as additional data quality considerations. These data quality requirements are consistent with those used in the U.S Environmental Protection Agency (2017) Quality Assurance Program Plan (QAPP) for the Cyanobacteria Monitoring Collaborative Program Rev 1, June 22, 2021.

Precision is a measure of how repeatable the result of an analysis is. It will be measured by splitting a single sample into two aliquots immediately after collection of the environmental sample. Split samples will be assigned unique sample identification numbers by field staff. This procedure will be completed for each batch of processed water.

Accuracy is a measure of how close the result of analysis is to the accepted and expected result. It will be measured by spiking at least 5% of the samples with a known aliquot of the parameter at the laboratory and carried through the analysis process as a matrix spike. The laboratory will also run the spiked reagent water blank (or Laboratory Control Sample, frequency = minimum 1 per Sample Delivery Group (SDG) to assess any bias in the analytical system. Sensors will be calibrated to the manufacturer's specification.

Representativeness of samples in this study is defined based on representing the conditions in the receiving waters. Samples will be taken from well-mixed samples as collected according to protocols.

Comparability is a measure of how data results can be compared between different sampling events at the same location, how data can be compared between different sampling locations, and how data can be compared to water quality standards. For this study, comparability will be achieved by following consistent sampling protocols (from event to event), sampling at the same locations, and obtaining analytical data following standardized methods for chemical analyses of water.

Completeness is a measure of the number of samples intended to be collected and analyzed compared to the number of samples actually collected and analyzed, expressed as a percentage. For this study ninety-five percent (95%) of samples at a site is the minimum acceptable level of completeness.

Data Quality Objectives

Zooplankton and Cyanobacteria:
The precision DQO is maintained such that the Percent Taxonomic Disagreement (PTD) is 15% or less and Percent Difference in Enumeration (PDE) is 15% or less. Bias is minimized and representativeness is maximized by homogenizing samples prior to subsampling. The comparability DQO is met by following standardized protocols and procedures for the identification and enumeration of zooplankton. Accuracy is achieved through use of standard taxonomic literature and by comparisons of taxa between specialists. No zooplankton or cyanobacterial taxonomic certification exists.

Table 4. Analytical Specifications and QA/QC

Parameter	Analytic Lab	Standard Method	Precision	Accuracy	Calibration	Blanks	Quantitation Limit
Field Measurements							
Temperature	In Situ	2550 B	± 1˚C	± 1.5˚C	N/A		
Dissolved Oxygen		4500-0 G	± 1%	± 6%	Daily	~	
pH		4500-H + B	± 0.05 SU	± 0.2	Weekly	~	
Conductivity		2510 B	± 0.001mS/cm	± 1%	Weekly	~	
Water Chemistry							
Phycocyanin -particulate and water (extracted)	UNH-CFB MVC	EPA (2017) (Fluorometric)		± 25%	~	Every 15 Samples	0.4 ug/L
Phycoerythrin-particulate and water (extracted)		EPA (2017) (Fluorometric)		± 25%	~		0.4 ug/L
Water Chemistry							
Microcystin-particulate and water	UNH-CFB	EPA 546	N/A	N/A	~	Every 15 Samples	0.1 µg/l
Other cyanotoxins-particulate and water	UNH-CFB	Abraxis (520060)					0.15 µg/l

Table 5. Biological Specifications and QA/QC Requirements

Parameter	Method	Lab	Precision	Accuracy	Calibration	Quantitation Limit
Zooplankton abundance	Microscopic Analysis	University of New Hampshire-Center for Freshwater Biology and Ecotoxicology	Percent Taxonomic Disagreement: 15% or less Percent Difference in Enumeration: 15% or less	N/A	N/A	N/A
Cyanobacteria composition	Microscopic Analysis	University of New Hampshire-Center for Freshwater Biology and Ecotoxicology	Percent Taxonomic Disagreement: 15% or less Percent Difference in Enumeration: 15% or less	N/A	N/A	N/A

A.8 Special Training/Certification

Principal Investigators will ensure that laboratory and field staff have the relevant training to fulfill safe and effective sampling.

