

## **SAMPLING AND ANALYSIS PLAN**

### **Sediment Phosphorus Flux in Three Massachusetts Lakes; Horn Pond, Wedge Pond, and Spy Pond**



#### **Prepared for:**

Watershed Planning Program  
Division of Watershed Management, Bureau of Water Resources  
Massachusetts Department of Environmental Protection



#### **Prepared by:**

GEI Consultants Inc.



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### **Cover Photo**


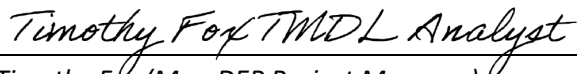
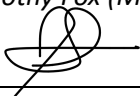
Spy Pond and various elements of a phosphorus flux study, Steven Wolosoff and Craig Wolf

### **Disclaimer**

References to trade names, commercial products, manufacturers, or distributors in this report constituted neither endorsement nor recommendation by MassDEP.

## Sampling and Analysis Plan - Sediment Phosphorus Flux in Three Massachusetts Lakes

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This SAP has also been appended to Sediment Phosphorus Flux in Massachusetts Lakes QAPP (MassDEP 2025).

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## Acronyms and Abbreviations

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cm	Centimeter
COC	Chain-of-Custody
CWA	Clean Water Act
cc/sec	Cubic Centimeter per Second
DQO	Data Quality Objective
DIW	Deionized Water
DO	Dissolved Oxygen
ft	Foot and Feet
HDPE	High Density Polyethylene
km	Kilometer
LCS	Laboratory Control Samples
LLRM	Lake Loading Response Model
MassDEP	Massachusetts Department of Environmental Protection
MWRA	Massachusetts Water Resources Authority
MS	Matrix Spikes
m	Meter
µm	Micrometer
mg/L	Milligrams per Liter
mL	Milliliter
MyRWA	Mystic River Watershed Association
N <sub>2</sub>	Nitrogen Gas
N/A	Not Applicable
OP	Orthophosphate
ppm	Parts per Million
pH	Potential of Hydrogen
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RPD	Relative Percent Difference
RL	Reporting Limit
SAP	Sampling and Analysis Plan
TMDL	Total Maximum Daily Load
TP	Total Phosphorus
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WPP	Watershed Planning Program

# **1. Introduction**

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Excess nutrients in freshwater lakes and ponds are the primary cause for eutrophication and associated impairment of Designated Uses for recreation, aquatic life, and drinking water. Sources of nutrients can be either external such as stormwater runoff, groundwater, point source discharges or atmospheric deposition, or internal such as releases from lake bottom sediment by diffusion or physical resuspension. Section 303(d) of the Clean Water Act (CWA) requires states to identify waters within their boundaries that are not meeting state water quality standards. For these impaired waterbodies, Section 303(d) further requires the United States Environmental Protection Agency (USEPA) and individual states to develop a Total Maximum Daily Load (TMDL) for the pollutant(s) violating or causing violation of water quality standards. A TMDL is used to determine the extent of nutrient loads a lake can receive and still meet water quality standards. Conducting a linkage analysis establishes the relationship between nutrient loads to lake water quality responses. The linkage analysis creates a nutrient budget for the lake accounting for external inflows, flushing to downstream waters, biological uptake, particulate settling to the lake bottom, and release of solubilized forms from the lake bottom. In lakes with a low watershed to lake surface area ratio, internal load from lake bottom sediments may account for most of the bioavailable phosphorus in the water column during the growing season. Thus, internal loads can significantly reduce the ability for lakes to assimilate external load and meet water quality standards.

The Commonwealth of Massachusetts Department of Environmental Protection (MassDEP) plans to develop new TMDLs for three freshwater lakes, Horn Pond, Wedge Pond, and Spy Pond and seeks to collect site-specific data to parameterize sediment phosphorus flux in a lake water quality model that will serve as the linkage analysis for the TMDLs. This Sampling and Analysis Plan (SAP) serves to guide the collection of lake sediment for experimental laboratory analysis of phosphorus flux rates to better define internal phosphorus loading. This SAP has also been appended to Sediment Phosphorus Flux in Massachusetts Lakes QAPP (MassDEP 2025).

## **1.1. Mystic River Watershed**

The headwaters of the Mystic River originate near Reading, Massachusetts, and encompasses a 76-square mile area called the Mystic River Watershed that drains into Boston Harbor (Figure 1). The watershed has been divided into three sub-watersheds, an upper, central, and lower, which includes 44 lakes and ponds that provide aquatic life, recreational, and aesthetic uses for 22 communities (USEPA 2020). Three of the 44 waterbodies—Horn Pond, Spy Pond, and Wedge Pond—are included on the 2022 Massachusetts CWA Section 303(d) list (MassDEP 2023) as impaired by total phosphorus, low dissolved oxygen, and harmful algal blooms, which also affect chlorophyll-a content and Secchi-disk transparency in each waterbody. There are other pollutant issues listed in Section 2.1 that may be tangentially linked to nutrient-related impairment but are not a focus of this SAP.

## **1.2. Waterbody Locations and Site Descriptions**

Horn Pond is in the upper watershed (Figure 2), within Woburn, Massachusetts, and receives tributary inflows from Fowle Brook and Sucker Brook as well as surface water runoff from the surrounding land use with outflows to Horn Pond Brook. Horn Pond has a surface area of 102 acres, a maximum water

depth of 40 feet (ft), a mean water depth of 10 ft, and mean residence time of 99 days. It is a Class B waterbody qualified as a Warm Water Fishery and identified as MA71019 (MassDEP Assessment Unit Identification code [AU\_ID]). Class B waters are designated as a habitat for fish, other aquatic life, and wildlife, including for their reproduction, migration, growth, and other critical functions, and for primary and secondary contact recreation. Class B waters shall also be suitable for irrigation and other agricultural uses, compatible for industrial cooling and process uses and shall have consistently good aesthetic value (MassDEP 2021).

Wedge Pond is also in the upper watershed (Figure 3) and located approximately 1.5 kilometers (km) downstream of Horn Pond, in Winchester, Massachusetts, and receives tributary inflows from Horn Pond Brook, as well as surface water runoff from the surrounding land use areas with outflow to Aberjona River. The tributary inflow and outflow are located near each other, on the northeast shoreline, which effectively circumvents flow past Wedge Pond. Wedge Pond has a surface area of 22 acres, a maximum water depth of 16 ft, a mean water depth of approximately 6 ft, mean residence time of 8 days, and is a Class B waterbody identified as MA71045.

Spy Pond is in the lower watershed (Figure 4) near East Arlington, Massachusetts, and primarily receives surface water runoff from the surrounding land use area with no well-defined tributary inflows. The outflow is connected to Little Pond that discharges to Little River (a.k.a. Alewife Brook) a tributary to Mystic River. Spy Pond has a surface area of 103 acres, a maximum water depth of 36 ft, a mean water depth of 12 ft, a mean residence time of 292 days, and is a Class B waterbody identified as MA71040.

Most of the land use areas surrounding each waterbody are single family residential units with a mixture of open space/greenbelt and inlet wetlands. Although the watershed contains multifamily residential units and commercial property that typically generate more surface water runoff than single family residential and open space land use.



Figure 1. Map of the Mystic River Watershed

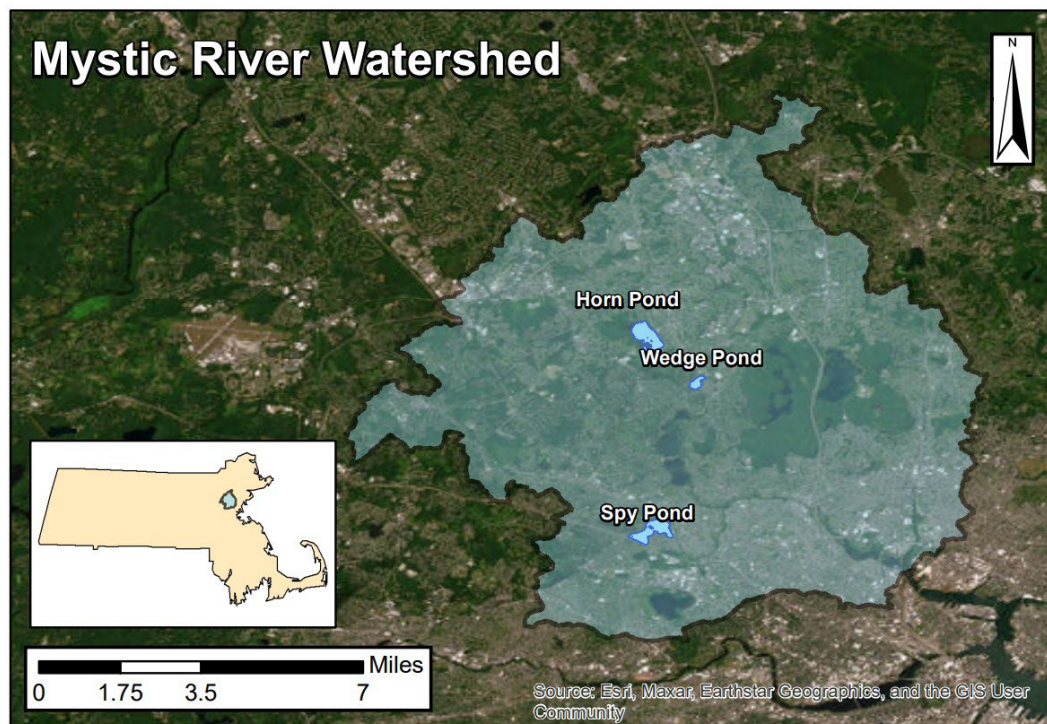


Figure 2. Bathymetry Map of Horn Pond with Sampling Locations and Extent of Anoxia

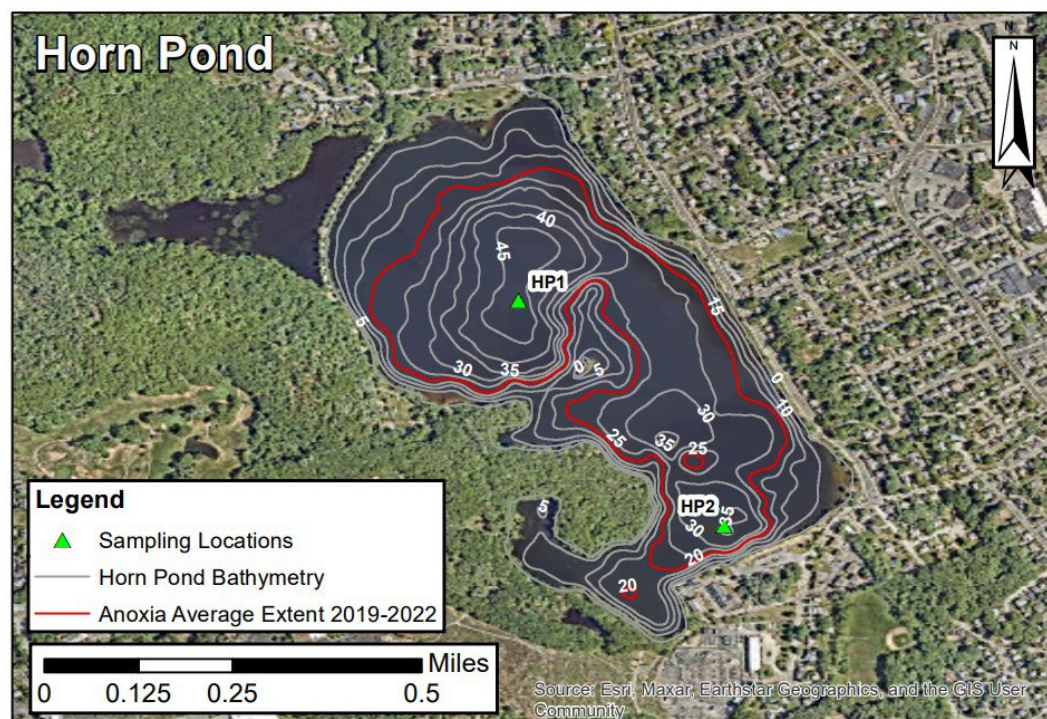




Figure 3. Bathymetry Map of Wedge Pond with Sampling Locations and Extent of Anoxia

