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 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

STEPHEN SHIVERS Digitally signed by STEPHEN SHIVERS Date: 2021.12.03 13:34:23 -05'00'

Signature *Date*

Branch Chief: Betty Kreakie

BETTY KREAKIE Digitally signed by BETTY KREAKIE Date: 2021.12.06 11:38:34 -05'00'

Signature *Date*

QA Manager: Joseph Livolsi

JOSEPH LIVOLSI Digitally signed by JOSEPH LIVOLSI Date: 2021.12.03 13:22:31 -05'00'

Signature *Date*

A2. Table of Contents

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) 782-9654
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 compared to extracted chlorophyll and phycocyanin.

A6. Project/Task Description

In this project, six fluorometers will be compared to solvent-based extracted methods: Turner Trilogy (*in vivo* chlorophyll module), Turner FluoroSense, Turner CyanoFluor, AmiScience FluoroQuick, bbe AlgaeTorch 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.

Samples from local ponds will be run on each fluorometer before and after being frozen to assess whether a freeze/thaw cycle generates more accurate results.

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 fluorometers 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. All the fluorometers will be calibrated according to the schedule their manuals recommend. Drift from the calibration will be tracked on each fluorometer using secondary standards. 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 on using fluorometers will be given by technicians or others who have expertise with the method. Some of the fluorometers are very simple and require only reading the manual to be used proficiently. Training will be documented via the ORD competency forms.

A9. Documents and Records

Sophie Fournier will be responsible for maintaining and updating this QAPP. All equipment calibrations will be logged in the lab 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 project repository as a csv file.

Field Tasks
Data acquisition in the field using sondes and fluorometers and site observations will be recorded in Rite in the Rain notebooks (see Sections B2 and B4 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). Data collected with the FluoroQuik and the CyanoFluor will be written on printed data sheets (see attachments 1 & 2). 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). Data output from the bbe PhycoProbe will be converted to .csv format 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

Samples will be collected in two batches, once toward the end of the summer and one during the early fall. This is when chlorophyll and phycocyanin will be high. The field instruments (the AlgaeTorch and the FluoroSense) will be used at the same time and place that the samples are taken. The sample water will then be run on each fluorometer before and after being frozen. The samples will be filtered, and the filters will be used for the extracted chlorophyll and phycocyanin method. Permanent mount slides will be created with each sample to determine composition which may be used to examine differences between the fluorometers.

B2. Sampling Methods

Samples will be collected from a few ponds known to have high levels of cyanobacteria (see table 1). Two liter surface samples will be collected in triplicate by wading into each pond and collecting the samples in acid-washed 1 L amber bottles at a depth of at least two feet to avoid sediment. Bottles will be labeled with site (pond name), date, triplicate number, and "Fresh." 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 collected in the samples in differing amounts. Bottles will be placed in a cooler on ice until returning to the lab. At the same time, chlorophyll and phycocyanin will be measured *in vivo* with the field instruments, the AlgaeTorch and the FluoroSense. A sonde will be used to collect temperature, dissolved oxygen, conductivity, pH and salinity. These measurements will be recorded in a dedicated Rite in the Rain notebook. The notebook page will also be labeled with the site and date.

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.

Within 24 hours of collection, each of the field samples will be analyzed in triplicate on the FluoroQuik, the PhycoProbe, the CyanoFluor and the Trilogy (chl-a in vivo module). The

preservative, gluteraldehyde, will be added to 125mL aliquots of each sample and will be stored in amber glass bottles for creation of permanent mounts (SOP in development). These will be labeled with site name, date collected, triplicate number and "Glut." 10mL aliquots will also be stored in glass scintillation vials at -20°C for potential microcystin analysis. These will be labeled with site name, date collected, triplicate number and "microcystin."

Next, each sample will be frozen for at least 4 hours and then thawed to be analyzed in the same way on the FluoroQuik, the PhycoProbe, the CyanoFluor and the Trilogy (chl-a *in vivo* module).

Within 24 hours of collection, samples will also be filtered onto 0.7 µm pre-ashed glass fiber (0.7 µm) filters, frozen, and stored at -20°C for later extraction and determination on the Trilogy.

Chlorophyll *a* filtering for Extraction Method*:* Samples will be filtered under reduced light conditions (no direct sunlight). 250 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. Filters will be wrapped in foil and stored frozen (below 0 °C) until extraction (within 60 days of filtering). The volume of water that was filtered will be written on the foil packet along with the site name, collection date, triplicate number and "chl." To start extraction, filters will be placed in 15 mL polystyrene tubes (prefilled with 90% acetone/10% DI water). The tubes will be stored in the freezer for a minimum of 12 hours before analysis.