A.9 Documents and Records

Physical Laboratory Data Sheets and Electronic Laboratory Data Collection
Hard copies of the laboratory data records are to be submitted to the Project Manager. Hand- recorded data records will be taken with indelible ink, and changes to such data records will be made by drawing a single line through the error and initialed by the responsible person. The Project Manager will have ultimate responsibility for any and all changes to records and documents. Similar controls will be put in place for electronic records. Laboratory data will be stored electronically on a secure server with access restricted to the Project Manager only.

Calibration Log Books
A calibration/maintenance log book is kept with each sensor as described by the manufacturer's maintenance and calibration procedures.

Analytical Laboratory Results
Complete data packages are required in order to provide data validation capability. For The PI will review the results and discuss any irregularities with QA staff.

Report format/information
Interim reports will be produced by the Project Manager and team, detailing the results of any laboratory analyses available at the time. All results will be summarized in a final report to be prepared by the Project Manager and team. The final report will include all laboratory QA/QC results including any blanks, lab duplicate analyses, matrix spike and field duplicates analyzed during this study. A summary section on how QA/QC objectives were or were not met will be included in the final report. The final report will include a summary and discussion of analytical results.

Document/record control

The Project Manager is responsible for maintaining updated versions of the QAPP and its distribution. Data will be maintained by University of New Hampshire-Center for Freshwater Biology and Ecotoxicology. Data will be available to EPA for up to three years beyond the completion of the project. The Project Manager shall retain copies of all management reports, memoranda, and all correspondence between EPA and project personnel.

B DATA GENERATION AND ACQUISITION

B1. Experimental Design

- **Experimental Design:** Replicate samples through time
  - Each experimental unit will be measured more than once.
- **Experimental Unit:** An aliquot of sample (extracted particulate, filtrate) measured pre- and post-treatment.
  - Treatment may represent installation of stormwater control measures.
  - Aliquots represent independent samples.
- **Statistic:** Paired t-test ($\alpha = 0.05$)
  - **General Hypothesis Structure:**
    - H0: The mean difference in measurements between pre- and post- treatment does not differ from zero.
    - HA: The mean difference in measurements between pre- and post- treatment differs from zero.
  - **Equivalence Testing Structure:**
    - H0: The mean difference in measurements between pre- and post- treatment do differ from zero.
    - HA: The mean difference in measurements between pre- and post- treatment do not differ from zero.

B2. Analytical Methods and Field Measurements

Monitoring will include measurements of integrated whole lake (WLW) and size fractionated particulate and water samples to obtain data on the following parameters:

1. Cyanotoxins (particulate and water)
2. Cyanobacterial biomass (particulate and water)
3. Cyanobacterial community composition (particulate and water)

Three (3) integrated WLW samples will be collected from the surface water at each of the four (4) sampling sites (Figure), including Lagoon Pond (LGP-2 and LGP-11) and Tashmoo Pond (TSH-Sen and TSH-Spring) for this study during each sampling round. These sites were selected for implementation of stormwater control measures (SCM) by a joint committee consisting of representatives from the UNH-Stormwater Center, Martha's Vineyard Commission and representatives from participating communities. Sampling rounds will occur within two-three (2-3) days following precipitation events that produce runoff, as confirmed by the real-time UV-Vis sensor monitoring data collected by the UNH Stormwater Center. The water collected for the integrated sample will be processed in the laboratory to create samples for each of the parameters listed below. (See Table 6 to Table 8). All samples will be filtered and size fractionated to prepare for proper analyses as described in the Project Task Description. Particulate cyanotoxin samples will be analyzed using enzyme linked immunosorbent assay (ELISA) techniques (Table 4). Cyanobacterial biomass measured as pigments phycocyanin (PC) and phycoerythrin (PC) will be analyzed using fluorometric analysis (Table 4). Cyanobacterial community composition will be analyzed using light microscopy (Table 5).

