# 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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## 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 in Barnstable, MA: Hamblin 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*

Hamblin Pond is a small kettle pond (46.4 ha) with a maximum depth of 19.1 m located in Barnstable, Massachusetts. Typical of a kettle pond, Hamblin Pond lacks a surface water connection but does have groundwater connectivity and receives groundwater inflow from an adjacent pond (Middle Pond). Hamblin Pond has history of algal blooms resulting from high internal phosphorus loading from agricultural sources near the pond. Alum treatments were performed in 1995 and 2015 and no HAB advisories have been issued after the last treatment.

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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| **CY 2021** | | | | | **CY 2022** | | | | **CY 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 on the sonde and field laptop (L26JHOLLIST). Upon completion these records will be exported to a csv file in the project repository. 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 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), otherwise data will be logged on the device. Data will be entered/downloaded and transferred to the project repository, 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 flow injection 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 in the project repository. Data output from the Turner Designs Trilogy fluorometer will be transferred to a local laptop, converted to .csv format using R, and stored in the project repository (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 “nearish” the center of Hamblin and Shubael ponds before the bloom season begins (installed by June 15) to capture baseline data before bloom occurrence. These buoys will remain in place through the end of the bloom season (approximately November/December). 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 every other week (depending on local weather conditions) in triplicate for lab analyses (chlorophyll *a*, phycocyanin, microcystin, TN, TP, NO3, PO4, and NH4) at the buoy location. Additionally, zooplankton and phytoplankton samples will be collected at the buoy location.

Water samples will also be collected at four perimeter sites (locations recorded on initial field visit and will have a minimum of 2m depth) in duplicate for chlorophyll *a* and phycocyanin. Opportunistic samples (e.g. corralled blooms not at a predetermined site) will be collected if necessary. Concurrent with water sample collection, the FLAMe system will follow a predetermined path and collect spatial data approximately every 20 meters covering the entire pond using the same sensors that are on the buoys.

## B2. Sampling Methods

Physical and biological parameters will be measured on the buoy using a YSI EXO2 multiparameter sonde. This sonde will be suspended approximately 1 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 from the buoy is first sent to the vendors data portal (<https://wqdatalive.com>) every 15 minutes, then sent to USEPA GoAnywhere SFTP twice daily. Finally, the data are downloaded to the project repository daily via scheduled R script. In addition, the data are stored on the buoys data logger and will be manually retrieved periodically. A depth profile will also be taken at the buoy location using a handheld YSI multiparameter sonde.

Secchi transparency will be measured at the buoy site 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. Sunglasses will be removed.

Water samples will be collected 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 buoy 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. 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.

Phytoplankton collection will follow a procedure developed by Ann St. Amand at Phycotech. Whole water samples will be decanted from the 1L Nalgene bottles into 125 mL amber glass bottles and preserved with glutaraldehyde (0.25-0.5%) for permanent mounting at a later date. The samples will be filtered onto 0.45 µm mixed cellulose filters and permanently mounted onto glass slides in triplicate using an HPMA resin.

## 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).

Samples will remain in USEPA ACESD custody at all times.

## B4. Analytical Methods

Chlorophyll a determination for the water samples 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 for the water samples 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).

Zooplankton identification and analysis will follow the EPA National Lake Assessment methodology (EPA 841-B-11-003) as well as methods described in Mack et al. 2012. Zooplankton samples will be rinsed with DI water in an appropriately sized sieve for the sample and then rinsed into a graduated cylinder. The cylinder will be rinsed and the total volume of water for the rinses will be recorded as the dilution volume. Aliquots will be counted until 200 individuals of a taxa are counted or until 5 aliquots are counted. Cladocerans will be identified to genus and copepods to order.

The phytoplankton counting method is in development and will be added when completed.

## 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 (flow injection 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. 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.

The YSI multiparameter sondes will be calibrated with known standards and checked before each use. The handheld and FLAMe mounted sonde will be calibrated prior to each sampling trip. The buoys will be deployed from May/June through November/December and thus can be calibrated only prior to initial deployment. Since the buoy mounted sondes will be calibrated only once, they will be checked for drift with a calibrated handheld sonde. Additionally, we will replace EXO2 sondes on the buoys with freshly calibrated sondes every 8-12 weeks or if the handheld sonde is showing greater than 20% deviation from the buoy sonde. Table with sensor specific deviation?

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## 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 and in an acceptable condition.

## B9. Non-direct Measurements

Hamblin and Shubael Ponds have been monitored in the past by various organizations (e.g. Town of Barnstable, Barnstable Clean Water Coalition, Association to Protect Cape Cod, and UMASS-Dartmouth). Best available data will be used as historical context and to aid in data interpretation.

## B10. Data Management

All manual field data will be recorded on Rite-In-The-Rain paper to prevent reduced legibility from contact with water and will be transferred approximately weekly to the project repository. Data from analytical equipment will be transferred to the project repository 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 local drives, backed up to the L:/ drive, and remotely on GitHub. Access to the project repository 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 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 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

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).

Data from the buoy sensors will be compared to the freshly calibrated handheld and FLAMe sensors to check for drift. Additionally, sensor-based chlorophyll *a,* phycocyanin, and nitrate measurements will be checked against fluorometery and autoanalyzer measurements for appropriateness of general trends. It is important to note that extracted chlorophyll and phycocyanin from the Trilogy fluorometer are inherently different measurements than the *in situ* chlorophyll and phycocyanin from the sensors, but they both should track general trends (e.g. increasing extracted chlorophyll will also show as increasing *in situ* chlorophyll).