Phycocyanin filtering for Extraction Method: An additional 250 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 (within 60 days of filtering). The volume of water that was filtered will be written on the foil packet along with the site name, collection date, triplicate number and "phyco." To start extraction, filters will be placed in 30 mL centrifuge tubes (prefilled with phosphate buffer). The tubes will be stored in the fridge for 2 hours and then at room temperature for 1 hour before analysis.

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

B4. Analytical Methods

Extracted 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).

Extracted 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.

Within 24 hours of collection, each of the field samples will be analyzed in triplicate on the FluoroQuik, the PhycoProbe, the CyanoFluor and the Trilogy (chl-a *in vivo* module). After being frozen for a minimum of 4 hours (and a maximum of 60 days) and then thawed completely in a room temperature water bath the samples will be run on the FluoroQuik, the PhycoProbe, the CyanoFluor and the Trilogy (chl-a *in vivo* module) again. 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.

The FluoroQuik will be blanked as needed (once per cuvette) with an empty 1cm polystyrene cuvette then the cuvette will be filled three-quarters of the way with whole sample water to be read. The fluorometer will read out chl-a and phycocyanin results in µg/L, Relative Fluorescence Units (RFUs), and the ratio between them. Three replicates will be run per field sample.

The PhycoProbe will be blanked once per pond with sample water filtered through a 0.22µm syringe filter to create a unique fingerprint for that waterbody. This aids in yellow substance correction. Then, 25mL of whole sample water is read 5 times while being stirred. The PhycoProbe results include measurements in µg/L and cell count. The parameters are unbound phycocyanin, green algae, bluegreen algae, diatoms, cryptophyta, Planktothrix, total chl-a concentration, yellow substances, turbidity and sample temperature.

The CyanoFluor will also be blanked once per triplicate with sample water filtered through a 0.22µm syringe filter to correct for yellow substances. Each field sample will be run in triplicate as whole water. The results of the CyanoFluor are given as phycocyanin and chlorophyll concentrations in RFU and the ratio between them.

The Turner Trilogy chl-*a in vivo* module will also be blanked once per triplicate with sample water filtered through a 0.22µm syringe filter to correct for yellow substances. Each field sample will then be run in triplicate as whole water. The results will be presented in RFUs.

Permanent mounts will be used for cell counts to verify the results of the fluorometers and to look at algal community dominance.

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.

B5. Quality Control

Field blanks will be filtered for extracted fluorometric analyses. A sample filtered through a 0.22µm syringe filter will be used as a blank on the CyanoFluor, Trilogy (*in vivo* module) and the PhycoProbe to correct for dissolved organic materials interference. Deionized water will be used as a blank on the FluoroQuik to correct for imperfections in the cuvette walls. Using three field duplicates and three replicate measurements for each of those duplicates will ensure the integrity and accuracy of the data being collected. Any replicates that deviate too much from each other will be rerun.

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

All analytical equipment (Turner Trilogy, Turner FluoroSense, Turner CyanoFluor, bbe PhycoProbe, bbe AlgaeTorch and AmiScience FluoroQuik), 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 bbe AlgaeTorch and bbe PhycoProbe are factory calibrated every two years per manufacturer recommendation. Manufacturer calibration of the AlgaeTorch 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. The AmiScience FluoroQuik is calibrated yearly per manufacturer recommendation. Accuracy of all fluorometers will be assessed before each sample run using a secondary calibration standard. The Turner FluoroSense only needs to be recalibrated if more than 3% drift is tracked from the secondary standard solution. The Turner CyanoFluor only needs to be recalibrated if the calibration check standard “fails” 3 out of 5 times. 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. secondary standards, filters, gluteraldehyde and syringes) are available when needed.

B9. Non-direct Measurements

There will not be any non-direct measurements used in this project. All the data will be collected firsthand.

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 instruments, lab instruments, and handwritten 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) 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.

All manually entered data will be inspected for potential problems (e.g. transpositions).

Any replicate fluorometer results, for the same duplicate sample, outside 20% of each other will be rerun to ensure instrument accuracy.

D3. Analysis and Reconciliation with User Requirements

The original question for this project asks how well a variety of fluorometers compare to the gold standard method of extraction for chlorophyll and phycocyanin quantification. We plan to use linear regressions to determine the relationship between the fluorometric measures and the extractions methods. Traditional measures of R^2 and p-values will be used to assesse the results of the regressions. Appropriate measures of uncertainty (e.g. confidence limits, etc.) will be used to convey appropriate limitations.

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

Rhode Island -Department of Environmental Management, RI DEM, Cyanobacteria (Blue-Green Algae). dem.ri.gov/programs/water/quality/surface-water/cyanobacteria.php.

