

<i>Title:</i> AOS Protocol and Procedure: Algae Sampling in Lakes and Non-Wadeable Streams		<i>Date:</i> 05/15/2015
<i>NEON Doc. #:</i> NEON.DOC.001203	<i>Author:</i> S. Parker	<i>Revision:</i> D

AOS PROTOCOL AND PROCEDURE: ALGAE SAMPLING IN LAKES AND NON-WADEABLE STREAMS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	02/10/2014	ECO-01132	Initial release
B	08/29/2014	ECO-02210	Minor updates based on feedback from the field
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D	05/15/2015	ECO-02666	Minor updates including changes to phytoplankton sample volume and preservation, addition of filter manifold, updates to sample shipping and labeling, and the addition of sampling dates to appendix.

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1 OVERVIEW

1.1 Background

Aquatic primary producer communities are dominated by algae in most systems (Lowe and LaLiberte 2006). Algae are photosynthetic organisms that produce energy (fix carbon) from sunlight using chlorophyll α , and often form the base of the food chain (Graham and Wilcox 2000). They differ from other aquatic producers, such as aquatic plants and mosses, due to a lack of true tissues and multicellular gametangia. Algae can be broken into three distinct groups: macroalgae (visible to the naked eye), phytoplankton (microscopic algae inhabiting the water column), and benthic microalgae (periphyton growing on submerged surfaces such as sediment or plants). Macroalgae will be sampled as part of the Aquatic Plant and Macroalgae Sampling in Lakes and Non-wadeable Streams Protocol (RD[10]), while phytoplankton and benthic microalgae will be sampled in this protocol.

Phytoplankton are free-floating single-cells or colonies of algae that can be highly diverse in many lakes and ponds (Wehr and Sheath 2003). Phytoplankton communities are primarily controlled by nutrient supply, light, and consumer grazing pressure. Phytoplankton are present in higher density above the **metalimnion** in **stratified** lakes (lakes with a **thermocline**) and within the **euphotic zone** (the region through which light penetrates) in **non-stratified** lakes (Figure 1).

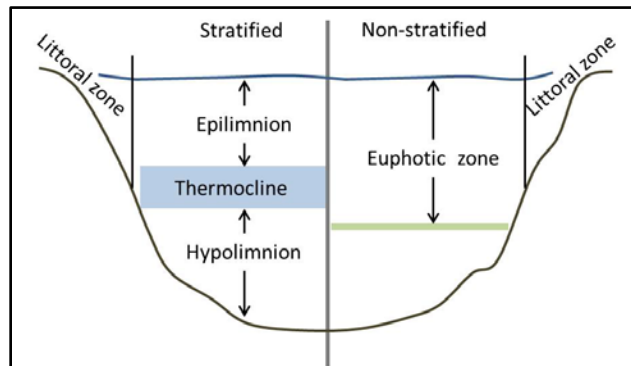


Figure 1. The zones in a stratified and non-stratified lake.

Benthic microalgae, along with a matrix of cyanobacteria, microbes, and detritus combine on surfaces in aquatic systems to form **periphyton**. Most surfaces that are exposed to light in the **littoral zone** of lakes sustain periphyton communities, and light penetration typically limits periphyton to the shallow littoral zone (Figure 1). Benthic microalgae communities can be classified based on the substrata that they colonize: **epiphyton** colonize plant surfaces, **epilithon** colonize cobbles and boulders, **epipelon** colonize silty sediments, and **epipsammon** colonize sand (Wehr and Sheath 2003). Epilithic communities (growing on rock surfaces) are typically well-studied in lakes while epiphyton and epipsammon/epipelon tend to be less understood. Diatoms compose the majority of the periphyton community, with structural attributes ranging from prostrate (closely adhered to the substratum), stalked, or colonial (often chain-forming, loosely associated with the periphyton mat; Figure 2).

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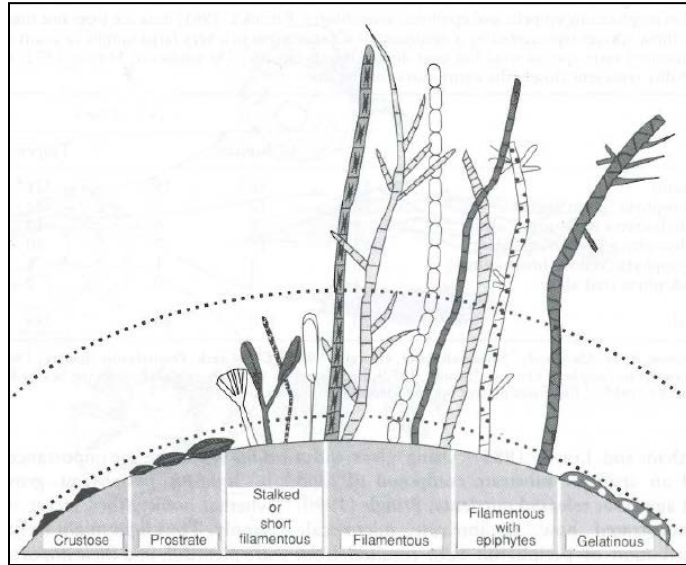


Figure 2. Growth forms of periphyton, ranging from prostrate types to long, filamentous types. Vertical layering differs among growth forms (from Allan 1995).

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON’s Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON’s document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

This document is based on the protocols of the US Environmental Protection Agency (USEPA) Environmental Monitoring and Assessment (EMAP) program (Baker et al. 1997), the Arctic Long-Term Ecological Research (LTER) Lakes Field Sampling Protocol (Bahr et al. 2002), the Minnesota EPA National Lake Assessment (NLA) protocol (USEPA 2009), the US Geological Survey (USGS) National Field Manual for the Collection of Water Quality Data (NAWQA; Hambrook Berkman and Canova 2007), and the USEPA Sampling Procedures for the Great Lakes (USEPA 2010).

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2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.014051	Field Audit Plan
AD[06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.002192	Datasheets for AOS Protocol and Procedure: Algae Sampling in Lakes and Non-Wadeable Streams
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling
RD[08]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[09]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[10]	NEON.DOC.001161	AOS Protocol and Procedure: Plant and Macroalgae Sampling in Lakes and Non-Wadeable Streams
RD[11]	NEON.DOC.001195	AOS Protocol and Procedure: Riparian Mapping in Lakes and Non-Wadeable Streams
RD[12]	NEON.DOC.001196	AOS Protocol and Procedure: Stream Riparian Mapping
RD[13]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[14]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[15]	NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[16]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

2.3 Acronyms

Acronym	Definition
AFDM	ash-free dry mass
C	carbon
DI	deionized water
EMAP	Environmental Monitoring and Assessment Program (USEPA)
FPOM	fine particulate organic matter
GF/F	glass fiber filter, grade F
HDPE	high-density polyethylene
hr	hour
km	kilometer
L	liter
LTER	Long Term Ecological Research Program
m	meter
mL	milliliter
µm	micrometer
mm	millimeter
NLA	National Lake Assessment
NAWQA	National Water Quality Assessment Program
N	nitrogen
PFD	personal flotation device
P	phosphorus
RTH	Richest Targeted Habitat (USGS NAWQA program)
S	sulfur
USEPA	US Environmental Protection Agency

2.4 Definitions

Bryophyte: Aquatic moss, liverworts, or hornworts lacking true vascular tissues (Figure 3).



Figure 3. Aquatic mosses (bryophytes) may grow on rocks in streams. Bryophytes may also have epilithic algae growing on its surface.

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Chlorophyll: Green pigments that are found in the chloroplasts of plants, chlorophyll *a* concentration is often used as a proxy for algal biomass.

Cobble: Medium-sized rocks in the lake/non-wadeable stream bottom, geologically defined as 64 to 256 mm diameter. Cobbles are larger than pebbles (4-64 mm), and smaller than boulders (>256 mm).

Epixylon: Algae colonizing woody substrata.

Epilimnion: Top layer of water of a stratified lake, denoted by highest temperatures and least dense water in the summer (Figure 1).

Epilithon: Algae colonizing rock substrata (Figure 4).



Figure 4. Algal epilithon colonizing the surface of a cobble.

Epipelon: Algae colonizing silt substrata.

Epiphyton: Algae colonizing surface of aquatic plants (Figure 5).



Figure 5. Algal epiphytes growing on the surface of an aquatic moss.

Epipsammon: Algae colonizing sand substrata.

