Quality Assurance Project Plan
For
Field Deployment of an Autonomous Nutrient Monitor in Casco Bay

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1.1 Title

Quality Assurance Project Plan for Field Deployment of an Autonomous Nutrient Monitor in Casco Bay

Approvals

06/24/2019

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6/26/2019

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06/24/2019

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Attachment 1. NuLAB User Manual version 1.3. Green Eyes, LLC. 2019.

Attachment 2. EcoLab and NuLAB Data Processing Guide. V1.0 Green Eyes, LLC. No date.

Attachment 3. Onset HOBO Salt Water Conductivity/Salinity Data Logger model U24-002 Manual.

Attachment 4. Quality Assurance Project Plan for Friends of Casco Bay Environmental Monitoring Program. Revision 5. July 10, 2017.

Attachment 5. QAPP for the Water Quality Analysis Lab at the University of New Hampshire, Department of Natural Resources, Durham, NH. Prepared by Jeff Merriam. Date of Last Revision: 1/10/2018. Revised by: Jody Potter.

Attachments 6 through 10 are checklists and datasheets.

List of Abbreviations and Acronyms

CBEP	Casco Bay Estuary Partnership
CSO(s)	Combined Sewer Overflow(s)
DIN	Dissolved Inorganic Nitrogen
DIW	Deionized water
EE WWTF	East End Wastewater Treatment Facility, Portland Water District
FOCB	Friends of Casco Bay
IV	Intravenous bags
Maine DEP	Maine Department of Environmental Protection
MCU	Microcontroller unit
MDL	Method detection limit
NOx	Nitrate (NO_3^-) + Nitrite (NO_2^-) concentration
NH4	Ammonium (NH_4^+) concentration
OBS	On-Board Standard
QA/QC	Quality Assurance and Quality Control
PSU	Practical Salinity Units (roughly equivalent to PPT, or parts per thousand)
RPD	Relative Percent Difference
SP WWTF	South Portland Wastewater Treatment Facility
TN	Total nitrogen
UMaine	University of Maine
UNH	University of New Hampshire Water Quality Analysis Laboratory
USM	University of Southern Maine

1.3 Distribution List and Contacts

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A. PROJECT MANAGEMENT

1.4 Project Organization

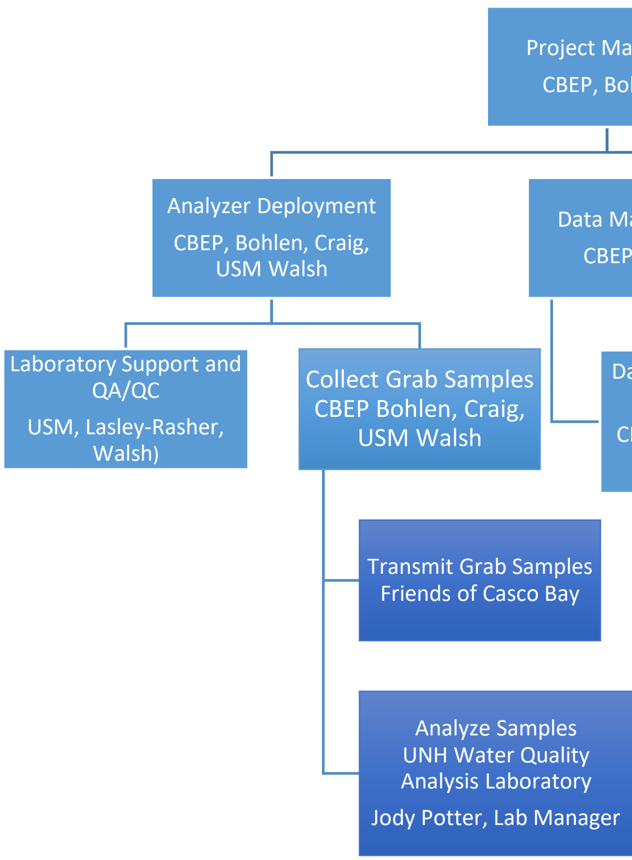
Casco Bay Estuary Partnership is testing an autonomous nutrient monitor – called the NuLAB analyzer -- in the Fore River Estuary in western Casco Bay. The project complements activities by a coalition of organizations (included in Table 1) that are monitoring nutrients in response to concerns about nutrient enrichment in Casco Bay.

Table 1. Key project personnel and organizations conducting nutrient monitoring in Casco Bay.

Organization	Key Contacts	Roles	Project organizations or monitoring organizations
Casco Bay Estuary Partnership	Curtis Bohlen, Director; Matt Craig, Habitat Program Coordinator Marti Blair, Program Coordinator	Project Manager, Instrument Deployment, Data QA/QC, Data review, visualization and storage, and reporting.	Project; monitoring
Friends of Casco Bay	Mike Doan, Staff scientist	Sends bottle grabs to University of New Hampshire (UNH) for analysis	Project; monitoring
Green Eyes, LLC	Vince Kelly, Director	Technical Assistance throughout project	Project
University of New Hampshire	Jody Potter, Lab Manager	Provides analysis of bottles samples	Project
University of Southern Maine	Rachel Lasley-Rasher Student assistant, Tyler Walsh	Laboratory support for instrument deployment	Project
Maine Department of Environmental Protection	Angie Brewer	Monitoring nutrients in Casco Bay in 2017 and 2018; not part of project in 2019	Monitoring
University of Maine	Damian Brady	Operated analyzer in 2018, models and estimates nutrient loads to Casco Bay; not part of project in 2019	Monitoring

CBEP Executive Director Curtis Bohlen serves as the Project Manager and will lead the operation of the nutrient analyzer. He will be assisted by CBEP staff Matt Craig and Marti Blair (Figure 1). Matt Craig will serve as the independent QA manager and Marti Blair will be responsible for document management. They will be assisted by a student (Tyler Walsh) from the University of Southern Maine (USM). Vince Kelly of Green Eyes, LLC -- the developer of the NuLAB analyzer -- will provide technical support throughout the project. Friends of Casco Bay will assist with collection of grab samples for validation purposes. These samples will be sent to the University of New Hampshire (UNH) Water Quality Analysis Laboratory[1] for analysis using equivalent methods as the nitrogen analyzer (but see section 2.4).

Figure 1. Project Organization. Boxes in dark blue represents data collected under separate data quality assurance procedures.