## D3. Analysis and Reconciliation with User Requirements

The original questions for this project are to quantify the temporal and spatial variation of HABs in two Cape Cod ponds, but there will likely be additional questions that these data may be used to address. Given the nature of these questions, it is not possible to determine appropriate analysis methods *a priori.* In general, we will use appropriate spatial and time-series statistical and visualization techniques to analyze the data for trends and associations. Appropriate measures of uncertainty (e.g. probability distributions, 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.

Mack, H.R., J. Conroy, K. Blocksom, R. Stein, and S. Ludsin. 2012. A comparative analysis of zooplankton field collection and sample enumeration methods. Limnology and Oceanography: Methods 10:41-53.

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 |
| Conductivity | 0-100000 µS/cm | ± (1.0 % of reading or 2 µS/cm) | Range-dependent (0.1 µS/cm -10 µS/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 | 0-999 FNU (0.3 FNU or ± 2% of reading)  1000-4000 FNU (± 5% of reading) | Range-dependent (0.01 FNU – 0.1 FNU) |

**Table 2: TriOS NICO sonde specifications**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Range | Accuracy | Resolution |
| 10 mm path length | 0.05 – 6 mg/L NO3-N | ± (5% + 0.1 mg/L NO3-N) | 0.01 mg/L |

**Appendix 1.** ISO Sensor Check List

**Information Security Officer – EPA Campus Sensor Check List**

*If your project incorporates sensors that report information to a cloud (online sensors), please include this filled-out template as an Appendix to your QAPP. This will be a standalone document transmitted to the ORD Information Security Officer (ISO), so please fill in each section. If there are sections where information is unknown or cannot be obtained by the manufacturer, please note this.*

1. Summary of project/effort/business justification:
   1. Project name, effort, and business justification:
      1. High frequency spatial and temporal dynamics of freshwater cyanobacterial HABs. Part of SSWR 4.3.1 and focus on high priority EPA research area of Harmful Algal Blooms
   2. POC(s):
      1. Jeff Hollister, Stephen Shivers
   3. QA Manager:
      1. Joseph LiVolsi
   4. Length of project/effort:
      1. May 2021 - TBD
2. Cloud/Manufacturer Server Use.
   1. Sensor cloud(s):
      1. https://wqdatalive.com
   2. The physical location of the servers (manufacturer or cloud) the data will be stored on:
      1. Suwanee, GA
   3. Will the data from the sensor device be immediately displayed on the manufacturer’s website?
      1. Yes If yes, see d.,
   4. How is the sensor /data logger depicted (i.e. name, number, or other)?
      1. Site Name (e.g. Shubael Pond) and buoy name (X2-CB-C-VZ4G-20193)
   5. What sensor data is depicted?
      1. All of it (see 3b)
   6. Does researcher/scientist have access to the data displayed on the cloud?
      1. Yes
   7. Does the cloud provider make the sensor data available to the public? If so, what information is the public able to see.
      1. It is password protected and only available to named users that researcher has identified.
3. Sensor Technology.
   1. Summary Description:
      1. Nexsens CB-150 data buoys and X2-CB logger with YSI EXO2, Trios NICO, and Airmar 200WX
   2. Identify/Name all Data Logger/Sensor Model(s) from this vendor that are utilized:
      1. Data Logger: Nexsens X2-CB
      2. Sensors;
         1. Trios NICO UV Nitrate
         2. Airmar 200WX with Rel Baromteric Pressure, Air Temperature, Relative Humidty, Dwpoint, Wind Direction, Wind Speed, Pitch, Roll
         3. YSI EXO2 With Temperature, pH, Specific Conductivity, Dissolved Oxygen, Turbidity, Chlorophyll, Phycocyanin, and FDOM
   3. Is the Vendor on the prohibited National Defense Authorization Act (NDAA) Section 889?
      1. No
   4. Does the vendors technology utilize components from NDAA Section 889 forbidden vendors?
      1. No (Section 889 form submitted as part of purchase)
4. Sensor Placement and communication.
   1. Where (EPA room number, building, etc) will the sensors be placed?
      1. These are field deployed and will not be in an EPA building. One will be in Shubael Pond, Barnstable, MA and the other in Hamblin Pond, Barnstable MA.
   2. Physical security of the device (describe any physical security implementations to protect the device from tampering).
      1. Buoys will be placed at the center of the ponds which limits access. Additionally, each buoy weights ~100lbs and will be moored with a 70lb anchor and 10 feet of 3/8in chain. Tampering with the buoys would be extremely difficult. Also we are working with local partners that will regularly check the buoys and EPA staff will be on site every other week.
   3. Description of sensor communication/connection (i.e. USB, internet, cell, satellite):
      1. USEPA Verizon cellular
5. Information Types.
   1. What type of Information is collected (i.e. air or water quality, temperature, etc)?:
      1. Water Quality and Weather
6. Official EPA Record.
   1. Can data be download directly from the sensor itself?
      1. Yes
   2. If so, is there a planned frequency for downloading this data (information)?
      1. Yes, but still to be determined
   3. Where will the data be kept/stored (i.e. ORD provided file share):
      1. Project leads will maintain files on OneDrive and once data approved for public release copies will be made available via USEPA Github.
7. Data Integrity.
   1. Describe briefly and in general terms how the integrity of the data will be verified before decision/action:
      1. Data from the buoys will be verified through comparison with data logged on the buoy itself. We will compare measurements from the buoys to data collected via handheld version of the same sensors on the buoy as well as compared to water samples collected at the buoy location and independently processed in the lab.
   2. If this effort is required to use a Quality Assurance Project Plan (QAPP), how is the integrity detailed in the related project?
      1. A QAPP is required and these details are documented in the appropriate sections in the QAPP, in particular Sections B and D.
8. Data Ownership.
   1. Please provide manufacturer terms and conditions or correspondence describing the ownership of the data, for the use of online sensors in your project
      1. <https://www.wqdatalive.com/privacy>
      2. See attached email