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Euphotic zone (or “Photic zone”): The upper layer of lake water where sunlight penetrates and photosynthesis can occur.

Eutrophic: Having high primary production. In lakes, this is often a response to nutrient enrichment leading to increased production of algae or algal blooms.

Hypolimnion: The dense bottom layer of a stratified lake that sits below the thermocline (Figure 1). This layer is denoted by cooler summer temperatures and slightly warmer winter temperatures relative to the epilimnion.

Metalimnion: The layer of water in a stratified lake that sits between the hypolimnion and the epilimnion. Often equated with the thermocline (Figure 1).

Oligotrophic: The ecosystem response to low nutrient content. In lakes, this often equates to very clear water and little algal production.

Pelagic: The part of the lake that is not near shore or close to the bottom.

Periphyton: Mixture of algae, cyanobacteria, microbes, and detritus that coats submerged surfaces in most bodies of water.

Phytoplankton: Microscopic photosynthesizing organisms that inhabit the upper layers of bodies of water that are exposed to sunlight.

Sand: Small sediment particles, 0.062-2.0 mm diameter.

Silt: Very small sediment particles, 3.9-62.5 µm diameter.

Stratified: Indicating the presence of a thermocline.

Thermocline: A distinct layer in a body of water where the change in temperature is more rapid than increasing depth - usually a change of more than 1°C per meter. The denser and cooler layer below the thermocline is defined by the hypolimnion. The warmer upper layer is termed the epilimnion.

Thalweg: The line of least resistance to water flow in a stream or river, often the line of maximum water velocity.

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3 METHOD

The goals of the Algae Sampling in Lakes and Non-wadeable Streams Protocol are to quantify biodiversity, richness, and biomass (or biovolume) at each lake and non-wadeable stream site. These variables will be used to build a database over time, so changes can be tracked in algal species presence/absence, community structure and function, and the introduction of invasive species.

Phytoplankton samples are collected as an integrated water column sample at the central location of the lake or in the thalweg of the non-wadeable streams. Two additional samples are taken at the inlet and outlet of the lake near water chemistry sampling locations, or from the river-left and river-right littoral habitats of the non-wadeable streams. Samples are taken three times per year in order to capture multiple species presence and abundance. Benthic periphyton samples are taken following the divisions set forth in the Lake Riparian Mapping Protocol (RD[11]) and the Stream Riparian Mapping Protocol (RD[12]) at sites where safety and permitting allows the field technicians to step out of the boat and wade.

Algal sampling occurs three times per year. Timing of sampling is site-specific and determined based on historical hydrological and meteorological data. Specific details on sample dates are provided in the NEON Aquatic Sample Strategy Document (RD[08]) and Appendix D. Sample bout 1 is an early-season date, representing a period of rapid biomass accumulation after winter, typically after ice-off (where applicable) and prior to leaf out. Sample bout 2 targets mid-summer conditions. Sample bout 3 represents the late growing season (typically autumn) during leaf-fall. These dates will differ on a site-by-site basis, but should always occur at or near baseflow conditions in the watershed

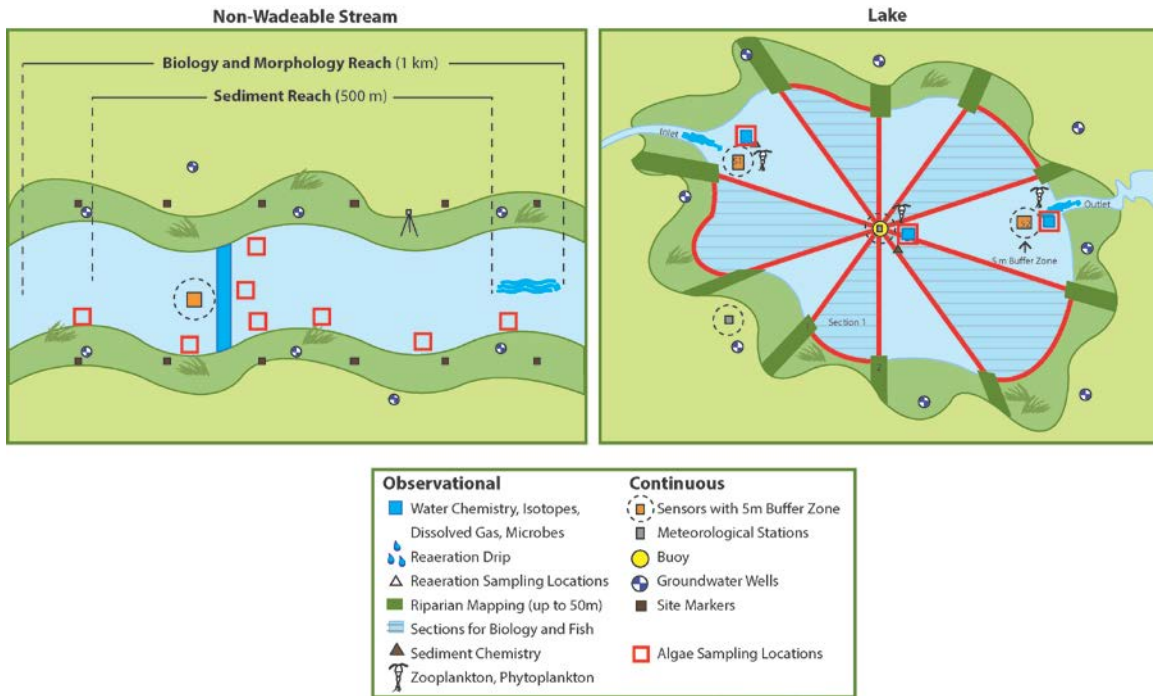


Figure 6. A generic site layout for lakes and non-wadeable streams with phytoplankton and benthic algae sampling locations.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON’s problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON’s problem tracking system.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[05]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[06]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Lake and non-wadeable algal sampling occurs three times per year at each site, roughly spring, summer, and autumn. Sampling must occur within the 1 month window specified in Appendix D, with a minimum

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of two weeks between sampling dates. Accommodations for local weather conditions (e.g., late ice-off) may be made that cause the sample date to fall outside of the pre-determined window.

4.2 Criteria for Determining Onset and Cessation of Sampling

A range of dates for each site were determined *a priori*, based on historical data including ice on/ice off, the accumulation of degree days, weather, and riparian phenology (Appendix D).

4.3 Timing for Laboratory Processing and Analysis

All lab processing must begin within 24 hours of field sampling:

1. Day 1: 3-8 hours for filtering samples and preserving taxonomy samples
2. Day 2: 1-2 hours for weighing dried filters
3. Day 3: 1-2 hours for weighing ashed filters

Dried samples may be stored between each lab processing day if necessary, days do not need to be consecutive. Taxonomy samples may be stored at 4 °C for up to 30 days. Filters may be stored at -20 °C for up to 14 days. For additional storage and shipping timelines see SOP E.

4.4 Sampling Timing Contingencies

All samples from one sampling bout must be collected within one day (i.e., all samples per site as detailed in this protocol). A minimum of 2 weeks between sample periods shall be observed.

Table 1. Contingent decisions

Delay/Situation	Action	Outcome for Data Products
Hours	If circumstances occur that impede sampling (e.g., wildlife, weather), discard samples and start over the next day that conditions permit.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the weather requirements below, continue to collect samples.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If weather conditions deteriorate and the lake/non-wadeable stream becomes too windy (>9 km hr ⁻¹) to hold the boat stationary over a sampling point, return to shore and wait in a safe location for 30 minutes. If wind subsides, resume sampling, if not, return to the Domain Support Facility and sample at another time.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If you are able to return to the lake/non-wadeable stream to sample within 24 hours, you may keep samples from the previous day. If you are not able to return within 24 hours, discard any previously collected samples in the lake/non-wadeable stream or at the Domain Support Facility and start over.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
5 or More Days	Samples shall be taken a minimum of 5 days following a major flow event (>25% change in flow within 15 minutes and/or turbidity levels are double the monthly average), allowing for recolonization before sampling occurs.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.

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4.5 Sampling Specific Concerns

1. Including bryophyte or aquatic plant leaves in periphyton samples artificially increases chlorophyll concentration. Take care not to scrub substrata with attached leaves.
2. Take care to keep track of the volume of water used to scrub the sample in the field and the volume of water used for filtering in the lab, these data are very important for conversion to higher data products.
3. Failure to completely mix sample before filtering can result in skewed results. All subsamples are meant to be representative of one-another, so careful mixing is a necessity.