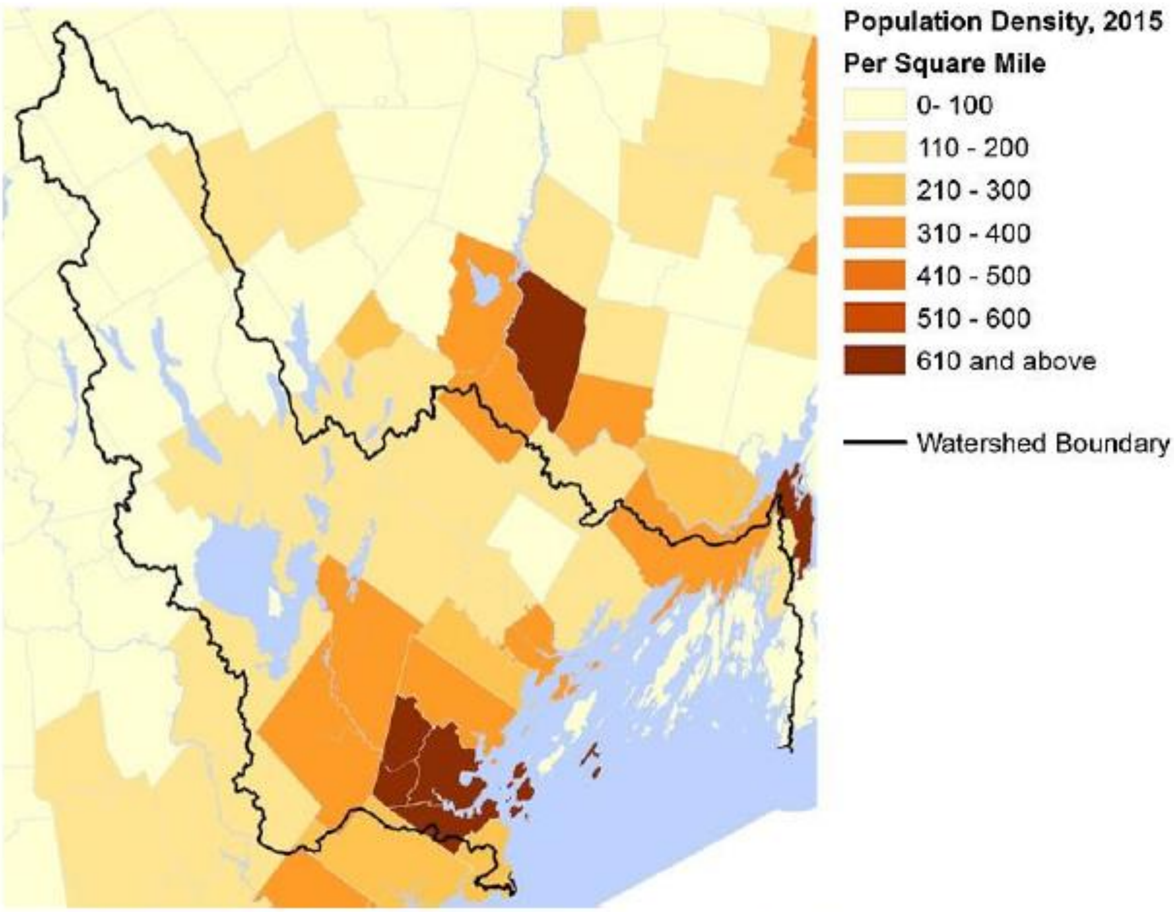
1.5 Problem Definition/Background

Casco Bay borders Portland and South Portland, Maine's principal economic and technological hub. The Casco Bay watershed region houses one quarter of Maine's population and one third of the total jobs and economic output in the state (CBEP, 2017; Figure 2). Although two thirds of the watershed remain

[1] https://wrrc.unh.edu/unh-water-quality-analysis-laboratory

forested, the most heavily developed portions of the watershed – about 10% of the landmass – border tributaries and the Bay itself, with extensive impervious surface (CBEP, 2015). Numerous streams in the Casco Bay watershed are impaired due to stormwater from impervious cover.

Figure 2. Population density by municipality in the Casco Bay region; American Community Survey (ACS) 5-year estimate 2011-2015. Source: CBEP, 2017.



Source: US Census, Population Estimates; ACS 5 year estimate 2011-2015.

In Casco Bay's marine waters, certain areas show signs of coastal eutrophication and hypoxia (CBEP 2015, CBEP 2016, CBEP 2019 (Nutrient Council report). Portland Harbor and the waters surrounding it have among the highest total nitrogen (TN) concentrations observed anywhere on the Maine coast, with median conditions exceeding 90% of coastal nitrogen measurements in Maine (Cadmus Group and Saquish Scientific, 2009; CBEP 2015). Recent evidence suggests that impacts from these high nitrogen loads are having increasingly negative consequences. Reports of algal overgrowth of tidal flats are more common (Miller, 2016). High coastal nutrient concentrations may also be leading to coastally enhanced acidification due to algal growth (Cai *et al*. 2011; Gledhill, *et al.*, 2015; Thornton and Mayer, 2015). The Maine legislature has formally recognized Casco Bay as a state-wide priority for addressing nutrient pollution and developing coastal nutrient criteria (Maine 123rd Legislature, 2007).

Recognizing these concerns, CBEP convened the Casco Bay Nutrient Council (the Council) to provide a forum for examining the impact of nutrient pollution on Casco Bay, and identifying effective and cost-effective strategies to address nutrient pollution in the Bay. The Council consists of a core group of 12 members, representing municipal government, wastewater treatment plant operators, stormwater engineers, regulatory agencies, advocacy organizations, and academics. Many of the recommendations of the council are to expand nutrient monitoring from unmeasured sources (e.g. stormwater); better estimate nutrient loads from tributaries to Casco Bay; and develop a nutrient monitoring plan with identified funding sources. As a member of the National Estuary Program, CBEP is required to develop a monitoring plan, and has convened a multi-agency monitoring network that shares information and generates priorities for monitoring on a regular basis.

Population growth in the Greater Portland area increases both point source and non-point source nitrogen loads to Casco Bay and growth is expected to increase in coming decades. At present, neither of the region's largest wastewater treatment plants is designed to remove nitrogen from the waste stream. The Portland Water District's East End Wastewater Treatment Facility (EEWWTF; the largest discharger in the state) will begin denitrification to reduce its nitrogen load by 20 to 40% over the next five years. The somewhat smaller South Portland Wastewater Treatment Facility (SP WWTF) – which discharges directly into the Fore River Estuary -- will explore ways to optimize nitrogen removal over the same period. Non-point sources are managed through the stormwater permits (e.g., Long Creek water district), but there are no regulatory limits for nitrogen discharges.