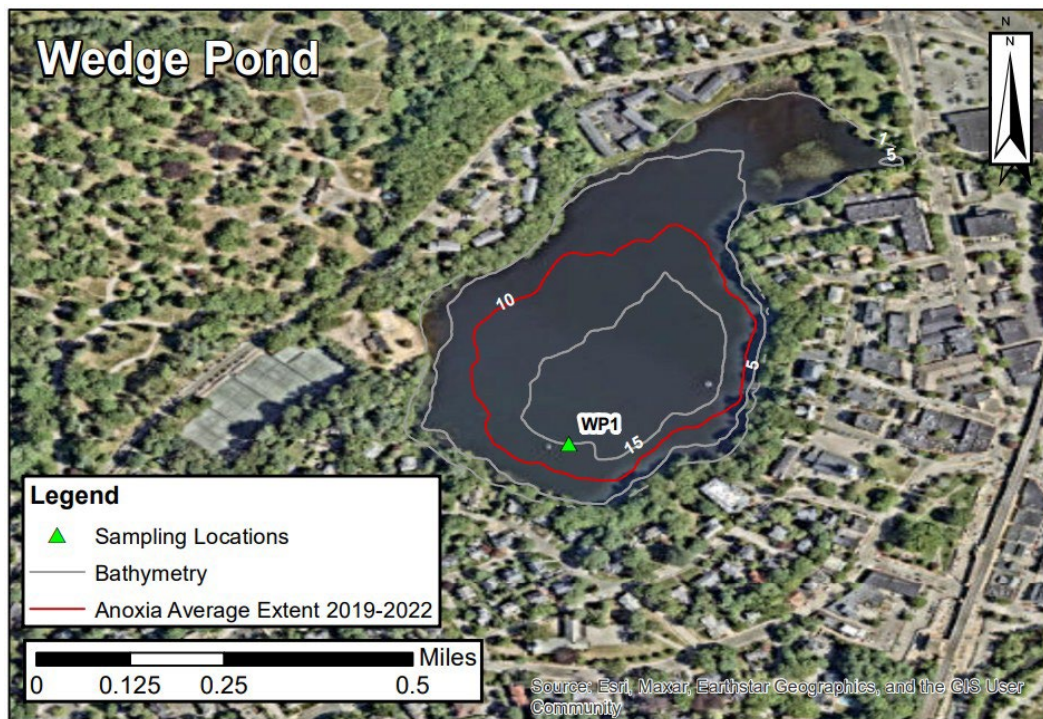
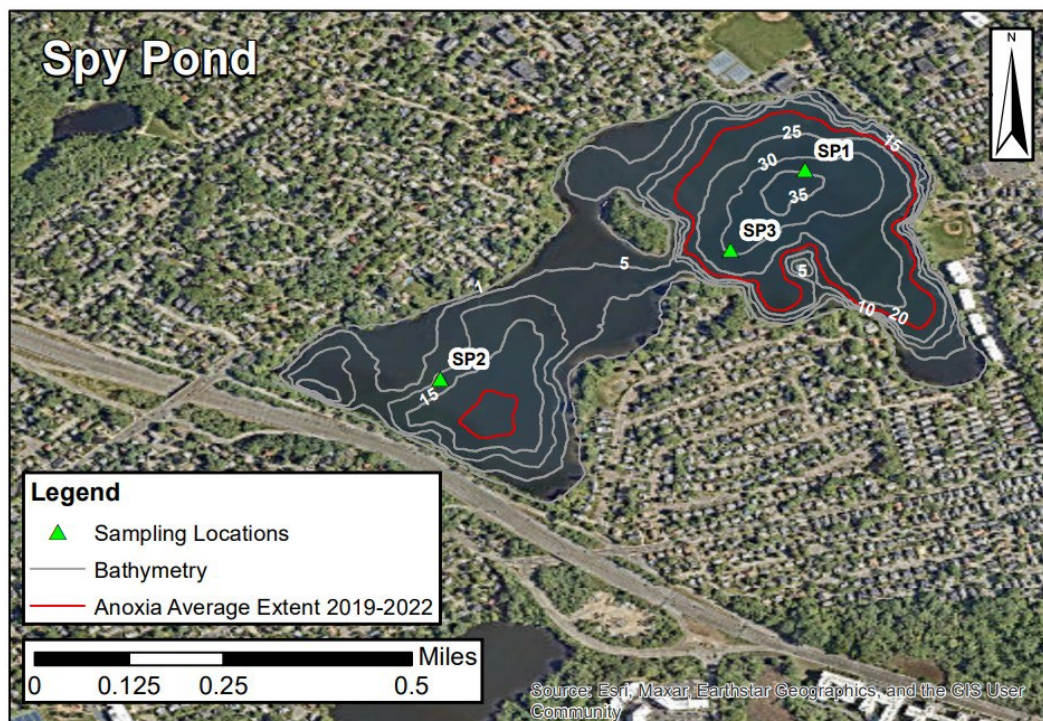


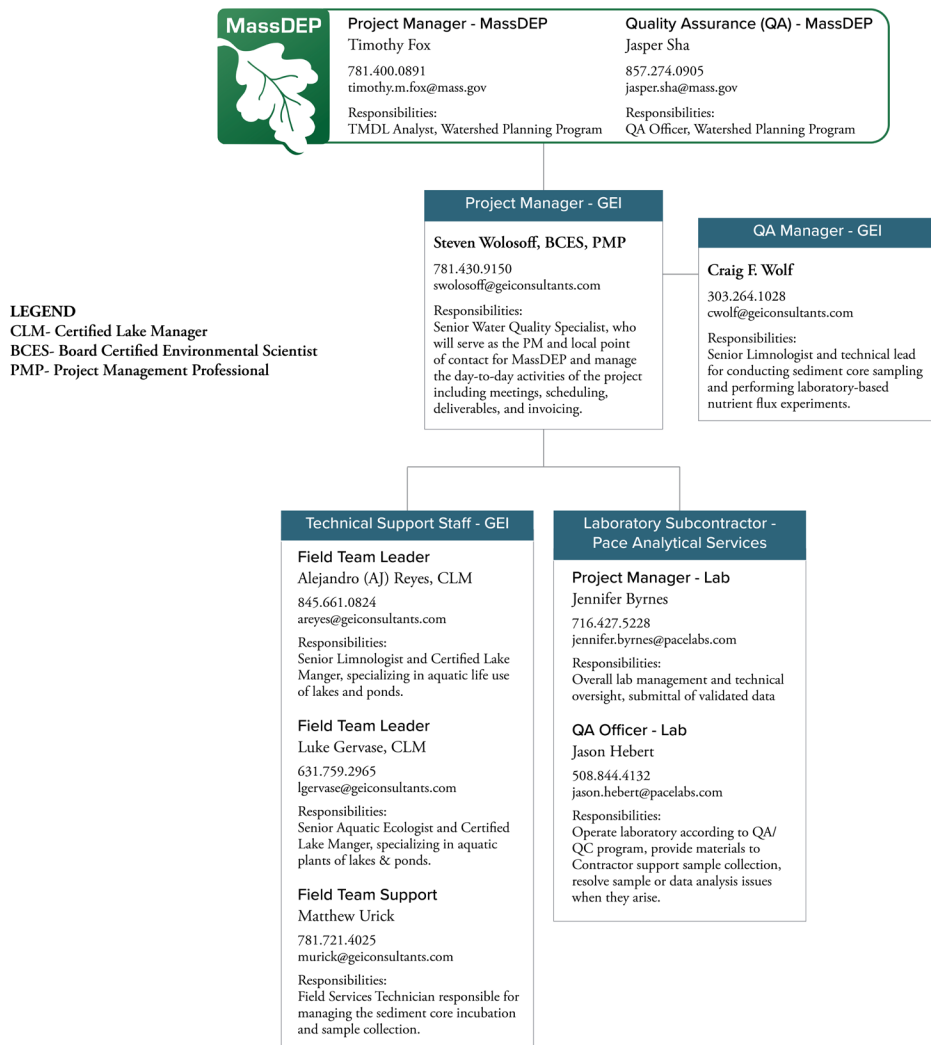
Figure 4. Bathymetry Map of Spy Pond with Sampling Locations and Extent of Anoxia.



### 1.3. Project Organization

The project team includes a group of experts with diverse experience in freshwater lake science involving monitoring, modeling, analysis to provide basis for regulatory actions, and development and implementation of lake management plans. In addition, the project team has experience with water quality monitoring of lakes and ponds and are responsible for overseeing the on-site geotechnical and environmental lab. An organizational chart for the MassDEP project team (Figure 5) is provided herein, showing the primary roles each member will serve to implement the project. The project distribution list is provided in Table 1.

**Figure 5. Organization Chart.**



## Sampling and Analysis Plan - Sediment Phosphorus Flux in Horn, Wedge, and Spy Ponds

**Table 1. Key Project Personnel, Contact Information, and Responsibilities**

<b>Organization</b>	<b>Title / Name</b>	<b>Contact Information</b>	<b>Responsibilities</b>
MassDEP	Project Manager, Timothy Fox	(781) 400-0891 timothy.m.fox@mass.gov	Oversee, manage development and distribution of project specific SAP and conduct of study.
MassDEP	Quality Assurance (QA) Officer, Jasper Sha	(857) 274-0905 jasper.sha@mass.gov	Overall technical oversight of project data QA/QC and management.
Contractor (GEI)	Project Manager, Steven Wolosoff	(781) 430-9150 swolosoff@geiconsultants.com	Primary person or entity responsible for project meeting materials and development of project specific SAP, conduct of study, and all reporting.
Contractor (GEI)	QA Manager, Craig Wolf	(303) 264-1028 cwolf@geiconsultants.com	Primary person responsible for project QA/QC procedures and is independent from project operations. This includes creation of electronic data deliverables and data validation and regularly communicating with the project manager.
Contractor (GEI)	Field Team Leader, Alejandro (AJ) Reyes	(845) 661-0824 areyes@geiconsultants.com	Under the direction of the Project Manager and Chief Scientist, the Project Scientist must follow all procedures for field sample collection and conduct of core-flux experiment.
Contractor (GEI)	Field Team Leader, Luke Gervase	(631) 759-2965 lgervase@geiconsultants.com	Under the direction of the Project Manager and Chief Scientist, the Project Scientist must follow all procedures for field sample collection and conduct of core-flux experiment.
Laboratory (Pace Labs)	Laboratory Project Manager, Jennifer Byrnes	(781) 427-5228 Jennifer.byrnes@pacelabs.com	Overall lab management and technical oversight regarding the performance of laboratory analysis and submittal of validated data to the Contractor Project Manager in compliance with contractual arrangements.
Laboratory (Pace Labs)	Laboratory QA Officer, Jason Hebert	(508) 844-4132 Jason.hebert@pacelabs.com	Operate laboratory according to QA/QC program, provide to the Contractor all the necessary containers, preservatives (if required), chain-of-custody (COC) forms to support sample collection, and resolve sample or data analysis issues when they arise.

## 2. Background

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The Final Massachusetts Integrated List of Waters for the Clean Water Act 2022 Reporting Cycle (MassDEP 2023) listed Horn Pond, Wedge Pond and Spy Pond as Category 5 impaired waterbodies requiring the development of a nutrient TMDL for each waterbody (Table 2). The TMDLs will define the maximum amount of phosphorus that a waterbody can assimilate while continuing to meet applicable water quality standards. The TMDLs will also allocate the maximum allowable load between internal and external sources and provide a framework for USEPA, MassDEP, and partner organizations to establish and implement nutrient control and management plans with the goal of achieving water quality conditions that support the beneficial uses. This SAP seeks to inform the internal phosphorus loading component of the lake TMDL development.

**Table 2. Mystic Lakes Pollutants of Concern Regarding Nutrient TMDL Development**

Waterbody	Pollutants of Concern	Supporting Information
Horn Pond	Total Phosphorus	5 sampling events 2019, 5 sampling events 2021; Range = 0.0069 - 1.000 mg/L
	Dissolved Oxygen	5 sampling events 2019, 5 sampling events 2021, 5 sampling events 2022; Range = 0.20 - 15.53 mg/L
	Harmful Algal Blooms	N/A
	Invasive Aquatic Plants	>25 % of surface area covered by non-rooted macrophytes and/or algal mats/films/clumps
	TP Load Estimate	2 sampling events to calculate load, 2000-2016
Wedge Pond	Total Phosphorus	5 sampling events 2019, 5 sampling events 2021; Range = 0.0090 - 0.1300 mg/L
	Dissolved Oxygen	5 sampling events 2019, 5 sampling events 2021, 5 sampling events 2022; Range = 0.20 – 12.74 mg/L
	Harmful Algal Blooms	N/A
	TP Load Estimate	33 sampling events to calculate load, 2000-2016
Spy Pond	Total Phosphorus	5 sampling events 2019, 5 sampling events 2021; Range = 0.0065 - 1.200 mg/L
	Dissolved Oxygen	5 sampling events 2019, 5 sampling events 2021, 5 sampling events 2022; Range = 0.20 – 12.37 mg/L
	Harmful Algal Blooms	N/A
	Invasive Aquatic Plants	N/A
	TP Load Estimate	17 sampling events to calculate load, 2000-2016

## 2.1. Previous Investigations and Regulatory Background

The Mystic River Watershed Alternative TMDL analysis for Phosphorus Management was conducted between 2017 and 2019 to estimate annual loadings of phosphorus; relate phosphorus loads to response variables in critical surface water reaches of the watershed; estimate the load reductions needed to improve water quality and attain water quality standards; and to introduce a pilot Opti-Tool analysis that demonstrates cost-effective and opportunistic stormwater load reduction strategies that communities can consider adopting (USEPA 2020).