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

Safety Data Sheets (SDS) shall be readily available and reviewed for all chemicals used during this task.

See Section 10 in the NEON Operations Field Safety and Security Plan (AD[02]) for aquatic-specific field safety requirements. In addition, the following safety requirements are sought:

1. Due to site-specific hazards that may be encountered, technicians may conduct sampling from the boat, without dismounting from the vessel. In addition, technicians are required to use extra caution in waters where alligators are present and to make sure a safe distance from hazards is maintained.
2. All personnel must be wearing a personal flotation device prior to entering the boat.
3. All employees shall have access to a form of communication with other team members such as a two-way radio.
4. Technicians should be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.)

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6 PERSONNEL AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Table 2. Equipment list – General equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Site-specific Bathymetry Map	Determining sampling locations	1	N
	R	Site-specific Riparian Vegetation Map	Determining sampling locations	1	N
	S	Clipboard	Recording data	1	N
	R	Cooler, 9-28 quart	Field sample storage; use size appropriate to samples being collected	1	N
	R	Knee boots or waders (pair)	Boating or wading	1 per person	N
Consumable items					
RD[06]	R	Aquatic Field Metadata Sheet	Recording metadata	1	N

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Item No.	R/S	Description	Purpose	Quantity	Special Handling
RD[07]	R	Secchi disc and Depth profile Data sheet	Recording depth profile data	1	N
RD[05]	R	Field data sheets (print on all-weather paper, write in pencil)	Recording data	2	N
	R	Pre-printed all-weather adhesive labels, 2"x4"	Labeling samples	15	N
	R	Pre-printed all-weather paper labels	Labeling samples	1 sheet	N
	R	Pencils	Recording data	4	N
	R	Permanent markers	Labeling samples	4	N
	R	Ice packs	Keeping samples cool	1-4	N

R/S=Required/Suggested

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Table 3. Equipment list – Phytoplankton sampling

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Handheld GPS unit (with batteries, ± 1 m accuracy) or Humminbird	Navigation to sampling location	1	N
MX100393	R	Kemmerer sampler with rope and messenger	Collecting water	1	N
MX100447	R	Secchi disk and weight	Determining the depth of the euphotic zone	1	N
	R	Braided polyester line, calibrated	Determining the depth of the euphotic zone	1	N
	R	Multisonde	Measuring water temperature for temperature profile	1	N
	R	4 L HDPE jug	Mixing integrated samples	1	N
	R	Amber HDPE wide-mouth sample bottles with caps, 1 L	Sample container	9	N
Consumable items					
		(None)			

R/S=Required/Suggested

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Table 4. Equipment list – Benthic sampling

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
	R	Template (35 mm plastic slide cassette)	Sampling area for rock and wood scrubs	Littoral cobbles and woody snags	1	N
	R	HDPE bottles with lids, amber wide-mouth (125 mL)	Sample container; container size is selected by technicians (either 125 mL or 250 mL)	All	15	N
	R	HDPE bottles with lids, amber wide-mouth (250 mL)	Sample container; container size is selected by technicians (either 125 mL or 250 mL)	All	5	N
	R	Wire-bristle brush (brass), toothbrush-size	Epilithon scrubbing	Littoral cobbles	1	N
	R	Nylon-bristle toothbrush	Epixylon scrubbing	Littoral woody snags	1	N
	R	Larval insect tray, plastic	Scrubbing container in which sample is collected	All	1	N
	R	125 mL unitary wash bottle	Rinsing substrate and larval tray into sample bottles	All	1	N

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Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	25 mL graduated cylinder, plastic	Measuring extra rinse water	All	1	N
	R	Petri dish lid, plastic, 47 mm diameter	Epipsammon and Epipelon collection	Littoral sand and silt	1	N
	R	Spatula (metal, offset)	Epipsammon and Epipelon collection	Littoral sand and silt	1	N
	R	Scissors	Epiphyton collection	Littoral plant surfaces	1	N
	R	Metric ruler	Epiphyton collection	Littoral plant surfaces	1	N
Consumable items						
	S	Razor blade	Epilithon scraping	Littoral cobbles	2	N
	R	Whirl-paks®, 24 oz.	Epiphyton container	Littoral plant surfaces	20	N
	R	Resealable bags, gallon	Organizing samples, Epiphyton container	Littoral plant surfaces	10	N
	R	DI water	Rinsing substrata	All	1 L	N

R/S=Required/Suggested

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Table 5. Equipment list – General boating

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Boat		1	Y
	R	Anchor with rope		2	N
	R	Oars		2	N
	R	Trolling Electric Motor		1	Y
	R	Battery (12 volt)		1	Y
	R	Safety kit for boat (e.g., flares, bailer, float with rope)		1	Y
	R	First Aid Kit		1	N
	R	Personal Flotation Devices (PFDs)		1 per person	N
Consumable items					
		(None)			

R/S=Required/Suggested

Table 6. Equipment list – General lab supplies

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Wash bottle, unitary, 125 mL	Rinsing the filter funnel	1	N
	R	Waste container (for non-hazardous liquids)	For rinse water and unused sample	1	N
Consumable items					
RD[05]	R	Lab data sheets	Recording data	1	N
	R	Adhesive weatherproof labels (~1"x2")	Labeling samples and filters	1 sheet	N
	R	Aluminum foil	Wrapping GF/F filters for shipment	1	N
	R	Zip-top resealable bags, quart size	Organizing and storing filters	2	N
	R	DI water	Rinsing the filter funnel	1 L	N

R/S=Required/Suggested

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Table 7. Equipment list – Epiphyte processing

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Soft toothbrush	Scrubbing epiphytes	1	N
	R	Ruler (metric) or calipers	Measuring length of epiphytes	1	N
	R	Larval tray, plastic	Scrubbing container in which sample is collected	1	N
Consumable items					
	R	Paper lunch bags	Drying plants associated with epiphyte samples	20	N

R/S=Required/Suggested

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Table 8. Equipment list – Filtering

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX100386	R	Filter funnel (25 mm diameter)	Filtering samples	1	N
MX100388	R	Vacuum filter flask (1L)	Filtering samples	1	N
	R	Vacuum pump	Filtering samples	1	N
	R	Filter forceps (flat ends)	Handling filters	1	N
	S	Hand-held stirrer (periphyton homogenizer)	Homogenizing periphyton and breaking up clumps of algae	1	N
	R	Graduated cylinder, 250 mL	Measuring and adding aliquots of sample to the filter funnel	1	N
	R	Adjustable pipette, 10 mL	Measuring and adding aliquots of sample to the filter funnel	1	N
Consumable items					
	R	Pipette tips, 10 mL	Measuring and adding the volume of sample into the filter funnel	1	N
MX106350	R	GF/F filters (25 mm diameter, pre-ashed)	Filters for AFDM and chemistry samples	90	N

R/S=Required/Suggested

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Table 9. Equipment list – Ash-free dry mass

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	S	Plastic weigh boats	Measuring dry weight	20	N
	R	Aluminum weigh boats	AFDM sample processing	20	N
	R	Analytical balance	Measuring weight	1	N
	R	Drying oven	Drying samples	1	N
	R	Muffle furnace	Burning organic matter for ash-free dry mass determination	1	N
	S	Aluminum baking pan	Sample storage in muffle furnace	2	N
	R	Heat-proof gloves (pair)	Safe handling of equipment in the muffle furnace and drying oven	1	N
	R	Crucible tongs	Safe handling of equipment in the muffle furnace and drying oven	1	N
	R	Desiccator (bench top)	Storing dried samples	1	N
Consumable items					
	R	Desiccant packs	For bench top desiccator	1-2	N

R/S=Required/Suggested

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Table 10. Equipment list – Algae preservation

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Freezer (-20 °C)	Sample storage	1	N
Consumable items					
	R	Glutaraldehyde, Grade II, 25% in H ₂ O	Preserving samples	1 L	Y
	R	High-iodine Lugol's solution	Preserving phytoplankton samples	1 L	Y
	R	HDPE bottles with lids, wide-mouth (60 mL)	Periphyton sample container	5	N
	R	HDPE bottles with lids, wide-mouth (1 L)	Phytoplankton sample container	3	N

R/S=Required/Suggested

Table 11. Equipment list – Shipping

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Dry ice shipping container	Shipping filters	1	N
	R	Non-dry ice shipping container (e.g., 9 qt cooler)	Shipping taxonomy samples	1	N
Consumable items					
	R	Dry ice	Shipping filters	1 lb	N
	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	TBD	N
	S	Cardboard box (~9"x7"x7")	Shipping taxonomy samples	1	N
	R	Shipping inventory (RD[16])	Provides sample information to external lab	1	N

R/S=Required/Suggested

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6.2 Training Requirements

Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]).

