Title and Approval Page (A1)

Quality Assurance Project Plan for Santuit Pond Baseline Monitoring and Sediment Coring Program

Lead Organization(s): Mashpee Wampanoag Tribe, Town of Mashpee, Kimberly Groff Consulting (Southeast New England Program [SNEP] Network Liaison)
Consultant: ESS Group, LLC (a TRC Company)
June 5, 2022

Rev. 1

Jason Steiding Jason Steiding (Sep 6, 2022 14:32 EDT)	Sep 6, 2022
Jason Steiding– Natural Resource Director, Mashpee Wampanoag Tribe	Date
Ashley Fisher	9-2-22
Ashley Fisher – Department of Natural Resources Director, Town of Mashpee	Date
Kimbelry Groff Kimbelry Groff (Jul 21, 2022 12:43 EDT)	Jul 21, 2022
Kimberly Groff – SNEP Network Massachusetts Liaison	Date
	Jul 20, 2022
Matt Ladewig - ESS Project Manager	Date
	6/14/2022
Jeff Hershberger – ESS QA Officer	Date
Jessica Iverson	9/30/22
Jessica Iverson – EPA Region 1 QA Reviewer	Date
Dombroski, Ian Digitally signed by Dombroski, Ian Date: 2022.09.30 17:11:44 -04'00'	
Ian Dombroski – EPA Project Officer	Date

This QAPP was developed in accordance with the following guidance documents: EPA Guidance for Quality Assurance Project Plans (EPA QA/G-5) (EPA, 2002), EPA New England Environmental Data Review Program Guidance (EPA, 2018), and EPA New England QAPP Guidance for Projects Using Secondary Data (EPA, 2009).

Table of Contents (A2)

Title and Approval Page (A1) 1

Quality Assurance Project Plan for Santuit Pond Baseline Monitoring and Sediment Coring Program ... 1
Distribution List (A3) 4

Section A: Project Management 5

A4: Project Task and Organization 5
A5: Problem Definition and Background 7
A6: Project Task Descriptions 11
A7: Quality Objectives and Criteria 14
A8: Special Trainings and Certification 17
A9: Documentation and Records 17
Section B: Data Generation and Acquisition 19

B1: Sampling Process Design (Experimental Design) 19
B2: Sampling Methods 20
B3: Sample Handling and Custody 21
B4: Analytical Methods 21
B5: Quality Control 22
B6: Instrument/Equipment Testing, Inspection and Maintenance 23
B7: Instrument/Equipment Calibration and Frequency 23
B8: Inspection/Acceptance of Supplies and Consumables 24
B9: Non-Direct Measurements (i.e., secondary data) 24
B10: Data Management 24
Section C: Assessment and Oversight 26

C1: Assessments and Response Actions 26
C2: Reports to Management 26
Section D: Data Validation and Usability 27

D1: Data Review, Verification, and Validation 27
D2: Verification and Validation of Methods 27
D3: Reconciliation with User Requirements 28
References 28

Appendix A: Field Sampling SOPs and Data Forms

Standard Operating Guidelines for Measurement of Water Clarity with a Secchi Disk
Guidelines for Measurement of Specific Conductance
Guidelines for Measurement of Dissolved Oxygen

Guidelines for Measurement of pH
Guidelines for the Measurement of Turbidity
Standard Operating Guidelines for Collection of Sediments from Freshwater Environments
Guidelines for Collection of Surface Water Samples

Appendix B: Laboratory SOPs

Alpha Analytical Chlorophyll A
BEC Sequential Fractionation of Phosphorus in Sediment
Phoenix Environmental Laboratories Phosphorus, All Forms

Distribution List (A3)

Organization	Contact(s)	Email Address(es)
Mashpee Wampanoag Tribe	**Jason Steiding** Natural Resource Director, Mashpee Wampanoag Tribe	Jason.steiding@mwtribe-nsn.gov
Town of Mashpee	**Ashley Fisher** Department of Natural Resources Director, Town of Mashpee	Afisher@mashpeema.gov
Kimberly Groff Consulting	**Kimberly Groff** Consultant (508) 932-5528	kimberlygroffma@gmail.com
ESS Group, LLC (a TRC Company)	**Matt Ladewig** Principal Water Resources Scientist (401) 330-1204 **Jeff Hershberger** Senior Hydrogeologist (781) 419-7702 **Anna Chase** Water Resources Scientist (781) 419-7716	mladewig@trccompanies.com jhershberger@trccompanies.com achase@trccompanies.com
US Environmental Protection Agency	**Jessica Iverson** QA Officer (617) 918-8630 **Ian Dombroski** Life Scientist and Project Officer (617) 918-1342 **Tom Faber** (617) 918-8672	Iverson.jessica@epa.gov Dombroski.ian@epa.gov Faber.Tom@epa.gov

Section A: Project Management

The following section provides information regarding the background of the Santuit Pond Baseline Monitoring and Sediment Coring Program, the tasks involved in completing the project, and the names and responsibilities of key project team members.

A4: Project Task and Organization

See **Table 1** for a list of the specific members from each organization and **Figure 1** for an organizational chart.

Table 1. Project Participants

Name	Title	Organization	Primary Responsibility
Ashley Fisher	Department of Natural Resources Director	Town of Mashpee	Project Partner
Jason Steiding	Natural Resource Director	Mashpee Wampanoag Tribe	Project Partner
Kimberly Groff	Consultant	Kimberly Groff Consulting	Project Liaison
Matt Ladewig	Principal Water Resources Scientist	ESS Group, LLC (a TRC Company)	Project Manager
Jeff Hershberger	Senior Hydrogeologist	ESS Group, LLC (a TRC Company)	Project QA Officer

Mashpee Wampanoag Tribe and Town of Mashpee (Project Partners)

The Mashpee Wampanoag Tribe are the stewards of 321 acres of Tribal lands in Mashpee, Middleborough, and Taunton, Massachusetts. Known as "People of the First Light," they strive to work in harmony with their vast local bounty of natural resources, having made their living from the land and the sea for thousands of years.

In July 2021, the SNEP Network released a "Call for Participants" to offer direct technical, financial, and training assistance to municipalities, tribes, and non-profits in the SNEP watershed. Assistance was offered through the SNEP Network's existing capacity and members of their pre-approved consultant pool.

Through a competitive process, the Mashpee Wampanoag Tribe request was one of the initial 14 applications that was approved to receive technical assistance. The Mashpee Wampanoag Tribe requested the evaluation and implementation of measures to address nutrient pollution in Santuit Pond as well as assistance in managing water quality issues related to stormwater on a watershed scale.

On its end, the Town of Mashpee is moving forward in addressing loading from septic systems and residential landscapes in proximity to the pond.

Therefore, the SNEP Network deemed appropriate for the Mashpee Wampanoag Tribe to partner with the Town of Mashpee to work together as Project Partners to optimize workflows and minimize duplication of efforts. This relationship was formalized under a Memorandum of Agreement in September 2021 to examine opportunities to reduce watershed sources and directly address in-pond management issues.

Kimberly Groff Consulting

Kimberly Groff Consulting will act as the SNEP Network liaison between the Project Partners, ESS, and the New England Environmental Finance Center at the University of Southern Maine. In this role, Dr. Groff will manage the project on behalf of the SNEP Network and assist the Project Partners in reviewing ESS's project deliverables.

ESS Group, LLC (a TRC Company)

ESS Group, LLC (ESS) is the primary environmental contractor responsible for the field implementation, data management and analysis, and reporting as part of the Santuit Pond Baseline Monitoring and Sediment Coring Program project. ESS Project Manager Matt Ladewig will serve as the contractor's primary point of contact for the project, overseeing implementation of the field program, management of laboratory subcontractors, and development of project deliverables. ESS QA Officer Jeff Hershberger will be responsible for quality assurance/quality control review of field and laboratory-generated data and ESS deliverables.

BEC Engineering & Geology, P.C

BEC Engineering & Geology, P.C. (BEC) of Minneapolis, Minnesota will provide laboratory support and analysis as a subcontractor of ESS. BEC will analyze sequential fractionation of phosphorus in sediment and work with ESS to develop the alum dosing recommendations. BEC is a full-service environmental consulting firm offering a wide variety of testing services under the standards of ASTM E1527-13.

Alpha Analytical, Inc.

Alpha Analytical, Inc. (Alpha) of Westborough and Mansfield, Massachusetts will provide laboratory support and analysis as a subcontractor of ESS. Alpha will provide analysis of Chlorophyll a in surface water samples. The MassDEP lab certification IDs for Alpha's laboratories are M-MA086 and M-MA030.

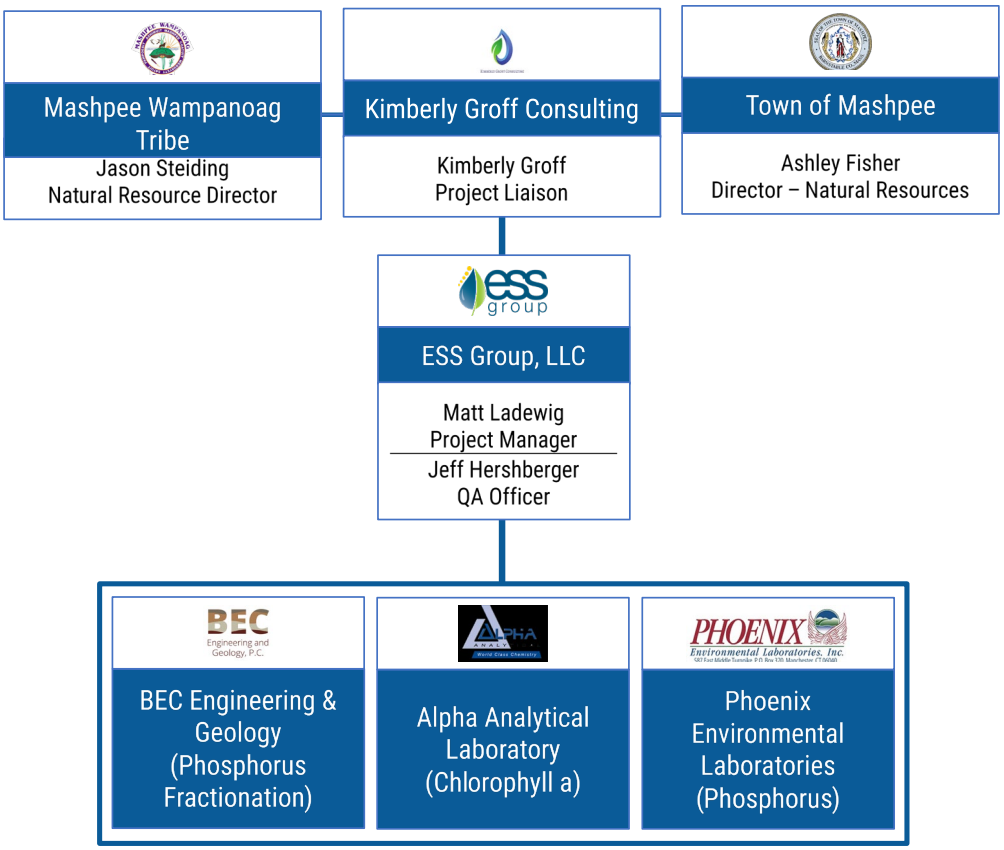


Figure 1. Project Organizational Chart

Phoenix Environmental Laboratories, Inc.

Phoenix Environmental Laboratories, Inc. (Phoenix) of Manchester, Connecticut will provide laboratory support and analysis as a subcontractor of ESS. Phoenix will provide analysis of Total Phosphorus in surface water samples. The MassDEP lab certification ID is M-CT007.

A5: Problem Definition and Background

Santuit Pond is an approximately 176-acre Great Pond located entirely in the town of Mashpee, Massachusetts. The pond serves as the headwaters of the Santuit River, which originates as the outlet at the pond's southernmost end and flows south into Shoestring Bay. As with most of Cape Cod, Santuit Pond is located in an area of thick glacial outwash deposits. As a result, the pond is primarily groundwater-fed and has

no permanent surface tributaries, although surface flows may reach the pond in the form of stormwater runoff.

Under the Final Massachusetts Integrated List of Waters for the Clean Water Act 2018/2020 Reporting Cycle (MassDEP 2021), Santuit Pond is listed as a Category 5 water body (i.e., needing a TMDL) and is impacted by the following impairments:

1. Fish passage barrier
2. Abnormal fish deformities, erosions, lesions, tumors
3. Chlorophyll-a
4. Harmful algal blooms
5. Nutrient/eutrophication biological indicators
6. High pH
7. Total phosphorus
8. Transparency/clarity

With the possible exception of the first two, these impairments are highly interrelated. For example, excessive total phosphorus is likely to result in eutrophication and harmful algal blooms, which likewise result in high concentrations of chlorophyll a. These impairments, in turn, reduce transparency and can lead to high pH levels. This suggests that the reduction of phosphorus concentrations in Santuit Pond could have cascading benefits to multiple impairments.

In the recently completed *Focused Feasibility Study of Santuit Pond* (ESS 2022) a number of in-pond management options were evaluated as means to improve water quality over the short term while watershed controls (septic and stormwater) come online to provide longer term reductions in nutrient loading loading. One of the primary recommendations was to investigate the potential benefits from nutrient inactivation (e.g., alum treatment) that targets internal release of phosphorus from the sediments.

Internal recycling of phosphorus through release of available sediment phosphorus fractions was documented in the 2010 *Santuit Pond Diagnostic Study* (AECOM). The key findings of this study related to internal loading were as follows:

- Despite shallow depths and frequent mixing, dissolved oxygen depletion still occurs in bottom waters. This allows release of phosphorus from sediments into the waters of Santuit Pond (internal loading).
- The total phosphorus load to Santuit Pond is approximately 380 kg/yr, which is equivalent to an annual in-pond phosphorus concentration of 80 µg/L. Most of this (78%) is from internal loading.

- Available fractions (i.e., iron-bound and loosely-sorbed fractions) of phosphorus in the sediment were found to be high (i.e., >300 mg/kg dry) in three of the four sediment core locations sampled.

While helpful, these observations are more than a decade old. Therefore, prior to advancing a sediment dose alum treatment, which is likely to cost several hundred thousand dollars, the Project Partners seek to reassess the levels of key phosphorus fractions present in Santuit Pod sediments.

Project Description

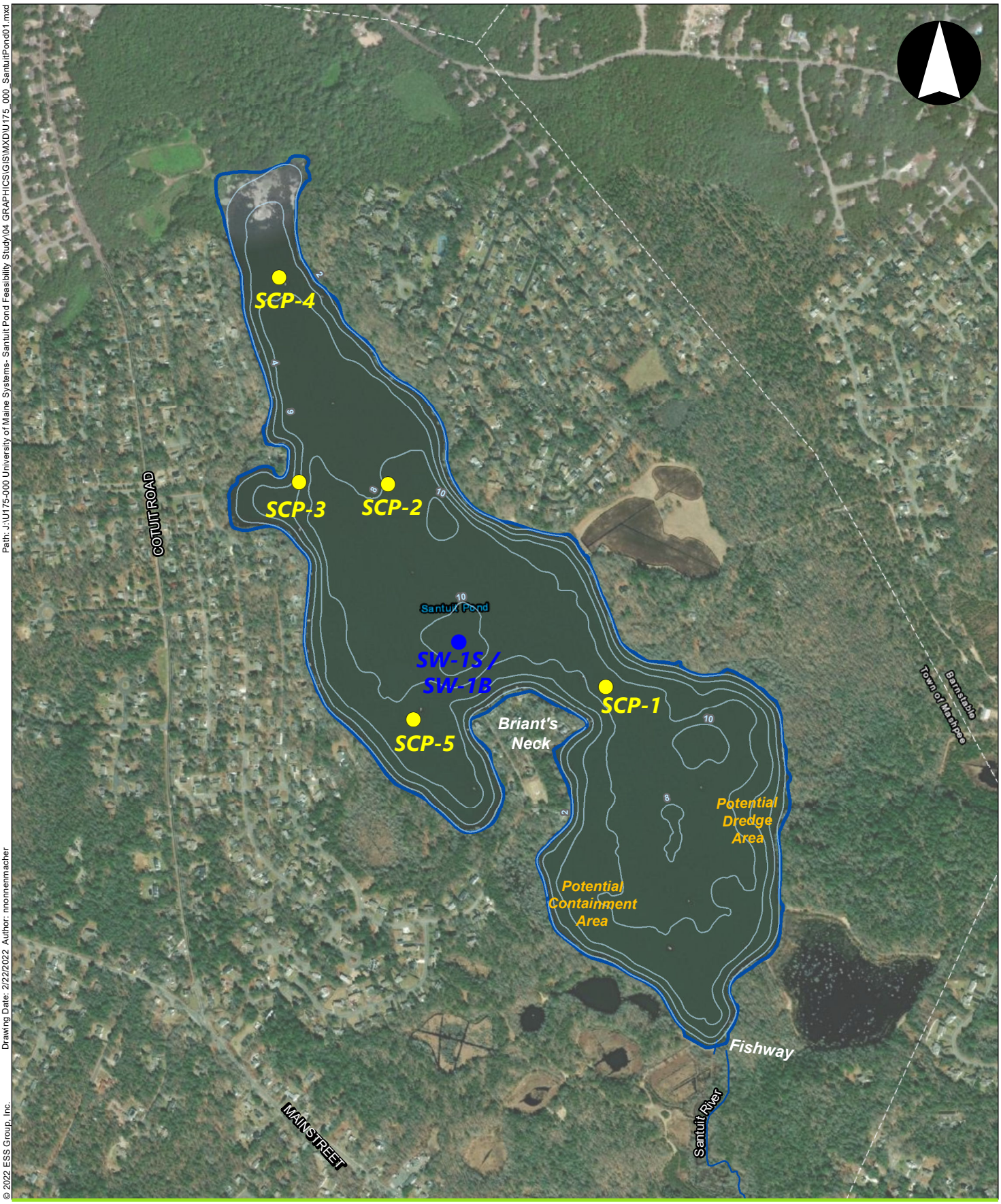
The Santuit Pond Baseline Monitoring and Sediment Coring Program seeks to investigate water quality and sediment conditions within the pond (**Figure 2**).

The overall goals of this Project are as follows:

1. Collect water quality data to document and build upon existing information that indicates water quality issues in Santuit Pond; and
2. Collect sediment data to optimize the dosage of alum for in-pond nutrient inactivation to address excessive phosphorus loading.

To support these goals, ESS will collect and analyze water quality and sediment data in the area of interest.

The area of interest includes portions of the pond located away from the potential dredge and fill areas identified by the New England District of the U.S. Army Corps of Engineers in their preferred preliminary alternative for a Section 206 aquatic ecosystem restoration project at Santuit Pond (USACE 2021).



Path: J:\U175-000 University of Maine Systems- Santuit Pond Feasibility Study\04 GRAPHICS\GIS\MXD\U175_000_SantuitPond01.mxd
Drawing Date: 2/22/2022 Author: nnonnenmacher
© 2022 ESS Group, Inc.

ess group
environmental consulting & engineering services

0 400 800 Feet

Santuit Pond QAPP - Baseline Monitoring and Sediment Coring Program

Mashpee, MA

Source: 1) ESRI, World Imagery, 2021
2) MassGIS, Bathymetry, 2019

- Proposed Water Quality Locations
- Proposed Sediment Core Locations
- Bathymetry Contours (2ft Intervals)
- Streams

Santuit Pond Proposed Sampling Locations

Figure 2

A6: Project Task Descriptions

An overview of project tasks and schedule is presented in **Table 2**.