The stakeholder driven process included EPA Region 1, Mystic River Watershed Association (MyRWA), MassDEP, United States Geological Survey (USGS), and Massachusetts Water Resources Authority (MWRA). The components of the alternative TMDL included:

- Develop conceptual model of hydrology and nutrient dynamics
- Evaluate existing water quality data
- Review modeling endpoint approaches
- Estimate watershed phosphorus loading
- Evaluate combined sewer overflow and sanitary sewer overflow data
- Develop BATHTUB model and calibrate results
- Determine critical period of interest for phosphorus load reduction analysis
- Evaluate watershed phosphorus load reduction analysis
- Develop nutrient stormwater management strategies using Opti-Tool

In 2019, MassDEP Watershed Planning Program (WPP) began an intensive water quality monitoring program to evaluate the current trophic status of Horn Pond, Wedge Pond, and Spy Pond to aid in the development of a Lake Loading Response Model (LLRM) to support TMDL development for each waterbody and the Mystic River Watershed Alternative TMDL. The water quality data included:

- Vertical water quality profiles for temperature, dissolved oxygen, pH, and conductivity
- Secchi disk transparency
- Nutrients (total nitrogen and total phosphorus)
- True color and turbidity
- Chlorophyll-a (depth integrated)
- Aesthetic observations
- Human disturbance observations
- Bathymetry

## **2.2. Hydrogeological Information**

The Mystic River Watershed has a complex hydrogeological condition that is typical of glaciated New England fluvial valleys with glacial till deposits of sand and gravel that overlie granitic bedrock (de Lima and Olimpio 1989). In wetland areas, peat deposits overlie stratified drift material. Shallow groundwater recharges and discharges fluctuate continuously within the stratified drift material and is primarily driven by storm events.

Horn Pond and Wedge Pond receive phosphorus loads primarily from stormwater runoff. Much of the upper watershed is serviced by separate sanitary sewer and storm sewer drainage systems, and there are no combined septic/storm sewer areas or wastewater treatment facility discharges upgradient of either waterbody (USEPA 2020). However, sanitary sewer overflows are known to occur infrequently during major storm events but still may be a source of short-term nutrient pulses to the waterbodies (USEPA 2020).

Spy Pond primarily receives phosphorus loads from stormwater runoff. Spy Pond is in the lower watershed, which is heavily influenced by combined septic/storm sewer overflows that increase phosphorus loads to those receiving waters. Although, recent management efforts have greatly reduced the hydrological load from these outfalls, and many outfalls have been closed along Alewife Brook and Mystic River, the legacy of combined septic/storm sewer overflows likely increased the organic matter load to the pond, which contributes to the internal phosphorus loading and poor dissolved oxygen conditions.



### **3. Project Objectives**

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The purpose of this SAP is to guide the field collection of sediment cores from Horn Pond, Wedge Pond, and Spy Pond to be used in laboratory-based experiments for the quantification of site-specific phosphorus release rates under anoxic and oxic conditions. MassDEP will incorporate the site-specific release rates in a LLRM to quantify the internal nutrient load for each waterbody.

#### **3.1. Field Quality Objectives**

Field collection of sediment cores shall be performed in a manner that preserves the sediment/water interface and maintains a consistent volume of sediment and water in each replicate core for a sample location. Sediment cores shall be collected in deep or mid-depth areas of the pond that experience low dissolved oxygen conditions (i.e., < 2 mg/L) during a portion of the summer.

Field collection of lake water representative of conditions near (i.e., within 1 meter) the sediment interface shall be collected in a manner that maintains the chemical characteristics of the “bottom” water at the sample location.

#### **3.2. Laboratory Quality Objectives**

Laboratory-based phosphorus flux experiments shall be performed in a temperature and light-controlled environment for the duration of the experiment, simulating deep water conditions in the ponds. The overlying water column in the incubated cores will be replaced with 0.2 micrometer ( $\mu\text{m}$ ) filtered water in a manner that preserve the integrity of the sediment/water interface.

#### **3.3. Data Quality Objectives**

The data quality objectives (DQOs) for this study will be achieved if the sediment cores and water quality data can be used to determine the phosphorus flux rate for lake sediment under controlled laboratory conditions. Data quality objectives can be described in terms of the following performance indicators: sensitivity, precision, accuracy and bias, representativeness, comparability, and completeness.

##### **3.3.1. Sensitivity**

Sensitivity refers to the capability of a method or instrument to discriminate between measurement responses within the expected ranges reported in the QAPP. In the field and core experiment laboratory, the sensitivity of water quality sonde instruments shall achieve the desired resolution for field or laboratory sondes as reported in the QAPP. In the analytical chemistry laboratory, the sensitivity of analytical methods identified in Section 2.5 (Analytical Methods) of the QAPP shall be sufficient to measure analytes at or above the detection and reporting limits identified for analytes listed in Table 3 of the QAPP to meet DQOs.



### 3.3.2. Precision

Precision is the degree of mutual agreement between or among independent measurements of a similar property. Analytical chemistry measurements will be sufficiently precise to ensure detection of phosphorus within the expected environmental and experimental range. Precision will be measured using Relative Percent Difference (RPD) between duplicates with a 20 percent acceptance range. Field duplicates will be collected, representing at least 10 percent of the total environmental chemistry samples collected. In the core experiment laboratory, duplicates will be collected, representing at least five percent of the total core-flux chemistry samples collected. The Project Manager will check other data to ensure that they are of sufficient precision to meet DQOs.

### 3.3.3. Accuracy and Bias

Accuracy is the degree of mutual agreement of a measurement with a known value and includes systematic error (bias) of both sampling and analytical operations. Bias results in the distortion of the measurement process, which results in errors in one direction. In the field, accuracy will be assessed through the adherence to all instrument calibration, sample handling, preservation, and sample hold time. The bias component will be assessed using equipment blanks, representing at least 10 percent of the environmental samples collected. In the core experiment laboratory, equipment blanks will be collected, representing at least five percent of the total core-flux chemistry samples collected. The Project Manager will check other data to ensure that they are of sufficient accuracy and do not contain an unreasonable level of bias to meet DQOs.

In the analytical chemistry laboratory, accuracy and bias will be assessed through the analysis of matrix spikes (MS), laboratory control samples (LCS), blanks, and the percent recovery criteria of control samples and matrix spiked samples. Acceptable criteria limits for this project are provided in Table 3.

**Table 3. Analytical Laboratory Methods for Analysis of Phosphorus Content in Water**

Analyte	Method	MDL <sup>1</sup>	RL	Units	LCS Criteria (%)	MS Criteria (%)	MS RPD (%)	Duplicate RPD (%)	Hold Time
Phosphorus, Total (TP) <sup>2</sup>	SM 4500P-E	0.004	0.01	mg/L	80-120	75-125	20	20	28 days
Phosphorus, Orthophosphate (OP) <sup>3</sup>	SM 4500P-E	0.001	0.005	mg/L	90-110	80-120	20	20	48 hours

Notes:

1. The QAPP specifies a TP and OP MDL of at least 0.010 mg/L or lower to achieve data quality objectives. Pace Laboratory in Westborough, MA can achieve the lower MDLs.
2. TP sample preserved with sulfuric acid.
3. OP sample unpreserved.

### 3.3.4. Representativeness

Representativeness is the degree to which data accurately and precisely represents an environmental condition targeted for sampling. In the field, sediment cores will be representative of internal nutrient

loading conditions if cores are collected in accordance with methodology described herein and from locations in each pond that experience low dissolved oxygen conditions during a portion of the year. Depending upon sediment composition, the upper 10-centimeter (cm) layer is typically considered the active layer where microbial-mediated reduction of iron-bound and manganese-bound phosphorus occurs. Therefore, each core should contain at least 15 cm of sediment to fully capture this layer. If the sediment thickness is less, then the core should be visually inspected to evaluate any layering, and collection of another core should be considered. If the substrate layer is less than 10 cm, then the core will be discarded, and collection of another core will be performed. Site-specific field conditions such as sediment composition (e.g., sand and gravel), organic debris, or depth of refusal may influence sediment core collection, which may require moving to a different location.

In the core-flux laboratory, sediment core incubation conditions are representative of natural conditions observed in each pond and the oxic and anoxic release rates from the sediment cores are representative of internal phosphorus loading conditions for each sampling location. The Project Manager will check other data to ensure that they are adequately representative of the environmental conditions targeted to sample and will meet DQOs.

### **3.3.5. Comparability**

Comparability expresses the confidence with which one data set can be compared to another and is dependent upon the study design and implementation of the project's quality assurance plan. Sediment cores collected from different sampling locations will be sufficiently comparable, provided samples are collected in accordance with the methodology described herein and that the integrity of the water/sediment interface is maintained during collection, transport, and the duration of the core-flux experiment. Deviations from the established methodology will be noted by field/laboratory personnel.

In the core-flux laboratory, the flux rates will be sufficiently comparable between and within locations/treatment factors if experimental conditions (e.g., oxic or anoxic) are maintained for each core for the duration of the experiment. The Project Manager will check other data to ensure that they are sufficiently comparable to meet DQOs.

### **3.3.6. Completeness**

Completeness is expressed as a percentage of valid and useable data obtained compared to the amount that was expected. In the field, the collection of sediment cores and bottom water will be considered complete if at least 80 percent of the desired number of samples are collected from each waterbody. If samples cannot be obtained as planned, the field personnel will notify the Project Manager, and alternatives will be considered to improve the completeness of the field data. Any deviations will be noted in the field book and report.

In the core-flux laboratory, the experiment will be considered complete if at least 80 percent of the desired number of samples are collected from each core over the duration of the experiment; however, all samples from Day 0 must be collected from the experiment to be considered complete. Deviations from the sampling design will be noted by field/laboratory personnel. The Project Manager will check other data to ensure that they are sufficiently complete to meet DQOs.

## 4. Sampling Rationale, Methods, and Procedures

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Sampling of each waterbody for the purpose of sediment phosphorus flux experiments includes multiple components. Initially, water quality profile data are collected from each sampling location to provide a snapshot of environmental conditions to be used for setting up the laboratory incubator and to establish baseline conditions. Second, bottom water samples are collected for experimental use and to establish baseline phosphorus conditions. Third, sediment core samples are collected for the flux experiment. Lastly, once the flux experiment is setup, water samples are collected from each core over the duration of the experiment to monitor the change in phosphorus concentrations for oxic and anoxic treatment.

### 4.1. Rationale for Sample Locations

Sediment cores for phosphorus flux analyses will be collected from selected MassDEP water quality sampling locations in each waterbody in May 2025 (Table 4, Figure 2, Figure 3, and Figure 4). This will help pair phosphorus flux results with water quality data and previous estimates of areal hypoxia based on dissolved oxygen content measured at each site by MassDEP. In Horn Pond, two deep water sites were selected for sediment core collection because each basin is relatively isolated from each other given the bathymetry of the waterbody (Table 5). In Wedge Pond, a single deep-water location was identified because the waterbody has one basin, and the areal extent of anoxia covered a large portion of that basin. In Spy Pond, two deep water sites and one mid-depth site were identified for sediment core collection because the two deep water sites are relatively isolated and separated by an island and sill. The largest areal extent of anoxia occurs in the northern basin. The mid-depth sampling location in the southern basin will provide a phosphorus flux that may be more relevant for late season hypoxia as conditions spread throughout the waterbody. The two deep water locations will provide flux measurements relative to early season hypoxia and as conditions progress to anoxia.

**Table 4. Sediment Sampling Locations in Each Waterbody**

Waterbody	Site Name	MassDEP ID	Latitude	Longitude
Horn Pond	HP1	W1087	42.46963022	-71.15765018
Horn Pond	HP2	NA	42.46533078	-71.15370619
Wedge Pond	WP1	W1226	42.45289418	-71.14158508
Spy Pond	SP1	W2837	42.40999949	-71.15199400
Spy Pond	SP2	W2839	42.40600014	-71.15899647
Spy Pond	SP3	NA	42.40846981	-71.15342687

**Table 5. Sediment Sampling Design and Rationale**

Site	Number of Sediment Cores	Rationale
HP1	4 sediment cores	Deep basin in northern portion of pond 1 oxic core, 2 anoxic cores, 1 spare
HP2	4 sediment cores	Deep basin in southern portion of pond 1 oxic core, 2 anoxic cores, 1 spare
WP1	4 sediment cores	Deep basin of pond 1 oxic core, 2 anoxic cores, 1 spare
SP1	4 sediment cores	Deep basin in northern portion of pond 1 oxic core, 2 anoxic cores, 1 spare
SP2	4 sediment cores	Mid-depth area in southern basin of pond 1 oxic core, 2 anoxic cores, 1 spare
SP3	4 sediment cores	Deep basin in northern portion of pond 1 oxic core, 2 anoxic cores, 1 spare

## 4.2. Methodology for Collection of Water

At each site, determine the maximum water depth and collect a water quality profile for temperature, dissolved oxygen, and pH using a calibrated multiparameter sonde from near surface to near sediment, without disturbing the sediment. Measurement depths shall begin at the 0.5 meter (m) below the surface, 1 m, 2 m, and every meter thereafter until the nearest 0.5 m increment closest to the sediment boundary. These data will provide a snapshot of environmental conditions prior to collecting a bottom water sample and provide information for setting up the phosphorus flux experiment.