All personnel required to operate a boat shall be trained through an approved program. All others shall be aware of boating safety procedures.

Personnel will be trained in the field protocols associated with this document, and trained in safe working practices for lake- and river-based field work. Technicians must also be trained in safe handling of glutaraldehyde (AD[03]).

6.3 Specialized Skills

Where applicable, personnel will be licensed to operate a boat and able to safely handle a motor and drive a boat safely.

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

Field sampling requires two technicians for three hours per site, plus travel to and from the site. Lab processing requires one technician for 3-8 hours within 24 hours of field sampling, one technician for 1-2 hours the second day, and one technician for 1-2 hours the third day.

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7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Labels

1. Print all-weather adhesive labels and adhere to sample bottles (Figure 7, RD[05]).

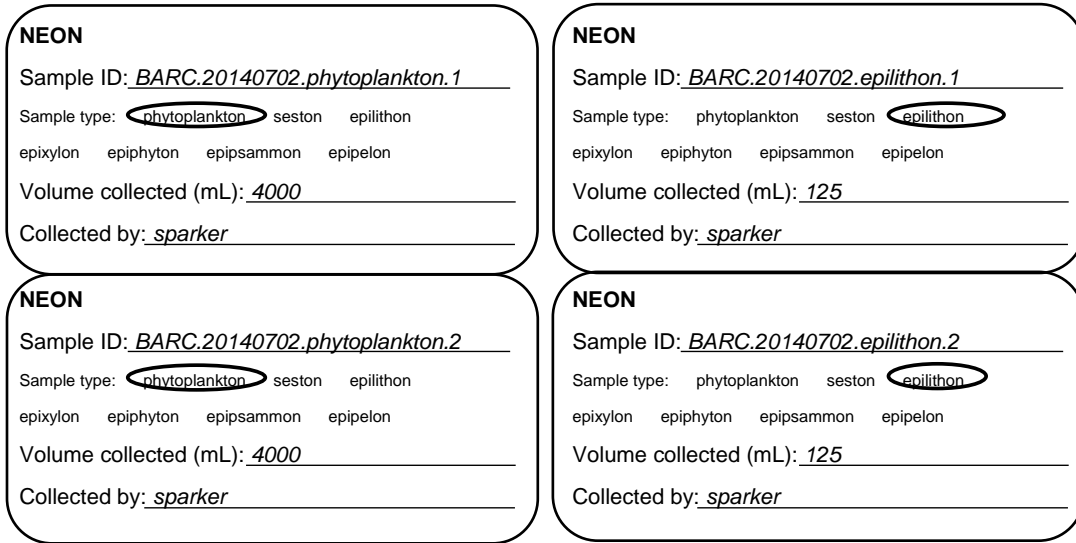


Figure 7. Example of field labels (2" x 4") for phytoplankton and periphyton sample bottles. These labels should be filled out with permanent marker.

2. Print all-weather paper labels for epiphyte samples. Cut labels apart using scissors. One label will be placed inside each Whirl-pak®.

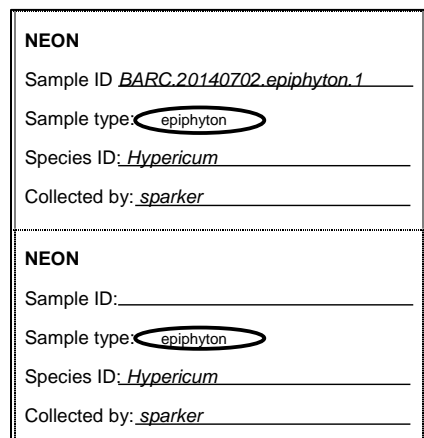


Figure 8. Example of field labels to be printed on all-weather paper. These labels should be filled out with pencil.

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3. Print field datasheets (RD[05]), aquatic general field metadata sheet (RD[06]), and Secchi and depth profile data sheets (RD[07]).

A.2 Equipment

1. Collect and prepare all equipment, including sample bottles, filters, and labels.
 - a. Load GPS sampling coordinates on handheld GPS unit.
2. Open 35 mm slide template (like a book) and separate into two halves (Figure 9). You will get two rectangular templates from each slide cassette.

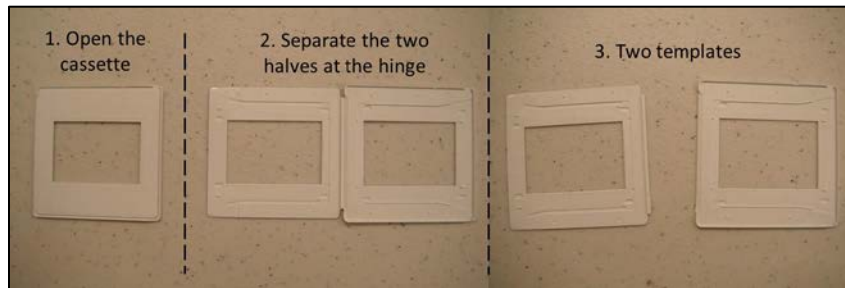


Figure 9. Separating the two halves of the 35 mm slide template.

3. Have ice or ice packs frozen and ready for cooler.
4. Check that all equipment is clean and in good condition and all batteries are charged.
5. See Laboratory Preparation procedures in SOP C.2 for additional pre-sampling activities (e.g., filter and weigh boat preparation).
6. Fill out general aquatic field metadata sheet (RD[06]) and Secchi and depth profile data sheet (RD[07]) upon every field sampling visit.

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SOP B Determining Habitat and Sampler Type

B.1 Decision Tree: Determining Habitat to Sample

1. If permits are not in place allowing the sampling crew to step out of the boat near the shoreline (see AD[02]), periphyton sampling will need to take place from the boat (SOP B.3).
2. Pelagic sampling
 - a. Lakes: Phytoplankton samples are collected at the center (buoy), inlet, and outlet.
 - b. Non-wadeable streams: Phytoplankton samples are collected at the sensor set, river right, and river left.
3. Littoral sampling
 - a. Determine which periphyton substrata to sample in the littoral areas (Appendix E). Substrata must account for >20% of littoral habitat.
 - 1) The habitat type chosen should be present during all sampling bouts.
 - 2) All 5 replicate samples must be taken from the same habitat type on each sampling bout, unless a major event (i.e., a flood) causes significant changes to the substrata.
 - 3) Replicates may be taken on either river right or river left (or a combination of the two) within the riparian transects.
 - 4) Targeted substratum types, in order of sampling preference (see Definitions, 2.5). If the a preferred substratum is present in high enough density, that takes precedence over a less preferred, more dominant substratum type (e.g., if silt is the dominant substratum type but cobbles are present in high enough density to sample consistently, cobble sampling takes priority).
 - a) Cobble (epilithon)
 - b) Woody snag (epixylon)
 - c) Plant surface (epiphyton)
 - d) Sand (epipsammon)
 - e) Silt (epipelon)

B.2 Phytoplankton Sampling

1. Sample in three locations per lake or non-wadeable stream.
 - a. Lakes (near water chemistry sampling locations):
 - 1) Deepest point in the lake, determine by bathymetric site map (RD[13]) and preloaded GPS coordinates
 - 2) Near the lake inlet
 - 3) Near the lake outlet
 - b. Non-wadeable streams (5-10 m below sensor set):
 - 1) Thalweg
 - 2) Half the distance between the thalweg and the right bank
 - 3) Half the distance between the thalweg and the left bank

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2. Navigate the boat to the sampling location.
3. Gently lower anchors at the bow and stern so as not to suspend sediments.
 - a. Allow ~5 minutes for sediments to settle after lowering the anchor; you can use this time to prepare the sampling equipment.
 - b. The boat must be anchored at the bow and stern in order to collect representative water column samples.
4. Always sample near the bow of the boat to minimize the effects of the motor on the water column.
5. Determine the total water depth from the sonar readings.
6. Determine the depth of the euphotic zone (depth to which 1% of light penetrates) using the Secchi disk.