Table 2. Project Tasks and Schedule

Task	Deliverable	Timeline	Relevant Details/Comments
1. Project Kick-off and Develop Quality Assurance Project Plan	Approved QAPP	April 2022	Assumes review by EPA
2. Baseline Monitoring	Water quality results to be incorporated into Task 4 deliverable	Baseline monitoring will occur by the end of May 2022 (or as soon as possible following QAPP approval)	Includes field-measured and laboratory water quality.
3. Sediment Coring and Phosphorus Fractionation Analysis	Sediment core analytical results and dosing recommendations (to be incorporated into Task 4 deliverable)	Sediment coring will occur by the end of May 2022 (or as soon as possible following QAPP approval)	Includes collection and analysis of sediment cores.
4. Baseline Monitoring and Alum Dosing Report	Data deliverables, tabular summaries, figures, and accompanying narrative documenting the results of Tasks 2 and 3 and describe the recommended alum dosing level to reduce phosphorus levels in the pond.	Draft report submitted for client review 12 weeks following sample collection. Report finalized within 2 weeks of receipt of client comments	

Task 1: Project Kick-Off and Develop Quality Assurance Project Plan

This task consists of project kick-off and coordination between the Project Partners, Kimberly Groff, and ESS's Project Manager, and the development of a Quality Assurance Project Plan (QAPP).

This QAPP describes the quality management system and procedures, as well as the roles and responsibilities of the Project Team. It also provides an overview of the project goals, data quality objectives, and quality assurance related to data collected and used as part of the project.

ESS will maintain the final version of the QAPP as part of the project documentation and distribute it electronically to designated recipients.

At the conclusion of the project, ESS will include an assessment of quality assurance/quality control measures implemented, their outcomes, and impacts on the usability of project data.

Task 2: Baseline Monitoring

In-pond sampling of water quality will be used to establish the current baseline conditions in Santuit Pond. The sampling will occur in spring of 2022.

During this event, samples will be collected within one meter of the surface and bottom of the deepest part of Santuit Pond (anticipated to be approximately just over three meters deep) for a total of two samples at one location (Figure 2). Additionally, water quality profiles will be measured in situ within the water column.

The geographic coordinates for each sampling location will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

Water quality samples will be sent to the laboratory for analysis of the following:

- Total Phosphorus (two grab samples)
- Chlorophyll a – (two grab samples)

Additionally, the following parameters will be field measured:

- Secchi Disk Transparency – surface only
- Water Temperature – full vertical profile at 0.3 m increments
- Specific Conductance – full vertical profile at 0.3 m increments
- Dissolved Oxygen – full vertical profile at 0.3 m increments
- pH – full vertical profile at 0.3 m increments

Task 3: Sediment Coring and Phosphorus Fractionation Analysis

Internal recycling is a potentially critical source of phosphorus loading in ponds that experience dissolved oxygen depletion and contain high levels of phosphorus in

redox-sensitive forms (e.g., iron-bound). The collection and analysis of sediment core samples will be used to assess the current levels of phosphorus in Santuit Pond sediments and inform recommendations for alum dosing to reduce internal recycling in the pond. This will be accomplished through a sediment phosphorus fractionation analysis that quantifies the different forms of phosphorus present in surficial sediment layers.

This work is planned for spring 2022, while water temperatures are still cool and before dissolved oxygen concentrations fall to anoxic (i.e., less than 2 mg/L) levels. This will help to capture conditions while phosphorus conditions in the sediment are at or near their seasonal maximum.

Under this approach, a gravity corer or similar device will be deployed from a boat to collect undisturbed and uncontaminated cores of 10 cm to 25 cm (depending on penetration and recovery depths achieved) for use in evaluating maximum phosphorus release rates. Collected cores will be extruded through the top of the upright corer and a sharp, clean blade will be used to section the material into 2-4 cm sections.

Five sediment cores will be collected and sliced into multiple 2-4 cm sections (for an anticipated total of up to 35 sections). Three cores will be collected from locations previously sampled by AECOM more than 10 years ago to allow for comparison of findings and assess whether the potential for phosphorus release has increased or decreased. These cores are labeled SCP-1, SCP-2, and SCP-3 (**Figure 2**). The other two cores will be located to balance geographic distribution of sampling locations in the pond. However, ESS will avoid collecting cores in the southernmost portion of the pond, where the US Army Corps of Engineers recent dredge study identified potential dredge and fill areas. All sediment cores will be collected from waters that are at least one meter in depth.

The geographic coordinates for each sampling location will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

These core samples will be sent to a specialty phosphorus fractionation laboratory (BEC) and analyzed for the following specialty parameters:

- Iron-bound phosphorus
- Aluminum-bound phosphorus
- Calcium-bound phosphorus
- Organically bound phosphorus
- Percent water
- Loss on ignition-organic carbon content
- Density

These data will help to define the spatial variability of phosphorus across the pond bottom, as well as vertically within the bottom sediment. This will help to provide some indication of how deep into the sediment phosphorus release occurs and will provide critical dosing information for future nutrient inactivation treatments.

Please refer to Section B1 for further details on the rationale and goals for sediment coring and phosphorus fractionation.

Task 4: Baseline Monitoring and Alum Dosing Report

At the conclusion of the Santuit Pond Baseline Monitoring and Sediment Coring Program, results and recommendations will be documented in a final report. This report will include data deliverables, tabular summaries, figures, and accompanying narrative documenting the results of Tasks 2 and 3 and describe the recommended alum dosing level to reduce phosphorus levels in the pond. It is anticipated that this report will serve as the technical basis for project permitting, should the Project Partners decide to implement an alum treatment.

A7: Quality Objectives and Criteria

Description of Data Acceptance

The data quality objectives (DQOs) for this project will be met if the data collected can be used to assess or determine the value or change in value of each of the water and sediment quality measures.

DQOs can be described in terms of the following performance criteria: precision, accuracy and bias, representativeness, comparability, and completeness.

Precision:

Water Quality. Water quality laboratory sample and field measurement results will be sufficiently precise to ensure reliable detection of each analyte or parameter within its expected environmental range.

A single field duplicate will be collected for each laboratory parameter and submitted as a blind duplicate (i.e., not labeled as "duplicate") for quality control purposes. Laboratory duplicates will be tested in accordance with the requirements of the laboratory SOPs and/or Quality Manual/Quality Assurance Manual.

Precision will be measured using Relative Percent Difference (RPD) between duplicates. The precision objective for field parameters is presented in **Table 3**. The precision objective for laboratory samples is presented in **Table 4**.

Sediment Cores. Sediment laboratory sample results will be sufficiently precise to ensure reliable detection of each analyte within its expected environmental range. Laboratory duplicates will be tested at a 5% rate.

Accuracy and Bias:

Water Quality. Water quality laboratory sample and field measurement results will be sufficiently accurate and unbiased to ensure results that reflect the true value of the analyte or parameter being measured.

Where applicable, laboratory accuracy will be measured using Percent Recovery of one or more of the following quality control samples: laboratory control samples (LCS) and matrix spikes (MS). Method blanks and other checks on accuracy may also be used. The accuracy objective for this project is presented in **Table 3** for field parameters and **Table 4** for lab parameters.

Sediment Cores. Sediment laboratory sample results will be sufficiently accurate and unbiased to ensure results that reflect the true value of the analyte or parameter being measured.

Table 3. Field Parameter Performance Objectives

Parameter	Units	Resolution	Expected Range	Precision Objective*	Accuracy Objective
Temperature	°C	0.01	0-30	+/- 0.2	+/- 0.15
Dissolved Oxygen	mg/L	0.01	0-14	+/- 0.2	+/- 0.2
Specific Conductance	µS/cm	4 digits	25-500	10%	+/- 5%
Water Depth	ft	0.1	0-75	10%	+/- 0.5
pH	SU	0.01	5-9	+/- 0.2	+/- 0.2
Transparency (Secchi Depth)	m	0.1	0-10	+/- 0.25	N/A

*As measured by RPD between duplicate measurements

Table 4. Laboratory Performance Objectives

Analyte	Units	Practical Quantitation Limit/ Reporting Limit	Expected Range	Precision Objective *	Accuracy Objective
Phosphorus (Aqueous)	mg/L	0.010	0.010-1.0	±20%	±15%
Chlorophyll a	mg/m^3	2	1-150	±35%	N/A

Analyte	Units	Practical Quantitation Limit/ Reporting Limit	Expected Range	Precision Objective *	Accuracy Objective
Phosphorus (Sediment - Wet)	mg/cm^3	0.002	0.002-0.25	±20%	±15%

*As measured by RPD between duplicate measurements

Representativeness:

Water Quality. Water quality samples will be collected at the deep hole location in Santuit Pond. Sample results are expected to be representative provided that samples are collected in accordance with relevant SOPs and analytical laboratory sampling protocols, including use of appropriate containers, collection of sufficient volume, use of preservatives as directed, and adherence to sample hold times. Deviations from the established protocols will be noted on the chain-of-custody form or field books by field personnel and included in data reporting.

Sediment. Sediment samples will be collected at five stations within Santuit Pond. Three cores will be collected near locations that were previously samples by AECOM more than 10 years ago. The remaining two cores will be located to balance geographic distribution of sampling locations in the pond.

Additionally, sediment cores will be considered representative of surficial sediments if the recovered core is undisturbed and at least 15 cm deep. If core recovery is less than 15 cm, a second core will be attempted at a location that is offset from the first by at least two meters. If the recovery of the second core is also less than 15 cm, the field crew will contact the Project Manager to consider alternative sampling locations or approaches to obtain a representative core.

Comparability:

Water Quality. Water quality samples collected from different monitoring locations will be sufficiently comparable provided that samples are collected, stored, and transported in accordance with relevant SOPs and the laboratory sampling protocols. Deviations from the established protocols will be noted by field personnel and included in data reporting.

Sediment. Sediment samples collected from different monitoring locations will be sufficiently comparable provided that samples are collected, stored, and transported in accordance with relevant field collection procedures and the laboratory analytical protocols. Deviations from the established protocols will be noted by field personnel and included in data reporting.

Completeness:

Water Quality. Data will be considered complete if water quality samples are collected during the anticipated sampling event and analyzed for the intended analytes. If data cannot be obtained as planned, this will be reported to the Project Manager in a timely fashion to determine whether it is likely to affect completeness of the dataset If the effect is determined to be substantive, the Project Manager will consider alternatives to improve completeness of the dataset.

Sediment. Sediment data will be considered complete if cores are collected from each of the proposed stations during the collection event. If data cannot be obtained as planned, this will be reported to the Project Manager in a timely fashion to determine whether it is likely to affect completeness of the dataset If the effect is determined to be substantive, the Project Manager will consider alternatives to improve completeness of the dataset.

A8: Special Trainings and Certification

No special training or certification courses were specifically attended in preparation for this project.

ESS personnel involved with this project have received education and training in necessary field methods, including water quality sampling and measurement, and hydrologic investigations. Knowledge and skills have been acquired from previous academic study, routine participation at conferences, and in-house training associated with similar projects throughout New England. Additional in-house training will be provided for ESS field staff as necessary to meet project requirements.

At a minimum, all personnel involved in field sample collection or data analysis will be provided with a copy of this QAPP and attachments (once the QAPP is finalized).

Analytical laboratories that are responsible for producing chemistry data for tests listed under MassDEP's laboratory certification program will maintain certification through MassDEP, or equivalent agency in other states, for analysis of water samples, as applicable to the analytes processed under this QAPP.

A9: Documentation and Records

ESS will maintain the current approved version of the QAPP. Should any change to the QAPP be necessary, the current version of the QAPP will be saved in the Project Manager's electronic archive folder for the project before any changes are made, and the updated version will be assigned the next consecutive version number. The Project Manager will distribute the new version of the QAPP to the individuals listed on the Title and Approvals page in Section A1 of this QAPP for approval before distributing the new version of the QAPP to the individuals identified on the Distribution List in Section A3.

The following data will be included as part of the Final Report:

- Chain-of-custody forms, laboratory analytical reports, and electronic data deliverables for samples analyzed in the laboratory
- Tabular data for field-measured parameters
- GIS data created to support development of map figures

The Project Manager will review analytical reports for accuracy and completeness and contact the party responsible for completing the form or report in the event that any omissions or suspected inaccuracies are found. In the event that any corrections are made, they will be written on the form, dated and initialed by the party responsible for completing the form, or a new report will be issued with a new version number and date. All project data will be retained in the Project Manager's electronic files for this project for a period of at least three years after the end of the project.

Section B: Data Generation and Acquisition

The description of data generation and acquisition contained in this QAPP was developed with guidance from EPA's Requirements for Quality Assurance Project Plans (QA/R-5).

B1: Sampling Process Design (Experimental Design)

Rationale for Design.

In-Pond Water Quality. A discrete sampling event will be completed in spring 2022 to build on existing data and provide a baseline for conditions in the pond prior to alum treatment.

During this event, samples will be collected from near the surface and near the bottom at the deep-hole location in the pond. A water quality profile will be measured in situ within the water column to provide a detailed profile of dissolved oxygen, temperature, specific conductance, and pH, which are considered to be important data for directly documenting the potential for internal recycling in Santuit Pond.

Sediment. Internal recycling is a potentially critical source of phosphorus loading in ponds that experience dissolved oxygen depletion near the sediment-water interface. The collection and analysis of sediment core samples will be used to confirm the impact of internal nutrient recycling on water quality within Santuit Pond. This will be accomplished through a sediment phosphorus fractionation analysis that quantifies the different forms of phosphorus present in the top sediment layers. Multiple cores will be collected from sediments in different parts of the pond and samples will be extruded from each core and analyzed to provide a vertical profile of phosphorus fractions in the sediments.

Data will be reported as both wet weight (mg/cm^3) and dry weight (mg/kg). Wet weight is particularly useful for exploring phosphorus release rates and determining dosage rates for nutrient inactivation management strategies to control internal loading. Data for each phosphorus fraction are reported separately in an electronic data deliverable.

Types and Number of Samples Required.

In-Pond Water Quality and Sediment. In-pond water quality and sediment samples at Santuit Pond will be analyzed by the laboratories indicated for the analytes identified in **Table 5**.

Table 5. Anticipated Sample Collection and Analysis

Analyte / Parameter	Sampling Position(s)	Number of Samples	Laboratory
Total Phosphorus	Surface, Bottom*	2	Phoenix
Chlorophyll a	Surface, Bottom*	2	Alpha
Phosphorus (Sediment)	Sediment	5 cores/ 35 samples anticipated	BEC

*Represents two sampling positions at one location.

Additionally, several water quality parameters will be measured at one location in the field, as identified in **Table 6.**

Table 6. Anticipated Measurement of Water Quality Field Parameters

Analyte / Parameter	Sampling Position(s)	Number of Measurements per Location
pH	Every 0.3 meter	10*
Secchi Disk Transparency	Surface	1
Water Temperature	Every 0.3 meter	10*
Specific Conductance	Every 0.3 meter	10*
Dissolved Oxygen	Every 0.3 meter	10*

*Estimated. Total will depend on actual water depth at the location on the date of sampling,

B2: Sampling Methods

Water Quality. Field sampling of water quality measurements will be conducted in a manner consistent with SOPs, including Standard Operating Guidelines for Measurement of Water Clarity with a Secchi Disk, Guidelines for Measurement of Specific Conductance, Guidelines for Measurement of Dissolved Oxygen, Guidelines for Measurement of pH, Guidelines for the Measurement of Turbidity, Standard Operating Guidelines for Collection of Sediments from Freshwater Environments, and Guidelines for Collection of Surface Water Samples (**Appendix A)**.

Laboratory sample containers, preservation methods, and hold times that are applicable to the analytes being sampled as part of the Santuit Pond Baseline Monitoring and Sediment Coring Program are summarized in **Table 7**. Additionally, laboratory analysis will be conducted in a manner consistent with the SOPs provided (**Appendix B**).

Table 7. Laboratory Requirements for Collected Samples

Analyte	Container	Hold Time	Preservative*	Comments
Total Phosphorus	250 mL plastic	28 days	H_2SO_4	
Chlorophyll a	1 L opaque plastic	24 hours	None	Collect 2 bottles per sample for improved lab QC analysis
Phosphorus (Sediment)	4 oz glass jar	7 days	None	

*All samples should be held at 4°C, unless otherwise specifically noted.

B3: Sample Handling and Custody

Water Quality. Water quality samples will be collected in accordance with relevant procedures or SOPs for water quality field sampling and handling (**Appendix A)**. Sample collection personnel will wear previously unused nitrile gloves when collecting and handling samples.

Sample collection personnel will use permanent marker to label all sample bottles with project ID, sample ID, date and time sampled, analyses requested, and initials of the sampler, at a minimum. Samples will be placed on ice or in a dedicated refrigerator to maintain sample temperature between 0°C and 4°C until transfer to the laboratory's custody. Samples will be transferred to the laboratory prior to exceeding the analytical hold time.

All samples transferred to separate laboratory custody will be accompanied by a completed chain-of-custody (COC) form (**Appendix B**). Personnel transferring samples to the laboratory will sign the COC and indicate the date and time of sample transfer.

B4: Analytical Methods

Laboratory analytical methods are summarized in **Table 8**. SOPs for each method are provided in **Appendix B**. These include Alpha Analytical's Chlorophyll a SOP, BEC's Sequential Fractionation of Phosphorus in Sediment SOP, and Phoenix Environmental Laboratories Phosphorus, All Forms SOP Field parameter methods are summarized in **Table 9**. SOPs for each method are provided in **Appendix A**.

Table 8. Anticipated Laboratory Analytical Methods

Analyte	Matrix	Laboratory	Analytical Method(s)
Phosphorus (Total)	Water	Phoenix	SM4500PE
Chlorophyll a	Water	Alpha	SM10200H
Phosphorus	Sediment	BEC	Sequential fractionation method*

*See description of method in **Appendix B**. Note that this analytical method description was developed by Sveriges lantbruksuniversitet (Swedish University of Agricultural Sciences).

Table 9. Anticipated Field Parameter Instruments and Analytical Methods

Parameter	Matrix	Instrument	Analytical Method(s)*
Temperature	Water	YSI Pro 2030	Manufacturer's guide
Specific Conductance	Water	YSI Pro 2030	Guidelines for Measurement of Specific Conductance
Dissolved Oxygen	Water	YSI Pro 2030 with polarographic sensor	Guidelines for Measurement of Dissolved Oxygen
Water Depth	Water	Vexilar LPS-1 Handheld Depth Sounder or Calibrated Sounding Rod/Line	Manufacturer's guide
Transparency (Secchi Depth)	Water	Secchi Disk	Standard Operating Guidelines for Measurement of Water Clarity Using a Secchi Disk
pH	Water	Oakton Waterproof Pocket Tester	Guidelines for Measurement of pH

*See **Appendix A** for Guidelines documents

B5: Quality Control

Laboratory analytical SOPs and quality control information relevant to each analyte are provided in **Appendix B**.

Field duplicates will be collected for water quality samples, and field water quality measurements at an overall 10% rate. These field duplicates will be used expressly for

quantifying the RPD between samples collected or measurements taken at the same location. To avoid biasing the dataset, duplicate sample data will not be included in the final data analyses.

The minimum field duplicate sampling rate for each laboratory analyte is summarized in **Table 10**. The minimum field duplicate sampling rate for each field parameter is summarized in **Table 11**.

Table 10. Analytical Sample Duplicates

Analyte	Minimum # Duplicate Samples
Total Phosphorus	1
Chlorophyll a	1
Phosphorus (Sediment)	4

Table 11. Field Parameter Duplicates

Parameter	Minimum # Duplicate Samples
Water Temperature	1
Specific Conductance	1
Dissolved Oxygen	1
Secchi Disk	1
pH	1

B6: Instrument/Equipment Testing, Inspection and Maintenance

Laboratory analytical equipment testing, inspection, and maintenance protocols are provided in **Appendix B**.

Field instrumentation will be cleaned, stored, and serviced in accordance with manufacturer recommendations.

B7: Instrument/Equipment Calibration and Frequency

Laboratory analytical equipment calibration protocols are provided in **Appendix B**.

Field instrumentation will be calibrated and/or checked in accordance with manufacturer recommendations. Where applicable, post-field checks will be conducted against the appropriate calibration standard for data validation/verification purposes.