This project will deploy a multichannel autonomous nutrient analyzer called NuLAB[2] in the Fore River Estuary on the South Portland side on a pier at a secure location. This urban sub-estuary of Casco Bay experiences the highest levels of nitrogen observed in Casco Bay due to discharges from wastewater, loadings from tributaries, and stormwater contributions. Treated wastewater is a major source; the Portland and South Portland WWTFs accounted for 81% of treated sewage discharged from the region's eight major wastewater treatment plants in 2017 (CBEP, 2019).

The SP WWTF typically discharges about 9.3 million gallons of secondary treated effluent per day (mgd), but the monthly average ranges from 4 mgd to 10 mgd, and its maximum allowable discharge is 22.9 mgd (Maine DEP, 2017).

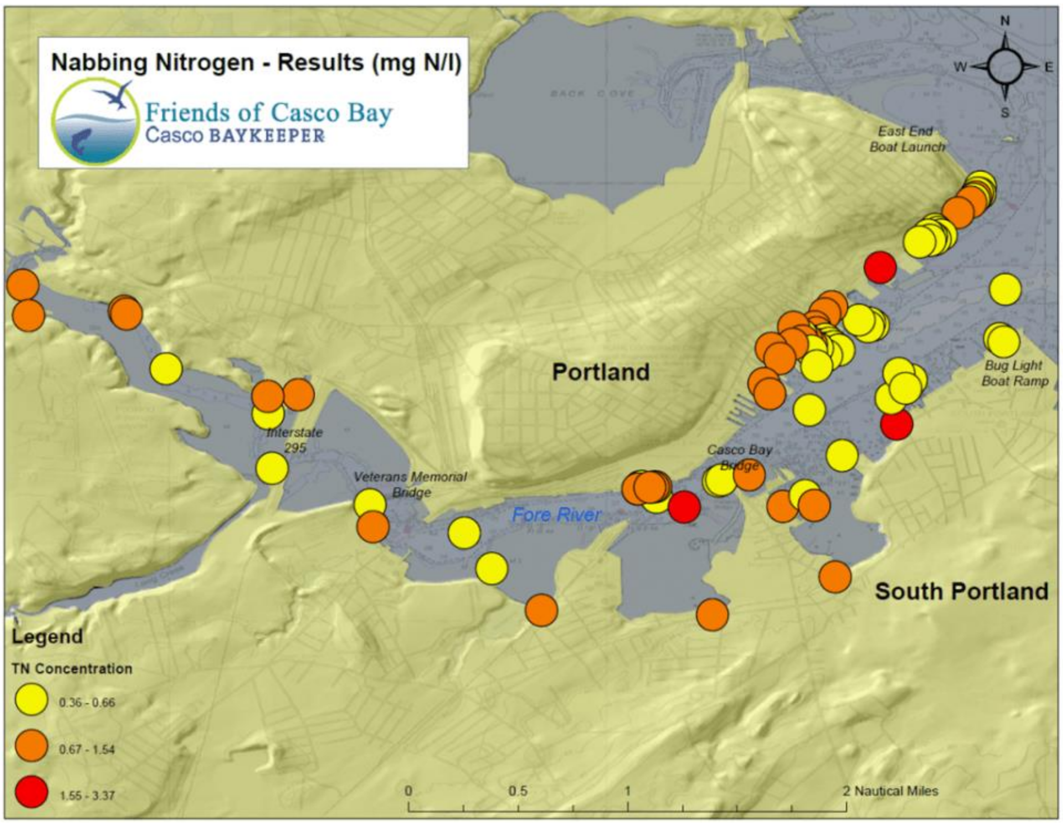
Upstream tributaries to the Fore River estuary (the Stroudwater River, Long Creek and Capisic Brook), and direct stormwater runoff from the urban landscape are the second most important source of nitrogen. The watershed of the Fore River is 16.5% impervious area. Based on measurements by University of Maine researchers in 2017 and 2018, typical levels of total nitrogen in Capisic Brook are about 1 mg per liter total nitrogen (TN) and are elevated compared to other major rivers in the Casco Bay watershed (e.g., the Royal and the Presumpscot rivers).

[2] http://gescience.com/

Another source of nutrients is combined sewer overflows (CSOs). Portland still has 30 active CSOs that, in the drought year of 2016, discharged 318.4 million gallons of untreated wastes to Casco Bay and its tributaries (Riley 2017).[3]

The Friends of Casco Bay's 2016 "Nitrogen Nabbing" event (a nutrient water sampling blitz) revealed highly spatially variable concentrations of nitrogen in Portland Harbor on a day following moderate rainfall (FOCB, 2016). High concentrations were especially common close to the shore and often exceeded 2 or 3 mg/l TN (Figure 3).

Figure 3. Nitrogen Nabbing event results. Source: Friends of Casco Bay, 2016.



1.5.1 Continuous monitoring and Project Objectives

Casco Bay has a diurnal tidal range on the order of three meters. Like many sub-estuaries, the Fore River estuary is a complex hydrodynamic environment and previous nutrient monitoring (relying on grab samples) lacks the temporal resolution to allow us to assess how water chemistry changes on small time scales. The FOCB sampling blitz in 2016 provided a one-time snapshot of conditions in this estuary but did not provide any temporal resolution. Time resolved data may allow us to correlate nitrogen concentrations with time-varying phenomena, such as tidal flux, precipitation, CSO events, and river discharge, thus helping clarify the relative contributions of different nutrient sources. These data will

[3] Although many of the CSOs discharge to Back Cove, in addition to the Fore River.

facilitate estimating non-point source nutrient loads to complement knowledge of point source loads and help prioritize nutrient reduction strategies. Thus, our primary goal (i.e. ***Project Objective***) is to clarify the relative importance of riverine inputs, wastewater discharges, stormwater, and combined sewer overflows as sources of nutrients to the waters around the Fore River estuary.

Our secondary goal is to test the efficacy of a novel analyzer for continuous monitoring. The NuLAB analyzer is a relatively new technology and its performance in real-world settings is not well understood. It relies on wet chemistry that is equivalent to standard methods for measurements of nitrate+nitrite, and ammonium. Specifically, nitrate+nitrite in water is reduced to nitrite through a copper coated cadmium column, and then reacts with reagents to produce a red color that is measured colorimetrically. It is equivalent to EPA method 353.2. Similarly, ammonium (NH_{4+}) in water reacts with specific reagents to produce a blue color which is quantified with a colorimeter. This method is equivalent to EPA method 350.1.