Table 1: Rhode Island Waterbodies with the Highest Number of Advisories in the Past Ten Years

Waterbody	Town	# of Advisories	Most Recent Advisory Posted
Mashapaug Pond	Providence	10	7/13/2021
J. L Curran Reservoir	Cranston	8	7/13/2021
Melville Ponds	Portsmouth	8	7/16/2021
Blackamore Pond	Cranston	7	7/13/2021
Slack Reservoir	Smithfield/Johnston	7	8/14/2020
Almy Pond	Newport	6	6/12/2021
Sisson Pond	Portsmouth	6	8/9/2019
Spectacle Pond	Cranston	6	7/13/2021
Watson Reservoir	Little Compton	6	11/8/2019
Pleasure Lake	Providence	5	8/21/2018
Paradise (Nelson's) Pond	Middletown	4	11/8/2019
Roger Williams Park Ponds	Providence	4	8/18/2017
Roosevelt Lake	Providence	4	8/9/2019
St. Mary's	Portsmouth	4	9/12/2016
Warwick Pond	Warwick	4	7/23/2021
Elm Lake	Providence	3	9/10/2020
Georgiaville Pond	Smithfield	3	7/8/2020
Japanese Gardens	Providence	3	8/10/2018
Lawton Valley Reservoir	Portsmouth	3	8/29/2017
Turner Reservoir	East Providence	3	7/13/2018

Attachment 1: CyanoFluor Data Sheet

CyanoFluor Run Date: _____

waterbody	site	dup	reps	PC:CHL	PC(RFU)	CHL(RFU)	PC BLK(RFU)	CHL BLK(RFU)	day	month	year	fresh/frozen1
na	std check	na	na							10	2021	na
yawgoo	1	1	1						6	10	2021	fresh
yawgoo	1	1	2						6	10	2021	fresh
yawgoo	1	1	3						6	10	2021	fresh
yawgoo	1	2	1						6	10	2021	fresh
yawgoo	1	2	2						6	10	2021	fresh
yawgoo	1	2	3						6	10	2021	fresh
yawgoo	1	3	1						6	10	2021	fresh
yawgoo	1	3	2						6	10	2021	fresh
yawgoo	1	3	3						6	10	2021	fresh
barber	1	1	1						6	10	2021	fresh
barber	1	1	2						6	10	2021	fresh
barber	1	1	3						6	10	2021	fresh
barber	1	2	1						6	10	2021	fresh
barber	1	2	2						6	10	2021	fresh
barber	1	2	3						6	10	2021	fresh
barber	1	3	1						6	10	2021	fresh
barber	1	3	2						6	10	2021	fresh
barber	1	3	3						6	10	2021	fresh
indian	1	1	1						6	10	2021	fresh
indian	1	1	2						6	10	2021	fresh
indian	1	1	3						6	10	2021	fresh
indian	1	2	1						6	10	2021	fresh
indian	1	2	2						6	10	2021	fresh
indian	1	2	3						6	10	2021	fresh
indian	1	3	1						6	10	2021	fresh
indian	1	3	2						6	10	2021	fresh
indian	1	3	3						6	10	2021	fresh

Attachment 2: FluoroQuik Data Sheet

URI FluoroQuik

Run Date: _____

waterbody	site	dup	reps	PC:CHL	PC(ug/l)	CHL(ug/l)	Ch1 Hi(RFU)	Ch1 Lo(RFU)	Ch2 Hi(RFU)	Ch2 Lo(RFU)	day	month	year	fresh/frozen1/2
na	200ppb	na	na									10	2021	na
na	800ppb	na	na									10	2021	na
yawgoo	1	1	1								6	10	2021	fresh
yawgoo	1	1	2								6	10	2021	fresh
yawgoo	1	1	3								6	10	2021	fresh
yawgoo	1	2	1								6	10	2021	fresh
yawgoo	1	2	2								6	10	2021	fresh
yawgoo	1	2	3								6	10	2021	fresh
yawgoo	1	3	1								6	10	2021	fresh
yawgoo	1	3	2								6	10	2021	fresh
yawgoo	1	3	3								6	10	2021	fresh
barber	1	1	1								6	10	2021	fresh
barber	1	1	2								6	10	2021	fresh
barber	1	1	3								6	10	2021	fresh
barber	1	2	1								6	10	2021	fresh
barber	1	2	2								6	10	2021	fresh
barber	1	2	3								6	10	2021	fresh
barber	1	3	1								6	10	2021	fresh
barber	1	3	2								6	10	2021	fresh
barber	1	3	3								6	10	2021	fresh
indian	1	1	1								6	10	2021	fresh
indian	1	1	2								6	10	2021	fresh
indian	1	1	3								6	10	2021	fresh
indian	1	2	1								6	10	2021	fresh
indian	1	2	2								6	10	2021	fresh
indian	1	2	3								6	10	2021	fresh
indian	1	3	1								6	10	2021	fresh
indian	1	3	2								6	10	2021	fresh
indian	1	3	3								6	10	2021	fresh