B8: Inspection/Acceptance of Supplies and Consumables

Sample collection personnel will complete a bottle order request form with each analytical laboratory to help ensure that the number, type, and condition of sample containers are appropriate for the analyses to be conducted. Additionally, sample collection personnel will also be responsible for visually inspecting sample containers and verifying their cleanliness, integrity, and consistency with this QAPP before collecting samples for laboratory analysis. Sample containers that are visibly damaged, soiled, or inappropriate for the required analysis will not be used for sample storage.

B9: Non-Direct Measurements (i.e., secondary data)

Geospatial data is the main type of secondary data that may be used to support the creation of map figures for the Santuit Baseline Monitoring and Sediment Coring Program. To ensure that the geospatial data are of sufficient quality to support the analyses identified in this QAPP, data will be obtained through official government sources or otherwise be of a quality meeting government standard for accuracy as documented in metadata or other supporting documentation.

Publicly available geospatial information, including high resolution orthophotography, will be used to develop map figures of the Project area. Where possible, this information will be obtained through MassGIS, Massachusetts's official geospatial data hub. Geospatial data available through MassGIS meet accepted metadata standards (https://www.mass.gov/massgis-standards-and-best-practices).

B10: Data Management

Field Data Collection and Records

Field notes, forms, and other documentation (e.g., photographs) will be saved for each round of field sampling or survey. A copy of each will be retained by the Project Manager for at least three years following project completion in the electronic records for the project.

Information to be recorded for each laboratory analytical sample will include the following: the individual performing the sample collection, date and time of sample collection, and laboratory analysis requested. This information will be recorded on a laboratory COC form (**Appendix B**). All COC forms will accompany samples for laboratory analysis and a copy of each will be retained by the Project Manager for at least three years following project completion in the electronic records for the project.

Analytical Laboratory Data

All electronic data deliverables, laboratory reports, case narratives, QC reports, email correspondence, or other information generated by an analytical laboratory will be retained by the Project Manager for at least three years following project completion

in the electronic records for the project. This will include preliminary or draft data, in addition to any revisions.

Section C: Assessment and Oversight

This section addresses the activities for assessing the effectiveness of the implementation of the quality assurance and quality control activities. The purpose of the assessment is to ensure that QAPP is implemented as described.

C1: Assessments and Response Actions

The Project Manager will provide oversight for field data collection to ensure that protocols described in this QAPP are being followed.

The Project Manager will review each batch of reported data to ensure that appropriate protocols are being followed and data are within the accepted range for each parameter or analyte. Any "outlier" data discovered will be identified and potential sources of error will be assessed to determine possible causes and avoid or minimize those going forward.

The Project Manager may also request data audits or reviews to assess conformance and compliance to the QAPP.

C2: Reports to Management

The Project Manager will provide regular updates to Kimberly Groff Consulting and the Project Partners related to project progress and results observed. These updates will include results of data quality assessments, as well as recommendations for resolving any issues associated with data integrity. The final report will be provided to the EPA Project Officer.

Section D: Data Validation and Usability

This section addresses the QA activities that occur after the data collection of the project has been completed. Implementation of these elements ensures that the data conform to the specified criteria and achieve the project objectives.

D1: Data Review, Verification, and Validation

Water Quality. Laboratories will use the control limits set forth in Section A7 to accept analytical data generated under conditions that meet the control limits for instrument precision and accuracy and reject or qualify analytical data generated under conditions that do not meet the control limits for instrument precision and accuracy.

The Project Manager or Quality Assurance Officer will review analytical reports to verify and validate the completeness and reasonableness of laboratory results using the record of samples submitted for analysis on the completed COC forms and the anticipated environmental range of values for the selected parameter and sample matrix.

Data will be considered complete when analytical values are reported for all analyses requested for all samples submitted to the laboratory. Data will be considered reasonable when all values measured for the sample and all values generated by the same analytical procedure fall within the expected range of values for that sample or that parameter.

Field-measured continuous and discrete data will be reviewed against the control limits provided in this document. The Project Manager, Quality Assurance Officer, or their designee will review field-measured data to verify and validate the completeness and reasonableness of field-measured results using the anticipated environmental range of values for the selected parameter and sample matrix.

Field-measured continuous and discrete data will be considered complete when values are reported for all parameters measured. Data will be considered reasonable when all values measured and all values generated by the same method fall within the expected range of values for that parameter.

Data that do not meet completeness or reasonableness criteria will be evaluated for rejection (if unusable) or qualification (if usable) and flagged accordingly.

D2: Verification and Validation of Methods

Water Quality. The Project Manager or Quality Assurance Officer will be responsible for verifying that all water quality samples collected are completely and accurately represented on COC forms prior to transfer to the laboratory for analysis. If there are data gaps or inaccuracies on COC forms, the forms will be corrected immediately upon noticing the error and the revised information will be communicated to the laboratory.

Laboratory technicians are responsible for validating instrument calibration by measuring the calibration standards for the selected parameter after accepting a new calibration and recalibrating if the measurements do not meet established control limits for accuracy and precision.

Analytical data provided by the laboratories will also be reviewed and validated by the Project Manager or Quality Assurance Officer to provide information on whether data are acceptable for use in developing project conclusions and deliverables. Results of the verification and validation processes will be addressed in project deliverables. The Project Manager will be responsible for final review of deliverables for completeness and adherence to method and quality control requirements.

D3: Reconciliation with User Requirements

Deliverables will include results of quality assurance activities and/or quality control outcomes. Results will be accompanied by an assessment of the impact of quality control anomalies or departures from assumptions, if any, on the ultimate usability of the data. Data qualifiers will also be described so that users are aware of these limitations.

References

AECOM. 2010. Santuit Pond Diagnostic Study; Mashpee, Massachusetts. Prepared for Town of Mashpee.

Environmental Protection Agency. 2001. EPA Requirements for Quality Assurance Project Plans. EPA QA/R-5.

Environmental Protection Agency. EPA R-5 Checklist for Review of Quality Assurance Project Plans.

Environmental Protection Agency. 2010. New England Quality Assurance Project Plan Program Guidance. EQAQAPP2005PG2.

ESS Group, LLC. 2022. Santuit Pond Focused Feasibility Study: Interim Recommendations for Water Quality Improvement. Prepared for Kimberly Groff, Massachusetts Liaison, SNEP Network.

Appendix A – Field Sampling SOPs

STANDARD OPERATING GUIDELINES FOR MEASUREMENT OF WATER CLARITY WITH A SECCHI DISK

1.0 INTRODUCTION

This Standard Operating Guideline (SOG) provides basic instructions for the routine measurement of water clarity in lakes and ponds with a Secchi disk. Water clarity is a function of the number of particles in the water (algae, sediment, etc) and the color of the water, which both have an impact on the depth of light penetration. The transparency of the water column can be used as an indicator of water body productivity, with certain exceptions (e.g., naturally sediment-laden waters, stained waters). Generally, the more productive a system is the more algae in the water column, and the lower the transparency. Water transparency can also be affected by suspended sediment particles which are related to water depth and wave action. Thus on any given day the transparency of a water body may be affected by its productivity, the season, wind speed and level of sunlight. The methods outlined below are intended (1) to standardize the use of a Secchi disk in the measurement of transparency; (2) to standardize recording of field data to assure proper documentation of weekly, monthly and seasonal patterns in transparency.

2.0 REQUIRED MATERIALS

The following materials are necessary for the measurement of transparency with a Secchi disk:

- Weighted Secchi disk with attached length of rope marked off in 0.1-meter increments with indelible ink.
- Field data sheets on weatherproof paper or field book
- Viewscope (optional – use if required by project plan or program)

3.0 METHODS

- A location will be selected from which to measure transparency. This location will stay constant throughout the study.
- Orient the vessel to minimize the influence of glare or shadow on the Secchi disk reading.
- The date, weather conditions, and personnel conducting the measurement will be recorded on the field sheet.
- The Secchi disk will be lowered slowly into the water by the rope so that the weight enters the water first and the disk follows, flat side parallel to the water surface.
- The disk will continue to be lowered through the water column until it is no longer visible.
- A note will be made of the depth of the disk at this point to the nearest 0.1 meter by reading where the surface of the water touches the rope.
- The disk will then be slowly raised until it is just visible again.
- Once again a note will be made of the depth of the disk at this point.
- An average of these two depths will be calculated to give the “Secchi depth”, i.e. a measure of the transparency of the water.

4.0 DOCUMENTATION

Secchi depth data will be reported on field data sheets for every day that a measurement is taken. Documentation for recorded data must include a minimum of the following:

- Date and time
- Weather conditions

© 2016 ESS Group, Inc.

- Signature or initials of person performing the measurement
- Depth measurements and average Secchi depth
- Field comments/observations on anything that may influence the Secchi depth measurement that day.

5.0 QUALITY CONTROL

- Duplicate measurements of a single sample will be performed at the frequency specified in the project plan. In the absence of project specific criteria, duplicate measurements should agree within ±0.25 meters.
- The Secchi disk rope should be checked at least annually against a tape measure to ensure the units of measurement are accurate.

©2016 ESS Group, Inc.

GUIDELINES FOR MEASUREMENT OF SPECIFIC CONDUCTANCE

1.0 INTRODUCTION

1.1 Purpose and Applicability

These Standard Operating Guidelines (SOG) provide basic instructions for routine calibration and operation of a variety of specific conductance meters. This SOG document also addresses estimation of total dissolved solids (TDS) and salinity by direct measurement of specific conductance (specific methods and capabilities for these parameters are outlined in the manufacturer's individual instrument manuals). This SOG is designed to be consistent with EPA Method 120.1 and Standard Method 2510 B which address specific conductance measurements of drinking, surface, and saline waters, domestic and industrial wastes, and acid rain.

1.2 Quality Assurance Planning Considerations

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. These quality assurance requirements will be defined in the site-specific workplan or Quality Assurance Project Plan (QAPP) (hereafter referred to as the project plan) or laboratory Quality Assurance Manual (QAM) and may include duplicate or replicate measurements or confirmatory analyses.

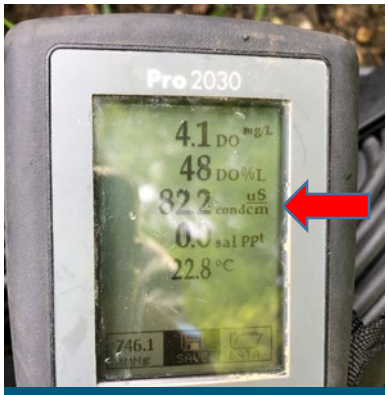
2.0 RESPONSIBILITIES

- The project manager is responsible for ensuring that project-specific requirements are communicated to the project team and for providing the materials, resources, and guidance necessary to perform the measurements in accordance with this SOG and the project plan.
- The analyst is responsible for verifying that the specific conductance meter is in proper operating condition prior to use and for implementing the calibration and measurement procedures in accordance with this SOG and the project plan.

3.0 REQUIRED MATERIALS

The following materials are necessary for this procedure:

- Specific conductance meter
- Specific conductance meter manufacturer's instruction manual
- Deionized water
- Conductivity standard at concentration that approximates anticipated range of sample concentrations
- Lint-free tissues
- Calibration sheets or logbook
- Laboratory or field data sheets or logbooks



Handheld YSI 2030 meter, red arrow showing where the specific conductance is measured

4.0 METHODS

4.1 Sample Handling, Preservation, and General Measurement Procedures

- Specific conductance measurements should be taken in situ or soon after sample collection since temperature changes, precipitation reactions, and absorption of carbon from the air can affect the specific conductance. If specific conductance measurements cannot be taken immediately (within 24 hours), samples should be filtered through a 0.45 µm filter, stored at 4°C and analyzed within 28 days.
- Report results as specific conductance, µmhos/cm or µS/cm at 25°C.

- Secondary standards may be purchased as a solution from commercial vendors. These standards should not be used after their expiration dates as provided by the manufacturer. An expiration date of one year from date of purchase should be used if the expiration date is worn or missing.

4.2. Calibration and Measurement Procedures

- The specific conductance meter must be calibrated (or the calibration checked) before any analyses are performed.
- Set up the instrument according to the manufacturer's instructions.
- Rinse the probe with deionized water prior to use. If necessary, dry with a lint-free tissue or cloth.
- Follow the manufacturer's recommendations for appropriate calibration receptacle and depth of immersion.
- Record the stabilized specific conductance reading of the standard and the temperature. Enter the calibration mode (according to manufacturer's instructions) and change the value on the primary display to match the value of the calibration standard. The meter may be adjusted to ±20% from the default setting. If the measurement differs by more than ±20%, the probe should be cleaned, serviced, or replaced as needed.
- An additional check may be performed, if required by the project plan, by placing the probe into an additional standard. This standard should be from a different source than the standard used for the initial calibration. This standard should read within 5% of the true value.



Use of YSI handheld device and probe

- Verify the calibration at least once a month or whenever the instrument has been moved from freshwater to saltwater environments or vice versa. Recalibrate or service the instrument, as needed, if the check value is not within 15% of the true (calibration standard) value.

4.3. Troubleshooting Information

If there are any performance problems with any of the specific conductance meters which result in inability to achieve the acceptance criteria presented in Section 5.0 or the project-specific acceptance criteria, consult the appropriate section of the meter instruction manual for troubleshooting procedures. If the problem persists, consult the manufacturer's customer service department immediately for further guidance.

4.4. Maintenance

- Instrument maintenance should be performed according to the procedures and frequencies required by the manufacturer.
- The probe must be stored and maintained according to the manufacturer's instructions.

5.0 QUALITY CONTROL

- The meter must be calibrated (or the calibration checked) before sampling, and will not be used for sample determinations of specific conductance unless the initial check standard value is within 5% of the true value.

- Duplicate measurements of a single sample will be performed at the frequency specified in the project plan. In the absence of project-specific criteria, duplicate measurements should agree within 10%.

6.0 DOCUMENTATION

- Meter calibration, temperature check, and maintenance information will be recorded in a calibration log. Specific conductance data may be recorded on the appropriate laboratory or field data sheets or logbooks.
- Calibration documentation should be maintained in a thorough and consistent manner. At a minimum, the following information should be recorded:
    - Date and time of calibration
    - Person performing the measurement
    - Instrument identification number/model
    - Expiration dates and batch numbers for all standards
    - Reading for standard before and after meter adjustment
    - Readings for all continuing calibration checks
    - Temperature of standards (corrected for any difference with reference thermometer)
    - Comments
- Documentation for recorded data must include a minimum of the following:
    - Date and time of analysis
    - Person performing the measurement
    - Sample identification/station location
    - Temperature (corrected for any difference with reference thermometer) and conductance of sample (including units and duplicate measurements).
    - Comments

7.0 TRAINING/QUALIFICATIONS

To properly perform specific conductance measurements, the analyst must be familiar with the calibration and measurement techniques stated in this SOG. The analyst must also be experienced in the operation of the meter.

Certain state certification programs require that specific conductance measurements be taken in the field by, or in the presence of, personnel that are qualified under the certification program.

GUIDELINES FOR MEASUREMENT OF DISSOLVED OXYGEN

1.0 INTRODUCTION

1.1 Purpose and Applicability

These Standard Operating Guidelines (SOG) provide basic instructions for routine measurement of dissolved oxygen using a polarographic sensor-equipped dissolved oxygen meter with a digital read-out (e.g., YSI Pro2030 Dissolved Oxygen, Conductivity, Salinity Instrument). Measurements are made in accordance with methods that address dissolved oxygen measurement of drinking, surface, and saline waters, and domestic and industrial wastes.

1.2 Quality Assurance Planning Considerations

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. These quality assurance requirements will be defined in the site-specific workplan or Quality Assurance Project Plan (QAPP) (hereafter referred to as the project plan) or laboratory Quality Assurance Manual (QAM) and may include duplicate or replicate measurements or confirmatory measurements.

2.0 RESPONSIBILITIES

- The project manager is responsible for ensuring that project-specific requirements are communicated to the project team and for providing the materials, resources, and guidance necessary to perform the measurements in accordance with this SOG and the project plan.
- The analyst is responsible for verifying that the dissolved oxygen measuring device is in proper operating condition prior to use and for implementing the calibration and measurement procedures in accordance with this SOG and the project plan.



Handheld YSI 2030 meter, Red arrow showing were the dissolved oxygen is measured

3.0 REQUIRED MATERIALS

The following materials are necessary for this procedure:

- Dissolved oxygen meter with digital read-out device
- Manufacturer's instruction manual for the instrument
- Manufacturer’s recommended operating solution and replacement membranes or caps
- Laboratory or field data sheets or logbooks

4.0 METHODS

4.1 Sample Handling, Preservation, and General Measurement Procedures

To achieve accurate dissolved oxygen measurements, samples should be analyzed in situ. Measurements in flowing waters should be made in relatively turbulent free areas. Measurements in standing waters may require gentle probe agitation to create water movement around the probe (check instrument manual to confirm).

4.2. Calibration and Measurement Procedures

To accurately calibrate some dissolved oxygen meters, it may be necessary to know the altitude of the region in which you are located and the approximate salinity of the water you will be analyzing. Fresh water has a salinity of approximately zero. Seawater has an approximate salinity of 35 practical salinity units

(psu). If uncertain, measure salinity first with an appropriate device. The instructions below are applicable to the YSI Model 55; for other instruments, consult the instruction manual.

- Ensure that the sponge inside the instrument's calibration chamber is wet then insert the probe into the chamber. Turn the instrument on and wait for readings to stabilize (as long as 15 minutes, depending on the model).
- To calibrate, enter the calibration menu by pressing and releasing both the up and down arrow keys at the same time. Enter the altitude (in hundreds of feet) at the prompt by using the arrow keys to increase or decrease the altitude (example: 12 = 1,200 feet). Press enter when correct altitude is shown.
- The meter should display CAL in the lower left of the display with the calibration value in the lower right of the display and the current D.O. reading (before calibration) should be on the main display. Once the D.O. reading is stable, press ENTER. Enter the salinity at the prompt by using the arrow keys. Press ENTER when finished and the instrument will return to normal operation.
- Calibration should be performed at a temperature within ± 10°C of the sample temperature. Recalibrate every 15 samples and whenever the unit is turned on.
- If calibration is out of range, erratic readings occur, bubbles appear, or if the membrane becomes damaged, wrinkled, or fouled refill the membrane solution and/or replace the membrane, per the manufacturer's manual.



Use of YSI handheld device and probe

- Avoid contact with environments containing substances that may attack the probe materials (e.g. acids, caustics, and strong solvents).

4.3. Troubleshooting Information

If there are any performance problems with the dissolved oxygen-measuring device, consult the appropriate section of the instruction manual for the checkout and self-test procedures. If the problem persists, consult the manufacturer's customer service department immediately for further instructions.

4.4. Maintenance

Instrument maintenance for meter-type dissolved oxygen measuring devices should be performed according to the procedures and frequencies required by the manufacturer. Rinsing the probe with distilled or deionized water and preventing exposure of the membrane to drying is typically all that is required on a day-to-day basis.

5.0 QUALITY CONTROL

Duplicate measurements of a single sample should be performed at the frequency specified in the project plan. In the absence of project-specific criteria, duplicate measurements should agree within ± 0.2 mg/L.

6.0 DOCUMENTATION

All dissolved oxygen meter calibration, checks, and maintenance information will be recorded in a calibration logbook. Dissolved oxygen data may be recorded on the appropriate field data sheets or field books.

- Calibration documentation must be maintained in a thorough and consistent manner. At a minimum, the following information must be recorded:
    - Date and time of calibration
    - Person performing the measurement
    - Instrument identification number/model
    - Readings for all continuing calibration checks
    - Comments
- Documentation for recorded data must include a minimum of the following:
    - Date and time of analysis
    - Person performing the measurement
    - Sample identification/station location
    - Dissolved oxygen, both in mg/L and percent saturation and temperature of sample (including units and duplicate measurements)
    - Comments

7.0 TRAINING/QUALIFICATIONS

To properly perform dissolved oxygen measurements, the analyst must be familiar with the calibration and measurement techniques stated in this SOG. The analyst must also be experienced in the operation of the meter.