In 2017, CBEP was partially funded through a winning proposal to EPA's Nutrient Sensor Action Challenge to deploy two "sensors" in Casco Bay.

(1) The National Oceanography Center's "Lab-on-a-chip" Nitrate+Nitrate Analyzer; and
(2) The Green Eyes NuLAB, Autonomous nutrient analyzer, configured to measure nitrate+nitrite and ammonium.[4]

Further information about the Nutrient Sensor Action Challenge is here:
https://www.epa.gov/innovation/nutrient-sensor-action-challenge and
https://www.epa.gov/innovation/nutrient-sensor-action-challenge-stage-i-winners#portland

The NOC Lab on a Chip sensor was selected as an Honorable Mention in the Nutrient Sensor Technology Challenge in 2016, organized by the Alliance for Coastal Technologies, and partially funded by the EPA (Alliance for Coastal Technologies. 2017; http://www.act-us.info/nutrients-challenge/Awards.php). The Green Eyes NuLAB analyzer did not compete in that challenge.

In 2018, CBEP worked with University of Maine to deploy both "sensors" in Casco Bay. Based on the results of this preliminary testing, CBEP decided that the Green Eyes NuLAB analyzer held the most promise and will deploy this analyzer in South Portland in the Fore River Estuary in 2019.

1.6 Project/Task Description and Schedule

We will deploy the NuLAB analyzer beginning June 2019 to collect dissolved nitrate+nitrate (sometimes called Nox) and ammonium on a two-hourly basis for two to three months in 2019 and longer in 2020, depending on funding. To provide hydrologic context for the nitrogen data, we will also:

[4] We are calling the system an analyzer instead of a sensor, because it is based on wet chemistry, not electronic sensing

(1) deploy HOBO[5] conductivity-temperature data loggers in the water immediately adjacent to the water inlet of the NuLAB analyzer at six-minute intervals synchronized to the Portland tide gauge;
(2) retrieve weather, tide and river discharge data from on-line sources; and
(3) receive related data, such as daily wastewater discharges, storm-related CSO discharges, and stream flow data from partner organizations.

The analyzer will be visited approximately every two weeks for servicing and to remove biofouling (if necessary) from the pump. Each time the instrument is serviced, a discrete grab sample of ambient water will be collected directly adjacent to the inlet tube for validation purposes. It is also possible that this sample will be collected directly from the instrument itself – there is a way to directly sample the water after intake, but before it is filtered. Once samples are collected, they will immediately be put on ice, and transported to the Friends of Casco Bay offices in South Portland, ME. FOCB will include these samples for analyses sent to UNH for nutrient analyses. Samples will be analyzed for nitrate+nitrite, and ammonium (described below). A HOBO conductivity and temperature data logger will be deployed in a stilling well at the dock and will log data at six-minute intervals. Ancillary water quality data including approximate salinity and temperature will be collected using a refractometer (Advanced Microscopy Group RHS-10ATC or similar) and a handheld pocket thermometer (LaMotte Model 545 armored, alcohol filled) when grab samples are collected. (These data will support field observations and serve as a rough check on the HOBO measurements.)

1.6.1 Project and Sampling schedule

Table 2 displays the Project and sampling schedule.

1.6.2 Sampling Location

The analyzer will be deployed at the Portland Street Pier, in South Portland (PSPSP; 43.65129, -70.24367; Figure 4). This site was selected because it receives contributions from upstream riverine sources, wastewater from the South Portland Wastewater Treatment Plant (SP WWTF), and offshore (i.e. Casco Bay) marine sources. It is also a relatively secure location but may not be available next year as it is slated for reconstruction**.**

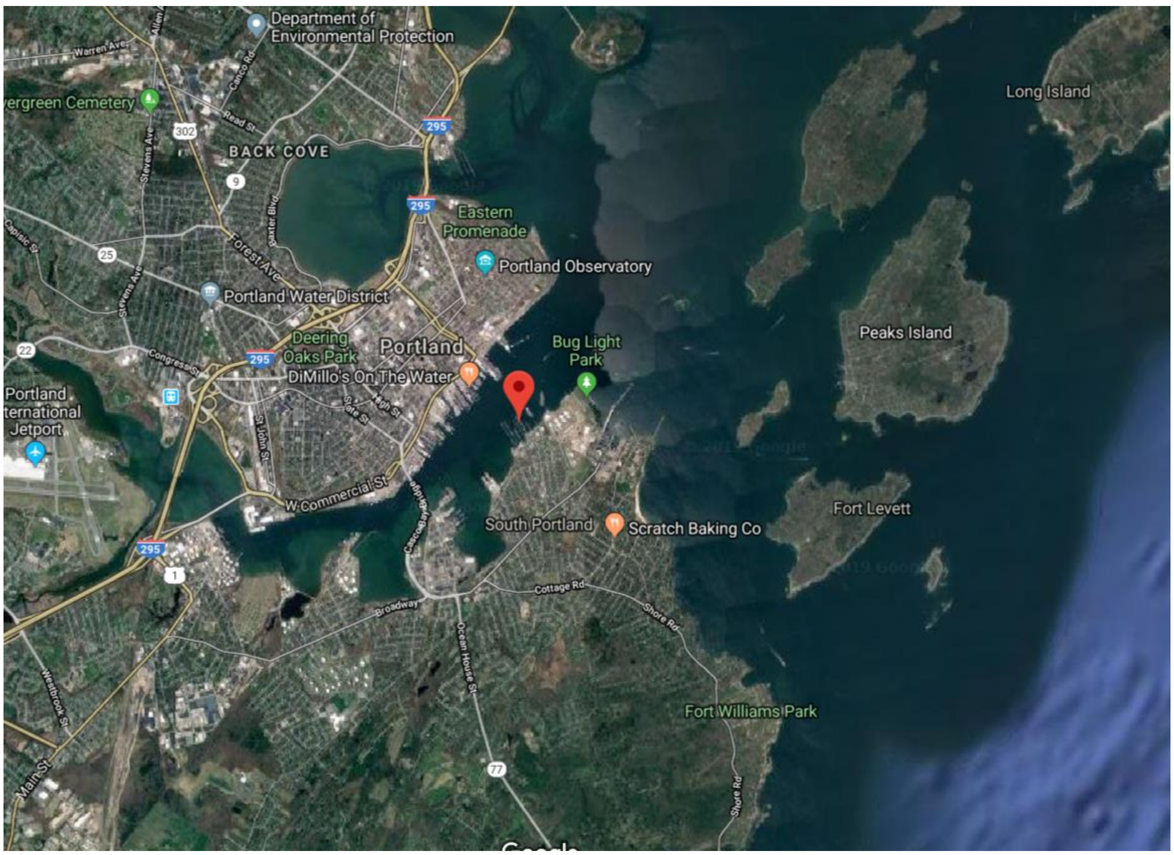
Table 2. Project and sampling schedule.