Table 6. Sample preparation requirements per site

Sample	# of samples	Filter	Volume Required	Container Type	Storage
Filters					
Cyanobacterial toxins	1	50µm nylon mesh: use ring net	Filtered volume	Darkened snap cap, insert into Whirlpak	Frozen
	1	5µm nylon mesh: use syringe	Filtered volume	Darkened snap cap, insert into Whirlpak	Frozen
	1	0.2 µm combusted GF/F filter: use syringe	Filtered volume	Swinnex filter holder, insert into Whirlpak	Frozen
Cyanobacterial biomass	1	50µm nylon mesh: use ring net	5	Darkened HDPE	Refrigeration (6 hour max) and frozen
	1	5µm nylon mesh: use syringe	5	Darkened HDPE	Refrigeration (6 hour max) and frozen
	1	0.2 µm combusted GF/F filter: use syringe	5	Darkened HDPE	Refrigeration (6 hour max) and frozen
Cyanobacterial Composition	1	50µm nylon mesh: use ring net	15	Darkened HDPE	Refrigeration (6 hour max) and formalin
	1	5µm nylon mesh: use syringe	15	Darkened HDPE	Refrigeration (6 hour max) and formalin
	1	0.2 µm combusted GF/F filter: use syringe	15	Darkened HDPE	Refrigeration (6 hour max) and formalin
Unfiltered Whole Lake Water					
Cyanobacterial biomass	1	None required	5	Darkened HDPE	Refrigeration (6 hour max) and frozen
Cyanobacterial Composition	1	None required	15	Darkened HDPE	Refrigeration (6 hour max) and formalin

Table 7. Sample preservation and storage requirements

Parameter	Container Type	Volume Required	Initial Preservation	Maximum Holding Time
Cyanobacterial toxins	Darkened snap cap, insert into Whirlpak	Filtered volume	Frozen	90 days, frozen
	Darkened snap cap, insert into Whirlpak	Filtered volume	Frozen	90 days, frozen
	Swinnex filter holder, insert into Whirlpak	Filtered volume	Frozen	90 days, frozen
Cyanobacterial biomass	Darkened HDPE	5	Refrigeration (6 hr max) and frozen	90 days, frozen
	Darkened HDPE	5	Refrigeration (6 hr max) and frozen	90 days, frozen
	Darkened HDPE	5	Refrigeration (6 hr max) and frozen	90 days, frozen
	Darkened HDPE	5	Refrigeration (6 hr max) and frozen	90 days, frozen
Cyanobacterial Composition	Darkened HDPE	15	Refrigeration (6 hr max) and formalin	90 days, formalin
	Darkened HDPE	15	Refrigeration (6 hr max) and formalin	90 days, formalin
	Darkened HDPE	15	Refrigeration (6 hr max) and formalin	90 days, formalin
	Darkened HDPE	15	Refrigeration (6 hr max) and formalin	90 days, formalin

Table 8. Laboratory analytical methods

Analyte	Laboratory	Method Type	Method Reference	Target Limits
Particulate and water cyanotoxins (Microcystins)	UNH-CFB or Tribe	ELISA	EPA 546	8 µg L-1
Particulate and water cyanotoxins (Anatoxin-a)	UNH-CFB or Tribe	ELISA	Abraxis 520060	No EPA requirement
Cyanobacterial biomass	UNH-CFB or MVC	Fluorescence	CMC-QAPP EPA (2017)	No EPA requirement
Cyanobacterial composition	UNH-CFB or MVC	Light microscope	CMC-QAPP EPA (2017)	No EPA requirement

B.3 Sample Handling and Custody

Sample handling and storage shall follow the CMC QAPP (EPA, 2017). Sample handling and storage for analysis of Microcystin-LR shall follow EPA Method 546. Sample handling and storage for analysis of anatoxin-a shall follow manufacturers recommendations (Abraxis) to include three freeze/thaw cycles for cell lysing. This procedure using the three freeze/thaw cycles will not degrade Anatoxin-a.

B.4. Analytical Methods

The analysis of microcystin-LR will follow EPA Method 546. The analysis for anatoxin-a has been suggested to follow EPA Method 545, however for this study UNH-CFB will use the recommendations for enzyme-linked immunosorbent assay (ELISA) per manufacturers recommendations (Abraxis 520060). This method, although not EPA approved, is a substantially lower cost and is easier to use. The evaluation of the ELISA can be performed using a. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a 4-Parameter standard curve by plotting the %B/B0 for each standard on a vertical linear (y) axis versus the corresponding Anatoxin-a concentration on horizontal logarithmic (x) axis

on graph paper. %B/B0 for the control and samples will then yield levels in ppb of Anatoxin-a by interpolation using the standard curve.