- a. Lower the Secchi disk slowly into the water on the shady side of the boat (or cut the glare from the sun using your hand or other object) until the white quadrants disappear from view. NOTE: Do not wear sunglasses as this will interfere with the readings.
- b. Record depth read from the lines on the Secchi rope to the nearest 0.1 m on the Secchi depth field data sheet (Figure 11, RD[07]).
- c. Lower the Secchi disk approximately 0.5 m deeper than the first reading.
- d. Slowly pull the disk up until the white quadrants reappear, record depth to nearest 0.1 m on field data sheet as “Secchi 2”.
- e. Take the mean the two depths and record on the data sheet (RD[07]). Multiply value by 2.5 to determine depth of the euphotic zone.
- f. Repeat at each sampling location.

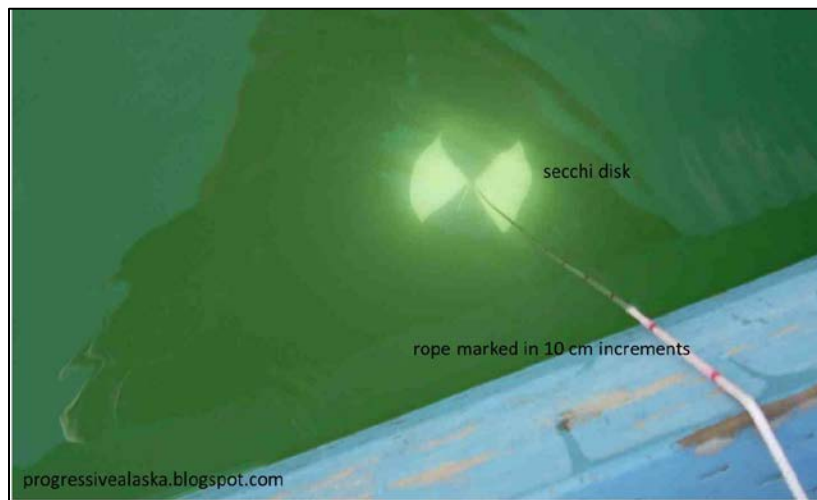


Figure 10. Example of a Secchi disk underwater.

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NEON Periphyton and Phytoplankton Collection							
Lakes and Non-wadeable Streams							
Site (4-letter code): <i>BARC</i>				Recorded by: <i>sparker</i>			
Date (YYYYMMDD): <i>20140702</i>				Collected by: <i>bjensen</i>			
Local time (HH:MM): <i>13:40</i>				Sampling protocol & Rev.: <i>NEON.DOC.001203vB</i>			
PHYTOPLANKTON							
Location ID	Sample type	Replicate	Phyto		Sample ID	Sample volume (mL)	Notes
			Phyto depth 1 (m)	depth 2 (m)			
<i>IN</i>	<i>phytoplankton</i>	<i>1</i>	<i>1.0</i>	<i>NA</i>	<i>BARC.20140702.phytoplankton.1</i>	<i>4000</i>	
<i>OT</i>	<i>phytoplankton</i>	<i>2</i>	<i>1.0</i>	<i>NA</i>	<i>BARC.20140702.phytoplankton.2</i>	<i>4000</i>	
<i>C0</i>	<i>phytoplankton</i>	<i>3</i>	<i>1.0</i>	<i>3.2</i>	<i>BARC.20140702.phytoplankton.3</i>	<i>4000</i>	
PERIPHYTON							
Location ID	Habitat	Substratum size class	Sample type	Replicate	Sample ID	Sample volume (mL)	Notes
<i>S1</i>	<i>littoral</i>	<i>sand</i>	<i>epiphyton</i>	<i>1</i>	<i>BARC.20140702.epiphyton.1</i>	<i>NA</i>	
<i>S3</i>	<i>littoral</i>	<i>sand</i>	<i>epiphyton</i>	<i>2</i>	<i>BARC.20140702.epiphyton.2</i>	<i>NA</i>	
<i>S5</i>	<i>littoral</i>	<i>sand</i>	<i>epiphyton</i>	<i>3</i>	<i>BARC.20140702.epiphyton.3</i>	<i>NA</i>	
<i>S7</i>	<i>littoral</i>	<i>silt</i>	<i>epiphyton</i>	<i>4</i>	<i>BARC.20140702.epiphyton.4</i>	<i>NA</i>	
<i>S9</i>	<i>littoral</i>	<i>sand</i>	<i>epiphyton</i>	<i>5</i>	<i>BARC.20140702.epiphyton.5</i>	<i>NA</i>	

Figure 11. Example field sheet for phytoplankton and periphyton collection. Blank field sheets can be found in RD[05].

7. Determine the depth of the thermocline (if present) at the sampling location using the handheld meter. Record on field data sheet (Figure 11, RD[07]).
 - a. Put the handheld meter into the water and slowly lower through the water column, stopping at 0.5 m intervals to note depth and water temperature.
 - b. Note the depth and water temperature every 0.5 m on the field data sheet.
 - c. Thermal stratification occurs where the rate of decrease in temperature with increasing depth is greatest (usually >1 °C per 0.5 m depth change).
8. If thermal stratification is present, proceed to Step 9 where sampling depth is based on the epilimnion. If stratification is not present, proceed to Step 10 where sampling depth is based on the euphotic zone.
9. For a stratified lake/non-wadeable stream, determine sample depths from the temperature profile (Figure 12). Measure depth to the bottom of the Kemmerer sampler (minimum depth for the Kemmerer sampler is 1 m). Record depths on field data sheet (Figure 11, RD[05]).
 - a. If epilimnion is ≤1 m deep:
 - 1) Surface (1 m below water surface) – integrate 2 Kemmerer samples
 - b. If epilimnion is >1 m deep, integrate the following samples:
 - 1) Surface (1 m below water surface) – 1 Kemmerer sample
 - 2) Depth (hypolimnion, 1 m below thermocline) – 1 Kemmerer sample

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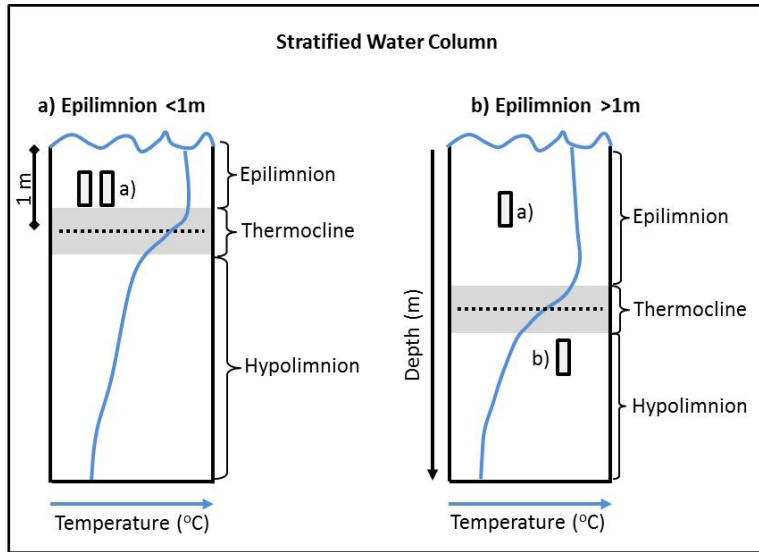


Figure 12. Examples of sample depths in a stratified water column. The blue line is a temperature profile indicating the presence of a thermocline (i.e., colder water near the lake/non-wadeable stream bottom). Regardless of total depth, a surface sample will be taken and integrated 1 m below the water surface (a). Where the epilimnion is >1m, an additional sample will be taken 1 m below the thermocline and integrated (b).