Grab environmental samples from the bottom water layer will be collected at each site prior to collecting any sediment cores. This field-based sampling effort will result in six phosphorus (TP and OP) environmental samples that characterize bottom water conditions. Six QC samples (three duplicates and three equipment blanks) will also be collected to evaluate the field sampling effort. Then an additional two gallons of water (replenish water) will be collected from each site to support the phosphorus flux experiments. A horizontal Van Dorn sampler or peristaltic pump will be used to collect the water samples and replenish water from each sediment sampling site. The water shall be collected within 1 m of the sediment interface. The environmental and QC samples will be processed the same and placed in their respective sample containers and stored in a cooler with ice until transport to the laboratory. The replenish water will be filtered through a 0.2  $\mu\text{m}$  filter to remove organic matter, algae, and bacteria. Collect the filtrate for the experiment and store in appropriately labeled 1-gallon cubitainers, in a cooler with ice, then placed inside the laboratory incubator set at the average bottom water temperature for all three waterbodies. Depending upon the clarity of the water, pre-filtration using either a 10  $\mu\text{m}$ , 5  $\mu\text{m}$ , or 1  $\mu\text{m}$  may be necessary to remove particulates before filtering through the 0.2  $\mu\text{m}$  filter. Plan accordingly, because filtering 12 gallons of water for six sites will take time and the filtered water is required for Day 0 analysis of phosphorus content and for laboratory setup of the phosphorus flux

experiment. The replenish water should be collected the day before sediment sampling occurs to provide sufficient time to setup the phosphorus flux experiment.

### **4.3. Methodology for Collection of Sediment Cores**

Field collection of sediment cores shall be performed in a manner that preserves the sediment/water interface and maintains a consistent volume of sediment in each core. A modified six-inch Ekman dredge (i.e., increased height and weighted) will be used to collect a sediment grab sample. The modified dredge sampler typically collects the upper 15 to 20 cm layer of the sediment and allows for subsampling to acquire four replicate cores of similar composition and volume to reduce variability among treatment replicates for each site.

Before lowering the dredge, determine water depth, and slowly lower the device until refusal (*Do Not* let the dredge free fall). Use a weighted messenger to activate the closure mechanism and slowly retrieve the dredge to the 0.5 m depth below the water's surface. Before removing the dredge from the water, position a 7-gallon bucket in the water below the dredge so that the dredge remains underwater while being brought aboard the boat. This procedure preserves the integrity of each sediment sample by eliminating drainage from the dredge and allows for sediment subsampling to occur underwater, eliminating exposure of the sediment to air. Gently lift the top flaps of the dredge and view the surface layer. If the surface layer is disturbed, then discard the sediment core into a bucket and collect another core. *Do Not* discard the sediment core into the lake until all cores are collected.

Depending upon sediment composition, the upper 10 cm layer is typically the active layer where microbial mediated reduction of iron-bound and manganese-bound phosphorus occurs under anoxic conditions. Therefore, each core should contain at least 15 cm of sediment to fully capture this layer. If the sediment thickness is less, then visually inspect the core to evaluate any layering, and consider collecting another core. If the substrate layer is less than 10 cm in the dredge, then discard and collect another dredge sample. Site-specific field conditions such as sediment composition (e.g., sand and gravel), organic debris, or depth of refusal may influence sediment core collection, which may require another dredge sample. When collecting multiple dredge samples, make sure to reposition the boat by at least 3 meters so it is not directly over the same location.

To subsample the dredge, gently insert four, 2 × 24 inch acrylic tubes into the sediment to the bottom of the sampler. Because the top end of the core tubes will be above the water line in the bucket, carefully add lake water to each core tube, without disturbing the sediment interface. This will remove any headspace in the core tube. Notably, the lake water in the core tube will be replaced in the laboratory so this water does not need to be collected from near the lake bottom.

Once all four core tubes are inserted into the sediment sample, the top end of each core tube is plugged with a #10 rubber bung to allow for the slight repositioning of each core for extraction. Hydrostatic suction will allow for the core tube to be partially released from the surrounding sediment without losing the sediment from the bottom of the core tube. Before completely removing the core from the surrounding sediment, gently insert a #9.5 rubber bung into the bottom of the core tube. This process requires coordination with a second person to release the top bung to balance the displacement volume of the bottom bung as it is inserted, allowing water to escape the top of the core tube. Once the bottom

bung is in place, fully remove the core tube and place an end cap on the core tube. Wipe off the core tube and use electrical tape to secure the end cap, then replace the top #10 bung. Repeat these steps to collect the three remaining sediment cores. One core will be used as an oxic control; two cores will be used as anoxic treatment replicates; and the fourth core is a spare in case the sediment interface of a core is disturbed during sample collection, transit, or during laboratory setup. If space allows, the fourth core can be used to monitor static conditions during the phosphorus flux experiment or discarded after the experiment is fully set up and operational.

In the field, core tubes shall be stored in an upright and secured position, within water near bottom water temperature (ice may be used to cool the water), and covered to minimize any heating or exposure to sunlight. Transport the cores to the laboratory.

#### **4.4. Methodology for Phosphorus Flux Experiment**

In the laboratory, the phosphorus flux experiment follows the methodology discussed in the Journal of Visualized Experiments (Ogdahl et al. 2014) to estimate the anoxic phosphorus release rate. An incubation period of 8 days has been chosen for the phosphorus flux experiment and sampling will be conducted in each core on setup (Day 0) as well as Day 1, 2, 3, 4, 6, and 8 of the experiment (Table 6).

Attention to the proper set up of the core incubation chamber is necessary. The core incubation chamber shall be setup with the gas line distribution system (ambient air and buffered nitrogen) prior to sediment sampling and the temperature set to approximate the average bottom water layer temperature in the ponds. The gas distribution lines shall be calibrated to deliver approximately 0.7 cubic centimeters per second (cc/sec) to each treatment. Nitrogen gas (N<sub>2</sub>) is buffered with 350 parts per million (ppm) of carbon dioxide to maintain stable pH condition in the anoxic treatments.

Once the sediment cores are in the laboratory, place the cores in the incubation chamber and allow to equilibrate to laboratory conditions. Use a peristaltic pump to remove the overlying water column from each core and replace with 0.2 µm filtered site water. Remove all but the last 2 to 3 cm of water in the core tube to maintain the integrity of the sediment interface, then slowly add filtered site water to the core tube. Do not disturb the sediment boundary when replacing water or bubbling gas in the overlying water. If a disturbance occurs, allow the sediment to settle and note in the laboratory book. Replace the top #10 rubber bung with one that contains two holes. In one hole, place either the ambient air line or buffered nitrogen gas line depending upon treatment factor, and in the second hole place the tubing for the sampling port. The sampling port shall be positioned in the overlying water column at mid-depth. Repeat with each core tube. The holes in the top bung allow for gas displacement without pressurizing the core tubes. Maintain gas flow, temperature, and dark environmental conditions for the duration of the study.

Phosphorus (TP and OP) samples will be collected from the replenish water at the time of water replacement for each core (i.e., Day 0), and then from the overlying water column in each core on Day 1, 2, 3, 4, 6, and 8 of the experiment. Three Quality Control (QC) lab duplicates and equipment blanks will be collected on Day 0, Day 3, and Day 8 (Table 6). This laboratory sampling regime will result in 114 samples for analysis of both TP and OP content and 12 Quality Assurance (QA) samples for analysis of

phosphorus content (six TP and six OP samples). This QA approach will reduce the influence of sample removal on any one core.

Use a 60 milliliter (mL) Luer-tip syringe to connect to the Luer-tip sampling port tube and withdraw the desired sample volume needed for analysis of TP (120 mL) and OP (60 mL) content. Two syringes shall be dedicated for each site, one for sample collection and one for replacement of 0.2  $\mu$ m filtered water. The sample collection syringe shall be rinsed with deionized water (DIW) between sample collection (e.g., oxic/anoxic treatment cores and sample days). For dissolved oxygen and pH analysis, use a field sonde that will fit into the core tube. Remove the top bung and measure dissolved oxygen and pH after the phosphorus samples are collected, but before the replenish water is added to the core. This will prevent displacement of water from the core tube when measuring dissolved oxygen and pH conditions. The analytical laboratory desires at least 120 mL for TP and 60 mL for OP analysis. Replace the total sample volume removed for analyses with 0.2  $\mu$ m filtered water and note the sample volume in the field book. Following the last day of sample collection, measure the height and volume of the overlying water column in each core (i.e., use volume of a cylinder), and areal cross-section of the core tube, and volume of sediment in each core. The water volume and cross-section measurements are required to estimate the flux rate, and the volume of sediment is used for informational purposes.

**Table 6. Number of Phosphorus Flux Samples (TP and OP) and Quality Control Samples (duplicate and blank) Collected for Each Site**

Site	DAY 0	DAY 0 QA*	DAY 1	DAY 2	DAY 3	DAY 3 QA*	DAY 4	DAY 6	DAY 8	DAY 8 QA*
HP1	1	1	3	3	3		3	3	3	1
HP2	1		3	3	3	1	3	3	3	
WP1	1	1	3	3	3	1	3	3	3	1
SP1	1	1	3	3	3		3	3	3	
SP2	1		3	3	3	1	3	3	3	
SP3	1		3	3	3		3	3	3	1
Blank		1				1				1

Notes:

- \* QA samples will be rotated among treatment replicates within a site to reduce the effects of sample volume replacement on any one core; six QA samples will be analyzed for TP content and six QA samples will be analyzed for OP content.

## 5. Analytical Measurements

### 5.1. Analyses Narrative

Measurement of phosphorus (TP and OP), dissolved oxygen, and pH shall be performed on the overlying water within each core to evaluate the effect of treatment controls (i.e., oxic versus anoxic) and whether environmental conditions are being maintained within each core (Table 7). Phosphorus (TP and OP) concentrations will be used to quantify the sediment release rate based on the overlying water concentration, duration of the experiment, slope of the relationship, and sediment surface area.

Measurement of dissolved oxygen content shall occur immediately after sample collection because atmospheric reaeration of the anoxic sample may influence the result. Phosphorus (TP and OP) samples will be submitted to Pace Analytical Services for analysis.

### 5.2. Analytical Laboratory

Pace Analytical Services (formerly Alpha Analytical) in Westborough, Massachusetts, will analyze phosphorus content in water samples. One of the limiting factors when identifying a laboratory for analysis of phosphorus flux samples is the small sample size restraint. Many contract laboratories request a 500 mL sample volume for each TP and OP analysis to achieve their desired quality control. However, the overlying water volume in each core tube typically ranges from 500 to 750 mL, thus removing a substantial volume for analysis is not practicable. Pace Analytical Services reviewed our sample volume limitations in context with our DQOs and determined they could provide TP and OP results that met the project's requirements. For laboratory SOPs see Appendix B.

**Table 7. Analytical Methods for Laboratory Nutrient Flux Experiments**

Parameter	Analytical Method	Containers	Preservation	Holding Times
Total Phosphorus	SM 4500P E	125 mL plastic bottle	Sulfuric Acid, H <sub>2</sub> SO <sub>4</sub> 4°C	28 days
Orthophosphate	SM 4500P E	125 mL plastic bottle	0.45 µm filtered, 4°C in the dark	48 hours
Dissolved Oxygen, Lab	SM 4500-O-G	In-situ	N/A	Immediate
pH, Lab	SM 4500-H+ B	In-situ	N/A	Immediate



## 6. Field Equipment

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### 6.1. List of Field Equipment

- Health and Safety Plan
- Boat with electrical trolling motor
- Boat battery (charged)
- Personal flotation devices (PFDs)
- Safety cushions or life ring
- Two boat anchors with rope
- Depth sonar
- Multiparameter water quality sonde (calibrated)
- Van Dorn horizontal water sampler or peristaltic pump
- Peristaltic pump
- Pump battery (charged)
- Polyethylene tubing with weight
- Silicone tubing for pump head
- 1-gallon Cubitainers + spares (15)
- Cooler with blue ice (3)
- 10  $\mu\text{m}$ , 5  $\mu\text{m}$ , 1  $\mu\text{m}$  and 0.2  $\mu\text{m}$  filters (6 each)
- Modified Ekman dredge with messenger and deployment line
- 7-gallon buckets (6), need at least two 7-gallon buckets and others may be 5-gallon
- Acrylic core tubes (2 inch  $\times$  24 inch) with orange end caps
- #9.5 bungs (for bottom of core tube)
- #10 bungs (for top of core tube)
- #10 bungs with holes (for top of core tube during incubation)
- Paper towels
- Electrical tape
- Zip ties
- Paracord
- Core tube cooler with tube holder
- Tube baffle for filling core with water
- Label tape
- Sharpie markers
- Pencils
- Field book

## **6.2. Decontamination Procedures**

Decontamination of limnological sampling equipment will occur prior to and after each use of a piece of equipment. The boat is inspected for aquatic invasive species (AIS) and removed before leaving a waterbody and the exterior is sprayed with a 10 percent bleach solution and allowed to dry before entering a different waterbody. All water sampling devices are cleaned with Liquinox, or similar phosphate free detergent, and rinsed with tap water, then DIW. Before collecting an environmental sample, the equipment is also rinsed with site water. Sediment sampling equipment (e.g., dredge, core tubes, bungs) are precleaned by removing any sediment, then cleaned with Liquinox and rinsed with tap water and DIW. When sampling equipment is moved between lakes on the same day, debris or sediment will be removed from the equipment, rinsed with site water, then rinsed with DIW before being used in the next waterbody. Equipment that is newly unpackaged for use (e.g., pump tubing) will have site water run through the tubing before collecting an environmental sample.