B.5 Quality Control

Quality control for cyanobacterial sample collection and processing for visual and fluorometric analysis shall follow the CMC QAPP (EPA, 2017). Quality control for analysis of Microcystin-LR shall follow EPA Method 546. Quality control for analysis of anatoxin-a shall follow manufacturers recommendations (Abraxis) to include the use of standards (as provided) and blanks.

B6/7. Instrument Equipment Testing, Inspection/Maintenance and Calibration Requirements

All equipment used for cyanobacterial sample collection, processing and analysis shall follow the CMC QAPP (EPA 2017). Instruments used for analysis of cyanotoxins Microcystin-LR and anatoxin-a shall be calibrated at the beginning of each test cycle using the standards provided by the manufacturer.

B.8 Inspection/Acceptance for Supplies and Consumables

Inspection of supplies and consumables must be made upon arrival of new materials and immediately before their use in the field or laboratory. For newly arrived supplies and consumables all materials must be in their original packaging and free of noticeable damages. For materials already obtained and about to be used no noticeable defects will be allowed.

B.9 Non-Direct Measurements

Non-direct measurements are also termed secondary or external data. No data of this type will be used in this project.

B.10 Data Management

Sample information (collection date, time) and field parameter measurements (temperature, dissolved oxygen, pH, conductivity) will be transferred from laboratory notebook to an Excel data sheet by institution staff or downloaded into CSV files from the logging device associated with the field sensors.

Analytic results from water quality laboratories (UNH-CFB or MVC) will be reported in a complete data document (SDG, package) that includes summaries of data validation conducted by the analytic laboratory.

Results from biological laboratories (UNH-CFB) will be reported in Excel spreadsheets with PDF data documents that includes summaries of data validation conducted by the laboratory.

For all labs, any inconsistencies in the data files are flagged for review and correction by the Project Manager. Once the sample collection information (date, time, and parameter) has been verified, the water quality result values are reviewed. Values are compared against assessment criteria, including established parameter-specific limits. If reported values exceed the established limit, the result is flagged for further investigation.

Investigation of laboratory values may result in confirmation of the results by the laboratory, comparison of the value against other results from the same site, inserting an appropriate data qualifier, and/or accepting the value without qualification. Data qualifiers have been established for laboratory values that are known to be suspicious, less than the reported value, or affected by QA/QC equipment blank contamination.

C. ASSESSMENT/OVERSIGHT

Project assessments will be conducted to evaluate the validity of the data collection and analytical activities conducted as part of this program. All laboratory and field staff will be briefed on appropriate project objectives and methods for the special project by the Program and Project Managers in advance of any work.

For laboratories performing analysis on parameters not covered UNH-CFB will conduct internal audits to meet demonstration of competency requirements. Audit reports are retained and available for USEPA review upon request.

C 1. Assessment and Response Actions

Revisions to the Quality Assurance Project Plan are to be approved by the Project Manager who will notify those on the distribution list of the revision.

Major sources of errors may include analytical and equipment problems and those resulting from the deviation from intended plans and procedures. If these problems occur in the laboratory, corrective actions should be taken and noted in the laboratory notebook.

Deviation from intended plans and procedures should be noted by the person observing the deviation and reported to project staff responsible for the operation or analysis in question. The appropriate project personnel shall (1) develop a corrective action plan within 2 hours of observing the deviation to ensure that future sampling, analyses, etc. are conducted in accordance with the QA procedures presented in this QAPP; (2) rerun procedures in the appropriate manner and re- analyze samples, if sufficient sample material is available and holding times are not exceeded; and (3) report all problems and deviations to the Project Manager, who will also be consulted during the development of any proposed corrective action plans. The Project Manager will determine whether the corrective action is sufficient to continue the project. All deviations from intended plans and procedures are to be recorded in the appropriate field or laboratory notes.

C 2. Reports to Management

Data validation reviews will be performed by the Project Manager in their initial review of the data. This evaluation together with the analysis of the completeness, precision, and accuracy of the study will provide a level of confidence to the data set and to the interpretations and conclusions drawn from the data.