10. For a non-stratified lake/non-wadeable stream, determine the depths at which to sample from the Secchi disk readings. Measure depth to the bottom of the Kemmerer sampler (minimum depth for the Kemmerer sampler is 1 m).
 - a. Determine the depth of the euphotic zone by multiplying the mean Secchi depth by 2.5.
 - b. If euphotic zone is ≤ 1 m deep:
 - 1) Surface (1 m below water surface) – integrate 2 Kemmerer samples
 - c. If euphotic zone is >1 m deep, integrate the following samples:
 - 1) Surface (1 m below water surface) – 1 Kemmerer sample
 - 2) Depth (1 m above bottom of the euphotic zone) – 1 Kemmerer sample

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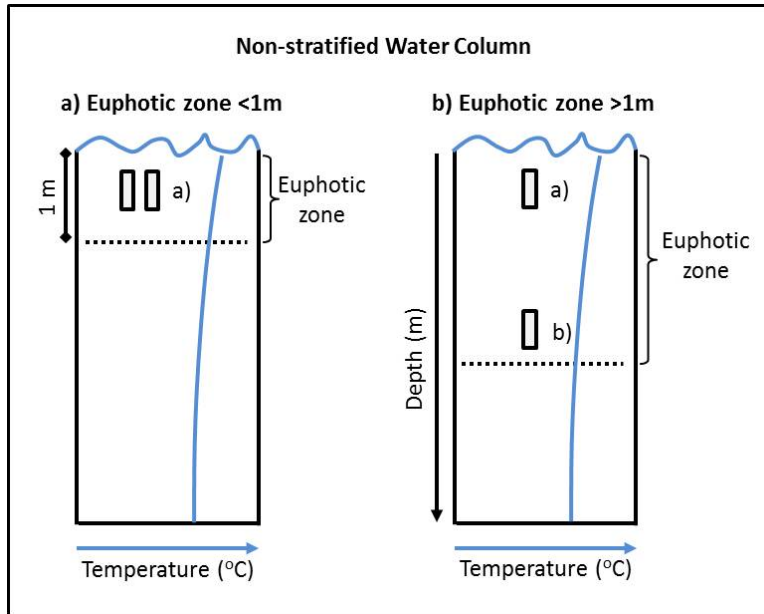


Figure 13. Examples of sample depths in a non-stratified water column. The blue line is a temperature profile indicating that a thermocline is not present (i.e., water is a similar temperature throughout). Regardless of total depth, a surface sample will be taken 1 m below the water surface (a). Where the euphotic zone is >1m, an additional sample will be taken 0.5 m above the bottom of the euphotic zone and integrated (b).

11. Rinse the 4 L HDPE jug and Kemmerer with lake/non-wadeable stream water over the opposite side of the boat from where you plan to sample. Discard rinse water into the lake/non-wadeable stream. Set 4 L jug aside.

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12. Cock the Kemmerer sampler by pulling the trip head into the trip plate by holding the top and bottom stopper and giving a short, hard pull to the bottom stopper (Figure 14).
 - a. NOTE: A short, hard pull is important to keep the stoppers open. If the stoppers don't stay open, pull harder.

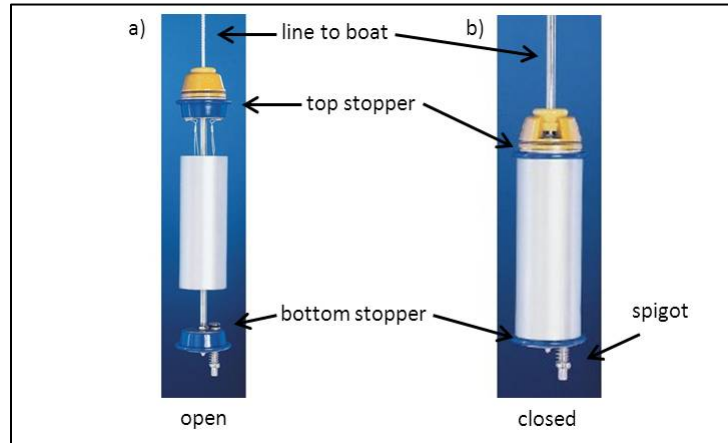


Figure 14. Vertical Kemmerer water sampler in the a) open or cocked position and b) closed (after dropping the messenger) position

13. Tie the free end of the Kemmerer line to a cleat on the boat to prevent losing the sampler.
14. Always start with the shallowest sample first to avoid disturbing the water column (i.e., sample surface water first and bottom of thermocline last).
15. Always sample near the bow of the boat to minimize the effects of the motor on the water column. When anchored, the bow of the boat tends to orient itself with the bow into the wind or current.
16. Carefully lower the sampler over the side of the boat into the water. Keep the messenger on the portion of the rope that remains in the boat. Hold the messenger in one hand. Ensure it is secured properly to the line.
17. Continue to lower the sampler until it reaches the desired depth (see Step 9), by using the depth markings on the line attached to sampler.
18. When the sampler has reached the desired depth, drop the messenger down the line to the sampler. The messenger will trigger the stoppers to close (Figure 14b).
19. Pull the sampler up by pulling the line into the boat, coiling it neatly.
 - a. Note: If the Kemmerer does not close properly when you pull it up, resample in the same location.
20. Uncap the 4 L HDPE jug.
21. Carefully open the spigot (Figure 14b) on the bottom of the Kemmerer sampler, allowing the contents of the cylinder to flow into 4 L jug.
 - a. Repeat Steps 12- 21 and combine both samples in the 4 L jug.
 - b. Mix integrated sample gently in 4 L jug.

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22. Rinse pre-labeled (Figure 7) sample bottles three times with lake/non-wadeable stream water, discard water back into the lake/non-wadeable stream.
23. Fill 4 1L amber HDPE bottles. Cap each bottle once filled.
 - a. Immediately place all sample bottles in cooler with ice packs until they can be refrigerated in the lab.
 - b. The 4 1L bottles = 1 composite sample.
 - c. Samples must be kept dark and cool until filtering.
24. Discard remainder of the water in the 4 L jug into the lake/non-wadeable stream.
25. Proceed to the next sample location and repeat above steps.

B.3 Periphyton (Benthic Algae) Sample Collection

1. Determine dominant substratum type (>50% of substrata around the entire lake/non-wadeable stream edge) in the shallow littoral area of the riparian sections.
 - a. If the a preferred substratum is present in high enough density, that takes precedence over a less preferred, more dominant substratum type (e.g., if silt is the dominant substratum type but cobbles are present in high enough sensity to sample consistently, cobble sampling takes priority).
 - b. The order of preference for sampling substrata is as follows (Porter et al. 1993):
 - 1) Epilithon (rock substrata) – proceed to SOP B.4
 - 2) Epixylon (wood substrata) – proceed to SOP B.4
 - 3) Epiphyton (plant substrata) – proceed to SOP B.5
 - 4) Epipsammon (sand substrata) – proceed to SOP B.6
 - 5) Epipelon (silt substrata) – proceed to SOP B.6
2. Determine sampling locations.
 - a. For lakes, refer to the site-specific riparian vegetation map (created in the Riparian Vegetation Mapping Protocol (RD[11]) which divides the lakeshore into 10 sections (Figure 15).
 - b. For non-wadeable streams (rivers), select 5 of the 10 riparian transects, and sample either in the river right or river left (or a combination of the two) littoral areas within those transects.

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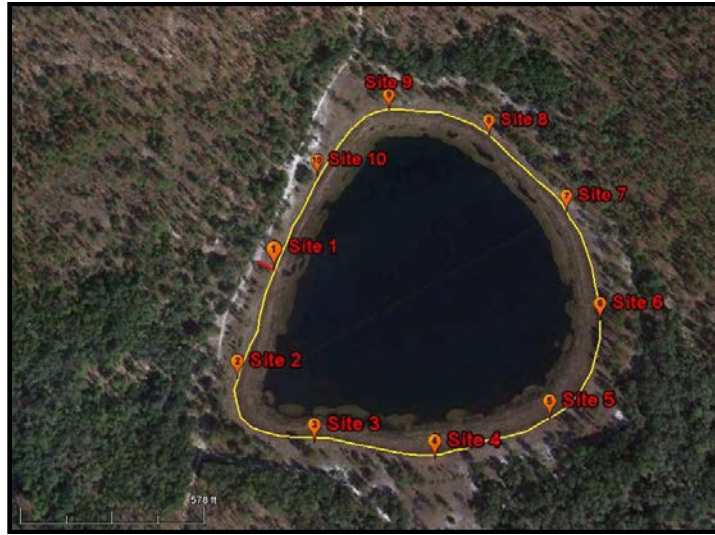


Figure 15. Example of lake perimeter subdivisions from the Lake Riparian Mapping Protocol (RD[11]).

3. Evenly partition the five samples around the Riparian Sections. If there is a section of the lake where the substrata changes, move to the next section where your chosen dominate substrata can be found.
 - a. Collect 1 sample per riparian section.
 - b. NOTE: All 5 samples must be sampled from the same type of substrata for all three sampling bouts.
 - c. The habitat/substratum type should be present during all sampling bouts.
4. Do not sample areas that have been or appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).