Activity	Date	Comments
NuLAB training	March 2019	Vince Kelley provided training to CBEP staff
CBEP familiarizing itself with equipment	April 2019	CBEP staff becoming familiar with instrument
QAPP development	April and May 2019	CBEP staff prepare QAPP
USM student hired	May 2019	CBEP staff train student

[5] https://www.onsetcomp.com/products/data-loggers/conductivity-and-salinity

QAPP approved	June 2019	
Deployment	June 2019	Validation samples collected
Maintenance	June through August, 2019	Every two weeks maintenance conducted
System recovered	September 2019	Validation samples collected
Repeat in 2020		

Figure 4. Monitoring Location in South Portland in the Fore River Estuary. (Specific site may change.)



1.7 Data Quality Objectives and Criteria for Measurement Data

1.7.1 Data Quality Objectives

Our project objectives are:

- Clarify the relative importance of riverine inputs, wastewater discharges, stormwater, and combined sewer overflows as sources of nutrients to the waters around Portland in alignment with the Casco Bay Nutrient Council.
- Test the performance of a novel field deployable analyzer using standard laboratory analyses

Thus, our first ***Data Quality Objective (DQO)*** is to *resolve differences* among different sources of nutrients as defined by tidal stage, waste water discharge events (e.g. CSOs), and weather conditions, e.g. storm events. Our second ***DQO*** is to determine whether the instrument can successfully operate in the field and provide laboratory-equivalent analyses of nutrients in water continuously and within an expected range based on discrete validation samples and historical observations.

1.7.2 Data quality indicators (Performance Goals)

Detection limits, precision and accuracy

A review of historic data in inner Casco Bay (Cadmus Group and Saquish Scientific, 2009) showed mean ammonium concentrations of 4.87 μM, and mean nitrate+nitrite levels of 4.21 μM. Data from Maine DEP monitoring in 2017 (personal communication from Angie Brewer, May, 2018) showed similar average levels, although with substantial variation. Observed concentrations ranged over roughly two orders of magnitude for both parameters (Table 3).

Table 3. Minimum and maximum nitrate+nitrite and ammonium concentrations observed in Casco Bay by Maine DEP in 2017.

Parameter	mg/l as N	uM
Nitrate+Nitrite		
Minimum	0.02 (detection limit)	1.43
Median	0.046	3.28
Maximum	1.8	128.51
Ammonium		
Minimum	0.005 (detection limit)	0.36
Median	0.0053	3.8
Maximum	0.92	65.68

Based on Friends of Casco Bay's historical water quality monitoring data (shared with CBEP for our 2015 State of the Bay report, CBEP 2015), summer (June, July, August) salinity and temperature conditions in Portland Harbor are relatively stable. At the Custom House Wharf, across Portland Harbor from the monitoring location, 50% of temperature observations were between 15.5°C and 18°C, with colder temperatures almost always observed in June. Fifty (50) % of salinity observations lie between 27.9 PSU and 31.1 PSU. Over more than 20 years (1993 through 2014), only three observations were less than 10 PSU and only nine observations were below 20 PSU. We therefore expect our ancillary temperature and salinity measurements to show moderate (and somewhat predictable) variation, and we should be able to detect changes in salinity or temperature caused by a major summer storm or overflow event.

Tables 4 and 5 list our data quality indicators and performance goals for the project. Our project objectives require us to measure a range of nutrient concentrations.

Table 4. Performance goals for sensitivity (detection limit), precision and accuracy for the NuLAB analyzer, HOBO data logger and handheld instruments. These are based on the standard ranges for the low sensitivity detectors for nitrate+nitrite and high sensitivity detectors for ammonium published in the NuLAB User Manual version 1.3 (Attachment 1); and NuLAB Data Processing Guide (Attachment 2), personal communication from Vince Kelley; Specifications for Onset HOBO Salt Water Conductivity/Salinity Data Logger model U24-002 (Attachment 3); and specifications for handheld instruments.

Parameter	Detection Limit and Range	Precision (RPD, or relative change of value)	Accuracy (RPD, or relative change of value)
Nitrate+Nitrite (NOx)	0.01 to 2.8 mg/l as N or 0.8 to 200 uM (MDL)	±2% relative difference or relative change of value based on one standard deviation at midrange of scale	±0.1 mg/l as N or 10% of value
Ammonium	0.005 to 1.0 mg/l as N or 0.3 to 50 uM (MDL)	±15% relative difference or relative change based on one standard deviation at midrange of scale	±0.005 mg/l as N or 20% of value
Salinity	2 to 35 PSU	±2 uS/cm (<< 0.1 PSU)	±3% of reading or 50 µS/cm, whichever is greater
Temperature	-2°C to 36°C (28°F to 97°F)	±0.01°C (0.02°F)	±0.1°C (0.2°F)
Salinity by Refractometer	0 to 50 PSU	±1 PSU	±1 PSU
Temperature by pocket thermometer	-5.0 to +45.0°C in 0.5°C	±0.5°C (0.9°F)	0.1°C (0.2°F)

For the NuLAB analyzer, the detection limits were calculated as three times the standard deviation of the method blank. The upper limit of the range is based on the established linearity of response of the detector. NuLabs has established that linearity extends to absorbance of 0.6, which equates to about 200 uM for nitrate+nitrite and 50 uM for ammonia which is above (for nitrate+nitrite) or near (for ammonia) the highest concentrations observed in 2017. Accuracy is based on the accuracy of the preserved On-board standard (OBS). Precision is estimated as one standard deviation at the midrange of the scale. The manual also says this:

It is also a convenient way to check the repeatability of the OBS, reagent blank and other standards fed through the inlet. Green Eyes strongly recommends running a few OBS macros and insuring stable results and then running a few reagent blanks with the inlet tube connected to nutrient free water (DIW or low nutrient seawater depending on deployment water).