Certain state certification programs require that dissolved oxygen measurements in the field be taken by, or in the presence of, personnel that are qualified under the certification program.

GUIDELINES FOR MEASUREMENT OF PH

1.0 INTRODUCTION

1.1 Purpose and Applicability

These Standard Operating Guidelines (SOG) provide basic instructions for routine calibration and operation of a variety of pH field pens. Although these meters may measure additional parameters (e.g., temperature, specific conductance, etc.), this SOG addresses pH measurement only (other capabilities are outlined in the appropriate SOG and manufacturer's individual instrument manuals). This SOG is designed specifically for the measurement of pH in accordance with EPA Method 150.1 and Standard Method 4500-H B which address electrometric pH measurements of drinking, surface, and saline waters, domestic and industrial wastes, and acid rain.

1.2 Quality Assurance Planning Considerations

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. These quality assurance requirements will be defined in the site-specific workplan or Quality Assurance Project Plan (QAPP) (hereafter referred to as the project plan) or laboratory Quality Assurance Manual (QAM) and may include duplicate or replicate measurements or confirmatory analyses.

2.0 RESPONSIBILITIES

- The project manager is responsible for ensuring that project-specific requirements are communicated to the project team and for providing the materials, resources, and guidance necessary to perform the measurements in accordance with this SOG and the project plan.
- The analyst is responsible for verifying that the pH meter is in proper operating condition prior to use and for implementing the calibration and measurement procedures in accordance with this SOG and the project plan.

3.0 REQUIRED MATERIALS

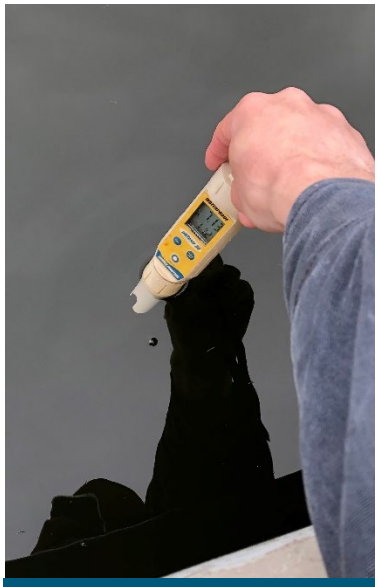
The following materials are necessary for this procedure:

- pH meter
- pH meter manufacturer's instruction manual
- Deionized or distilled water
- 4.0, 7.0, and 10.0 buffer solutions
- Lint-free tissues
- Mild detergent
- Manufacturer’s recommended storage solution
- Manufacturer’s recommended cleaning solution
- Field data sheet or logbook
- Calibration sheet or logbook

4.0 METHODS

4.1 Sample Handling, Preservation, and General Measurement Procedures

- To achieve accurate pH measurements, samples should be analyzed immediately in the field, or as soon as possible after collection. Sample should be measured *in situ* or collected in plastic or glass containers.
- As temperature can affect the pH measurements obtained, both the pH and the temperature of the sample must be recorded, unless the meter is capable of automatic temperature correction (ATC).
- Primary standard buffer salts available from NIST can be purchased and are necessary for situations where extreme accuracy is required. Secondary standard buffers may be purchased as a solution from commercial vendors and are recommended for routine use. Buffers should not be used after their expiration dates as provided by the manufacturer. An expiration date of one year should be used if the manufacturer does not supply an expiration date or if the buffers are prepared from pH powder pillows, etc.
- Keep the probe elevated off the bottom to avoid disturbing sediments. Allow readings to fully stabilize before recording the pH measurement. This may take several minutes, especially if the pH is drastically different from the last reading or the bulb has been allowed to dry out between readings.
- Rinse the electrode with deionized or distilled water between samples and wipe gently, if needed, with a lint-free tissue. If a more thorough cleaning is required, use a mild detergent (e.g., dish soap) or the manufacturer's recommended cleaning solution.
- Store the probe in the manufacturer's recommended storage solution or, if this is not available, tap water. Do not use distilled or deionized water for storage purposes.



Handheld pH meter

4.2. Calibration and Measurement Procedures

- The pH meter should be checked weekly before any analyses are performed. Otherwise, the meter should be checked or calibrated at the frequency specified in the project plan.
- Calibration should include a minimum of one point but ideally, a two point calibration that brackets the expected pH of the samples to be measured is desirable. Calibration measurements should be recorded in the calibration logbook.
- Choose either 7.0 and 10.0 (high range) or 4.0 and 7.0 (low range) buffers, whichever will bracket the expected sample range. Pour each buffer into a clean glass beaker. The volume should be sufficient to fully submerse the pH bulb and thermistor. If the pH is being measured in a laboratory, place the beaker on the magnetic stirrer and place the stirring bar in the beaker. Measure and record the temperatures of the buffers using a calibrated thermometer or automatic temperature compensation (ATC).
- Follow the manufacturer's calibration instructions.
- Once calibration is complete, discard the buffer and rinse the beaker (and stirring bar, if used) thoroughly with distilled or deionized water.

- An additional check may be performed, if required by the project plan, by placing the electrode into an additional buffer solution. This buffer should be from a different source than the buffers used for the initial calibration. This buffer should read within ±0.2 pH units of the buffer's true pH value.
- Recalibrate the instrument if any of the following apply:
  - the check value varies more than 0.2 pH units from the true value
  - the expected pH of the sampled water body is outside the current calibration range
  - readings are erratic or do not stabilize
  - the instrument has just been cleaned or otherwise disturbed for maintenance

4.3. Troubleshooting Information

If there are any instrument performance problems that result in the inability to achieve the acceptance criteria presented in Section 5.0, consult the appropriate section of the meter instruction manual for troubleshooting procedures. If the problem persists, consult the manufacturer's customer service department immediately for further guidance.

4.4. Maintenance

- Instrument maintenance should be performed according to the procedures and frequencies required by the manufacturer.
- The electrode should be stored and maintained according to the manufacturer's instructions.

5.0 QUALITY CONTROL

- Duplicate measurements of a single sample will be performed at the frequency specified in the project plan. In the absence of project-specific criteria, duplicate measurements should agree within ±0.2 pH units.

6.0 DOCUMENTATION

- All pH meter calibration, temperature check, and maintenance information will be recorded in a calibration logbook.
- pH data may be recorded on the appropriate laboratory or field data sheets or logbooks.
- Calibration documentation must be maintained in a thorough and consistent manner. At a minimum, the following information must be recorded:
  - Date and time of calibration
  - Person performing the measurement
  - Instrument identification number/model
  - Expiration dates and batch numbers for all buffer solutions
  - Reading for pH 7.0 buffer before and after meter adjustment
  - Reading for pH 4.0 or 10.0 buffer before and after meter adjustment
  - Readings for all continuing calibration checks
  - Temperature of buffers (corrected for any difference with reference thermometer), including units
  - Comments

- Documentation for recorded data must include a minimum of the following:
    - Date and time of analysis
    - Person performing the measurement
    - Sample identification/station location
    - Temperature and pH of sample (including units and duplicate measurements)
    - Comments

7.0 TRAINING/QUALIFICATIONS

To properly perform pH measurements, the analyst must be familiar with the calibration and measurement techniques stated in this SOG. The analyst must also be experienced in the operation of the meter.

GUIDELINES FOR THE MEASUREMENT OF TURBIDITY

1.0 INTRODUCTION

1.1 Purpose and Applicability

These Standard Operating Guidelines (SOG) provide basic instructions for routine measurement of turbidity using a nephelometric turbidity meter with a digital read-out device (e.g., LaMotte 2020we Turbidimeter). Measurements are made in accordance with EPA Method 180.1 that addresses nephelometeric turbidity measurement of drinking, surface, and saline waters, and domestic and industrial wastes.

1.2 Quality Assurance Planning Considerations

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. These quality assurance requirements will be defined in the site-specific workplan or Quality Assurance Project Plan (QAPP) (hereafter referred to as the project plan) or laboratory Quality Assurance Manual (QAM) and may include duplicate or replicate measurements or confirmatory measurements.

2.0 RESPONSIBILITIES

- The analyst is responsible for verifying that the turbidity measuring device is in proper operating condition prior to use and for implementing the calibration and measurement procedures in accordance with this SOG and the project plan.
- The project manager is responsible for ensuring that project-specific requirements are communicated to the project team and for providing the materials, resources, and guidance necessary to perform the measurements in accordance with this SOG and the project plan.

3.0 REQUIRED MATERIALS

The following materials are necessary for this procedure:

- Turbidity meter with digital read-out device
- Manufacturer's instruction manual for the instrument
- Turbidity tubes/cuvettes
- Mild detergent
- Lint-free cloth
- Distilled water
- Nephelometric Turbidity Unit (NTU) calibration standards
- Laboratory or field data sheets or logbooks



Example of a sample cuvette

4.0 METHODS

4.1 Sample Handling, Preservation, and General Measurement Procedures

To achieve accurate turbidity measurements, samples should be analyzed immediately upon collection (preferably within 15 minutes). Samples should be collected in glass or plastic containers.

4.2. Calibration and Measurement Procedures

- Select a turbidity standard in the range of the samples to be tested (typically 0.00 NTU, 1.00 NTU or 10.0 NTU) or as recommended by the manufacturer. Fill a turbidity tube or cuvette with the standard, cap, and wipe the tube with the clean lint-free cloth.

- Place the sample into the turbidity meter such that the indexing arrow or line on the turbidity tube is aligned with the indexing arrow or notch on the meter face. Close the lid and press the “READ” button. If the displayed value is not the same as the value of the standard (within 10%), continue with the calibration procedure.
- Follow the calibration procedures outlined by the manufacturer’s manual.
- Verify the calibration every 15 samples and at the end of the day. Recalibrate the instrument if the check value varies more than 10% from the true value.
- Rinse cuvettes with deionized or distilled water and wiped gently with a lint-free tissue between sample analysis.
- Recalibrate the instrument with the appropriate NTU standard if the standard is not of the same order of magnitude as the samples being tested.
- The meter must be re-calibrated following any maintenance activities and prior to the next use.
- Record the turbidity reading to the nearest 0.01 NTU for measurements less than 11 NTU and to the nearest 0.1 for measurements greater than 11 NTU but less than 110 NTU. For values greater than 110 NTU record to the nearest 1 NTU.



Turbidity meter

4.3. Troubleshooting Information

If there are any performance problems with any of the meter-type turbidity measuring devices, consult the appropriate section of the meter instruction manual for the checkout and self-test procedures. If the problem persists, consult the manufacturer's customer service department immediately for further instructions.

4.4. Maintenance

Instrument maintenance for meter-type turbidity measuring devices should be performed according to the procedures and frequencies required by the manufacturer.

5.0 QUALITY CONTROL

The turbidity measuring tubes will, at a minimum, be checked against NTU calibration standards at the frequency stated in Section 4.2. This verification procedure will be performed in accordance with the manufacturer’s manual.

Duplicate measurements of a single sample will be performed at the frequency required in the project plan. In the absence of project-specific criteria, duplicate measurements should agree within + 5% for readings below 10 NTU and + 10% for readings above 100 NTU.

6.0 DOCUMENTATION

All turbidity meter calibration, checks, and maintenance information will be recorded on the daily calibration sheet or logbook. Turbidity data may be recorded on the appropriate laboratory or field data sheets or logbooks.

Calibration documentation must be maintained in a thorough and consistent manner. At a minimum, the following information must be recorded:

- Date and time of calibration
- Person performing the measurement
- Instrument identification number/model
- Expiration dates and batch numbers for all standard solutions
- Reading for each standard before and after meter adjustment
- Readings for all continuing calibration checks
- Comments

Documentation for recorded data must include a minimum of the following:

- Date and time of analysis
- Person performing the measurement
- Sample identification/station location
- Turbidity of sample (including units and any duplicate measurements)
- Comments

7.0 TRAINING/QUALIFICATIONS

To properly perform turbidity measurements, the analyst must be familiar with the calibration and measurement techniques stated in this SOG. The analyst must also be experienced in the operation of the meter.

.

STANDARD OPERATING GUIDELINES FOR COLLECTION OF SEDIMENTS FROM FRESHWATER ENVIRONMENTS

1.0 INTRODUCTION

1.1 Purpose and Applicability

These Standard Operating Guidelines (SOGs) provide basic instructions for the collection of bottom sediments from freshwater environments. Collections are to be performed in accordance with methodologies generally accepted by the Massachusetts Department of Environmental Protection (MADEP), Rhode Island Department of Environmental Management (RIDEM), or other agency, as applicable. In some cases, deviation from these SOGs may be necessary to comply with particular local, state, or federal program requirements.

1.2 Quality Assurance Planning Considerations

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. These quality assurance requirements may be defined in a site-specific workplan or Quality Assurance Project Plan (QAPP) (hereafter referred to as the project plan) and may include duplicate or replicate measurements or confirmatory measurements.

2.0 RESPONSIBILITIES

- The project manager is responsible for ensuring that project-specific requirements are communicated to the project team and for providing the materials, resources, and guidance necessary to perform the measurements in accordance with this SOG and the project plan.
- Field personnel are responsible for verifying that all sampling equipment is in proper operating condition prior to use and for implementing the sampling procedures in accordance with this SOG and any specific project plan.

3.0 REQUIRED MATERIALS

The following materials may be necessary for this procedure:

- Sediment coring or grab sampling device
- Stainless steel or Teflon mixing bowl
- Stainless steel or Teflon mixing spoon or tool
- Nitrile gloves
- Decontamination agents (e.g., Alconox, isopropyl alcohol, distilled/deionized water)
- Scrub brush (to remove sediment and debris)
- Paper towels
- Pre-cleaned sample jars provided by laboratory
- Pencil and indelible marker
- Field data sheets or logbooks
- Digital camera
- GPS receiver
- Field map (optional)

4.0 METHOD

Samples should be collected at locations specified in the project plan. A GPS may be used to navigate to planned locations, as well as to record the final sampling location. Field personnel should collect sediment cores or grabs in accordance with the instructions provided with each specific sampling device deployed. At a minimum, nitrile gloves should be worn at all times during these procedures to prevent direct contact with sampled sediments.

At each sampling location, a pre-cleaned grab sample dredge or corer should be deployed from a stable platform, typically an anchored vessel or ice cover. Sampling equipment should be decontaminated using alconox, isopropyl alcohol, and distilled or deionized water before the collection of each discrete sample. A scrub brush and paper towels may be used to loosen or remove sediments that are stuck to the equipment.

© 2016 ESS Group, Inc.

Upon retrieval, the core or grab should be checked to ensure a complete, representative sample has been collected. Each sample should be photographed to document physical characteristics such as sediment color, moisture, and grain size. Grab samples may be photographed from above with the sampler flaps open. Core samples may be photographed with the core laid down and a measuring tape fastened parallel to the core. This will allow physical characteristics to be documented over the full profile of the core, with identification of any discrete vertical layers.

Advanced core logging may be required for certain projects. Requirements relevant to core logging are not included in this SOG. Typically, these are provided in a separate document, such as a project-specific sampling and analysis plan.

If specified by the project plan, multiple samples may be composited in a pre-cleaned mixing bowl and mixed thoroughly with a pre-cleaned spoon before being transferred to the glass sampling jars provided by the laboratory. However, volatile organic compound (VOC) samples should be collected from individual, undisturbed samples (i.e., prior to compositing).

Samples should be collected in accordance with the specific sample volumes and holding times required by each laboratory analytical method, unless the project plan has outlined other project-specific requirements.

The sample jar should be labeled with project name or number, sample identification, date, time, and any other project-specific requirements. This information should also be recorded in a field book or GPS data dictionary at the time of sampling along with other essential information such as water depth, sample coordinates, and notes on the physical nature of the sediment collected.

5.0 QUALITY CONTROL

Duplicate field samples or split samples may be collected if specified by the project plan. Once samples have been retrieved and placed into jars, the samples should be kept on ice or refrigerated until the laboratory can analyze them.

6.0 DOCUMENTATION

Documentation for recorded data must include a minimum of the following:

- Date and time of collection and analysis
- Signature or initials of person performing the collection or measurement
- Sample identification/station location
- Sample digital photographs
- Pertinent comments

7.0 TRAINING/QUALIFICATIONS

To properly perform sediment collections, the field personnel must be familiar with the techniques stated in this SOG and experienced in the operation of the sampling equipment.

© 2016 ESS Group, Inc.

GUIDELINES FOR COLLECTION OF SURFACE WATER SAMPLES

1.0 INTRODUCTION

These guidelines provide basic instructions for the routine acquisition of surface water from lakes, ponds, and streams. The methods outlined below are intended to (1) standardize water sample collection methods; (2) ensure that samples delivered to the laboratory represent field conditions as accurately as possible; (3) assure proper documentation of sample collection; and (4) minimize cross contamination between sampling sites.

2.0 REQUIRED MATERIALS

The following materials are necessary for the acquisition of surface water samples:

- Nitrile gloves
- Labeled sample bottles provided by contracted laboratory (appropriately sanitized and containing the necessary preservative for desired analyses, see Table 1.0 for examples)
- Field data sheets or logbooks, including list of sites or locations to be sampled, and pencil
- Cooler with ice packs for sample storage
- Integrated depth sampler (if collecting algae sample)
- Secchi disk (if collecting algae samples)
- Laboratory Chain of Custody

Table 1.0 Example Container Types, Preservative Requirements, and Hold Times for Water Quality Samples.

Analysis	Bottle Type	Preservative	Hold Time
Total Phosphorus	plastic	H_2SO_4	28 days
Dissolved Phosphorus	plastic	As Is	analyze immediately*
Total Suspended Solids (TSS)	plastic	As Is	7 days
Nitrate/Nitrite	plastic	As Is	48 hrs
Total Kjeldahl Nitrogen (TKN)	plastic	H_2SO_4	28 days
Metals - Total	plastic	HNO_3	6 months**
Metals - Dissolved	plastic	As Is	6 months**
Algae	opaque plastic	Lugol's iodine	>1 year
Chlorophyll-a	opaque plastic	As Is	analyze immediately
Bacteria	sterile plastic	As Is	6 hrs

* = 24 hrs with field filtration, ** = 28 days for mercury

3.0 METHODS

3.1 General Sampling Instructions

- Testing methods, sample containers, preservation techniques, and sample volumes should be selected in consultation with the laboratory to ensure that samples obtained will provide the desired results.
- Hold times vary considerably between different analytes and must be taken into consideration when planning field sampling efforts and lab courier pickups to assure the validity of analytical results.
- Field filtration of certain samples (dissolved phosphorus) is recommended. The laboratory can supply syringes and filters for use in the field.



Using a pre-cleaned (as is) bottle to fill a sample bottle containing preservative.

- In general, surface water samples should be collected via direct grab methods.
- Sample collection should precede the measurement of physical field parameters (including pH, apparent color, turbidity, conductivity, and dissolved oxygen) in order to minimize the risk of sediment disturbance and/or sample contamination.
- Clean rubber gloves should be worn at each sampling location. When sampling multiple sites on the same day, gloves may be rinsed in the immediate area of the waterbody to be sampled (downstream at flowing sites).
- Approximately 1-inch of air space should be left when filling sample bottles (except for dissolved oxygen, alkalinity, and BOD samples), so that bottles may be shaken (if needed) before analyses (EPA, 1997; Simpson 1991).
- Sample containers with preservatives should not be used to collect water samples. If using containers with preservatives, a pre-cleaned container of similar type (an as is bottle) should be used to collect and subsequently transfer the sample to the preserved container.
- Ensure that all sample bottles are correctly and completely labeled before storage. Sample bottles should be stored in a cooler with ice packs (it is best to avoid ice, as meltwater could potentially contaminate samples) or in a refrigerator until they are submitted to a lab courier.