B.4 Epilithon (Rock Scrubs) and Epixylon (Wood Scrubs)

1. Label (2" x 4" adhesive labels) five 125 mL wide-mouth amber HDPE bottles with domain, date, site, sample number, location, habitat type, type of sample (e.g., rock scrub or epilithon), and collector's name (Figure 7; RD[05]).
 - a. Write in permanent marker on all-weather adhesive labels (RD[05]).
 - b. Adhere labels to bottles before bottles get wet.
 - c. **NOTE:** Sample bottles may be rinsed with DI and reused from the last sampling bout.
2. Select a sampling location with shallow (<1 m) water that appear to be historically wetted (i.e., usually underwater). Avoid areas that have been recently dried (signs of recent drying include: extremely shallow areas, rocks that have nothing growing on them, and rocks that are not slippery to the touch).
3. Select 3 cobbles or pieces of woody debris for each composite sample that meet the following requirements [after Richest Targeted Habitat (RTH) requirements; Porter et al. 1993 and Moulton et al. 2002]:



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- a. Representative of the periphyton cover of the area (i.e., not extremely dense or extremely sparse cover relative to nearby substrata).
- b. Stable in the lake/non-wadeable stream bottom (i.e., have not recently tumbled).
- c. Larger than the scrubbing template (i.e., >2 inches diameter).
- d. Avoid cobbles/pieces of woody debris that are heavily colonized with aquatic plants, bryophytes, invertebrates, or have leaf litter clinging to the surface (see Definitions, Section 2.4 for details on identifying aquatic plants and bryophytes).
- e. Avoid cobbles/woody debris that have noticeably tumbled or been recently disturbed.
- f. Avoid cobbles/woody debris that you or other observers may have recently stepped on.
- g. Do not choose all of the cobbles/woody debris from the same location.
4. Place cobbles/woody debris and right-side up (the side exposed to the sun) in the white larval tray. Take care to keep the cobble right-side up, this is the surface that is exposed to the sunlight, and will be the portion of the cobble that you sample.
5. Keep cobbles/woody debris moist with lake/non-wadeable lake/river water until scrubbing.
6. Proceed to a safe location on the bank or in the boat to process the sample. This location should be out of direct sunlight if possible.
 - a.
7. Rinse and fill the 125 mL wash bottle with DI water to “fill line” (as marked on bottle).
 - a. DI must be used to prevent additional phytoplankton from entering the sample.
 - b. Make sure that bottom of the meniscus lines up with the “fill line”.
 - c. **Since this is a volume-based sampling technique, it is important to keep the sample volume consistent and record the volume of water used.**
 - d. Depending on the productivity of the site, >125 mL may be necessary to meet the external lab filtering requirements. See Table 13 for recommended filter volume and adjust water volume and amber HDPE bottle size as necessary.
8. Rinse the inside of the amber wide-mouth HDPE sample bottle with lake/non-wadeable stream water – fill bottle ~1/4 full, cap, and shake vigorously. Discard rinse water into the lake/non-wadeable stream or on the bank. Repeat until bottle has been rinsed 3 times. Recap bottle and set aside.
 - a. Samples do not need to be numbered in any particular order (i.e., you do not have to fill bottles in order from 1-5), however the Riparian Section numbers must match the replicate number.
 - b. You can rinse all sample bottles for that site at the same time and set aside, or rinse them separately.
9. Holding cobble/woody debris underwater, briefly sweep any leaves and/or visible invertebrates from surface (you can use your hand to gently sweep insects off the cobble/woody debris surface, but take care not to scrub hard and dislodge periphyton).
 - a. Recheck cobble, if there are still more than 10 insects attached to the substratum within your template, discard and choose a new cobble/piece of woody debris.



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- b. If there is growth of aquatic plants on the surface and will fall within your template, discard and choose a new cobble.
10. Place cobble/woody debris right-side up (the side exposed to the sun) in white tray and pour any excess water out of the tray.
11. Place white slide template on top of cobble/woody debris (surface that was exposed to light at the stream bottom; Figure 16). Check cobble/woody debris again for colonization of invertebrates, bryophytes, or plants.
12. Holding the template firmly in place on the cobble/woody debris, begin scrubbing inside the template (scrub gently if woody debris).
 - a. If the substratum is colonized by a thick mat of algal material (e.g., *Didymosphenia geminata*), first scrape the inside of the template with a razor blade before scrubbing with a brush. Place scraped material in samples bottle with remainder of scrubbed samples.
 - b. Use the wire-bristled brush for cobbles, and use the toothbrush for woody debris.
 - c. Be sure to hold the template in place, as slipping would change the area you are sampling (Figure 16).
 - d. Scrubbing should be similar to brushing your teeth.



Figure 16. Template placement for epilithon protocol. (Note: Samples should not be processed in direct sunlight.)



13. Periodically rinse the inside area of the template using the 125 mL wash bottle **while holding the template in place**. Allow water to run into the white tray – **DO NOT DISCARD** rinse-water.
14. Continue scrubbing until the inside of the template is clean (scrub cobble longer than woody debris).
15. Remove template. There should be a clean rectangle left from your scrubbing.
16. Using the wash bottle, completely rinse the template (front and back), the cobble/woody debris.
17. Discard cobble/woody debris in the water if you are finished collecting substrata at that location.
18. Repeat Steps 12-18 until all 3 cobbles/woody debris collected for the composite sample have been scrubbed and rinsed.

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19. Rinse scrub brush and fingers over the tray. If there is any remaining water in the 125 mL wash bottle, dump this into the tray. All rinse-water should now be in the white tray and should total 125 mL, **do not discard as this is your sample.**

20. Discard cobble/woody debris.

- a. If you are finished at that location, you may discard the cobble/woody debris in the lake/non-wadeable stream.
- b. If you are not done at that location, hold the cobble/woody debris on the bank and discard in the lake/non-wadeable stream when you leave the site.

21. Carefully swirl contents of tray (scrubbed material + rinse-water) to re-suspend the sample.

22. Carefully pour sample water into 125 mL amber wide-mouth HDPE bottle. Take care not to spill any of the sample.

- a. If you do spill a small amount (i.e., <10 mL) of the sample at this point, it is ok because this is a volume-based approach.
- b. If you spill a significant amount (i.e., >10 mL) of sample, dump the entire sample and start over at Step 1.

23. There may be some sand left in the bottom of the tray after swirling and pouring out the sample. This is ok.

24. If you feel that your sample wasn't properly mixed and some has remained in the tray, you may pour the sample back in the tray and repeat Steps 22-24.

25. If you need more water to rinse with, refill the 125 mL wash bottle and use for rinsing. If you do this, be sure to use the entire 125 mL or measure using the 25 mL graduated cylinder, and make a note of this change in volume on the sample label and on the field data sheet. **It is very important to keep track of any changes in sample volume.**



- a. Additional water will also require that you use a larger sample bottle.

26. Cap bottle tightly and place in a cool storage location out of direct sunlight until sampling is finished.

- a. Place sample bottles in the cooler or submerge the bottles in stream water to keep them cool. If submerged, be sure that they will not float away. Place bottles in the cooler upon returning to the car.

27. Fill out field data sheet in pencil (Figure 11, RD[05]).

28. Rinse tray, brush, and template with lake/non-wadeable stream water before starting next sample.

29. Move to next lake/non-wadeable stream section and repeat above until all 5 lake sections have been sampled.

B.5 Epiphyton (Aquatic Plant Surfaces)

1. Label five all-weather paper labels (Figure 8, RD[05]).
2. Select plants for sampling that are well-colonized with epiphytes (Figure 17) and that meet the following requirements:

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- a. Plants should be exposed to ambient light (e.g., not under a log or cut bank).
- b. Plants should not be covered by sediments.
- c. Have not been recently disturbed or trampled.



Figure 17. Example of epiphytes growing on reeds in a Colorado stream.



3. Select a 10 x 10 cm area of lake/river bottom to sample where plants are rooted.
 - a. NOTE: If plant cover is thick, plants may be growing across the area and not necessarily rooted. Collect all rooted material within the selected quadrat.
 - b. Use a metric ruler to estimate the area to sample.
4. Cut all plants within the 10 x 10 cm area at their bases using scissors or clippers and carefully place them inside a Whirl-pak[®] bag.
 - a. Cut off tops of plants growing above the waterline and discard, it is not necessary for these to be part of the sample.
 - b. Be gentle with the plants as epiphytes may be easily dislodged.
 - c. Do not add water to the Whirl-pak[®].
5. Close Whirl-pak[®] bag and place in a cool, dark location (e.g., cooler).
 - a. Close the Whirl-pak[®] by holding the wire tabs at either side of the bag (Figure 18), then whirl the bag at least 3 complete revolutions to form leakproof seal. Rather than whirling, you may also fold the top over as tightly as possible at least 3 times. Bend the wire ends over onto the bag to complete.