Table 5. Summary of Data Quality Indicators, QC activities and Performance Goals for NuLAB analyzer, validation grab samples, and HOBO conductivity-temperature data logger.

Data Quality Indicator	Quality control activities	Performance goal
Detection limits (sensitivity) and range	Calculate the standard deviation of a method (DIW) reagent blank.	See Table 4.
Precision	Compare consecutive results taken immediately before and after high or low tides (slack tide), in the absence of rain within 24 hours. In addition, we can periodically run one sample 3 to 5 times out of a bottle in manual mode. Duplicate validation grab samples will be collected.	See Table 4 for Performance Goals related to NuLAB analyzer and HOBO performance. For comparison of consecutive samples, 90% of paired observations should be less than 25% RPD, especially at lower levels. Precision of bottle samples in manual mode, less than 20% RPD. Precision of validation grab samples should be similar to precision observed by FOCB.
Accuracy	Compare to co-located validation grab samples analyzed by UNH; Run On-board standards with each water sample, and Deionized water (DIW) reagent blanks to establish standard curve.	See Table 4 for Performance Goals related to NuLAB analyzer. For co-located validation grab samples, less than 25% RPD. On-board standards, DIW and HOBO do not drift.
Representativeness	Plot histograms to evaluate temporal variability of results in relation to tidal conditions and storm events and to historical results for Fore River estuary.	Distributions within ranges from historical data, if available for DIN, and extrapolated from FOCB and Maine DEP data.

Comparability	Compare results to measurements collected by Friends of Casco Bay and Maine DEP at nearby station ***but see caveat below.***	Within 50% of nearby measurements at appropriate scales and periods.
Completeness	Compare to project objectives; two-hour intervals capture a range of conditions; analyzer operates as expected and provides usable data during the sampling window.	Two-hour intervals detect changes in nutrients related to tidal and storm events. Expect greater than 80% of measurements within the project period or within the tidal and storm conditions. Minimum of 3 to 5 two-week periods, and 3 significant rainfall events.

Caveat on comparison between the NuLAB analyzer and discrete samples for dissolved inorganic nitrogen: It is important to point out that the system pumps water through a 70-micron filter (to improve performance), which is typically larger than filters (0.45-micron) used to separate out particulate from dissolved inorganic nitrogen. Based on a comparison of the NuLAB analyzer to 0.45 micron filtered samples, Stroud Water Research Center found that results were comparable, at least for dissolved phosphorus.[6] Thus, we need to caveat our description of nitrate+nitrite and ammonium as dissolved. We will, however, conduct a small study to test whether 70-micron filtered samples are equivalent to .45 micron. We will collect a minimum of five samples that are split between 70-micron 0.45-micron filter and include these samples in a batch with other grab samples for analysis at UNH. These samples can be collected by turning on the pump and sampling from the waste end.

Temporal Resolution

Timing of sample collection needs to be sufficiently accurate to allow us to correlate observations with other time-varying phenomena. Most ancillary data have low temporal resolution (daily or hourly). Two important ancillary data sources, however, have higher temporal resolution. River discharge data from the USGS Presumpscot River gauge is reported every 15 minutes.[7] There is no USGS river gauge for the Fore River, but one of the tributaries to the Fore River is Long Creek, and some discharge data are available. Tidal elevations are reported at NOAA's Portland Tide station on a six-minute interval. Our data quality goal, therefore, is to ensure that sample collection times occur within six minutes of the nominal sampling time.

[6] Memorandum on NuLAB-Plus performance from Marc Peipoch, Ph.D, Assistant Research Scientist, Stroud Water Research Center. Undated.

[7] We recognize that we may have to estimate time of travel of Presumpscot discharge to the Fore River estuary.

1.8 Special Training Requirements/Certification

All staff collecting field samples have significant prior experience collecting water quality samples collected for nutrient analyses and working under stringent data quality control practices. CBEP will train the student in operation of analyzer. FOCB will train the student to collect water samples as per FOCB sampling protocols described in section 2.2 and in the FOCB QAPP (Attachment 4; RFA number 17014). Vince Kelly trained CBEP staff in March 2019. Laboratory analyses will all be carried out by experienced laboratory technicians at the University of New Hampshire Water Quality Analysis Laboratory, as part of a contract established by FOCB.

1.9 Documents and Records

Curtis Bohlen will distribute the QAPP to all participants listed in the distribution list. We have identified five key documents and checklists, as described in detail in Section 2.6. Specifically, the following documents will be stored by CBEP project manager, Curtis Bohlen. These checklists are hard copy and will be collected in a three-ring notebook managed by the USM student and Curtis Bohlen. Ultimately, Curtis Bohlen and Marti Blair will be responsible for permanently storing these records in the CBEP office. They are:

- Pre-deployment Laboratory checklist
- Field equipment checklist
- Field Deployment / Maintenance checklist
- “Daily” remote data check logging sheet
- Validation Sample with Chain of Custody datasheet

CBEP will also receive an electronic data package from UNH through Friends of Casco Bay. Curtis Bohlen will store these data packages.

B. DATA GENERATION AND ACQUISITION

2.1 Experimental Design

The analyzer is deployed off a pier in South Portland. A measurement is taken every two hours. The two-hour interval was selected as a trade-off between the need to resolve temporal changes, and costs for the reagents. Every two weeks, the system will be visited to clean biofouling, and grab samples are taken to validate the analyzer measurement. This site was selected because it receives contributions from upstream riverine sources, wastewater from the SP WWTF, and offshore (i.e. Casco Bay) marine sources. It is also a relatively secure location**.** Table 6 lists the number of data records and QC samples taken.

Water is pumped into the system through a 70-micron filter and then into a reaction vessel. Syringes deliver precise volumes of sample water, standards, and reagents into the vessel through a rotary valve. After reaction of the reagents (delivered from IV bags) in the vessel, the water is delivered by the rotary valve to a flow cell, where it is exposed to a LED light source. Absorbance by the color in the reaction is than quantified by a photodiode and associated electronics and read as a voltage (Figure 5).