## **7. Sample Containers, Preservation, Packaging and Shipping**

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### **7.1. Water Samples**

High density polyethylene (HDPE) 125 mL bottles shall be used for the collection of water samples for the analysis of phosphorus (TP and OP) content. The analytical laboratory shall provide the sample bottles and follow their decontamination and cleaning protocol and at a minimum shall include a pre-rinse with tap water, wash with Liquinox or other non-phosphate laboratory detergent, rinsed with DIW, a 5 percent hydrochloric acid soak, and rinsed with DIW, then allowed to dry.

If sulfuric acid preservative is used by the laboratory, then the bottles shall contain the acid prior to sample collection. Dispense the sample into the bottle with preservative then cap and lightly shake to mix the preservative. The bottle will be capped and lightly shaken to mix in the preservative. Samples will be chilled to 4 °C immediately upon collection until transferred to the laboratory.

### **7.2. Sediment Samples**

Acrylic core tubes (2-inch outside diameter, 1.75-inch inside diameter) shall be used for sediment core collection with the use of #9.5 and #10 rubber bungs to close the bottom and top ends of the core tube, respectively, along with a 2-inch end cap for the bottom to prevent release of the bottom bung. Sediment cores tubes are transferred to the lab for phosphorus flux incubation experiments. The core tubes and rubber bungs are precleaned with Liquinox, rinsed with tap water, and DIW before collecting a sediment core sample.

### **7.3. Packaging and Shipping**

All sample containers will be placed in a cooler for transfer to the laboratory. The following outlines the packaging procedures that will be followed for low concentration samples.

- Preferably blue ice should be used to keep samples cool, but when wet ice is used, pack it in zip lock, double plastic bags. Seal the drain plug of the cooler to prevent melting ice from leaking out of the cooler.
- Check screw caps for tightness.
- Ensure sample labels adhere to the bottles
- Place samples in a sturdy cooler(s) lined with a large plastic trash bag. Enclose the appropriate Chain-of-Custody (COC) form(s) in a zip lock plastic bag affixed to the underside of each cooler lid.
- Fill empty space in the cooler with bubble wrap or packing paper to prevent movement and breakage during shipment.

- Each ice chest will be securely taped shut with strapping tape with appropriate shipping label attached.

#### **7.4. Disposal of Residual Materials**

Once the phosphorus flux experiment is complete and the physical measurements of each core (i.e., sediment volume and overlying water volume) are collected, the sediment can be extruded into a 5-gallon bucket for recycling and preferably mixed into a composting bed of organic materials or disposed of within the landfill. To our knowledge the pond sediments are not contaminated such that special requirements need to be taken for disposal.

## **8. Sample Documentation**

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### **8.1. Field Notebook**

A field notebook shall document the locations where sediment grab samples were collected, methodology used, and whether there were any difficulties that required deviations from this SAP. The field notes should be complete and accurate enough to permit reconstruction of field activities with field staff names, waterbody names, site names, sample times, GPS locations, observations of environmental conditions, field data collected on-site or notations if data were electronically logged using field equipment, and photo documentation. Logbook entries should be complete.

At a minimum, the following information will be recorded during the collection of each sample:

- Field staff names
- Waterbody and site names
- GPS sample locations and description
- Date and time of sample collection
- Method of sample collection (soil, sediment, or water)
- Type of sampling equipment used
- Field instrument readings and calibration or notation of data electronically stored on the instrument
- Field observations and details related to analysis or integrity of samples (e.g., weather conditions, noticeable sulfide sediment odors, colors, etc.)
- Deviations from sampling plans, site safety plans, and Quality Assurance Project Plan (QAPP) procedures
- Sample preservation and storage details

### **8.2. Photographs**

Photographs shall be taken at the sampling locations and at other areas of interest on the site or sampling areas. They will serve to verify information entered in the field notebook. Note the number and types of photographs taken in the field book and ensure electronic files are correctly labeled in the office file directory.

### **8.3. Labeling**

All samples collected will be labeled in a clear and precise way for proper identification in the field and for tracking in the laboratory. The labels will contain the appropriate information for the sample and depending on sample type, this information may include station location, date of collection, incubation day, treatment factor, analytical parameter(s), and method of preservation.

#### **8.4. Chain-of-Custody Form**

All sample shipments or transfers of coolers containing samples shall be accompanied by a COC form. Request a PDF fillable COC form from the analytical laboratory and accurately complete the form (Appendix A). If multiple coolers are sent to a single laboratory on a single day, form(s) will be completed and sent with the samples for each cooler.

The COC form will identify the contents of each shipment and maintain the custodial integrity of the samples. Generally, a sample is in someone's custody if it is either in someone's physical possession, in someone's view, locked up, or kept in a secured area. The sampling team leader or designee will sign the COC form in the "relinquished by" box and note date and time.

## 9. Quality Control

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The following QC samples shall be collected to support the analysis of the sampling activity and phosphorus (TP and OP) flux samples collected during the incubation experiment. The site locations, core treatment, and type of QC sample shall be identified on the sample label. A total of 120 environmental samples/phosphorus (TP and OP) samples should be collected during the study (6 field samples and 114 core-flux samples), with an additional 18 QA/QC samples collected to evaluate both field activities and the laboratory incubation process of the study.

### 9.1. Field Quality Control Samples

Field QC samples shall be submitted as separate samples to the analytical laboratory and reported accordingly on the data reports. Because the Van Dorn water sampler will be rinsed with DIW between waterbodies and not fully decontaminated, an Equipment Blank and a Field Duplicate sample shall be collected at each waterbody (6 QA/QC samples). Equipment Blank samples shall be collected at the first site sampled in each waterbody before environmental samples are collected. Field Duplicate samples shall be collected from the first site sampled following the collection of the environmental sample. Specific requirements are outlined below.

**Equipment Blank** – A blank is prepared in the field or lab by pouring 1 liter (L) of DIW into the sampling equipment and ensuring contact with all parts of the device and sampling process, then collecting sample aliquots in prelabeled sample bottles. Blank samples are preserved, if any, then sealed, handled, stored, and analyzed for phosphorus (TP and OP) content. The OP field blank sample is processed (e.g., 0.45 µm filtered) the same as an environmental sample. Equipment blanks provide the best overall means of assessing contamination arising from the equipment, ambient conditions, sample containers, transit, and the laboratory. Measurable results reported for the equipment blanks will be examined on a case-by-case basis and adjusted for during the analysis of the phosphorus flux data.

**Field Duplicate** – A second sample is collected from the same location/core, in immediate succession, using identical techniques. Duplicate samples are preserved, if any, sealed, handled, stored, and analyzed in the sample manner as the primary sample. Precision of duplicate results is calculated by the relative percent difference (RPD) as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, D1 and D2, the RPD is calculated from the following equation and should be within ± 20 percent.

#### Equation 1. Duplicate Relative Percent Difference

$$RPD = \frac{|D1 - D2|}{\frac{(D1 + D2)}{2}} \times 100$$

## **9.2. Laboratory Quality Control Samples – Phosphorus Flux Incubation**

Laboratory QC samples will be collected during the incubation phase of the sediment core study and includes Laboratory Blanks and Laboratory Duplicates. A total of 12 QC samples will be collected during sediment core incubation. Specific requirements are outlined below.

**Laboratory Blank** - A blank is prepared in the laboratory by using a dedicated sampling syringe to collect and dispense DIW into prelabeled sample bottles. Blank samples are preserved, if any, then sealed, handled, stored, shipped, and analyzed for either TP or OP content. In the laboratory, the OP sample is filtered using a 0.45  $\mu\text{m}$  filter to collect the filtrate. Process the laboratory blank the same as a phosphorus flux sample. These blank samples provide information regarding sample bottle preparation, including analytical variability. Blanks will be collected on Day 0, Day 3, and Day 8 of the incubation experiment (three laboratory blanks).

**Laboratory Duplicate** – A duplicate sample is collected from the same core, immediately following the collection of the phosphorus flux sample. This sample will vary between sites and treatment factors to provide context on the analytical variability of the overlying water in each core. Process the laboratory duplicate the same as a phosphorus flux sample and filter the OP sample. Laboratory duplicates will be collected on Day 0, Day 3, and Day 8 of the incubation experiment (nine laboratory duplicates). To reduce the sample volume removed from each core, the duplicate samples will only be analyzed for TP or OP content, not both. Between the 12 QA samples collected (three blanks and nine duplicates), six TP and six OP analyses will be performed.

## **9.3. Field Variances**

As conditions in the field or laboratory may vary, it may become necessary to implement minor modifications to sampling as presented in this SAP. When appropriate, the Project Manager and QA Officer will be notified of the modifications made to the approved plan and these modifications will be documented in the sampling report.



## **10. Field Health and Safety Plan**

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A Health and Safety Plan (HASP) will be developed that establishes policies and procedures to protect Contractor personnel from the potential hazards posed by the activities at Horn Pond, Wedge Pond, and Spy Pond (site) in the Mystic River Watershed as well as in the Contractor Laboratory. Reading, understanding, and compliance with the contents of the HASP is required for on-site Contractor personnel. The HASP will identify measures to minimize accidents and injuries that may result from site conditions or activities, including within the laboratory. A copy of the HASP will be maintained onsite for the duration of the work.

## 11. References

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## Appendix A Pace Chain-of-Custody

[illegible]

## Appendix B Laboratory SOPs

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Alpha Analytical, Inc.

Facility: Westborough

Department: Wet Chemistry

Title: ENV-SOP-WES2-0085 v01 Orthophosphate - Colorimetric, Combined Reagent

ID No.:224611

Revision 2

Published Date: April 16, 2025

Page 1 of 8

### ENV-SOP-WES2-0085 v01 Orthophosphate - Colorimetric, Combined Reagent

Reference: SM 4500P-E, Standard Methods for the Examination of Water and Wastewater.  
APHA-AWWA-WEF. 2021.

#### 1. Scope and Application

**Matrices:** Water and wastewater samples.

**Definitions:** Refer to Alpha Analytical Quality Manual.

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particles or detritus, or in the bodies of aquatic organisms.

These forms of phosphate arise from a variety of sources. Small amounts of certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added when the water is used for laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm run-off and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues, and also may be formed from orthophosphates in biological treatment processes or by receiving water biota.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate the growth of photosynthetic aquatic micro- and macro-organisms in nuisance quantities.

Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.

Phosphorus analyses embody two general procedural steps: (a) conversion of the phosphorus form of interest to dissolved orthophosphate, and (b) colorimetric determination of dissolved orthophosphate. The separation of phosphorus into its various forms is defined analytically but the analytical differentiations have been selected so that they may be used for interpretive purposes.

Filtration through a 0.45- $\mu$ m-pore-diameter membrane filter separates dissolved from suspended forms of phosphorus. No claim is made that filtration through 0.45- $\mu$ m filters is a true separation of suspended and dissolved forms of phosphorus; it is merely a convenient and replicable analytical technique designed to make a gross separation.

Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample are termed "reactive phosphorus." While reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present usually is hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both dissolved and suspended forms.

The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for

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Department: Wet Chemistry

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the matrix. Approval of all method modifications is by one of the following laboratory personnel before performing the modification: Area Supervisor, Laboratory Director, or Quality Assurance Officer.

This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method by performing an initial demonstration of capability.

## 2. Summary of Method

The ascorbic acid method is used for the determination of orthophosphate in environmental samples. Ammonium molybdate and potassium antimonyl tartrates react in acid medium with orthophosphate to form a heteropoly acid-phosphomolybdic acid that is reduced to intensely colored molybdenum blue by ascorbic acid. Samples are analyzed at 880nm using a spectrophotometer.

### 2.1 Method Modifications from Reference

None.

## 3. Reporting Limits

The Reporting Limit is 0.005mg/L.

## 4. Interferences

**Correction for Turbidity or Interfering Color:** The natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract the blank absorbance from the absorbance of each sample.

