# 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:** Comparison of various fluorometers for the measurement of chlorophyll and phycocyanin

**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*

## A2. Table of Contents

[A. Project Management 1](#_Toc531072403)

[A1. Title and Approval Sheet 1](#_Toc531072404)

[A2. Table of Contents 2](#_Toc531072405)

[A3. Distribution List 4](#_Toc531072406)

[A4. Project Organization 4](#_Toc531072407)

[A5. Problem Definition and Background 4](#_Toc531072408)

[A6. Project/Task Description 5](#_Toc531072409)

[A7. Quality Objectives and Criteria for Measurement Data 6](#_Toc531072410)

[A8. Special Training/Certifications 6](#_Toc531072411)

[A9. Documents and Records 7](#_Toc531072412)

[B. DATA GENERATION AND ACQUISITION 7](#_Toc531072413)

[B1. Experimental Design 7](#_Toc531072414)

[B2. Sampling Methods 8](#_Toc531072415)

[B3. Sample Handling and Chain of Custody 8](#_Toc531072416)

[B4. Analytical Methods 9](#_Toc531072417)

[B5. Quality Control 10](#_Toc531072418)

[B6/B7. Instrument/Equipment Calibration, Testing, Inspection, Maintenance 10](#_Toc531072419)

[B8. Inspection/Acceptance of Supplies and Consumables 11](#_Toc531072420)

[B9. Non-direct Measurements 11](#_Toc531072421)

[B10. Data Management 12](#_Toc531072422)

[C. ASSESSMENTS AND OVERSIGHT 12](#_Toc531072423)

[C1. Assessments and Response Actions 12](#_Toc531072424)

[C2. Reports to Management 13](#_Toc531072425)

[D. DATA VALIDATION AND USABILITY 13](#_Toc531072426)

[D1/D2. Data Review, Verification, and Validation/Verification and Validation Methods 13](#_Toc531072427)

[D3. Analysis and Reconciliation with User Requirements 13](#_Toc531072428)

[E. References 14](#_Toc531072429)

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

## A3. Distribution List

Joe LiVolsi, QA Officer, ACESD

Timothy Gleason, Branch Chief MAB, ACESD

Jeffrey Hollister, ACESD

Betty Kreakie, Branch Chief WEDB, ACESD

Stephen Shivers, ACESD

Sophie Fournier, ACESD, ORISE

## A4. Project Organization

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name and Organization** | **Role** | **Email** | **City,State** | **Phone** |
| Stephen Shivers, ACESD | ORD Project Co-lead | shivers.stephen@epa.gov | Narragansett, RI | (401) 782-9629 |
| Sophie Fournier, ACESD/ORISE | ORISE Project Lead | fournier.sophie@epa.gov | Narragansett, RI | (401) |
| Jeff Hollister, ACESD | ORD Project Member | hollister.jeff@epa.gov | Narragansett, RI | (401) 782-9655 |
| Betty Kreakie, ACESD | ORD Project Member | kreakie.betty@epa.gov | Narragansett, RI | (401) 782-3067 |
| Tim Gleason, ACESD | ORD Collaborator | gleason.tim@epa.gov | Narragansett, RI | (401) 782-3033 |

## A5. Problem Definition and Background

Fluorometers are a key tool for measuring algal pigments in water and are often used to analyze water samples for the presence of cyanobacteria and assess for Harmful Algal Blooms (HABs). There are many fluorometer options available for this, yet little is known about how they compare. The objective of this project is to compare six different fluorometers and assess their precision and accuracy as compared to extracted chlorophyll and phycocyanin.

## A6. Project/Task Description

In this project, six fluorometers will be compared: Turner Trilogy (in vivo module), bbe AlgaeTorch, Turner, FluoroSense, AmiScience FluoroQuick, Turner CyanoFluor, and bbe Phycoprobe. The gold standard for fluorometry based methods of estimating chlorophyll and phycocyanin is extraction and this will be run on a Turner Trilogy using the chla-na (chlorophyll) and orange (phycocyanin) modules. The bbe AlgaeTorch and the Turner FluoroSense are field instruments that measure in vivo chlorophyll and phycocyanin. The Amiscience FluoroQuik and the Turner CyanoFluor are comparable units that quickly and easily measure chlorophyll, phycocyanin and the ratio between them from a small amount of fresh, whole water. The bbe Phycoprobe can be used as a field instrument or in the lab in “workstation mode”. We will be using it in its workstation mode. It quantifies chlorophyll, multiple classes of algae and unbound phycocyanin.

*Sample collection:*

Samples will be collected from a few ponds known to have high levels of cyanobacteria. Three, two liter surface samples will be collected from the edge of each pond. If any surface scum is present, it will be cleared before samples are taken to remove the possibility of differences occurring due to scum being in the samples in differing amounts. At the same time, measurements will be taken with the field instruments, the AlgaeTorch and the FluoroSense. A sonde will be used to collect temperature, dissolved oxygen, conductivity, pH and salinity.

*Sample preparation and analysis:*

Within a day after collection, each of the field samples will be analyzed in triplicate on the FluoroQuik, the Phycoprobe, and the CyanoFluor; samples will also be filtered onto 0.7 µm pre-ashed glass fiber filters, frozen, and stored at -20°C for later extraction and determination on the Trilogy. A filtered sample will be used as a blank on the CyanoFluor to correct for dissolved organic materials interference. Gluteraldehyde will be added to 125mL aliquots of each sample and will be stored in amber glass bottles for creation of permanent mounts. Permanent mounts will be used for cell counts to verify the results of the fluorometers and to look at community dominance. A 10mL aliquot will also be stored in glass scintillation vials at -20°Cfor potential microcystin analysis.

Next, each sample will be frozen for at least 4 hours and then thawed to be analyzed in the same way on each instrument. From these results a comparison between running fresh and frozen samples on all the instruments can be made. In addition, the samples will go through two more freeze/thaw cycles before being run on the FluoroQuik once again. This data will be used to look for increased consistency in analysis and normalization of samples across and within waterbodies after three freeze/thaw cycles.

In order to gain a deeper understanding of the Phycoprobe, a few other variables will be compared. Gluteraldehyde will be added to some samples to determine whether they can be preserved before being run. A time series of fluorescence measurements will be taken to examine degradation due to light exposure. Lastly, any variability due to the sample being stirred while measurements are taken will be observed.

## A7. Quality Objectives and Criteria for Measurement Data

The overall quality objective for this project is to generate data to evaluate the precision and accuracy of 6 commercially available fluormeters used to measure chlorophyll and phycocyanin. The quality objectives will be maintained by utilizing appropriate quality control measures in both the lab and the field. Regular calibration of 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*

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

NEED STUFF HERE

## B2. Sampling Methods

NEED STUFF HERE

## 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.

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

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.

## 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 (LIST FLUOROMETERS HERE), sondes, pipettes, and balances are maintained in accordance with manufacturer standards by ACESD.

Instrument calibration is critical for ensuring data quality and will be performed frequently. 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

NEED STUFF HERE

## 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 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

The project co-leads (Shivers and Fournier) 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

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

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.

USEPA. 2012. 2012 National Lakes Assessment, Laboratory Operations Manual. EPA 841-B-11-004. U.S. Environmental Protection Agency, Washington, D.C.

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 |  |