The complete data packages provided to the Project Manager by the analytical laboratory will report on analytical methods, sample holding times and laboratory preparation techniques that have deviated from the methods contained in this QAPP.

Interim progress reports, and a project final report will be prepared. Progress reports will note the status of project activities and identify whether any QA problems were encountered (and, if so, how they were handled). The project final report will analyze and interpret data, present observations, draw conclusions, identify data gaps, and describe any limitations in the way the data may be used.

D. DATA REVIEW AND EVALUATION

D1. Data Review, Verification and Validation

This QAPP shall govern the operation of the project at all times. Each responsible party shall adhere to the procedural requirements of the QAPP and ensure that subordinate personnel do likewise. This QAPP shall be reviewed at least annually to ensure that the project will achieve all intended purposes. All the responsible persons shall participate in the review of the QAPP. The Project Manager and the Quality Assurance Officer are responsible for determining that data are of adequate quality to support this project. The project will be modified as directed by the Project Manager. The Project Manager shall be responsible for the implementation of changes to the project and shall document the effective date of all changes made.

It is expected that from time to time ongoing and perhaps unexpected changes will need to be made to the project. The Project Manager shall authorize all changes or deviations in the operation of the project. Any significant changes will be noted in the next progress report and shall be considered an amendment to the QAPP. All verification and validation methods will be noted in the analysis provided in the final project report.

D2. Verification and Validation Methods

Water Chemistry Parameters

Water Chemistry results generated by the analytical laboratories are reviewed at three separate stages. First, analytic laboratory staff will follow specific laboratory protocols to ensure the quality and validity of the data. Second, the Project Manager reviews data results during the input and processing of data files. As previously discussed, this review includes confirmation of suspect values and the possible qualification of data results.

D.3 Evaluating Data in Terms of User Needs Meeting and reporting needs of the project

Uncertainty in the data allowed for use in the experimental end-product will be limited to that found acceptable in the data verification and validation process. After the QC calculations and examinations have been performed for all media, the results will be summarized in a final report. The QA/QC section of the final report will include a discussion and summary of the accuracy, precision, completeness, comparability, and representativeness observed during the study.

Mathematical and statistical methods for hypothesis and efficacy testing:
The hypothesis tests will be conducted using the statistical software SigmaPlot Systat. for the Shapiro-Wilk test, two-sided paired t-test, analysis of variance (ANOVA) and the Wilcoxon Signed Rank test. For equivalence testing, two one-sided t-test will be conducted and the Mann-Whitney Test for equivalence will be conducted.
Normality Test
Samples collected in this study are paired (i.e., dependent pre-treatment and post-treatment samples). The Shapiro-Wilk test will be used to assess if the difference in the paired samples are normally distributed ($\alpha = 0.05$). p-values that are ≥ 0.05 will be considered normally distributed, while p-values < 0.05 will be determined to not follow a normal distribution.
Standard Hypotheses (H0: $\mu = 0$; HA: $\mu \neq 0$)
If the paired differences are normally distributed, a two-side paired t-test will be conducted or an analysis of variance ($\alpha = 0.05$). If the paired differences are not normally distributed, a Wilcoxon Signed Rank test will be conducted ($\alpha = 0.05$).
Equivalence Testing (H0: $\mu \neq 0$; HA: $\mu = 0$)
If the paired differences are normally distributed, a two one-sided t-test will be conducted ($\alpha = 0.05$). If the paired differences are not normally distributed, a Mann-Whitney Test for Equivalence ($\alpha = 0.05$) will be conducted.

Evaluation of user experience:
Interviews with the Project Quality Assurance Officer and Field Coordinator will be conducted throughout the sampling season to determine the usability of the protocol. Of particular interest will be the ease of using pre-packaged filtering devices, documenting filtered volumes and sample preparation for fluorometric analyses. Laboratory sample processing times will be documented to determine if any cost savings have been achieved by the use of this protocol.

Approach to managing unusable data
It is expected that data collected as part of this project will meet the requirements for usability. Data that do not meet requirements for precision, accuracy, completeness or comparability will be carefully evaluated by the Project Manager for deviations from laboratory and accepted paradigms. If warranted these data will be removed from the data set, by the Project Manager, with appropriate comments regarding decision process for r
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