3.1.1. Lake and Pond Sampling

- Grab samples from lakes and ponds should be collected at approximately 8 to 12 inches beneath the water surface or mid-way between the surface and the bottom if the waterbody is shallow (EPA 1997). Samples should not be collected in close proximity to the lake shoreline or submerged obstacles.

- To collect water samples, hold an as is bottle near the base, remove the lid, and plunge it into the water with the opening facing downward. Invert the bottle and allow it to fill before bringing it to the surface. Decant sufficient water from the bottle to allow for the required headspace and replace the cover, or carefully pour the contents into a bottle containing preservative. Repeat the above process to refill the as is bottle as many times as necessary.

Algae Samples

- Algae samples should be stored in opaque bottles with a small amount of Lugol's iodine for preservative (~1-2 drops in a 250 mL bottle). Algal taxonomy labs can provide opaque plastic bottles, but standard plastic as is bottles covered in aluminum foil can also be used.



Integrated depth sampler for collection of algae samples.

- Algae samples should be collected using an integrated depth sampler. An integrated depth sampler consists of a length of tubing (~1in diameter, at least 2 m long) with a weight attached to one end. Sample collection procedures using the depth sampler should proceeds as follows (procedure adapted from EPA 2012):

    - Determine the euphotic zone:

        - Lower the secchi disk over the shaded side of the boat until it disappears. Lower the disk a bit further, then slowly raise the disk until it reappears. Record the reappearance depth. The euphotic zone is calculated by multiplying the reappearance depth by 2.

    - Holding onto the non-weighted end of the sampler, lower the tube into the water column. Rinse the sampler by submerging it three times.

    - Lower the sampler so that it is submerged to the depth of the euphotic zone, or fully submerged if the euphotic zone is deeper than the length of the sampler. Cover the opening at the non-weighted end with a gloved thumb.

- Lift the sampler completely out of the water and cover the opening at the weighted end with a gloved thumb (both ends should be covered). Repeatedly lift each end of the sampler to mix the water sample within the tube.
- Fill the algae sample bottle with the required volume of water from the sampler (the bottle will contain Lugol's solution as preservative so be careful not to over-fill).
- Unlike samples for most other analytes, preserved algae samples can be stored at room temperature before submission to a lab.

3.1.2. Stream Sampling

- Samples should be collected from the center of small streams (i.e., 10-20 feet wide with a maximum depth of less than 2 feet), and at a location where water depth is 2-3 feet in larger streams.
- Always approach a sampling location from downstream, traveling so as to minimize the disturbance of bottom sediments and upstream waters.
- Stand downstream of the desired sampling location, hold the sample bottle near its base and plunge it below the water surface with the opening (mouth) downward. The opening of sample bottles should always be directed away from the sampler in an upstream direction.
- To inform investigations about nutrient inputs, stream flow should be measured whenever water quality samples are collected (see Guidelines for Measuring Stream Flow)

4.0 DOCUMENTATION

Report surface water field data on sheets or in notebooks. Any unanticipated site-specific information, which requires deviation from the above guidelines, should be recorded. Field notes for surface water sampling should include a minimum of the following:

- Name or initials of person collecting the samples
- Sample identification/station location
- Date and time of sample collection
- Environmental conditions (e.g. wind, weather)
- Other comments or observations about water quality and site conditions (e.g. visible algae bloom, dead fish nearby, sample has noticeable odor or color, etc.)

Photographic evidence of any notable conditions is also desirable.

5.0 REFERENCES

EPA, 2012. 2012 National Lakes Assessment Field Operations Manual. Version 1.0, May 15, 2012. United States Environmental Protection Agency. Office of Water. EPA-841-B-11-003. Accessed January 22, 2020 at https://www.epa.gov/sites/production/files/2013-11/documents/nla2012_fieldoperationsmanual_120517_final_combinedqrg.pdf

Appendix B – Laboratory SOPs

CHAIN OF CUSTODY

PAGE_____ OF _____

WESTBORO, MA
TEL: 508-898-9220
FAX: 508-898-9193

MANSFIELD, MA
TEL: 508-822-9300
FAX: 508-822-3288

Date Rec'd in Lab:

ALPHA Job #:

Client Information

Client:

Address:

Phone:

Fax:

Email:

❑ These samples have been previously analyzed by Alpha

Project Information

Project Name:

Project Location:

Project #:

Project Manager:

ALPHA Quote #:

Turn-Around Time

❑ Standard ❑ RUSH *(only confirmed if pre-approved!)*

Date Due: **Time:**

Report Information - Data Deliverables

❑ FAX ❑ EMAIL

❑ ADEx ❑ Add'l Deliverables

Billing Information

❑ Same as Client info PO #:

Regulatory Requirements/Report Limits

State /Fed Program	*Criteria*

Other Project Specific Requirements/Comments/Detection Limits:

ANALYSIS

SAMPLE HANDLING

*Filtration*_____
❑ Done
❑ Not needed
❑ Lab to do

Preservation
❑ Lab to do
(Please specify below)

TOTAL # BOTTLES

ALPHA Lab ID (Lab Use Only)	Sample ID	Collection Date	Collection Time	Sample Matrix	Sampler's Initials	Sample Specific Comments

Container Type	
Preservative	

Relinquished By:	Date/Time	Received By:	Date/Time

Please print clearly, legibly and completely. Samples can not be logged in and turnaround time clock will not start until any ambiguities are resolved. All samples submitted are subject to Alpha's Terms and Conditions. See reverse side.

FORM NO: 01-01 (rev. 14-OCT-07)

CHAIN OF CUSTODY -
Customizable Form

ESS Group, LLC - A TRC Company
10 Hemingway Drive, 2nd Floor, East Providence, RI 02915
Phone: 401-330-1204

Report To (if different than above)

Name _____
Organization _____
Address _____
_____
Phone _____
email _____

Receiving Lab Contact Information

Name _____
Organization _____
Address _____
_____
Phone _____
email _____

Project: **Project #:**

Sample ID	Date	Time	Sample Matrix	Analysis Requested	Type of Container	Preservative	# of Containers
Total							

CLIENT Comments or Requests:

RECEIVING LAB Notes/Comments:

Sampled by:	Date/Time:
Relinquished by:	Date/Time:
Received by:	Date/Time:
Relinquished by:	Date/Time:
Received by:	Date/Time:

Cooler: Yes ☐ No ☐
Coolant: IPK ☐ ICE ☐ No ☐

CHAIN OF CUSTODY RECORD

PHOENIX
Environmental Laboratories, Inc.

587 East Middle Turnpike, P.O. Box 370, Manchester, CT 06040
Email Makrina Nolan: makrina@phoenixlabs.com Fax (860) 645-0823
Client Services (860) 645-1102

Temp ° C **Pg** of

Data Delivery/Contact Options:
☐ Fax: _____
☐ Phone: _____
☐ Email: _____

Customer: _____ Project: _____ Project P.O: _____
Address: _____ Report to: _____
_____ Invoice to: _____
_____ QUOTE # _____

This section MUST be completed with Bottle Quantities.

Client Sample - Information - Identification

Sampler's Signature _____ Date: _____

Matrix Code:
DW=Drinking Water **GW**=Ground Water **SW**=Surface Water **WW**=Waste Water
RW=Raw Water **SE=**Sediment **SL**=Sludge **S**=Soil **SD**=Solid **W**=Wipe **OIL**=Oil
B=Bulk **L**=Liquid **X =** _____(Other)

Analysis Request

PHOENIX USE ONLY SAMPLE #	Customer Sample Identification	Sample Matrix	Date Sampled	Time Sampled	MS/MSD *	GL Amber 8 oz. [ ] H3PO4 [ ] NAHSO4	Soil VOA Vials [ ] methanol [ ] H2O	GL Soil container ( ) oz	GL Soil container ( ) oz	40 ml VOA Vial [ ] As is [ ] HCL	GL Amber 1000ml [ ] As is [ ] H2SO4	PL As is [ ] 250ml [ ] 500ml [ ] 1000ml	PL H2SO4 [ ] 250ml [ ] 500ml	PL HNO3 250ml	PL NaOH 250ml	Bacteria Bottle w/thio	Bacteria Bottle as is

Relinquished by:	**Accepted by:**	**Date:**	**Time:**

Comments, Special Requirements or Regulations:

**MS/MSD are considered site samples and will be billed as such in accordance with the prices quoted.*

Turnaround Time:
☐ 1 Day*
☐ 2 Days*
☐ 3 Days*
☐ Standard
☐ Other
* **SURCHARGE APPLIES**

RI
☐ (Residential)
☐ (Comm/Industrial) Direct Exposure
☐ GA Leachability
☐ GB Leachability
☐ GA-GW Objectives
☐ GB-GW Objectives

CT
☐ RCP Cert
☐ GW Protection
☐ SW Protection
☐ GA Mobility
☐ GB Mobility
☐ Residential DEC
☐ I/C DEC
☐ Other

MA
☐ MCP Certification
☐ GW-1 ☐ MWRA eSMART
☐ GW-2 ☐ S-1 10% CALC
☐ GW-3
☐ S-1 GW-1 ☐ S-1 GW-2 ☐ S-1 GW-3
☐ S-2 GW-1 ☐ S-2 GW-2 ☐ S-2 GW-3
☐ S-3 GW-1 ☐ S-3 GW-2 ☐ S-3 GW-3
☐ SW Protection

State where samples were collected: _____

Data Format
☐ Excel
☐ PDF
☐ GIS/Key
☐ EQuIS
☐ Other

Data Package
☐ Tier II Checklist
☐ Full Data Package*
☐ Phoenix Std Report
☐ Other

* **SURCHARGE APPLIES**

Chlorophyll A

References: **SM 10200 H,** Standard Methods for the Examination of Water and Wastewater, 18th Edition

1. Scope and Application

Matrices: Waters

Definitions: Refer to Alpha Analytical Quality Manual.

The concentration of photosynthetic pigments is used extensively to estimate phytoplankton biomass. All green plants contain chlorophyll *a*, which constitutes approximately 1 to 2% of the dry weight of planktonic algae. Other pigments that occur in phytoplankton include chlorophylls *b* and *c*, xanthophylls, phycobilins, and carotenes. The important chlorophyll degradation products found in the aquatic environment are the chlorophyllides, pheophorbides, and pheophytins. The presence or absence of the various photosynthetic pigments is used, among other features, to separate the major algal groups. This method describes the spectrophotometric method for determining chlorophyll *a* in phytoplankton.

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 or more of the following laboratory personnel before performing the modification: Area Supervisor, Department 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 completing the record of training.

2. Summary of Method

Chlorophyll pigment is extracted from the plankton concentrate using aqueous acetone and the Optical Density (Optical Density (OD) = Absorbance) of the extract is determined with a spectrophotometer. The ease with which the chlorophylls are removed from the cells varies considerably with different algae. To achieve consistently the complete extraction of the pigments, the cells are disrupted with a tissue grinder. All work is conducted in subdued light to avoid degradation.

2.1 Method Modifications from Reference

None.

3. Reporting Limits

The Reported Detection Limit is 2 mg/m^3.

4. Interferences

4.1 Turbidity: Turbidity is removed by filtration of the extract through a 1.0µm solvent-r resistant disposable filter.

4.2 Light Exposure: Light exposure to the sample will degrade the chlorophyll. Therefore collection and analysis are to be conducted in subdued lighting conditions.

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.

NOTE: When using the tissue grinder, be sure to hold the glass cuvette on the sides and not on the bottom. This is to ensure that the grinder will not injure the chemist, should the cuvette accidentally break.

6. Sample Collection, Preservation, Shipping and Handling

6.1 Sample Collection

Samples are collected in dark glass or dark plastic containers. Containers may be covered with aluminum foil to minimize degradation of the sample by light.

6.2 Sample Preservation

None.

6.3 Sample Shipping

No specific requirements.

6.4 Sample Handling

Samples are filtered as soon as possible after sample collection. Filters are stored in the dark, refrigerated at 4ºC for no more than 3 days.

7. Equipment and Supplies

7.1 Glass Cuvettes: 1 cm and 2.5 cm

7.2 Aluminum Foil

7.3 Tissue Grinder

7.4 Glass Fiber Filters: Gelman, P/N 61631, 47mm

7.5 Filtration Equipment: Vacuum pump; membrane filter funnel.

7.6 Forceps: Smooth-tipped, without corrugations on the inner sides of the tips.

7.7 Pipets: 1mL, glass

7.8 10mL Solvent-resistant syringe: with 1.0µm PTFE filter membrane.

7.9 Centrifuge

7.10 Centrifuge tubes: 15mL , graduated, screwcap

7.11 Genesys 5 Spectrophotometer, UV: with a narrow band-width (0.5 to 2.0nm)

7.12 Amber Glass Jars: 250mL, with covers

7.13 Filter discs: 1.0um pore size, solvent resistant

7.14 10mL syringe: Solvent resistant

7.15 Membrane Filter Funnels: 0.45 µm filter

7.16 Refrigerator: 4 °C

8. Reagents and Standards

8.1 Saturated Magnesium carbonate solution: Add 1g fine powdered $MgCO_3$ to 100mL DI. Undissolved material is not uncommon.

8.2 Acetone: Reagent grade BP 56 ºC.

8.3 90% Aqueous Acetone/10% $MgCO_3$ working solution: 90 parts acetone (Section 8.2): 10 parts saturated $MgCO_3$ solution (Section 8.1). Store in a 250mL glass amber jar.

8.4 $MgCO_3$: Powder form, Lab grade (USP-FCC)

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

The Blank consists of 10mL of 90% Aqueous Acetone/10% $MgCO_3$ working solution in a 15mL centrifuge tube covered with aluminum foil. It is steeped for at least 2 hours at 4ºC alongside of the samples and is filtered through a 1.0µm solvent-resistant disposable filter.

The Spectrophotometer is zeroed at OD750 with the Blank.

9.2 Laboratory Control Sample (LCS)

Not Applicable

9.3 Initial Calibration Verification (ICV)

Not Applicable

9.4 Continuing Calibration Verification (CCV)

Not Applicable

9.5 Matrix Spike

Not Applicable

9.6 Laboratory Duplicate

A sample is analyzed in duplicate (if sample volume is available) once per batch of 20 samples or less

9.7 Method-specific Quality Control Samples

None.

9.8 Method Sequence

- Filter the sample onto a glass fiber filter.
- Wrap the filter in foil, secure in airtight bag, and place in 4 C refrigerator (if not immediately grinding).
- Grind the glass fiber filters in a tissue grinder with 90% Aqueous Acetone/10% $MgCO_3$ working solution.
- Steep the samples and Blank for 2 hours at 4ºC.
- Clarify the sample extract and Blank by centrifuging and filtering.
- Zero the Spectrophotometer at 750, 664, 647, and 630 respectively, with Blank
- Determine OD's of the samples
- Perform calculations.

10. Procedure

10.1 Set-up

Perform all analysis in subdued light, with all containers covered in aluminum foil to avoid degradation of the chlorophyll.

10.1.1 Sample Filtration/Preparation

Concentrate the chlorophyll by filtering the whole volume of sample onto a glass-fiber filter as soon as possible after sample collection.

The sample container should be gently swirled before each portion poured to ensure the sample aliquots are homogeneous. If the sample has too much sedimentary material present, and the filtering process becomes sluggish, do not add further sample volume to the filtering unit. Make sure to note correct volume of sample filtered in the Micro logbook.

If the sample is to be analyzed immediately, record in the laboratory notebook the sample ID, date and time of filtration, sample volume filtered, and the chemist's initials. Also record the lot # of the glass-fiber filters and the filter funnels used. Proceed to Section 10.3.1.

Otherwise, if the sample is not to be analyzed at this time, the filter is carefully (to avoid piercing of the filter) placed onto a piece of aluminum foil and folded in half so the chlorophyll extract is folded onto itself. (This is to avoid the chlorophyll adhering to the aluminum foil.) The folded filter/foil is stored in a sealed plastic bag at 4ºC until pigment extraction takes place (not to exceed 3 days). Note on the plastic bag the sample ID, date and time of filtration, sample volume filtered, and the initials of the chemist.

10.2 Initial Calibration

The spectrophotometer is zeroed at each wavelength with a Blank sample. The Blank sample is prepared as in Section 9.1.

10.3 Equipment Operation and Sample Processing

If the filter containing the chlorophyll has been stored in the refrigerator, transfer the following information into the laboratory notebook from the plastic bag in which the filter has been stored: the date and time of filtration, sample volume filtered, and the chemist's initials.

10.3.1 Pigment Extraction

Using forceps, carefully fold the glass-fiber filter containing the extract and place it in a 2.5cm glass cuvette that is covered with aluminum foil. Cover the filter with 5mL of 90% Aqueous Acetone/10% $MgCO_3$ working solution and place onto the tissue grinder. Grind at 500rpm for 1 minute. (Note: Whenever the 90% Aqueous Acetone/10% MgCO3 solution is used, the bottle it is contained within should be gently swirled first to ensure all undissolved material in the solution is uniformly dispersed.)

Transfer the slurry to a screwcap centrifuge tube that is covered with aluminum foil. Into the cuvette, rinse the grinder with a few mLs of 90% Aqueous Acetone/10% $MgCO_3$ working solution, and swirl to combine any leftover slurry. Add the rinse to the extraction slurry in the centrifuge tube. If there is still remaining slurry in the cuvette, use a 1mL glass pipette to transfer to the centrifuge tube. Adjust the total volume to a constant level

of 10mL with 90% Aqueous Acetone/10% $MgCO_3$ working solution. (Avoid excessive dilution of pigments.) Cover the centrifuge tube and swirl to ensure all of the slurry is mixed with the Aqueous Acetone.

If additional samples are to be ground, rinse the tissue grinder with Acetone (Section 8.2) and allow to dry before each sample.

Steep in darkness for at least 2 hours at 4ºC, not to exceed 2 ½ hours. Record the times in the laboratory notebook.

Place the 15mL centrifuge tube that contains the sample into a 50mL centrifuge tube that has a KimWipe in the bottom. (The KimWipe is used to minimize the movement of the smaller centrifuge tube within the larger centrifuge tube.)

Clarify the extract by centrifuging in the closed centrifuge tube for 20 minutes at 2800rpm. Then filter the supernate through a 1.0µm solvent-resistant disposable filter. (Note: Make sure to not over-centrifuge, since acetone will break down integrity of centrifuge tube over time).

Decant the clarified extract into a clean, calibrated 15mL screwcap centrifuge tube covered with aluminum foil.

10.3.2 Spectrophotometric Determination of Chlorophyll *A* – Trichromatic Method

Transfer the Blank sample into a DI-rinsed 1cm glass cuvette. Also, transfer the clarified extract(s) into separate DI-rinsed 1cm glass cuvette(s).

Zero the Spectrophotometer with the Blank sample at 750nm. Read the Optical Density (OD) of the samples at 750nm. Record OD in the laboratory notebook.

Zero the Spectrophotometer with the Blank sample at 664nm. Read the Optical Density (OD) of the samples at 664nm. Record OD in the laboratory notebook. (The OD664 should be between 0.1 and 1.0, therefore prepare dilutions with 90% Aqueous Acetone/10% $MgCO_3$ working solutions as necessary to provide an OD within this range.)

Zero the Spectrophotometer with the Blank sample at 647nm. Read the Optical Density (OD) of the samples at 647nm. Record OD in the laboratory notebook.

Zero the Spectrophotometer with the Blank sample at 630nm. Read the Optical Density (OD) of the samples at 630nm. Record OD in the laboratory notebook.

10.4 Continuing Calibration

Not Applicable.

10.5 Preventive Maintenance

An instrument service company calibrates the Spectrophotometer-UV on an semi-annual basis. Certificates are kept on file.

11. Calculations

11.1 Calculations are automatically performed on an Excel Spreadsheet for the Chlorophyll A analysis. Input the following information into the Spreadsheet:

- Extract Volume (mL), as determined in the second paragraph of Section 10.3.1.
- Volume of Sample (L), as determined prior to filtration in Section 10.1.1
- OD 750
- OD 664
- OD 647
- OD 630

11.2 The actual calculation performed on the Spreadsheet is as follows:

The OD750 is a correction for turbidity. Therefore, subtract the 750nm OD value from the readings at the other wavelengths ***before*** using them in the equations below.