The NuLAB analyzer is managed via a Raspberry Pi Controller[8]. During analyses, the valve, syringe and detector are all controlled by macros, which are text files of instructions loaded into the microcontroller unit (MCU) board of each NuLAB analytical channel; thus, the analyzer can be run from a PC or a data logger. Each chemistry (NO_3 + NO_2, NH_4) has specific macros which are in turn specific to the detector style. The controller allows the user to invoke specific macros, set up long-term logging operations, and to access recorded results. A cell phone WiFi hotspot communicates with the controller via Bluetooth, allowing remote access to the controller over the internet. Remote access allows near real-time data downloads, periodic checking of instrument status, reprogramming timing of logging, and remote troubleshooting.

Figure 5. The system opened and being set up in March 2019 at the USM Lasley-Rasher lab space. You can see reagent bags connected via tubes to the rotary valves which are connected to syringes. The small black boxes are the colorimeters. The touch screen at the top displays the macros and system status. There are also containers for waste.

[8] https://www.raspberrypi.org/

Table 6. Number of data records, samples or QC samples assuming a two-month (~60 days) deployment in 2019.

Sample type	Frequency	Number of samples, data records or QC samples	Type of sample
NuLab (Nitrate+nitrite and ammonia)	Every two hours	~720	Data record
NuLab OBS	Every two hours	~720	OBS (standards)
NuLab DIW	Every two weeks	~10	DIW (blank)
Validation grab samples	Every two weeks	~20 (each validation sample will be collected in duplicate)	Validation
HOBO conductivity and temperature	Every six minutes	~14,400	Data record
HOBO conductivity and temperature	Before and after deployment	2	Check for drift

2.2 Sampling methods

The system will pump surface water at approximately one-half meter depth, depending on the final wiring and plumbing for the submersible pump. The Onset HOBO conductivity-temperature data logger will be deployed immediately adjacent, in a stilling well.

To compare the continuous monitor analyses to standard lab analyses, CBEP staff will take validation grab samples at the same depth, soon after the NuLAB begins collecting or processing a sample (the pumps and stepping motors are readily audible). The NuLAB processes nitrate+nitrite (sometimes called NOx) and NH_4 samples together in approximately 30 minutes, so grab samples will be collected within 15 minutes of at least one measurement. In practice, grab samples will be collected between the NOx and NH_4 sample draws, reducing difference in sample timing further.

Validation grab samples are collected as per the FOCB QAPP (Attachment 4). Once water is collected (either through the pump outlet, or with a water sampler (bucket or Van Dorn) the water will be processes as follows: rinse a 500ml amber Nalgene bottle three times with sample water; fill it to a minimum of three-quarter full; cap and set in cooler to be filtered. Mix the Nalgene bottle and slowly draw up a small amount of the sample water (10 ml±) into a syringe, remove from bottle, pull plunger back, and rinse three times with sample water, then attach to a filter holder and rinse a small glass scintillation vial three times, and then fill the vial two-thirds full leaving room for expansion upon freezing. Cap and place in cooler (out of direct sunlight), and record on datasheet. All bottles will always be stored vertically and retightened after freezing. Before analysis, they will be shaken after thawing with the lid tight.

2.3 Sample Handling and Custody

Validation grab samples will be placed in a labelled freezer bag and delivered to the FOCB office with a checklist/field datasheet that includes Chain of Custody information and relinquished to a FOCB staff member. The sample vials will be placed in the FOCB freezer immediately and stored for no more than 4 weeks prior to delivery to the UNH Water Quality Analysis Laboratory. They will be checked for completeness and delivered frozen with a Chain of Custody form. The UNH QAPP (Attachment 5) requires samples are expected to be delivered frozen and can be held indefinitely.

2.4 Analytical Methods

For both the NuLAB nutrient analyzer, and the UNH Water Quality Analysis Laboratory, the analytic chemistry used is the same (Table 7). Sample collection and processing, however, differs. The NuLAB analyzes each sample immediately upon collection, and thus does not preserve or freeze the sample. The NuLAB samples are filtered through a 70-micron filter, rather than the 0.45-micron filters used for preparing samples to ship to UNH.

Table 7. EPA Methods and MDLs established by the UNH Water Quality Analysis Lab and equivalent to NuLAB automated analyses. Detection limit is based on user experience and previous analysis (not statistically calculated). For more information, see Attachment 5.

Analyte	Method	MDL	Source
Nitrate+nitrite	EPA method 353.2	0.005 mg/l as N	https://www.epa.gov/sites/production/files/2015-08/documents/method_353-2_1993.pdf
Ammonium	EPA method 350.1	0.005 mg/l as N	https://www.epa.gov/sites/production/files/2015-06/documents/epa-350.1.pdf

2.5 Quality Control

The following quality control samples are routinely performed by the project team or automatically by the NuLAB analyzer. See Attachments 1 and 2 for additional information on instrument operation and data processing.

The quality control practices for the UNH Water Quality Analysis Laboratory are listed in Attachment 5. They include preparation and analysis of laboratory control samples, blanks, laboratory fortified blanks, duplicates, and internal and external audits.

Table 8. Quality control sample types for NuLAB analyzer.

Quality Control sample type	Purpose	Frequency
Deionized water (DIW) reagent blank	To estimate background absorbance due to reagents, cuvettes, etc.	Biweekly; because the system does not have a dedicated DI water source, DI samples will be run at deployment and at each field visit.
On-board standard	To calibrate analyzer with known value	Every observation
Discrete bottle sample	To compare analyzer results to laboratory analyses (validation samples).	Every two weeks, duplicate samples will be collected.

2.6 Instrument/Equipment Testing, Inspection, and Maintenance

We will follow the instructions in the NuLABs manual (Attachment 1) before deployment and daily. The data files will be interrogated no less than three times per week. The instrument will be visited in person every two weeks to check for biofouling, replace reagents, remove used reagents and check on other operational functions as described in Table 8. At each visit, information will be recorded on checklists or datasheets listed in Table 9.

Table 9. Equipment inspection, setup and calibration steps will be recorded on checklists and datasheets.

Field checklists	Purpose	Staff person	Attachment
Pre-deployment Laboratory Checklist	Ensure instrument is calibrated and functioning correctly	Bohlen, USM student Walsh	6
Field Deployment / Maintenance Checklist	Check instrument is functioning appropriately, check ports, filters, pump, replace reagents	Bohlen, USM student Walsh	7
"Daily" remote data check logging sheet	Periodic check on equipment function review of recent data, and check on stability of absorbance of OBS	USM student, Walsh, Bohlen	8
Field equipment checklist	Ensure equipment, reagents, DIW, and spare parts are available in field for instrument inspection and maintenance	USM student Walsh, Bohlen	9
Validation Sample datasheet and Chain of Custody	Ensure validation samples for UNH are properly collected, along with ancillary data for salinity and temperature	USM student Walsh, Bohlen	10

2.7 Instrument/Equipment Calibration and Frequency

The NuLAB analyzer measures light absorbance along an optical path inside separate optical detectors for the nitrate+nitrite and ammonium (NH_4) channels. Absorbance values are converted to estimates of concentration using a linear relationship calibrated based on a two-point calibration (see Attachment 2) of the DIW and the On-board standards (OBS).