In some cases, the background color or turbidity can be eliminated by a dilution, but this will raise the reporting limit.

## 5. Health and Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

## 6. Sample Collection, Preservation, Shipping and Handling

### 6.1 Sample Collection

Samples are collected in 250mL plastic containers.

### 6.2 Sample Preservation

Samples are not preserved. Do not add either acid or  $\text{CHCl}_3$  as a preservative.

### 6.3 Sample Shipping

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No specific requirement.

#### 6.4 Sample Handling

Samples are stored under refrigeration at  $4 \pm 2$  °C. Analysis must be performed within 48 hours of collection.

### 7. Equipment and Supplies

**7.1 Spectrophotometer:** with infrared phototube for use at 880nm, providing a light path of 2.5cm.

**7.2 Acid-washed Glassware:** Use acid-washed glassware for determining low concentration of phosphorus. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with 1:1 HCl and rinse well with distilled water. The glassware should only be used for phosphate determination.

**7.3 Centrifuge Tubes:** 50mL volume. (Must be new and disposable.)

**7.4 0.45µm Acrodisc filters** with disposable syringes

**7.5 Pipets or Pipettor:** Various sizes, new and disposable or acid-rinsed glass

### 8. Reagents and Standards

**8.1 Stock Phosphate Standard: 1000 mgP/L** This stock solution is certified and purchased commercially prepared. Store at room temperature. Expires upon manufacturer's specified date.

**8.2 Intermediate Phosphate Standard: 50 mgP/L** Dilute 50.0mL stock phosphate solution to 1000mL with DI water, 1.00mL = 2.50µg P. Store refrigerated at  $4 \pm 2$  °C. Expires 6 months from date of preparation.

**8.3 Working Standard: 1.0 mgP/L** Add 2mL of 50 mgP/L intermediate standard (Section 8.2) to 100mL volumetric flask and dilute to volume with DI water. Prepare fresh on each day of use.

**8.4 Calibration Standards:** Follow table below for preparation instructions. Prepare fresh on each day of use. Use a calibrated pipettor and bring up to 25mL with DI.

Volume of 1.0 mg/L Working Standard (Section 8.3)	Final Volume (mL)	Calibration Standard Final Concentration (mgP/L)
0	25	0.0
0.125 mL	25	0.005
0.250 mL	25	0.01
1.250 mL	25	0.05
2.50 mL	25	0.10
12.5 mL	25	0.50
25 mL	25	1.00

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**8.5 ICV-LCS-CCV Stock Standard: 1000 mgP/L** Second source standard than that used in Section 8.1.

**8.6 ICV-LCS-CCV Intermediate Standard: 25 mgP/L** Add 5mL of 1000 mgP/L stock standard (Section 8.5) to 200mL volumetric flask and dilute to volume with DI water. Store refrigerated at  $4 \pm 2^\circ\text{C}$ . Expires 6 months from date of preparation.

**8.7 ICV-LCS-CCV Working Standards:** Prepare fresh on each day of use.

**8.7.1 0.5 mgP/L:** Add 0.25mL of 50 mg/L intermediate standard (Section 8.6) to 25mL volumetric flask and dilute to volume with DI water.

**8.8 Matrix Spike Solution:** Intermediate Phosphate Standard (Section 8.2) is utilized for matrix spike solution. 0.25mL of the 50 mgP/L standard added to 25mL of sample will afford a 0.5mgP/l matrix spike concentration.

**8.9 Sulfuric Acid, H<sub>2</sub>SO<sub>4</sub>, 5N:** Dilute 70mL concentrated sulfuric acid to 500mL with DI. Store at room temperature. Expires 6 months from date of preparation. Alternatively, 1L of 5N H<sub>2</sub>SO<sub>4</sub> can be made: dilute 140 ml of concentrated sulfuric acid to 1000mL with DI. Expires 6 months from date of preparation.

**8.10 Potassium Antimonyl Tartrate Solution:** Dissolve 1.3715g K(SbO) C<sub>4</sub>H<sub>4</sub>O<sub>6</sub> · ½H<sub>2</sub>O in 400mL DI water in a 500mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle at room temperature. Expires one month from date of preparation.

**8.11 Ammonium Molybdate Solution:** Dissolve 20g (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> · 4H<sub>2</sub>O in 500mL DI water. Store in a glass-stoppered bottle at room temperature. Expires one month from date of preparation.

**8.12 Ascorbic Acid, 0.1M:** Dissolve 3.52g ascorbic acid in 200mL DI water. The solution is stable for about 1 week at  $4 \pm 2^\circ\text{C}$ . Alternatively, 100 ml of Ascorbic Acid can be made: Dissolve 1.76g ascorbic acid in 100mL DI water. The solution is stable for 1 week at  $4 \pm 2^\circ\text{C}$ .

**8.13 Combined Reagent:** Mix the above reagents in the following proportions for 100mL of the combined reagent: 50mL 5N H<sub>2</sub>SO<sub>4</sub> (Section 8.9), 5mL potassium antimonyl tartrate solution (Section 8.10), 15mL ammonium molybdate solution (Section 8.11), and 30mL ascorbic acid solution (section 8.12). Mix after addition of each reagent. Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 hours. Discard reagent if it turns blue or black in color.

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## 9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

### 9.1 Blanks

**9.1.1 ICB or Method Blank** - One method blank, which consists of 25mL DI water filtered through a 0.45 micron Acrodisc filter, must be analyzed per batch of 20 samples or less. Evaluate Method Blanks for contamination that may be introduced into the analytical or preparation process.

Positive sample results are suspect if the analytes in the reagent blank are  $> \frac{1}{2}$  MRL (or reporting limit). Samples analyzed with a contaminated Method Blank must be reprepared and re-analyzed unless concentrations are  $\geq 10$  times the concentration found in the method blank or the samples are nondetect (ND).

Samples must be qualified with a "B" flag if the Method Blank is  $> \frac{1}{2}$  MRL.

**9.1.2 Continuing Calibration Blank** - The ICB should be re-read after every 10 samples (thus becoming the CCB) and at the end of the batch. The CCB result must be less than the Reporting Limit. If the CCB is greater than the RL, all samples analyzed since the last passing CCB must be recolored and reanalyzed.

### 9.2 Laboratory Control Sample (LCS)

Analyze one LCS per batch of 20 samples or less. The calibration curve must be verified by a second source standard prior to performing any sample analysis. The LCS must be recovered at 90 – 110% of the true value.

If the LCS fails, re-analyze. If failure remains, stop analysis, correct problem and perform re-calibration.

### 9.3 Initial Calibration Verification (ICV)

The calibration curve must be verified by a second source standard prior to performing any sample analysis. The ICV must be recovered at 90 – 110% of the true value.

If the ICV fails, re-analyze. If failure remains, stop analysis, correct problem and perform re-calibration.

### 9.4 Continuing Calibration Verification (CCV)

The calibration curve must be verified by a second source standard prior to performing any sample analysis. The CCV must be recovered at 90 – 110% of the true value.

If the CCV fails, re-analyze. If failure remains, stop analysis, correct problem and perform re-calibration.

The CCV must be re-read every 10 samples and at the end of the batch.

### 9.5 Matrix Spike

Analyze one per batch of 20 samples or less. Concentration is 0.5 mg P /L. The matrix spike must be recovered at 80 – 120% of the true value. If the MS recovery is outside of acceptance limits, the sample and its spike are reanalyzed. If the MS failure continues, a narrative is submitted with the data for inclusion on the Client report.

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## 9.6 Laboratory Duplicate

Analyze one sample in duplicate, per batch of 20 samples or less. The RPD must be  $\leq 20\%$ . If the RPD is outside of acceptance limits, the sample and its duplicate are reanalyzed. If the RPD failure continues, a narrative is submitted with the data for inclusion on the Client report.

## 9.7 Method-specific Quality Control Samples

### 9.7.1 Minimum Reporting Limit (MRL) Check

With each analytical batch, analyze a reagent-water sample spiked at the MRL. Analyze the MRL check after the Initial Calibration Verification and Initial Calibration Blank. The MRL check must recover between 50-150% of the expected value. If the MRL is outside of criteria, reanalyze once. If the MRL is outside of criteria a second time, recalibrate and re-analyze all associated samples. If the MRL is biased high, nondetect (ND) samples can be reported.

## 9.8 Method Sequence

- Calibration curve generation or verification of existing curve.
- Acid-rinsing of glassware
- Sample filtration including blank
- Add sample aliquot to a new centrifuge tube
- Add combined reagent to samples
- Read sample absorbance after 10-30 minutes
- Analyze CCV and CCB after every 10 samples to verify curve
- End sequence with CCV and CCB
- Calculate results

## 10. Procedure

### 10.1 Equipment Set-up

- 10.1.1 Preparation of calibration curve:** Prepare individual calibration curve from a series of six standards and DI (0.005 mgP/L to 1.0 mgP/L) on a daily basis when samples are to be analyzed. Use a DI water blank to zero the instrument before taking photometric readings for the calibration curve. Plot a curve of absorbance vs. phosphate concentration. The curve is acceptable if the calibration coefficient is  $\geq 0.995$ . The calibration curve is prepared fresh each day of analysis.

All calibration points are back calculated and should be within 10% from true concentration, except 2 lowest points of calibration curve. %recoveries for low range will be wider, but shouldn't exceed 100% and correlation coefficient will not be worse than 0.995.

### 10.2 Initial Calibration

See Section 9.3

### 10.3 Equipment Operation and Sample Processing

- 10.3.1** Filter samples and QC samples through 0.45µm Acrodisc filters. 1 µm Acrodisc filter may be used to prefilter hard-to-filter samples.
- 10.3.2** Pour 25mL of each clear filtered sample, a duplicate and matrix spike sample into a corresponding new (never used) centrifuge tube.

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- 10.3.3 Add 4.0mL combined reagent to all 25mL samples and QC sample aliquots and mix thoroughly.
- 10.3.4 After at least 10 minutes but no more than 30 minutes, measure absorbance of each sample at 880nm with a 2.5cm cell. Follow the procedure described in the Wet Chemistry Electronic Notebook Work Instructions (WI/2516).
- 10.3.5 Compare with a standard curve prepared from a range of standards and carried through this procedure. Sample concentration must fall within the range of the calibration curve.
- 10.3.6 If the sample concentration is greater than the highest calibration standard concentration, dilute the original sample with DI water and recolor and reanalyze as outlined above (Section 10.3.1 – 10.3.6).

#### 10.4 Continuing Calibration

See Section 9.4.

#### 10.5 Preventive Maintenance

The Spectrophotometers are calibrated on a semi-annual basis by an instrument service company. Certificates are kept on file.

### 11. Data Evaluation, Calculations and Reporting

$$\text{mg P/L} = \text{mg P (from calibration curve)} \times \text{dilution}$$

### 12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

Holding time exceedances or improper preservation are noted on the nonconformance report form.

Perform routine preventative maintenance following manufacturer's specification. Record all maintenance in the instrument logbook.

Review of standards, blanks and standard response for acceptable performance occurs for each batch of samples. Record any trends or unusual performance on a nonconformance action form.

If the CV or LCS recovery of any parameter falls outside the designated acceptance range, the laboratory performance for that parameter is judged to be out of control, and the problem must be immediately identified and corrected. The analytical result for that parameter in the unspiked samples is suspect and is only reported for regulatory compliance purposes with the appropriate nonconformance action form. Immediate corrective action includes reanalyzing all affected samples by using any retained sample before the expiration of the holding time.

### 13. Method Performance

#### 13.1 Detection Limit Study (DL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ)

The laboratory follows the procedure to determine the DL, LOD, and/or LOQ as outlined in Alpha SOP ID 1732. These studies performed by the laboratory are maintained on file for review.

#### 13.2 Demonstration of Capability Studies

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Refer to Alpha SOP ID 1739 for further information regarding IDC/DOC Generation.

**13.2.1 Initial (IDC)**

The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method, prior to the processing of any samples.

**13.2.2 Continuing (DOC)**

The analyst must make a continuing, annual, demonstration of the ability to generate acceptable accuracy and precision with this method.

**14. Pollution Prevention and Waste Management**

Refer to Alpha's Chemical Hygiene Plan and Hazardous Waste Management and Disposal SOP for further pollution prevention and waste management information.

**15. Referenced Documents**

2121 Chemical Hygiene Plan

1732 Detection Limit (DL), Limit of Detection (LOD) & Limit of Quantitation (LOQ) SOP

1739 Demonstration of Capability (DOC) Generation SOP

1728 Hazardous Waste Management and Disposal SOP

2516 Electronic Laboratory Notebook Work Instructions

**16. Attachments**

None.

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Title: ENV-SOP-WES2-0102 v01 Total Phosphorous, Dissolved Phosphorus - Colorimetric, Combined  
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## Total Phosphorous Dissolved Phosphorus Colorimetric, Combined Reagent

References: **SM 4500P-E**, Standard Methods for the Examination of Water and Wastewater. APHA-AWWA-WEF. Standard Methods Online.