Using the ***corrected*** values, calculate Chlorophyll *a* in the extract as follows:

$$C_a \text{ (mg/L)} = (11.85 \times OD664 \times \text{dilution factor}) - (1.54 \times OD647) - (0.08 \times OD630)$$

After determining the concentration of pigment in the extract, calculate the amount of pigment per unit volume as follows:

$$\text{Chlorophyll } a, \text{ mg/m}^3 = \frac{C_a \times \text{extract volume, mL}}{\text{Volume of sample, L}}$$

- The *extract volume* is determined in the second paragraph of Section 10.3.1.
- The *volume of sample* is determined prior to filtration in Section 10.1.1.

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

Holding time exceedance and 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 blanks for acceptable performance occurs for each batch of samples. Record any trends or unusual performance on a nonconformance action form.

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.

Not applicable to this method.

13.2 Demonstration of Capability Studies

Refer to Alpha SOP/1734 and 1739 for further information regarding IDC/DOC Generation.

Not applicable to this method.

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/1734, 1739 IDC/DOC Generation

SOP/1728 Waste Management and Disposal SOP

16. Attachments

None.

Sequential fractionation of phosphorus in sediment

To assess phosphorus in sediment a sequential chemical extraction can be used, commonly called phosphorus (P) fractionation. Different pools, with varying solubility and reactivity, are examined. This method description is developed from Psenner & Pucsko (1988) and Hupfer *et al.* (1995, 2009).

The pools are arbitrarily defined as in figure 1.

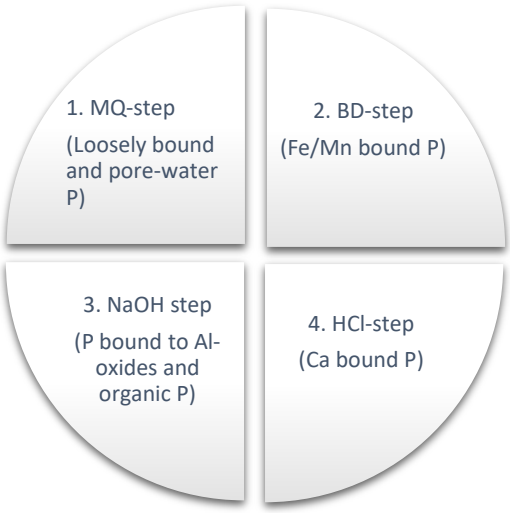


Figure 1. P pools generally extracted using the method described herein.

Quantification range: 0-800 µg/l

Equipment

Scale:
Spectrophotometer:
Autoclave:
Centrifuge:

Appendix 1: Chemical and solution preparation
Appendix 2: Phosphorus extraction with spectrophotometer
Appendix 3: Dry weights and loss on ignition
Appendix 4: Methodological background and references
Appendix 5: Adaptations of the method
Appendix 6: Quality assurance

Edited by: SS, O A-N, EL
Approved by:

Procedure

General advice

- For the pipettes, never turn the pipette upside down and be careful tilting it when it's full, to avoid contaminaton inside of the pipette. If the pipette is spraying a lot, sometimes the pipette tip needs to be changed if it's old or adjust it so the tip sits firmly on the pipette.
- Be careful not to contaminate any of the glass/plastic containers with detergent. Use only acid wash (HCl) and/or high purity (MQ) water for cleaning.
- It is preferred to use the same glass/plastic containers for the same solutions. If a container is contaminated, wash it with acid.
- Do not put the spatula inside the chemical containers when weighing chemicals for making the reagents. Never pour exessive chemicals back into the container but dispose according to safety protocols.
- Make sure that all working places, scales, sink and fume hood is clean and tidy at the end of the day. Don't leave traces of sediment and chemicals should be stored properly.
- When pipetting with a new or dry pippett tip, fill it up once and discard the solution. Up to 10% of the volume is lost as the pippett tip becomes "wet" with solution the first time it is used.
- Check the calibration of the pipettors monthly by pipetting water at 20 C (ca. room temperature) and weighing in a weigh boat.
- **You are responsible for your own safety, use gloves, safety glasses and lab coat accordingly. Be especially careful when handling $K_2S_2O_8$ and strong acids/bases.**

General routine

Days 1-3 describe the steps of the fractionation procedure. Basically what you do each day for three days

Appendix 1
Describes how to make the chemicals used in the fractionation procedure and for making reagents so the samples can be read on the spectrophotometer

Appendix 2
Describes how to analyze the samples on the spectrophotometer

Appendix 3
Describes how to analyze dry weight and loss on ignition

The description of days 1 to 3 and appendix 1 and 2 are all used during the fractionation procedure. Appendix three is used for dry weight and loss on ignition which can be done any time during the fractionation or before/after.

Edited by: SS, O A-N, EL
Approved by:

Day 1

- Add 100 to 150 mg (up to 200 mg for 0-1cm layer)[1] of wet sediment to 15 ml centrifuge tubes. Mix sediment well before putting the subsample in the tube. If sample is very wet, a pipette can be used, otherwise use a regular spatula. Rinse the pipette between each sample, and change rinsing water between each sediment core or sampling site. After rinsing with water, pipette and discard two samples (one if sample is limited) so that any remaining water from rinsing is removed. Then pipette the third sample into the centrifuge tube.

1. MQ-step
- Add 10ml of double de-ionized (MQ) water to the centrifuge tubes, shake to make sure all the particles end up in solution and wait 2 hours.
- Add 5 ml of MQ to 3 vials to be used for blanks in the analysis
- Make the BD solution during the 2 hours (**appendix 1**)
- Centrifuge the tubes at 3000 rpm for 10 minutes and pipette 5 ml of the supernatant from each tube into scintillation vials.
- Take remaining **5 ml** of supernatant out carefully **without** disturbing the sediment, pour the solution out by tilting the centrifuge tubes **once**.
- Add 5 ml of MQ water to the scintillation vials with MQ extract to make 10 ml total.
-Cover the vials with parafilm and place in a cupboard.

2. BD-step
- Add 10 ml $Na_2SO_3/NaHCO_3$ (BD) to the centrifuge tubes, mix sediment with spatula, shake carefully (avoid forcing air into the solution) to make sure all the particles end up in solution and wait 1 hour.
- Add 2 ml of stock BD solution to 3 vials to be used as blanks in the analysis
- Make 0.1 M NaOH during the hour (**appendix 1**).
- Centrifuge the tubes at 3000 rpm for 10 minutes and pipette **2 ml** of the supernatant from each tube to scintillation vials.
- Take remaining 8 ml of supernatant out carefully **without** disturbing the sediment, pour the solution out by tilting the centrifuge tubes **once**.
- Let BD-samples in scintillation vials sit over night at 4 C in a refrigerator. Do not cover the BD-samples to allow the samples to oxidize, which happens over night.

3. NaOH-step
- Add 10 ml NaOH (0.1 M) to the centrifuge tubes, mix sediment with spatula, shake to make sure all the particles end up in solution and wait 15-18 (16 hours is typical) hours. Whichever time is chosen must be consistent for all samples from the same lake. The samples can be stored at room temperature with parafilm on top.

[1] The amount is depending on the watercontent and the detection limit of the standard curve

Edited by: SS, O A-N, EL
Approved by:

Day 2

- Take out BD vials from the fridge and cover with parafilm. Store in a cupboard. If you have more than one tray, you can stack them and have parafilm on only the top/uppermost tray.
- Make $K_2S_2O_8$ (**appendix 1**)
- Make HCl solution (0.5 M) (**appendix 1**)
- Add 2 ml of NaOH (0.1 M) to 3 scintillation vials and 3 digestion tubes to be used as blanks in the analysis.
- Centrifuge tubes with NaOH for 10 minutes at 3000 rpm and follow table 1.

Table 1. Distribution order of chemicals. (All volumes should add up to 10 ml).

Reagent	**$NaOH_{rp}$ (scint. vials)**	**$NaOH_{nrp}$ (digestion tubes)**
Supernatant	1 ml	1 ml
HCl (0.5 M)	0.25 ml	0.25 ml
$K_2S_2O_8$	-	2 ml
MQ-water	8.75 ml	6.75 ml

- Place caps on digestion tubes and put in autoclave for 30 mins at 120 C (Liquid B waste program)
-If the samples are very yellow, take an additional 2 mL out of the tubes to use as color blinds (talk to Brian about this for help)
- Take remaining 6 ml of supernatant out carefully **without** disturbing the sediment, pour the solution out by tilting the centrifuge tubes **once**.
- Cover the NaOH-sample scintillation vials with parafilm and store at room temperature if not analysing them the same day.
-Remove digestion tubes with NaOH from the autoclave and let stand over night to cool.

4. HCl step
-Add 10 ml of HCl (0.5 M) to the centrifuge tubes, mix sediment with spatula, shake to make sure all the particles end up in solution, and wait 16-24 hours. The samples can be stored in room temperature.

Edited by: SS, O A-N, EL
Approved by:

Day 3

- Make NaOH (**2 M**) if needed (**appendix 1**)
- Pour some P-standard solution into the beaker labelled “low P” to let the solution get to room temperature. This can be done by covering with parafilm and waiting (best solution), or heating carefully on a hot plate if you need to do it quickly.
- Centrifuge tubes with HCl at 3000 rpm for 10 minutes and pipette 2 ml of the supernatant to scintillation vials.
- Add 2 ml of HCl (0.5 M) to 3 scintiallation vials to be used as blanks in the analysis
- Add 0.5 ml NaOH (**2 M)** to all HCl sample scintillation vials, including blanks.
- Add 7.5 ml MQ to make 10 ml.

- Add 8 ml MQ water to the BD samples (from step 2) to make 10 ml.

- Analyze MQ-samples, BD-samples, $NaOH_{rp}$, $NaOH_{nrp}$ (from autoclave), and HCl-samples using the spectrophotometer according to “Measuring sediment P extractions on the spectrophotometer” (**appendix 2**) for methods.

-Clean out all vials and centrifuge tubes 3 rinses of de-ionized water and one rinse MQ water.

-Analyze dry weights and loss on ignition (**appendix 3**). This can also be started during days 1 and 2 when you have time.

Appendix 1 - Chemicals and solutions

For fractionation

BD-solution (Na_2SO_3/$NaHCO_3$)
NaOH (0.1 M)
NaOH (2 M)
$K_2S_2O_8$
HCl (0.5 M)

Preparation

BD-solution (500 ml, 40-45 samples)

Lasting quality: Make new for each fractionation
Storage: Room temperature
Ingredients:

- $Na_2S_2O_4$ (solid)
- $NaHCO_3$ (solid)
- MQ-water

Procedure:

- Take a 500 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Take a new weighing boat for 4.6 g of $NaHCO_3$
- Pour the $NaHCO_3$ into the 500 ml flask
- Weigh 8.7 g $Na_2S_2O_4$
- Pour the $Na_2S_2O_4$ into the same flask
- Fill the flask with MQ-water up to the line to make 500 ml
- Put a cap on the flask and turn it (don't shake) to make sure the chemicals are dissolved completely.
- The solution volume might be reduced, fill up with more MQ-water to the line again.

NaOH (0.1 M)

Ingredients:

- NaOH-concentrated (solid)
- MQ-water

Procedure:

- Take a 1000 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Weigh 4 g NaOH
- Pour the NaOH in the flask
- Fill the flask with MQ-water to the line

Edited by: SS, O A-N, EL
Approved by:

- Put a cap on the flask and turn it to make sure the solution is mixed
- The solution volume might be reduced, fill up with more MQ-water to the line again.

NaOH (2 M)
Lasting quality: approx. 6 months
Storage: Room temperature
Procedure:

- Take a 250 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Weigh 20 g NaOH
- Pour the NaOH into the flask
- Fill the flask with MQ-water to the line
- Put a cap on the flask and turn it to make sure the solution is mixed.
- The solution volume might be reduced, fill up with more MQ-water to the line again.

$K_2S_2O_8$ (Potassium persulfate)
Lasting quality: 2 weeks
Storage: Room temperature
Ingredients:

- $K_2S_2O_8$ (solid)
- MQ-water

Procedure:

- Take a 250 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Weigh 12.5 g $K_2S_2O_8$
- Fill the flask with MQ-water to the line to make 250 ml
- Put a magnetic stir bar in the flask
- Put the flask on a heater and carefully heat the solution until all chemicals are dissolved
- Either pour the solution into a dark flask, or cover the flask with aluminum foil to keep it from light

HCl (0.5 M)
Lasting quality: approx. 6 months
Storage: Room temperature
Ingredients:

- HCl concentrated (liquid)

- MQ-water

Procedure:

- **Note**: **Always be careful when using concentrated acids, use protective gloves, lab coat and glasses**
- Mix the solution in a fume hood
- Use a 1 L flast
- Add MQ water (about 500 mL)
- Pour 40 ml HCl in a measuring cylinderPour the HCl from the measuring cylinder into the volumetric flask
- Then fill up with MQ-water in the 1 L flask to the line
- Put on a cap and turn the flask to make sure the solution is mixed
- Rinse the outside of the bottle with MQ-water, and wash the used measuring glass while still wearing gloves to make sure no acid gets in contact with skin

For spectrophotometer process

Ascorbic acid
Ammonium molybdate
KSb
H_2SO_4 (2.5 M)
Reagent-solution (the combination of the above 4 chemicals)
P-standard solution
Comment: The amount of these solutions depends on how many samples that will be analyzed

Ascorbic acid

Lasting quality: Make fresh for each analysis occasion
Storage: Room temperature
Ingredients:

- Ascorbic acid (solid)
- MQ-water

Procedure:

- Take a 100 ml volumetric flask (or 200 mL)
- Put a weighing boat on the scale and tare the scale
- Weigh 1.75 g ascorbic acid (3.5 g for 200 mL flask)
- Pour the ascorbic acid into the flask, rinse the weighing boat with MQ-water and pour the water in the flask to make sure all chemicals are transferred from the weighing boat to the flask
- Fill the flask with MQ-water to the line
- Put on a cap and turn the bottle, make sure the chemicals are solved
- Check volume, if reduced add more MQ water to the line

Ammonium molybdate

Lasting quality: Make fresh for each analysis occasion
Storage: Room temperature
Ingredients:

- Ammonium molybdate (solid)
- MQ-water

Procedure:

- Take a 50 ml volumetric flask (or 100 mL for more reagent)
- Put a weighing boat on the scale and tare the scale
- Weigh 2.0 g ammonium molybdate (or 4 g for 200 mL flask)
- Fill the flask with MQ-water to the line
- Put on a cap and turn the bottle, make sure the chemicals are solved
- Check volume, if reduced add more MQ water to the line

KSb (Sodium antimony)

Lasting quality: 1 month
Storage: Fridge, dark (sensitive to light)
Ingredients:

- KSb ($K(SbO)C_4H_4O_6$-$0.5H_2O$) (solid)
- MQ-water

Procedure:

- Take a 250 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Weigh 0.6855 g KSb
- Pour the KSb into the flask, rinse the weigh boat with MQ-water and pour the water in the flask to make sure all chemicals are transferred from the weighing boat to the flask
- Fill the flask with MQ-water to the line to make 250 ml
- Put on a cap and turn the bottle, make sure the chemicals are solved
- Check volume, if reduced add more MQ water to the line
- Pour into and store in a dark, non-clear glass bottle.

H_2SO_4 (2.5 M)

Lasting quality: approx. 6 months
Storage: Room temperature
Ingredients:

- H_2SO_4 concentrated (liquid)
- MQ-water

Procedure:

- **Note**: **Always be careful when using concentrated acids, use protective gloves, lab coat and glasses. H_2SO_4 is corrosive and gets heated when in contact with water**
- Make a water bath to prepare the solution in
- Measure and pour 140 ml H_2SO_4 in a measuring cylinder
- Pour some MQ-water into the volumetric flask (ca. 500 mL)
- Pour the H_2SO_4 from the measuring cylinder into the volumetric flask slowly and let the acid react with the MQ-water without shaking (the flask will get heated). Place stopper in the flask
- Put a 1000 ml volumetric flask in the water bath
- When the flask is starting to cool, slowly turn flask upside down and then back up (with stopper in!) one time. Check temperature and wait till cool

again. Repeat until flask does not get hot (you can also see the acid reacting with the water).
- Carefully add more MQ-water into the volumetric flask up to the line and let the acid and MQ-water react. Repeat previous step.
- Rinse the outside of the bottle with MQ-water, and wash the used measuring glass while still wearing gloves to make sure no acid gets in contact with skin

Reagent solution

Lasting quality: 4 hours
Storage: Room temperature
Ingredients:

- H_2SO_4 (2.5 M)
- Ammonium molybdate
- KSb
- Ascorbic acid

Procedure:

- ***Note: The following amounts are examples depending on how many samples you are analyzing, see the sheet with different volumes based on number of samples to be measured***
- Use a 500 ml beaker and add the following, in order:
- Measure 125 ml H_2SO_4 in a measuring cylinder
- Measure 37.5 ml ammonium molybdate in a measuring cylinder
- Measure 12.5 ml KSb in a measuring cylinder
- Measure 75 ml ascorbic acid in a measuring cylinder
- Add the ingredients to the beaker in the same order they are measured: H_2SO_4, ammonium molybdate, KSb and last ascorbic acid.

P-standard solution (from liquid solution) (1 mg/L)

Lasting quality: Approx. 6 months
Storage: Fridge
Ingredients:

- Phosphate Standard solution (1000 mg/L PO_4)
- MQ-water

Procedure:

- Pour some of the stock standard solution (usually in a plastic beaker in the fridge) in a plastic beaker (marked high P in red) and leave it in room temperature for at least one hour, to get the solution to room temperature
- Use either a glass pipette or the regular pipette (with tip marked high P).
- Take 3 ml stock solution in the pipette and waste it

- Pipette 3 ml stock solution in a 1000 ml glass bottle marked LP
- Fill up the rest of the bottle to the line with MQ-water.
- This will in reality give a concentration of 0,978 mg/L, not 1 mg/L
- Make a standard curve and measure on the spectrophotometer to make sure the concentration is right

P-standard solution (from dry chemical) (1 mg PO_4-P/L):

Lasting quality: Approx. 6 months
Storage: Fridge
Ingredients:

- KH_2PO_4 (dried at 105 ^{0}C for 24 hours, allow to cool in a desiccator before using)
- MQ-water

Procedure:

- Weigh 0.4392 KH_2PO_4
- Dissolve it in MQ-water in a 1000 ml glass bottle
- Add 1 mL chloroform to prevent bacterial growth
- Fill up the rest of the bottle to the line with MQ-water.
- Make a standard curve and measure on the spectrophotometer to make sure the concentration is right (see below).

Appendix 2 - Measuring sediment P extractions on the spectrophotometer - Ascorbic acid- ammonium molybdate method

- Make the reagent using ascorbic acid, ammonium molybdate, KSb, H2SO4 (2.5 M) and MQ-water. See appendix 1 for how to make the reagent solutions.

- Turn on the spectrophotometer and set the wavelength to 880 nm. Blank the machine by putting MQ water in the cuvette, and placing the cuvette in the machine. Press the auto-zero button.

- Make 6 **standards** with following amounts (table below) of P-standard and then fill to 10 ml with MQ water. See **appendix** 1 for how to make and make the stock the P-standard solution.