The zero-concentration value in the calibration is established periodically by running three or more DIW blanks through the NuLAB and recording observed absorbances. Mean absorbance is entered manually into the NuLAB operating software. We will establish the zero-concentration in the laboratory before deployment.[9] The zero-concentration is expected to be stable during deployment. We will check, however, for drift approximately every two weeks during field maintenance visits. At that time, we will run a DIW blank calibration check.[10] If the observed zero-concentration absorbance is not within 3 standard deviation of the pre-deployment mean, we will run two more DIW blanks, and reset the zero-concentration calibration in the NuLAB operating software to the mean absorbance of the three. Instrument readings are automatically calibrated against the OBS with each observation. The NuLAB records absorbance of the OBS and uses it to estimate the calibration curve relating the absorbance of the sample to concentrations of the analyte.

Periodic remote checks on the instrument will be carried out at least three times a week. During those remote checks, we will record the most recent three OBS absorbances for both NOx and NH_4 and evaluate whether values are showing signs of drift (change of more than 3%) or other anomalies. Absorbance is expected to be relatively stable.

The calibration procedure for the Onset HOBO U24 is based on measured points for conductivity and temperature at the beginning and end of a deployment which are used to post-process the data. Conductivity and temperature readings are plugged into HOBOWare's "Conductivity Assistant" tool. The tool is designed to compensate for drift and fouling over a deployment. See Attachment 3.

The refractometer will be occasionally checked against deionized water and seawater with known salinity. The handheld pocket thermometer will be checked against a NIST calibrated thermometer if available.

2.8 Inspection/Acceptance of Supplies and Consumables

The reagents are provided by Green Eyes, LLC and will be used before they expire. The system is housed in a box and is not exposed to light, thus minimizing degradation. Green Eyes also recommends that the box be painted a reflective color, which it is. According to Green Eyes, the reagents are stable at the

[9] Vince Kelley recommends running the DIW blank for ammonium outside because some laboratory air is contaminated with ammonia from cleaning products.

[10] Running three DIW blanks through the system on each channel, at more than 15 minutes per sample, takes over an hour and a half. Thus, running a full DIW blank calibration mid-deployment is likely to force us to skip at least one round of observations.

temperatures expected during the sampling window. Systems have been deployed in Florida and Chesapeake Bay. In addition, the filter and tubing for pumping samples will be examined every two weeks.

2.9 Non-direct Measurements

The following datasets will be used in the analysis of results.

- Rainfall data at the National Weather Service's Portland Jetport weather station will be acquired via NOAA's Climate Data Online (CDO) portal.[11]
- Tidal data will be accessed from NOAA's Portland Tide Station.
- Data on Presumpscot River Discharge will be accessed from USGS Presumpscot River Gauge.
- Daily discharge data from the South Portland WWTF will be acquired from the South Portland WWTF.
- Daily discharge data from the East End WWTF will be acquired from Portland Water District.
- Data on CSO discharges in Portland is released by the City of Portland monthly.
- We will work with the City of South Portland to access data on CSO discharges in South Portland.

2.10 Data Management

All data generated by the NuLAB are recorded on the Raspberry Pi Controller as text files and can be accessed and downloaded remotely. A log file is also produced, which logs every action. Data will be downloaded no less than three times per week and stored on a CBEP computer at USM. The Log files will be downloaded every two weeks, after the instrument is checked in the field.

C. ASSESSMENT, OVERSIGHT AND REPORTING

3.1 Assessment and Oversight

3.1.1 Corrective Actions

The project manager and USM student will evaluate measurements and performance of the analyzer routinely against the quality control samples listed in Table 8. If duplicates are outside the expected range, additional duplicates may be performed. If the OBS or DIW drifts, the instrument may need to be re-calibrated, or new DIW may need to be prepared. If instrument is not operating as expected, troubleshooting will be conducted. Additional data review will be conducted routinely as described in section 4.1

[11] NOAA's CDO portal has better archiving capability but less temporal resolution than the National Weather Service portal.

3.1.2 Deviations from QAPP

The QA manager will conduct periodic assessments of the project for deviations of the QAPP, such as inspection and maintenance of the instrument, and preliminary data reviews described in section 4.1.

3.2 Reporting

All usable data will be included in summary data reports. These data reports will be provided to participants on the project, and partners in the monitoring network. Synthesis reports and summaries will be provided to the public (via the program website), the Nutrient Council and incorporated into the CBEP State of the Bay report.

D. DATA REVIEW AND USABILITY

4.1 Data Review

The instrument will be accessed remotely at least three times per week to check on equipment function and review data. Recent data will be inspected to confirm that absorbance of the OBS and DIW reagent blanks are within expectation, and that measured field nitrogen concentrations are reasonable, based on prior observations. Any questionable observations or OBS absorbances will trigger assessment and trouble shooting. These preliminary data will be graphed and checked for continuity and extreme values. Data that are questionable will be flagged and may be omitted from further analyses. Preliminary data may be shared in graphical form on-line or for scientific presentations but will be clearly labeled as preliminary.

At the end of deployment, the data will again be graphed, and checked for continuity, extreme values, outliers and drift. Correlation between nitrate+nitrite and ammonium will be examined, looking for bivariate outliers. We will check data (including absorbances) and instrument logs associated with extreme values or outliers, to see whether there is any indication of equipment malfunction or contamination. We will cross-reference extreme values with ancillary data like rainfall and sewer discharges, to see whether high values may simply reflect short-term phenomena like summer storms. Results will also be compared to discrete samples analyzed by UNH. Measures of comparability will include calculations of Relative Percent Difference (RPD), and graphical analyses of differences over time.

4.2 Data Usability

Data will be evaluated against the project goals and the Data Quality Objectives. The project manager will evaluate whether the detection limits and other DQIs goals were met, and whether adequate tidal stages and weather conditions were sufficient to evaluate differences among different potential sources of nutrients to the Fore River estuary.

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