**SM4500P-B**, Section 5 (Persulfate Digestion), Standard Methods for the Examination of Water and Wastewater. APHA-AWWA-WEF. 2021.

AQ2 method: **EPA-119-A** Rev. 7, equivalent to EPA 385.1, version 2(1993) **SM4500-P-B**, F(18-20)

### 1. Scope and Application

**Matrices:** Water and wastewater samples and soils.

**Definitions:** See Alpha Laboratories Quality Manual Appendix A

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particles or detritus, or in the bodies of aquatic organisms.

These forms of phosphate arise from a variety of sources. Small amounts of certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added when the water is used for laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm run-off and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues, and also may be formed from orthophosphates in biological treatment processes or by receiving water biota.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate the growth of photosynthetic aquatic micro- and macro-organisms in nuisance quantities.

Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.

Phosphorus analyses embody two general procedural steps: (a) conversion of the phosphorus form of interest to dissolved orthophosphate, and (b) colorimetric determination of dissolved orthophosphate. The separation of phosphorus into its various forms is defined analytically but the analytical differentiations have been selected so that they may be used for interpretive purposes.

Filtration through a 0.45- $\mu$ m-pore-diameter membrane filter separates dissolved from suspended forms of phosphorus. No claim is made that filtration through 0.45- $\mu$ m filters is a true separation of suspended and dissolved forms of phosphorus; it is merely a convenient and replicable analytical technique designed to make a gross separation.

Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample are termed "reactive phosphorus." While reactive phosphorus is largely a measure of

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orthophosphate, a small fraction of any condensed phosphate present usually is hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both dissolved and suspended forms.

Acid hydrolysis at boiling-water temperature converts dissolved and particulate condensed phosphates to dissolved orthophosphates. The hydrolysis unavoidably releases some phosphate from organic compounds, but this may be reduced to a minimum by judicious selection of acid strength and hydrolysis time and temperature. The term "acid-hydrolyzable phosphorus" is preferred over "condensed phosphate" for this fraction.

The phosphate fractions that are converted to orthophosphate only by oxidation destruction of the organic matter present are considered "organic" or "organically bound" phosphorous. The severity of the oxidation required for this conversion depends on the form of, and to some extent on the amount of, the organic phosphorus present. Like reactive phosphorus and acid hydrolyzable phosphorus, organic phosphorus occurs both in the dissolved and suspended fractions.

The total phosphorus as well as the dissolved and suspended phosphorus fractions each may be divided analytically into the three chemical types that have been described: reactive, acid hydrolyzable, and organic phosphorus. Determinations usually are conducted only on the unfiltered and filtered samples. Suspended fractions generally are determined by difference.

The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for the matrix. Approval of all method modifications is by one of the following laboratory personnel before performing the modification: Area Supervisor, Laboratory Director, or Quality Assurance Officer.

This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method by performing an initial demonstration of capability.

## 2. Summary of Method

**Digestion Method:** Because phosphorus may occur in combination with organic matter, a digestion method to determine total phosphorus must be able to oxidize organic matter effectively to release phosphorus as orthophosphate. This digestion is performed by using the persulfate oxidation technique.

**Colorimetric Method:** The ascorbic acid method is used for the determination of orthophosphate in environmental samples. An extraction step is recommended for the lower levels and when interferences must be overcome. Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid-phosphomolybdic acid that is reduced to intensely colored molybdenum blue by ascorbic acid. The absorbance of this complex is measured photometrically at 880nm.

### 2.1 Method Modifications from Reference

Glassware is acid rinsed with room temperature 1:1 HCl, instead of hot dilute HCl.

Initial testing of samples with phenolphthalein has been eliminated since samples are received already preserved with H<sub>2</sub>SO<sub>4</sub> and are pH checked by the Login Department upon receipt. Soil samples are analyzed using the same digestive procedure.

## 3. Reporting Limits

The Reported Detection Limit is 0.01mg/L for waters and 5.0mg/kg for soils

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## 4. Interferences

**Correction for Turbidity or Interfering Color:** The natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the digested sample aliquot. Subtract the blank absorbance from the absorbance of each sample.

Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1mg As/L interfere with the phosphate determination.

Hexavalent chromium and  $\text{NO}_2$  interfere to give results about 3% low at concentrations of 1mg/L and 10 to 15% low at 10mg/L.

Sulfide ( $\text{Na}_2\text{S}$ ) and silicate do not interfere at concentrations of 1.0 and 10mg/L.

## 5. Health and Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material data handling sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

## 6. Sample Collection, Preservation, Shipping and Handling

### 6.1 Sample Collection

Water samples are collected in 500mL plastic bottles, soil samples may be collected in plastic or glass jars.

### 6.2 Sample Preservation

If samples are for Dissolved Phosphorus analysis, filtration must take place prior to preservation with  $\text{H}_2\text{SO}_4$  to a pH < 2.

All samples are preserved with  $\text{H}_2\text{SO}_4$ .

### 6.3 Sample Shipping

No special shipping requirements.

### 6.4 Sample Handling

Samples are stored under refrigeration at  $4 \pm 2^\circ\text{C}$ . Analysis must be performed within 28 days of collection. All samples should be analyzed as soon as possible after digestion. If a prolonged period passes in between, sample extracts are refrigerated at  $4 \pm 2^\circ\text{C}$ .

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## 7. Equipment and Supplies

**7.1 Spectrophotometer**, with infrared phototube for use at 880nm, providing a light path of 2.5cm.

**7.2 Acid-washed Glassware**: Use acid-washed glassware for determining low concentration of phosphorus. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with 1:1 HCl two times followed by two DI water rinses. Preferably, reserve the glassware only for phosphate determination. Only disposable syringes and filters are to be used for filtering samples for Dissolved Phosphorus analysis.

**7.3 Centrifuge Tubes**: 50mL volume. (Must be new and disposable.)

**7.4 Hot Plate**: A 30cm x 50cm heating surface is adequate.

**7.5 Scoop, 0.5gm** To hold required amounts of persulfate crystals.

**7.6 Erlenmeyer Flasks**: 125mL volume.

**7.7 0.45µm membrane filters**: For Dissolved Phosphorus sample preparation.

**7.8 Borosilicate Glass beads**

**7.9 SEAL AQ2 Discrete Analyzer**, with all associated reagent wedges, sample tubes, and reaction segments. The SEAL has a light and filter capable of maintaining a 880nm wavelength.

**7.10 Boiling Chips** ultra-pure, non-reactive.

**7.11 Syringes** to use with membrane filters.

**7.12 Pipettes** Class A glass or automated.

## 8. Reagents and Standards

**8.1 Calibration Curve and Spike, Stock Complex Phosphate Standard: 1000 mgP/L** This stock solution is certified and purchased commercially prepared. Stored at room temperature per manufacturer's specifications. Expires upon manufacturer's specified date.

**8.2 Calibration Curve and Spike, Intermediate Complex Phosphate Standard: 50 mgP/L** Dilute 5.0mL stock complex phosphate solution to 100mL with DI water. Store at  $4 \pm 2^{\circ}\text{C}$ . Expires 6 months after date of preparation.

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**8.3 Calibration Curve, Working Standard: 1.0 mgP/L** Add 2mL of 50 mgP/L intermediate standard (Section 8.2) to 100mL volumetric flask and dilute to volume with DI water. Prepare fresh on each day of use.

**8.4 Calibration Standards:** Follow table below. Prepare fresh on each day of use.

Volume of 1.0 mg/L Working Standard (Section 8.3)	Final Volume (mL)	Calibration Standard Final Concentration (mgP/L)
0 mL	50	0
0.5 mL	50	0.010
2 mL	50	0.040
5 mL	50	0.100
25mL	50	0.500
50mL	50	1.000

**8.5 ICV-LCS-CCV Stock Complex Phosphate Standard: 1000 mgP/L** Second, independent, source standard. Stored at room temperature per manufacturer's specifications. Expires upon manufacturer's specified date.

**8.6 ICV-LCS-CCV Intermediate Complex Phosphate Standard: 50 mgP/L** Add 5mL of 1000 mgP/L stock standard (Section 8.5) to 100mL volumetric flask and dilute to volume with DI water. Store at  $4 \pm 2^\circ\text{C}$ . Expires 6 months after date of preparation.

**8.7 ICV-LCS-CCV Working Standard:** Prepare fresh each day of use.

**8.7.1 0.5 mgP/L:** Add 0.5mL of 50 mg/L intermediate standard (Section 8.6) to 50mL centrifuge tube and dilute to the 50mL mark with DI water.

**8.8 Matrix Spike:** Intermediate Phosphate Standard (Section 8.2) is utilized for matrix spike solution. Pipet 0.5mL of the 50 mgP/L standard into 50mL of sample to result in a 0.5mg/L spike concentration.

**8.9 Sulfuric Acid,  $\text{H}_2\text{SO}_4$ , 5N:** Dilute 140mL concentrated sulfuric acid to 1L with DI. Store at room temperature. Expires 6 months from date of preparation.

**8.10 Potassium Antimonyl Tartrate Solution:** Dissolve 1.3715g  $\text{K}(\text{SbO}) \text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$  in 400mL DI water in a 500mL volumetric flask and dilute to volume. Store at  $4 \pm 2^\circ\text{C}$ . Expires one month from date of preparation.

**8.11 Ammonium Molybdate Solution:** Dissolve 10g  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in 250mL distilled water. Store at  $4 \pm 2^\circ\text{C}$ . Expires one month from date of preparation.

**8.12 Ascorbic Acid, 0.1M:** Dissolve 3.52g ascorbic acid in 200mL DI water. The solution is stable for about 1 week at  $4 \pm 2^\circ\text{C}$ .

**8.13 Orthophosphate 1000ppm solution** Independent, source standard. Store at  $4 \pm 2^\circ\text{C}$ . Expires upon manufacturer's specified date.

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## Sampling and Analysis Plan - Sediment Phosphorus Flux in Horn, Wedge, and Spy Ponds

Alpha Analytical, Inc.

ID No.:224628

Facility: Westborough

Revision 2

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**8.14 Orthophosphate 25ppm spike solution** Add 2.5mLs 1000ppm Orthophosphate solution to a clean, glass 100mL volumetric and dilute to volume with DI. Store at  $4 \pm 2^{\circ}\text{C}$ . Expires 6 months from date of preparation.

**8.15 SEAL Working Ascorbic Acid, 15g/L (with orthophosphate spike):** Dissolve 1.5g of ascorbic acid in about 80mL DI water. Spike with .15mL 25ppm Orthophosphate standard to produce a spike level of .025mg P/L. Dilute to 100mL and mix well. The solution is stable for one week if stored at  $4 \pm 2^{\circ}\text{C}$ . Discard if the solution becomes yellowed.

**8.16 Combined Reagent:** Mix 8.9, 8.10, 8.11, and 8.12 in the following proportions for 100mL of the combined reagent: 50mL 5N  $\text{H}_2\text{SO}_4$  (Section 8.9), 5mL potassium antimonyl tartrate solution (Section 8.10), 15mL ammonium molybdate solution (Section 8.11), and 30mL ascorbic acid solution (section 8.12). Mix after addition of each reagent. Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 hours. Discard reagent if it turns blue or black in color.

**8.17 SEAL Working Coloring Reagent:** To a clean 100mL volumetric flask, add 40mL sulfuric acid (8.9), followed by 6.5 mL antimony potassium tartrate (8.10) and swirl to mix. Then, add 20 mL ammonium molybdate (8.11). Swirl the contents, fill the flask up to the mark with DI water and mix well. Expires three weeks from day of preparation if stored at  $4 \pm 2^{\circ}\text{C}$ . Discard if the reagent turns blue or becomes turbid.

**8.18 Sodium Hydroxide, 6N:** Dissolve 240 grams of NaOH pellets in 1000mL of DI water. Store at room temperature. Expires one month from date of preparation.

**8.19 Phenolphthalein Indicator:** Aqueous solution, commercially available. Store at room temperature. Expires upon manufacturer's specified date.

**8.20 11N Sulfuric Acid Solution:** Dilute 308mL concentrated sulfuric acid to 1000mL with DI. Store at room temperature. Expires 6 months from date of preparation.

**8.21 Potassium Persulfate ( $\text{K}_2\text{S}_2\text{O}_8$ ):** Commercially available. Store at room temperature. Expires upon manufacturer's specified date.

**8.22 Deionized Water**

**8.23 Soil LCS/SRM** ERA Standard Reference Material for Nutrients in soil, catalog no. 542

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## 9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

### 9.1 Blank(s)

**Method Blank/Calibration Blank** - One method blank, which consists of DI water brought through the entire method, must be analyzed per batch of 20 samples or less. The CCB is analyzed after every 10 samples and at the end of the sequence.

Method Blanks for contamination that may be introduced into the analytical or preparation process.