Add ml of P standard	**MQ water**	**Concentration P (µg/L)**	Approximate absorbance value
0	10	0	0
0.5	9.5	50	30
1	9	100	60
2	8	200	120
4	6	400	240
8	2	800	480

-Note, the above concentrations are for 1 mg/L P standard. If the P standard in different, the concentrations above will be different. But still add the same amount of standard to each vile as shown above.
- Mix the reagent solution with the pipette tip and then add 2 ml of reagent to the standards **only** and wait 15 mins to make sure you get blue colour. The higher concentration of the standard, the darker blue. If no blue color, unexpectedly low blue color, or the color “runs away” (i.e. turns too blue), remake reagent solution and try again.
-Read the MQ water sample occasionally while you are waiting for the reagent to turn blue in the samples to make sure the machine is not drifting. If it does, zero it again.
- If the standards look ok after 15 mins (have turned blue) measure the standards and then rinse the cuvette carefully with MQ-water until the signal is around zero. If the standards look ok (see approximate absorbance in above table) and the values of the standard curve seem correct, add 2 ml of reagent to the first tray with samples and blanks. Shake the tray a little bit to help mix the samples and reagent, and wait at least 15 mins before starting to measure on the spec if the samples are at room temperature. If they are cold wait 30 mins.

- Start each sample series by measuring the three blanks in that series followed by the samples.
- Check the cuvette between samples to make sure there is no dirt/water on the cuvette.
- Measure your samples. If there is a large difference in blue colour between samples, put some sample in the cuvette to rinse it, then pour it out and put more in for measurement.
-Add reagent to each tray in the same pace that you read them, so each tray sits with reagent for at least 15 mins.

- Measure a standard (similar to your sample values) every 20 samples to make sure the machine isn't drifting.
- When done, pour out the samples and rinse the scintillation vials 2 times with deionized water
-Rinse the cuvette with MQ water at least three times, wipe with chemwipe to clean any smudges and store upside down (using an empty tray on top to keep the vials from falling out) to dry.

Appendix 3 - Dry weights and loss on ignition

In order to calculate P concentrations and P mass, quantification of dry weight and density of the sediment samples is needed.

Dry weight (DW) determination using freeze drier

Material: Freeze dryer, freezer, furnace, plastic scintillation vials with caps, spatula, scale (accuracy: 0.1µg)

1. Label all scintillation vials
2. Weigh the first empty scintillation vial without cap, record that value.
3. Homogenize the wet sample with a spatula and transfer at least 7-8g to the scintillation vial, record the new weight. If other analyses will be done (like metals, TOC, TN, etc.) make sure to find out how much dry sample is needed. Often you will need to add more wet sample if other analyses will be done.
4. Continue for all samples
5. Place caps on samples tightly
6. Freeze the samples in (-20°C or colder) for at least 24 hours.
7. Run the frozen samples in the freeze drier for 4 days, make sure the lids are loosely placed (unscrew cap ½ turn) to allow for vapor to escape container.
8. Transfer the freeze dried samples to a oven (60°C for 24h) to get rid of residual moisture.
9. Weigh the scintillation vial with dry sample (without cap) and note the value. Place the cap back on the vial tightly.

Calculations:

$$Water\ content = \frac{Mass(wet\ sample) - Mass(Dry\ sample)}{Mass(Wet\ sample)} \times 100$$

$$Dry\ weight\ (\%) = 100 - Water\ content$$

Loss on ignition (LOI) on freeze dried sediment

Material: Muffle oven, crucibles, spatula, scale (accuracy: 0.1µg)

1. Make sure crucibles are clean. If unsure, wipe with paper towels.
2. Place the empty crucible on the scale and note that value, also make sure the crucible is numbered and also record that to keep track of the sample.
3. Homogenize the freeze dried sample directly in the scintillation vials using a spatula to crush the any sediment clusters, wipe off spatula with paper towel between samples.
4. Pour approximately 1g of dry sample in the crucible and note the new value. Less can be used for samples with very little sediment (minimum 200 mg).

Edited by: SS, O A-N, EL
Approved by:

5. Gently transfer all samples to the muffle oven and burn the sediment for at least 2h in 550°C. Move slowly when transporting, because the dry sediment can be blown out of the crucibles quite easily.
6. Let the samples cool down to room temperature in the oven and measure the weight of the crucibles with burnt sample. Crucibles can be placed in a desiccator if they are not weighed immediately.

Calculation:

$$LOI(\%\ of\ Dry\ weight) = \frac{Mass(Dry\ sample) - Mass(Ignited\ sample)}{Mass(Dry\ sample)} \times 100$$

Appendix 4 – Description of sequential phosphorus fractionation method

To assess phosphorus in sediment a sequential chemical extraction can be used, commonly called phosphorus (P) fractionation. Different pools of P, with varying solubility and reactivity, that are separated and examined. Typically the procedure begins with dilute extractants, first removing loosely bound/pore water P, and then proceeding stepwise towards stronger extractants that dissolved/release more strongly bound forms (Lukkari *et al*., 2007).

In the late 1950s Chang & Jackson (1957) proposed a sequential extraction technique for P fractionation on soil. The scheme was later adapted to sediment by Williams *et al.* (1967, 1976). In the following decades the procedure was altered in different ways. For example, it was adapted for analysis of calcareous sediment by Hieltes & Lijklema (1980), Golterman (1977, 1982) wanted to further separate the bioavailable forms of P and developed a method using chelating agents in the procedure, and Ruttenberg (1992) focused on marine sediment using the SEDEX method. Psenner & Pucsko (1985, 1988) developed their P fractionation scheme in which they divided the different P forms into loosely sorbed P, redox sensitive (Fe/Mn bound P), ligand exchangeable P (Al bound and organic P), Ca bound P, and refractory P.

Due to lack of a harmonized procedure for determining forms of P in sediment, the European Commission (through the Standards, Measurements and testing – SMT) developed the SMT-protocol. This extraction procedure has been evaluated by different authors, e.g. Ruban *et al.* (2000) and Pardo *et al.* 2013.

However, the scheme by Psenner & Pucsko (1988) has not been abandoned, but further used, evaluated and adapted by e.g. Jensen & Thamdrup (1993) for marine sediment, Hupfer *et al.* (1995, 2009), Paludan & Jensen for sediment rich in humic material, and Jan *et al.* (2012, 2015) who came up with a strategy to better quantify P easily affected by low redox conditions. The fractionation protocol by Psenner & Pucsko (1984, 1988), and adapted by Hupfer *et al.* (1995, 2009), are used in this method description (Table 1).

Table 1. The differences in methods by Psenner & Pucsko to Hupfer et al (1995, 2009).

Psenner & Pucsko, 1985	a. H_2O (water soluble P), 10 min, centrifuge 3000 laps/10 min b. BD 0.11 mol/l (reductant soluble P), 40°C water bath, 30 min, centrifuge, wash with BD solution again, centrifuge. c. NaOH 1 mol/l (Fe and Al bound P), room temperature, 16 h, centrifuge, wash with NaOH, centrifuge. d. HCl 0.5 mol/l (Ca bound P), room temperature, 16 h, centrifuge, wash with HCl, centrifuge. e. NaOH 1 mol/l (refractory P), 85°C water bath, 24 h, centrifuge, wash with

	NaOH, centrifuge.
Hupfer *et al.* 1995, Hupfer *et al.* 2009	All analyses are conducted at room temperature. a. Changed H_2O for deoxygenated NH_4Cl (1M), extraction time 30 min. b. Changed reaction time to 1 h. c. Added a step for determining NRP from the NaOH-step (persulfate and autoclave). d. No change. e. Last step emitted.
This document	a. MQ water, room temperature, 2 h. b. BD 0.11 M, pH 7.2, room temperature. 50 min. c. NaOH (1 M), room temperature, 16 h (one set of samples NRP, addition of persulfate and autoclaved) d. HCl (0.5 M), room temperature, 16 h e. Last step omitted.

References

Chang, S. C. and M. L. Jackson. (1957). Fractionation of soil phosphorus. *Soil Science*, 84, pp 133–144.

Golterman, H. L., 1977. Sediments as a source of phosphate for algal growth. In: Golterman, H. L. (Ed.) Interactions between Sediments and Freshwater, pp. 286-293. Junk, The Hague.

Golterman, H. L. (1982). Differential extraction of sediment phosphates with NTA solutions. *Hydrobiologia*, 91, pp 683–687.

Hieltjes, A. H. M. and L. Lijklema. (1980). Fractionation of inorganic phosphates in calcareous sediments. *Journal of Environmental Quality*, 9, pp 405–407.

Hupfer, M., Gächter, R. & Giovanoli, R. (1995). Transformation of phosphorus species in settling seston and during early sediment diagenesis. *Aquatic Sciences-Research Across Boundaries*, 57(4), pp 305–324.

Hupfer, M., Zak, D., Roβberg, R., Herzog, C. & Pöthig, R. (2009). Evaluation of a well-established sequential phosphorus fractionation technique for use in calcite-rich lake sediments: identification and prevention of artifacts due to apatite formation. *Limnology and Oceanography: Methods*, 7(6), pp 399–410.

Jan, J., Borovec, J., Kopáček, J. & Hejzlar, J. (2013). What do results of common sequential fractionation and single-step extractions tell us about P binding with Fe and Al compounds in non-calcareous sediments? *Water Research*, 47(2), pp 547–557.

Jan, J., Borovec, J., Kopáček, J. & Hejzlar, J. (2015). Assessment of phosphorus associated with Fe and Al (hydr)oxides in sediments and soils. *Journal of Soils and Sediments*, 15(7), pp 1620–1629.

Jensen, H. S. & Thamdrup, B. (1993). Iron-bound phosphorus in marine sediments as measured by bicarbonate-dithionite extraction., 1993. pp 47–59. Springer.

Lijklema, L. (1980). Interaction of orthophosphate with iron (III) and aluminum hydroxides. *Environmental Science & Technology*, 14(5), pp 537–541.

Lukkari, K., Hartikainen, H. & Leivuori, M. (2007). Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. *Limnology and Oceanography: Methods*, 5(12), pp 433–444.

Paludan, C. and Jensen, H. (1995) Sequential extraction of phosphorus in freshwater wetland and lake sediment: Significance of humic acids, Wetlands, 15 (4), pp 365-373.

Pardo, P., López-Sánchez, J. F. & Rauret, G. (2003). Relationships between phosphorus fractionation and major components in sediments using the SMT harmonised extraction procedure. *Analytical and Bioanalytical Chemistry*, 376(2), pp 248–254.

Psenner, R., R. Pucsko, and M. Sager. (1984). Die fractionerung organisher und anorganisher Phosphorverbindungen von Sedimenten. *Archiv fur Hydrobiologie—Supplementband*, 70, pp 111–155.

Psenner, R. and R. Pucsko. (1988). Phosphorus fractionation: advantages and limits of the method for study of sediment P orgins and interactions. *Archiv fur Hydrobiologie Beiheft—Ergebnisse der Limnologie,* 30, pp 43–59.

Ruban, V., LópezSánchez, J. F., Pardo, P., Rauret, G., Muntau, H. & Quevauviller, P. (2001). Development of a harmonised phosphorus extraction procedure and certification of a sediment reference material. *Journal of Environmental Monitoring*, 3(1), pp 121–125.

Ruttenberg K. C. *(*1992*).* Development of a sequential extraction method for different forms of phosphorus in marine sediments*, Limnology and Oceanography, 37 (7), pp 1460-1482*

Schimel, D. S.2 1 Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543; email: sdoney@ whoi. edu 2 Climate

and Global Dynamics. *National Center for Atmospheric Research, Boulder Colorado*, 80307, pp 31–66.

Williams, J. D. H., J. K. Syers a T. W. Walter, 1967. Fractionation of soil inorganicphosphate by a modification of Chang and Jackson procedure. Soil Sci. Sot. Amer. Proc. 3 1: 736-739.

Williams, J. D. H., Jaquet, J. M. & Thomas, R. L., 1976. Forms of phosphorus in the surficial sediments of Lake Erie. J. Fish.Res. Bd Can. 33: 413-429.

Appendix 5 - Adaptations of the method

Yellow samples in MQ-step

When encountering strong yellow colour in the supernatant (before reagent added) it might be useful to quantify this background signal.

-Take an extra sub sample batch of MQ supernatant (4 ml) and read the samples in the spectrophotometer (880nm) without adding any reagent. Subtract this absorbance value from the MQ-samples analysed according to the ascorbic acid – ammonium molybdate method.

Brown/yellow samples in NaOHrp-step

During the extraction with NaOH there is a possibility that precipitates and colouring of the samples may occur. Often this occurs when analysing sediment samples from humic lakes.

-Quantify the background signal (brown colour) according to the description in “Yellow samples in MQ-step”.

If precipitate is present it might disturb the light beam in the spectrophotometer and thus overestimate the absorbance.

-Take an extra sub sample batch of NaOH supernatant (2 ml), add the reagent and then centrifuge the samples for 10 minutes at 3000rpm. Further read the samples on the spectrophotometer at 880nm.

Appendix 6 - Quality assurance

Reference material

In order to monitor the quality of the complete procedure it is needed to use a “reference sediment”, this is treated as any other sample in the batch. The reference sediment is created by pooling residual freeze dried sample and homogenizing it thoroughly. All data from different runs of the reference sediment should be saved in a database to create the possibility to target any errors in the method handling.

Replicated samples

It is recommended to replicate samples to assess the measurement error for the method, especially when the method handles low P concentrations.

PHOENIX
Environmental Laboratories, Inc.
587 East Middle Turnpike, P.O. Box 370, Manchester, CT 06040
Telephone: 860.645.1102 • Fax: 860.645.0823

SOP No.: 321.4500PE

Title: Phosphorus, All Forms

Scope and Application: This method covers the determination of all forms of phosphorus, including o-phosphate, organic phosphate, and total phosphorus. This method is applicable to all drinking water, surface water, ground water, industrial and domestic waste, and soil samples and their leachates. The range for this procedure is 0.010-1.0 mg/L. Samples above 1.0 mg/L can be analyzed by sample dilution prior to digestion. The limit of detection is 0.010 mg/L.

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates, 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 runoff 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.

I. Summary of Method

A. Ammonium molybdate and potassium antimony tartrate react in an acid medium to form a heteropoly acid (phosphomolybdic acid) which is reduced to an intensely blue-colored complex by ascorbic acid. The intensity of the blue color is directly proportional to the concentration of orthophosphate in the sample.

B. Since the above reaction only occurs with the o-phosphate form of phosphorus, pretreatment of condensed and organically bound is required for their

determination. Condensed phosphates must undergo acid hydrolysis at boiling-water temperature. Organically bound phosphates must undergo a digestion using acid, heat, and an oxidizing agent. These treatments transform the bound phosphates to o-phosphate, where they can be quantified.

C. For dissolved phosphate, filter the sample through a 0.45um filter before analysis. If only particulate phosphate is to be determined, analyze for dissolved phosphate and for total phosphate, and subtract dissolved from total.

D. Total Phosphorus (P)- all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure.

1. Total Orthophosphate (P, ortho) or Total Reactive Phosphorus- inorganic phosphorus [$(PO_4)^{-3}$] in the sample as measured by the direct colorimetric analysis procedure.
2. Total Hydrolyzable Phosphorus (P, hydro) – phosphorus in the sample as measured by the sulfuric acid hydrolysis procedure, and minus pre-determined orthophosphates. This hydrolyzable phosphorus includes polyphosphorus. [$(P_2O_7)^{-4}$, $(P_3O_{10})^{-5}$, etc.] plus some organic phosphorus.
3. Total Organic Phosphorus (P, org) – phosphorus (inorganic plus oxidizable organic) in the sample measured by the persulfate digestion procedure, and minus hydrolyzable phosphorus and orthophosphate.

E . Dissolved Phosphorus (P-D)- all of the phosphorus present in the filtrate of a Sample filtered through a phosphorus-free filter of 0.45 micron pore size and measured by the persulfate digestion procedure.

1. Dissolved Orthophosphate (P-D, ortho) or Dissolved Reactive Phosphorus- as measured by the direct colorimetric analysis procedure.
2. Dissolved Hydrolyzable Phosphorus (P-D, hydro)- as measured by the sulfuric acid hydrolysis procedure and minus pre-determined dissolved orthophosphates.
3. Dissolved Organic Phosphorus (P-D, org)-as measured by the persulfate digestion procedure, and minus dissolved hydrolyzable phosphorus and orthophosphate.

F. The following forms, when sufficient amounts of phosphorus are present in the sample to warrant such consideration, may be calculated:

1. Insoluble Phosphorus (P-I) = (P)-(P-D).
2. Insoluble orthophosphate (P-I, ortho)=(P,ortho)-(P-D,ortho).
3. Insoluble Hydrolyzable Phosphorus (P-I,hydro)=(P,hydro)-(P-D,hydro).
4. Insoluble Organic Phosphorus (P-I, org)=(P,org) – (P-D,org).

G. See Appendix B for flow chart.

II. Interferences

A. Glassware must be scrupulously cleaned, as carry over from phosphates is common. Refer to Appendix A in this SOP for the Phosphorus Glassware Cleaning Instructions.

B. Arsenate present in the sample to be tested is a positive interference, as it reacts with the molybdate reagent to produce a blue color similar to the blue formed by o-phosphate. Concentrations as low as 0.1 mg/L are enough to cause interference. By adding sodium bisulfite to the sample, arsenate is reduced to arsenious acid.

C. High concentrations of iron in a sample will give erroneously low results, as it will take away some of the reducing agent. Sodium bisulfite treatment will eliminate this interference.

D. Hexavalent chromium and nitrite interfere to give low results. Concentrations of these ions need to be at least 10.0 mg/L to interfere signifcantly.

E. Particulates may interfere with spectrophotometric analysis by absorbing light. Samples may be filtered after hydrolysis or digestion but not once the color reagent is added.

F. In the determination of orthophosphate, color can interfere. A color blank may be performed.

III. Sample Collection, Preservation and Storage

A. Samples can be collected in plastic or glass. They must be stored at 4°C until time of analysis.

B. Orthophosphate samples must be unpreserved and analyzed within 48 hours from time of collection.

C. Total Phosphorus samples must be H_2SO_4 preserved to pH<2 and analyzed within 28 days of collection.

IV. Equipment and Supplies

A. Spectophotometer, 880nm.

B. Environmental Express 54-position Hot Block with custom glass digestion vials.

C. Pro Clean Environmental Containers glass 60mL vials, P/N GV60/PCLB-72.

D. Graduated cylinder, 50mL.

E. Volumetric flasks, 50mL.

F. Class "A" volumetric pipets; assorted.

G. Hengar granules, boiling stones

V. Reagents and Standards

A. Sulfuric Acid, 5N. Dilute 139mL conc H_2SO_4 to 1000mL with DI water.

B. Potassium antimony tartrate solution. Dissolve 1.3715 g $K(SbO)C_4H_4O_6$ 1/2 H_2O in 400 mL DI water in a 500ml volumetric flask and dilute to volume. Store in a plastic, stoppered bottle.

C. Ammonium molybdate solution. Dissolve 20 g $(NH_4)_6$ Mo_7O_{24}*$4H_2O$ in 500ml DI water. Store in plastic, stoppered bottle.

D. Ascorbic acid solution, 0.01M. Dissolve 3.52g ascorbic acid, reagent grade, in 200mL DI water. Make fresh daily.

E. Combined color reagent. Mix 100mL 5N H_2SO_4, 10mL potassium antimony tartrate, 30mL ammonium molybdate, and 60mL ascorbic acid solution in a clean 200mL erlynmeyer flask. If more reagent is needed, increase volumes of each solution proportionally. **Add reagents in the order given, and mix after the addition of each reagent.** Combined reagent is only stable for 4 hours.

F. Sulfuric acid, 11N. Slowly add 300mL conc. H_2SO_4 to 600mL DI water. Cool in a water bath, and dilute to 1 liter. Alternatively, make from 18N (1:1) H_2SO_4 by diluting 600mL to 1 liter of DI water.

G. Ammonium persulfate $(NH_4)_2\ S_2O_8$ solid, reagent grade. Potassium persulfate may be used as an oxidizing reagent.

H. Sodium bisulfite solution. Dissolve 5.2 g of $NaHSO_3$ in l00mL of l N H_2SO_4.

I. Sodium hydroxide solution, 2N. Dissolve 80 g NaOH into 1 liter DI water.

J. Phenolphthalein indicator solution, alcohol-based. Dissolve 5g of phenolphthalein in approximately 500mL 95% ethyl or isopropyl alcohol. Dilute to 1 L with DI water.

K. Stock phosphorus solution. Dissolve 0.4393g of oven-dried and desiccated KH_2PO_4 in 1L DI water. 1.0mL = 100ug P. Record preparation of standards in standards logbook.

L. Standard phosphorus solution. Dilute 100mL of stock phosphorus solution to 1L with DI water. 1.0mL= 10ug P. Record preparation of standards in standards logbook.

VI. Definitions

A. Laboratory Control Sample (LCS)- A purchased standard of a known concentration that is from a different source than the calibration standards. The LCS is used to determine laboratory performance.

B. Preparation Blank (Prep Blank)- An aliquot of reagent water that is treated exactly as a sample. The prep blank is used to determine if method analytes are present in the lab environment, reagents or glassware.

C. Calibration standard- A solution prepared from the dilution of stock standard solutions. The calibration solutions are used to calibrate instrument response with respect to analyte concentration.

D. Matrix Spike (MS)- An aliquot of environmental sample to which a known quantity of the method analytes are added in the laboratory. The MS is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results.

VII. Procedure

A. Determination of total phosphorus (TP-WM), all forms of phosphate.

1. Check pH of the sample and record in Phos batching program.

2. Into a digestion vessel, measure 50mL sample or an aliquot diluted to 50mL, hengar granules, and add a drop of phenolphthalein solution. If it turns pink, add 11N H_2SO_4 drop-wise until color disappears.
3. Add 0.4g solid ammonium or potassium persulfate.
4. Add lmL 11N H_2SO_4. Use disposable pipets to reduce chances of contamination.
5. Set the Hot Block to approximately 140°C, enough to get the samples to 100°C. Boil gently under a ventilation hood until a final volume of less than 10mL is achieved. Do not allow sample to go to dryness.
6. For samples suspected to contain iron interference, add 5mL of sodium bisulfite and mix. continue heating for 20 min. Do not allow sample to go to dryness.
7. Cool, dilute to 30mL in the digestion vessels (filter sample, if needed), add 1 drop phenolphthalein solution, and neutralize to a faint pink color using 2N NaOH solution. *Do not filter while sample is neutral, as phosphate-containing precipitates form that will dissolve under acid conditions.*
8. Bring sample up to 50 mL volume with DI water in digestion vessel and mix well. Transfer a 25 mL aliquot to 60 mL glass vessel. If a larger dilution is necessary, transfer a small volume to glass vial and bring to 25 mL final volume with DI water.
9. Determine phosphorus as outlined in the determination of orthophosphate, C.2. and C.3. below.

B. Determination of total phosphates (code "TPFATE") hydrolyzable and condensed phosphates.

1. Into a digestion vessel, measure 50mL sample or an aliquot diluted to 50mL and add a drop of phenolphthalein solution. If it turns pink, add 11N H_2SO_4 drop-wise until color disappears. Record pH of original sample in Phos Batching sheet.
2. Add 1 mL of H_2SO_4 to sample. Use a disposable pipet to reduce risk of contamination.
3. Set the Hot Block to approxiamately 140°C, enough to get the samples to 100°C. Boil gently with hengar granules under a ventilation hood until a final volume of less than 10mL is achieved. Do not allow sample to go to dryness.
4. Treat samples containing interferences, as above.
5. Cool samples, filter if needed, then add 1 drop phenolphthalein solution, and neutralize to a faint pink color using 6N NaOH solution. *Do not filter while sample is neutral, as phosphate-containing precipitates from that will dissolve under acid conditions.*
6. Bring sample up to 50 mL volume with DI water in digestion vessel and mix well. Transfer a 25 mL aliquot to 60 mL glass vessel. If a larger dilution is necessary, transfer a small volume to glass vial and bring to 25 mL final volume with DI water.

7. Determine phosphorus as outlined in the determination of orthophosphate, C.2. and C.3. below.

C. Determination of orthophosphate (OP-WM).

1. Decant 25mL of unpreserved sample or an aliquot diluted to 25mL into a glass vial, add 1 drop of phenolphthalein solution. If a pink color appears, add 5N H_2SO_4 drop-wise until color disappears.
2. Add 4 mL combined color reagent and mix thoroughly.
3. After 10 (but before 30) minutes, measure the absorbance of each sample at 880nm, using DI water as the reference solution. The absorbance is correlated to a point on a linear regression which correlates to a concentration of phosphorus.

D. For other forms of Phosphorus, see Section 1.C – 1.F.

VIII. Calculations

A. Refer to the Standards Logbook for the most current calibration curve. To generate a new calibration curve, use phosphorus standard solution. Make the following dilutions: blank (DI water), 0.01mg/L, 0.025 mg/L, 0.05 mg/L, 0.10 mg/L, 0.20 mg/L, 0.50 mg/L and 1.0 mg/L. Add phenolphthalein and 5N H_2SO_4 to each, as you would a sample. Add 8 mL combined color reagent, and read at 880 nm after 15 minutes. Record absorbances, and plot them on the x-axis against the corresponding concentrations. Follow with a linear regression, and use the following calculation:

$Y = (MX + B) * DF$, where: Y = concentration (mg/L)
M = slope of the linear regression
X = absorbance at 620nm
B = y-intercept of linear regression
DF = dilution factor, if any

Note that M and B values will differ with each calibration.

B. Calculating RPD:

The RPD is the relative percent difference, and is a measure of the consistency of the sample replicate for the sample set. First, calculate the results for both the sample and the sample replicate. Then apply the two results to the following calculation:

$$RPD, \% = \frac{(R1 - R2)}{(R1+R2)/2} \times 100$$

where: R1 = value achieved for sample, mg/L
R2 = value achieved for sample replicate, mg/L

C. Calculating the sample spike recovery:

The sample spike is a test if the sample matrix is suitable for accurate testing. To calculate the % recovery, first calculate both the sample and the spiked sample. Then apply the two results to the following calculation:

$$\% \text{ Recovery, } \% = \frac{(SR - R)}{STV} \times 100$$

where: SR = value of sample with added spike, mg/L
R = value of sample, mg/L
STV = true value of spike added to sample, mg/L

D. Calculating the LCS:
The LCS is the laboratory control standard and is a test of the accuracy of the calibration and the test overall. To calculate the % recovery of the LCS, calculate the LCS sample, and then apply it to the following calculation:

$$\% \text{ Recovery, } \% = \frac{(RL)}{(LTV)} \times 100$$

where: RL = achieved value for LCS sample, mg/L
LTV = true value of LCS, mg/L

E. Calculating poly-phosphate, as P is done during data transfer, automatically, when needed.

IX. Quality Control

A. All reagents and standards are to be labelled with the following information: date prepared, date expires, preparer's initials, analyte, contents, and concentration of contents. Record preparation of all reagents in the reagent prep logbook.

B. A sample prep blank of dilution water must be analyzed with each batch of twenty or fewer samples. Prep blanks must be determined to be BRL (below reportable level).

C. Two purchased, second source, laboratory control standards (LCS) are run with each batch of twenty or fewer samples. One is a "complex" solution, which goes through the digestion process, while the other is a "simple" solution, which is not digested. The acceptable recovery of a LCS is 85-115%.

D. An in-house standard (CC) made from the standard solution used to calculate the curve is run after every ten samples. The true value of the CC is 0.25mg/L. The acceptable recovery of an in-house standard is 90-110%.

E. A DI water blank, (CB), is analyzed after the CC every ten samples. The CB must be BRL (below reportable level).

F. A sample spike and a sample duplicate are run with every batch of twenty or fewer samples. The acceptable recovery for a sample spike is 75-125%. The acceptable relative percent difference (RPD) for a sample duplicate is less than or equal to 20%.

G. A new calibration curve must be analyzed annually or whenever a new stock solution is made. The correlation coefficient of the curve must be >0.9975.

H. Samples labeled as trip blanks, field blanks, or equipment blanks are ineligible samples for spiking and duplicating.

X. Safety

A. The toxicity and carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. A reference file of material data handling sheets are available to all personnel involved in the chemical analysis.

B. Always wear safety glasses for eye protection as well as lab coats.

C. When diluting H_2SO_4, always add acid to water.

D. Refer to Phoenix SOP #805: Hazardous Chemical and Laboratory Safety Procedures

XI. Pollution Prevention

A. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation.

B. Reagents and chemicals should be purchased and/or prepared in volumes consistant with laboratory use to minimize the volume of disposal.

XII. Waste Management

A. It is the laboratories responsibility to comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

XIII. Method Performance

A. This method was validated through internal QA/QC monitoring, including annual method detection limit studies, precision and accuracy studies, initial and continuing calibration verifications, blank analysis, laboratory control samples and matrix spikes and duplicates.

B. See section IX Quality Control in this SOP for acceptable limits.

XIV. Corrective Action for Out-of-Control or Unacceptable Data

A. Should the calibration curve have a correlation coefficient of <0.9975, remake and reanalyze curve before processing samples.

B. Should the preparation blank, LCS or inhouse standard fail acceptance criteria, redigest and reanalyze batch.

C. Should the matrix spike or sample duplicate analysis fail acceptance criteria, a non-conformance report must be generated or the sample QC must be reanalyzed.

XV. Batching Program for Phosphorus

A. Double click on Phoenix MDB (2K MDE) to get to main menu.

B. Click on QA/QC Batching and double click on Prep Batching.

C. Go to the Batch square and type in "Phos-T".

D. Go to New Batch and click, this brings up the last batch made.

E. Click again and this brings up a new list of samples to pick for each batch to be made.

F. Click on numbers under Batches to build for the number needed.

G. Click on Build Batch.

H. Finish making the batch by filling in QC sample, weight/volume, initials and finish date.

I. All samples MUST be batched. They cannot be added to the Sequence Program if they are not in a batch.

J. All batches must have Initial sample weight/volume entered by the analyst before the batch can be added to the Sequence Program.

K. Samples cannot be transferred if % Solid is still pending.

L. Samples cannot be transferred if the batch LCS fails.

M. Changing the QC Sample:

1. A batch cannot be loaded in the Sequence Program until it has been assigned a QC sample. However, a QC sample can be changed.
2. To change a QC sample, select the batch in Prep Batching and Clear the finish date.
3. Change the QC sample to the desired QC sample. The LCS and Blank will automatically change in the Sequence Program.
4. The original spike and dup will remain. Do not select them for transfer and the results will go nowhere.
5. The spike and dup for the new QC sample will have to be added manually to the end of the sequence.

N. Updating the Calibration:

1. When recalibrating the instrument, change the dates in the set-up to reflect the effective ending date of prior calibration.

2. Enter a new record. This can be done by copying an existing record and pasting it on a new line.
3. Update the appropriate date, Slope, y-intercept, LCS true value and source, spike true value, etc.

O. Selecting or Deselecting Samples for Transfer:

1. Before transferring samples, the Batch Blank and Batch LCS must be selected. This is necessary so when a sample is selected, the program knows which QC is associated with it.
2. If a Blank or LCS is deselected, all samples in the batch will be deselected.

P. Rerunning QC Samples:

1. QC can be re-analyzed by re-adding the samples and appropriate QC record to the end of the sequence.
2. Deselect the original run(s) and select the new one, and the corresponding samples.

XVI. Data Transfer Program for Phosphorus

A. Click on Phoenix MDB (2K MDE) to go to the main menu.
B. Click on Data Transfer.
C. Double Click on Phosphate Sequence and Data Transfer.
D. Select "P" on Run Type drop down box.
E. Click on New Sequence or Add Batch for however many to be done.
F. After batches are completed, add analysts initials to bottom and click on Transfer to Labworks.
G. Click on Present Log to print a copy and exit page.

XVII. References

A. Standard Methods for the Examination of Wastewater, 22nd ed., 2011.
Methods used: 4500-P B2 for hydrolysis; 4500P B5 for digestion; 4500-P E for orthophosphate and color formation.
B. Phosphorus, All Forms, Environmental Express Hot Block Standard Procedure.
Method used: Colorimetric, Ascorbic Acid, Single Reagent.
C. USEPA Method 365.3, Phosphorus, All Forms, 1978.

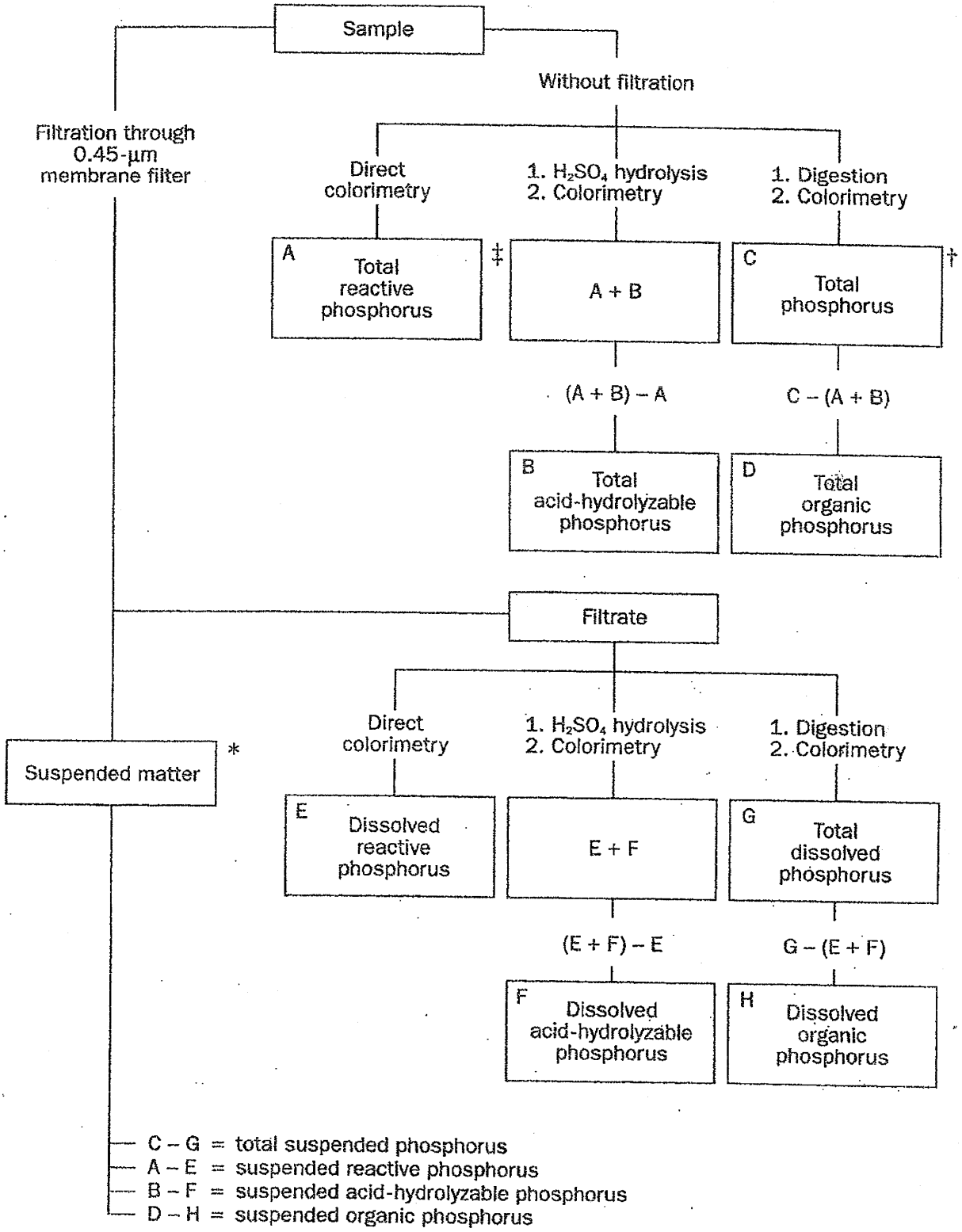
Appendix A- Phosphorus Glassware Cleaning Instructions

1) Make new 10% HCl solution at least once a week.
   a) Dump old solution in acid waste.
   b) Rinse bottles and caps thoroughly with deionized water.
      i) Bottles are usually in hood #8.
   c) Fill bottles to 900 mL line with deionized water.
   d) In a hood, fill bottles to 1000 mL line (the bump near the top), with concentrated HCl.
   e) Cap bottles and CAREFULLY invert several times to mix.

2) PUT 10% HCl bottles on stir/heat block in Hood #8 to heat.
   a) Turn on power switch for block.
   b) Turn heater switch to high. (Downward is low, upward is high, middle is off.)
   c) Set the heat knob to 150°C. Bottles should heat up within the hour.
   d) Monitor bottles to make sure they don't boil.

3) Handwash the 60 mL vessels (NOTE: Some vessels have a 50mL volumetric line for digestions, while others have a 25mL volumetric line for color developement. They are washed the same way!)
   a) Pour out the water that the vessel has been soaking in, while in the white floats.
   b) Fill with hot water, add a squirt of Phosphate free detergent and scrub with brush.
      i) Brush is the clean, white bristled brush hanging to the right of the sink.
      ii) The Phosphate free detergent is in a squeeze Nalgene bottle near brush.
      iii) Remove any sharpie marks on the outside of the vessel while scrubbing.
   c) Rinse washed vessels numerous times with DI water.
   d) Place the scrubbed / rinsed vessels upright in the plastic storage trays.
      i) Trays can usually be found piled up in the phosphorus area.
         (1) Blank trays are for "25 mL" vessels
         (2) Numbered trays are for "50 mL" vessels.
   e) In a hood, carefully fill each vessel to the brim with HOT 10% HCl solution, which was heated in Step #2.
      i) Use a hotglove borrowed from the ammonia setup if necessary; the acid solution should be hot enough to be slightly uncomfortable, but not boiling hot.
   f) Let the vessels soak in HCl solution for a few minutes
      i) The time necessary to pour another tray should be sufficient.
   g) Using the funnel that was stored with the bottles, pour the hot HCl solution back into its bottle. Cap it and store in hood #8 when finished.
   h) Take the tray of acid washed vessels to the sink for rinsing.
      i) Rinse three times with deionized water and pour out.
      ii) Fill vessels to the brim with deionized water
   i) Store clean deionized water filled tray of vessels either on the phosphorus countertop or in the shelves in the rear storage room.
      i) Cover vessels with clean paper towel to protect them.
   j) Dump DI water in vessels and rinse once more with DI before use.

Appendix B


Sample
Without filtration
Filtration through 0.45-µm membrane filter
Direct colorimetry
1. H_2SO_4 hydrolysis 2. Colorimetry
1. Digestion 2. Colorimetry
A Total reactive phosphorus
‡
A + B
C Total phosphorus
†
(A + B) – A
C – (A + B)
B Total acid-hydrolyzable phosphorus
D Total organic phosphorus
Filtrate
Direct colorimetry
1. H_2SO_4 hydrolysis 2. Colorimetry
1. Digestion 2. Colorimetry
Suspended matter
*
E Dissolved reactive phosphorus
E + F
G Total dissolved phosphorus
(E + F) – E
G – (E + F)
F Dissolved acid-hydrolyzable phosphorus
H Dissolved organic phosphorus
C – G = total suspended phosphorus
A – E = suspended reactive phosphorus
B – F = suspended acid-hydrolyzable phosphorus
D – H = suspended organic phosphorus

Revision History Log for SOP #321 TP

Date:	Revision #:	Summary of Changes:	Submitted By:	Approved By:	Effective Date:
1/6/14	5	Simple & Complex LCS added, recording pH of sample, updated vessel cleaning procedure	Kathy Cressia	Eric Geyer	1/6/14
10/04/16	5.1	Section 1.0- added D, E, F, G (forms of phos), Section VII- added D, added EPA method to references, just referring to forms of Phos, added Appendix B- flow chart	Kathy Cressia	Eric Geyer	10/04/16
3/15/18	5.2	Doubled combined reagent recipe, updated method reference year for MUR	Kathy Cressia	Eric Geyer	3/15/18

Expiration Date