Positive sample results are suspect if the analytes in the reagent blank are  $> \frac{1}{2}$  MRL (or reporting limit). Samples analyzed with a contaminated Method Blank must be reprepared and re-analyzed unless concentrations are  $\geq 10$  times the concentration found in the method blank or the samples are nondetect (ND).

Samples must be qualified with a "B" flag if the Method Blank is  $> \frac{1}{2}$  MRL

Soil blanks are made with 0.1gm boiling chips and 50mLs of DI water and are analyzed like water blanks.

### 9.2 Laboratory Control Sample (LCS)

Analyze one per batch of 20 samples or less. The calibration curve must be verified by a second source standard prior to performing any sample analysis. For Total and Dissolved Phosphorus, the LCS is the ICV.

The ICV/LCS for aqueous samples must be recovered within 90-110% of the true value. If the ICV/LCS fails, re-analyze. If failure continues, stop analysis, correct problem and re-calibrate.

The Soil LCS made with approximately 0.15g of Standard Reference Material (SRM) brought up to 50mL with DI water. The soil LCS recovery criteria will vary per Lot of SRM, according to the SRM manufacturer's criteria listed on the certificate of analysis.

### 9.3 Initial Calibration Verification (ICV)

The calibration curve must be verified by a second source standard prior to performing any sample analysis. For Total and Dissolved Phosphorus, the ICV is the LCS.

The ICV/LCS must be recovered within 90-110% of the true value for water samples and be within vendor criteria for SRM. If the ICV/LCS fails, re-analyze. If failure continues, stop analysis, correct problem and re-calibrate.

### 9.4 Continuing Calibration Verification (CCV)

The calibration curve must be verified by a second source standard. The CCV is analyzed after every 10 samples and at the end of the sequence to verify the curve.

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The CCV must be recovered within 90-110% of the true value. If the CCV fails, re-analyze. If failure continues, stop analysis, correct problem and re-calibrate.

### 9.5 Matrix Spike

Analyze one per batch of 20 samples or less. Concentration is 0.5 mgP/L. The matrix spike must be recovered within 75 – 125% of the true value. If the matrix spike recovery is outside acceptance criteria, and the LCS is acceptable, a narrative is submitted with the data for inclusion on the client report.

### 9.6 Laboratory Duplicate

Analyze one sample in duplicate per batch of 20 samples or less. The RPD must be  $\leq 20\%$ . If this criterion is not met, a narrative is submitted with the data for inclusion on the client report.

### 9.7 Method-specific Quality Control Samples

#### 9.7.1 Minimum Reporting Limit (MRL) Check

With each analytical batch, analyze a reagent-water sample spiked at the MRL. Analyze the MRL check after the Initial Calibration Verification and Initial Calibration Blank. The MRL check must recover between 50-150% of the expected value. If the MRL is outside of criteria, reanalyze once. If the MRL is outside of criteria a second time, recalibrate and re-analyze all associated samples. If the MRL is biased high, nondetect (ND) samples can be reported.

### 9.8 Method Sequence

#### 9.8.1 Using spectrophotometer:

- Acid-rinse all glassware
- Calibration curve generation.
- Filter samples if analysis is for Dissolved Phosphorus, then preserve with  $\text{H}_2\text{SO}_4$ .
- Add 50mL of water sample or 0.1g of soil sample and 50mL DI water to an Erlenmeyer flask.
- Add 1mL  $\text{H}_2\text{SO}_4$  solution and scoop solid  $\text{K}_2\text{S}_2\text{O}_8$  and glass beads
- Boil down to 10mL or less.
- Cool and dilute to 30mL with DI.
- Add 1 drop phenolphthalein indicator solution.
- Neutralize to faint pink color with NaOH.
- Add sample aliquot to a new centrifuge tube and bring up to 50mL with DI.
- Add 4 mL combined reagent to a 25mL aliquot of sample.
- Read sample absorbance after 10-30 minutes.
- Analyze CCV and CCB after every 10 samples to verify curve.
- End sequence with CCV and CCB.
- Calculate results.

#### 9.8.2 Using SEAL AQ2 analyzer:

- Acid-rinse all glassware
- Filter samples if analysis is for Dissolved Phosphorus, then preserve with  $\text{H}_2\text{SO}_4$ .
- Add 50mL of water sample (soils are not done on the SEAL) to an Erlenmeyer flask, along with a 1.0ppm calibration standard for the calibration curve.
- Add 1mL  $\text{H}_2\text{SO}_4$  solution and scoop solid  $\text{K}_2\text{S}_2\text{O}_8$  and glass beads
- Boil down to 10mL or less.

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- Cool and dilute to 50mL with DI.
- Turn on the SEAL AQ2 analyzer.
- Fill out a run sequence and save it.
- Fill cups, including a cup of 1.0ppm digested standard for the curve
- Start the analysis.
- Change names of blank and LCS to be what the LIMS will recognize (ie, what is on the batch sheet).
- Export the data to LIMS by dropping it into the "SEAL on bowzer" folder.

## 10. Procedure

### 10.1 Equipment Set-up

#### 10.1.1 Sample Preparation for Dissolved Phosphorus Analysis

Prior to preservation, samples to be analyzed for dissolved phosphorus are filtered using new disposable syringes and new 0.45um filter discs. 100mL of sample is filtered, poured into two new centrifuge tubes and preserved with H<sub>2</sub>SO<sub>4</sub>.

### 10.2 Initial Calibration

#### 10.2.1:

**Preparation of calibration curve, with spectrophotometer:** Prepare individual calibration curve from a series of six digested standards (0 mgP/L to 1.0 mgP/L) on each day of analysis. The curve must be digested. Use DI water without the combined reagent to zero the Spectrophotometer. Plot absorbance vs. phosphate concentration to give a straight line. The correlation coefficient must be 0.995 or greater for the curve to be considered valid. Analyze at least one phosphate standard with each batch of 20 samples or less.

All calibration points are back calculated (on excel) and should be within 10% from true concentration, except 2 lowest points of calibration curve. %recoveries for low range will be wider, but shouldn't exceed 50% and correlation coefficient will not be worse than 0.995.

#### 10.2.2:

**Preparation of calibration curve, with SEAL AQ2 analyzer:** Prepare and digest a 1.0 mgP/L standard and put it into the first slot in the auto-sampler. When prompted, click on "Auto-calibrate" to start calibration. Once the curve is finished, it may be checked in the "calibration" section. The correlation coefficient must be 0.995 or greater for the curve to be considered valid.

. All calibration points are back calculated by SEAL software and should be within 10% from true concentration, except 2 lowest points of calibration curve. %recoveries for low range will be wider, but shouldn't exceed 50% and correlation coefficient will not be worse than 0.995.

### 10.3 Equipment Operation and Sample Processing, with spectrophotometer

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- 10.3.1 Add 50mL or a suitable portion of thoroughly mixed sample to a prepared (Section 7.2) 125mL Erlenmeyer flask. Use 0.1g of soil sample with 50 ml of DI for soil samples.
- 10.3.2 Add 1mL H<sub>2</sub>SO<sub>4</sub> solution, one scoop solid K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and 3 to 5 glass beads.
- 10.3.3 Boil gently on a preheated hot plate until a final volume of 10mL or less is reached. Organophosphorus compounds such as AMP may require as much as 1-1/2 to 2 hours for complete digestion.
- 10.3.4 Cool, dilute to 30mL with DI water.
- 10.3.5 Add 0.05mL (1 drop) phenolphthalein indicator solution.
- 10.3.6 Neutralize to a faint pink color with NaOH.
- 10.3.7 Pour pink liquid sample into a new (unused) centrifuge tube and bring volume to 50mL with DI. Pour back into a 125mL Erlenmeyer flask.
- 10.3.8 Swirl the sample to mix and pour off 25mL digested sample into centrifuge tube.
- 10.3.9 Add 4.0mL combined reagent to all 25mL sample and QC sample aliquots and mix thoroughly.
- 10.3.10 After at least 10 minutes but no more than 30 minutes, use DI as the reference solution to zero the spectrophotometer at 880nm. Measure absorbance of each sample and record in the electronic laboratory notebook. If samples seem to have high background color before the addition of the coloring reagent, a background color may be checked for (see section 4).  
  
If the sample concentration is greater than the highest concentration of the calibration curve (1.0mg/L), the digested sample is diluted with DI water to a concentration within the range of the calibration curve.

### 10.4 Equipment Operation and Sample Processing, with SEAL AQ2 Analyzer

- 10.4.1 Acid rinse all glassware twice with 1:1 hydrochloric acid and then twice with DI water.
- 10.4.2 Pour out 50mLs of mixed samples and QC samples, including a 1.0ppm calibration standard.
- 10.4.3 Add 1mL H<sub>2</sub>SO<sub>4</sub> solution, one scoop solid K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and 3 to 5 glass beads.
- 10.4.4 Boil gently on a preheated hot plate until a final volume of 10mL or less is reached. Organophosphorus compounds such as AMP may require as much as 1-1/2 to 2 hours for complete digestion.
- 10.4.5 Cool and dilute to 50mLs.
- 10.4.6 Turn on SEAL AQ2 analyzer by flipping first the small, and then the large switch in the back.
- 10.4.7 Give the instrument at least half an hour to warm up.
- 10.4.8 If it has not yet been done that day, go through daily start-up, check voltages, and test aspiration.

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- 10.4.9 Double click on scheduling to open the schedule form, and insert samples, Method Blanks, LCSs, Duplicates, and Matrix Spikes (the CCVs and CCBs populate automatically) NB: leave the first spot for the calibration curve.
- 10.4.10 Pour a small (approximately 1 mL) aliquot into small tubes and put them into the instrument. Put the 1.0ppm standard into the first spot.
- 10.4.11 Check to see that all the reagent wedges are in the correct spots and that there are sufficient reaction segments.
- 10.4.12 Save the sequence and double click on "run"; check the boxes for curve analysis.
- 10.4.13 Once the instrument is done analyzing the run, check and approve the results in the Data Review section. Then, change the Blank and LCS names to be whatever they are on the batch sheet (ex: WG123456-1) and save the run in the "out" folder.
- 10.4.14 Open the "SEAL on bowzer" folder and drop the run into it from the "out" folder. This saves the data to LIMS.

### 10.5 Continuing Calibration

The method blank and LCS are used as the CCB/CCV and should be read after every ten samples and at the end of the batch. Recovery for the CCV must be between 85-115% of the true value. Recovery for the CCB must be between the RL and its negative, (i.e: within -.01mg/L and .01mg/L for waters).

### 10.6 Preventative Maintenance

The Spectrophotometers are calibrated on a semi-annual basis by an instrument service company. Certificates are kept on file.

## 11. Data Evaluation, Calculations and Reporting

Calculate the concentration value of the sample directly from the standard curve. (Section 10.2).

$$\text{mg P}_{\text{Total}}/\text{L} = \frac{\text{absorbance} - \text{y-intercept}}{\text{slope}} \times \text{Dilution factor}$$

If samples were filtered prior to preservation, report as mg P<sub>Dissolved</sub> / L.

For soil samples, convert results to mg/kg, by multiplying result in mg/l by extraction volume and dividing by weight. All results must be reported based on dry weight.

## 12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

Holding time exceedances or improper preservation are noted on the nonconformance report form.

Perform routine preventative maintenance following manufacturer's specification. Record all maintenance in the instrument logbook.

Review of standards, blanks and standard response for acceptable performance occurs for each batch of samples. Record any trends or unusual performance on a nonconformance action form.

If the CCV or LCS recovery of any parameter falls outside the designated acceptance range, the laboratory performance for that parameter is judged to be out of control, and the problem must be

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immediately identified and corrected. The analytical result for that parameter in the unspiked samples is suspect and is only reported for regulatory compliance purposes with the appropriate nonconformance action form. Immediate corrective action includes reanalyzing all affected samples by using any retained sample before the expiration of the holding time.

## 13. Method Performance

### 13.1 Method Detection Limit Study (MDL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ)

The laboratory follows the procedure to determine the MDL, LOD, and/or LOQ as outlined in Alpha SOP/1732. These studies performed by the laboratory are maintained on file for review.

The quarterly method reporting limit (MRL) verification requirement is satisfied by the quarterly LOQ analysis, as per SOP/1732.

### 13.2 Demonstration of Capability Studies

Refer to Alpha SOP/1739 for further information regarding IDC/DOC Generation.

#### 13.2.1 Initial (IDC)

The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method, prior to the processing of any samples.

#### 13.2.2 Continuing (DOC)

The analyst must make a continuing, annual, demonstration of the ability to generate acceptable accuracy and precision with this method.

## 14. Pollution Prevention and Waste Management

Refer to Alpha's Chemical Hygiene Plan and Waste Management and Disposal SOP for further pollution prevention and waste management information.

## 15. Referenced Documents

Chemical Hygiene Plan  
SOP/1732 MDL/LOD/LOQ Generation  
SOP/1739 IDC/DOC Generation  
SOP/1728 Waste Management and Disposal SOP

## 16. Attachments

None.

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