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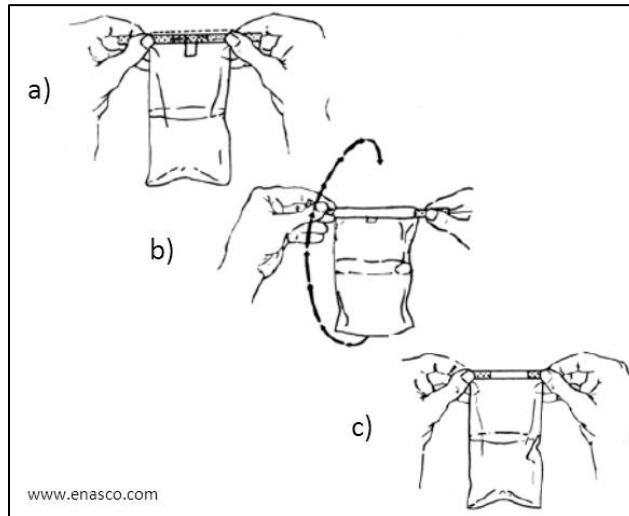


Figure 18. How to close a Whirl-pak[®] bag: a) hold the wire tabs; b) whirl the bag 3 complete revolutions (or fold the top over); and c) fold the wire ends over to close.


6. Fill out field data sheet in pencil (Figure 11, RD[05]).
7. Move to the next habitat station and repeat above steps until all 5 lake/non-wadeable stream sections have been sampled.
8. Return samples to the Domain Support Facility for further processing within 24 hours (SOP C).

B.6 Epipsammon (Sand) and Epipelon (Silt)

1. Label (2"x4" all-weather adhesive labels) five 250 mL amber HDPE bottles (Figure 7).
 - a. Write in permanent marker on all-weather adhesive labels.
 - b. Adhere labels to bottles before bottles get wet.
 - c. **NOTE:** sample bottles may be rinsed with DI and reused from the last sampling trip.
2. Choose sampling locations with relatively shallow (<1 m) water that appears to be historically wetted (i.e., are usually underwater). Avoid areas that have been recently dried (e.g., extremely shallow areas).
3. Select locations to sample that meet the following requirements [after Richest Targeted Habitat (RTH) requirements, Porter et al. 1993 and Moulton et al. 2002].
 - a. Representative of the epipsammon/epipelon habitat and periphyton cover of the sites (i.e., not extremely dense or extremely sparse periphyton cover).
 - b. Exposed to ambient light (e.g., not under a log or cut bank).
 - c. Avoid areas that are heavily colonized with aquatic plants, invertebrates, or have leaf litter covering the surface.
 - d. Have been recently disturbed (e.g., stepped on or sampled for another protocol).
 - e. Do not take all of the samples from the same location. Collect samples from different Riparian Sections.



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4. Rinse the inside of the 250 mL amber HDPE sample bottle with lake/river water – fill bottle ~1/4 full, cap, and shake vigorously. Discard rinse water into stream away from the location where you intend to sample. Rinse 3 times. Recap bottle and set aside.
 - a. Samples do not need to be numbered in any particular order (i.e., you do not have to fill bottles in order from 1-5).
5. Rinse white plastic sampling tray with lake/river water.
6. Rinse the lid of a 47 mm plastic petri dish in lake/non-wadeable stream water. Holding lid upside-down underwater, rub the inside of the lid with your fingers to remove air bubbles.
7. Lightly press the lid into the substratum to be sampled (like a cookie cutter). Take care not to disturb the substratum before placing lid on bottom. If substratum is disturbed, find a new sampling location.
8. Slide spatula under lid to enclose the sample. Holding the petri lid tightly to the spatula, lift out of water. Make sure that water and sediment do not leak out. Gently rinse excess silt not enclosed by petri lid from spatula with lake or non-wadeable stream water with a wash bottle. Do not count this rinse water in the final sample volume.
 - a. If sample leaks out of the petri lid when lifting the spatula out of the water, discard and resample.
9. Place spatula + sample + petri lid in white sampling tray and invert lid.
10. Repeat Steps 7-9 until you have collected 3 samples to combine into one composite sample.
11. Rinse and fill 125 mL wash bottle with DI to “fill line” (as marked on bottle).
 - a. Make sure that bottom of the meniscus lines up with the “fill line”.
 - b. Since this is a volume-based sampling technique, it is important to keep the sample volume consistent and record the volume of water used. If additional water is necessary for rinsing, use 25 mL graduated cylinder to add in increments. Keep track of volume used on field data sheet.
 - c. Depending on the productivity of the site, >125 mL may be necessary to meet the external lab filtering requirements. See Table 13 for recommended filter volume and adjust water volume and amber HDPE bottle size as necessary.
-  12. Using the 125 mL wash bottle, rinse petri lid and spatula into tray. Pour excess water from wash bottle into tray. **DO NOT DISCARD RINSE WATER.**
13. Carefully pour sample water into 250 mL amber HDPE sample bottle. Take care not to spill sample.
 - a. Use your fingers and the remaining water from the 125 mL wash bottles to help get the sample, including sand/silt, into the bottle.
 - b. If there is any water left in the 125 mL wash bottle after the tray is empty, pour this into the sample bottle to maintain a constant volume.
 - c. If additional water is needed, measure using the graduated cylinder and record total rinse volume on the field datasheet.

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14. Cap bottle tightly, double check labels, and place in a cool storage location out of direct sunlight until sampling is finished.
 - a. Place sample bottles in the cooler or submerge the bottles in lake/river water to keep them cool. If submerged, be sure that they will not float away. Place bottles in the cooler upon returning to the car.
15. Fill out field data sheet in pencil (Figure 11, RD[05]).
16. Rinse tray, petri lid, and spatula with lake/non-wadeable stream water before starting next sample.
17. Repeat above steps until 5 composite samples have been collected.

B.7 Sample Preservation

1. Samples must remain dark (in amber HDPE bottles) and cool (4 °C) until they are processed in the domain lab.
2. Samples must be processed in lab within 24 hours of field sampling; see lab protocol (SOP C).

B.8 Ending the Sampling Day

1. Refreshing the sampling kit
 - a. Remove old labels from HDPE bottles that will be reused.
 - b. Print and fill out new adhesive labels (RD[05]). Attach labels to bottles before going out in the field.
2. Equipment maintenance, cleaning and storage
 - a. Check depth markings on Kemmerer sampler and Secchi ropes, refresh markings if necessary.
 - b. Wash all equipment that has come in contact with lake/non-wadeable stream water according to the NEON Aquatic Decontamination Protocol (RD[09]).
 - c. Clean boat and motor; remove aquatic plants by hand to prevent spread of invasive taxa. Allow boat and motor to dry completely.
 - d. Rinse amber HDPE bottles with DI, these will be rinsed again in the field thoroughly during the next field collection.
 - e. Only reuse Amber HDPE bottles at the same site. Keep a separate set of bottles dedicated to each site.

Dry all items (except amber HDPE bottles) thoroughly between sites and before storage.

- f. Discard and replace any broken templates, petri dishes, or worn brushes.

SOP C Laboratory Sampling and Analysis

At the Domain Support Facility, phytoplankton and benthic algae samples will be processed for shipping to analytical facilities for the following parameters: chlorophyll *a* and pheophytin concentration, ash-free dry mass (AFDM), C, N, and P content, isotopes, and algal cell count and identification.

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C.1 Sample Processing Time

All lab processing must begin within 24 hours of field sampling:

1. Day 1: 3-8 hours for filtering samples and preserving taxonomy samples
2. Day 2: 1-2 hours for weighing dried filters
3. Day 3: 1-2 hours for weighing ashed filters

C.2 Preparation

1. Pre-ash GF/F filters (Table 12):
 - a. Place layers of 25 mm GF/F filters on aluminum foil using filter forceps or while wearing nitrile gloves. Use multiple layers of foil if needed, filters can be touching and placed on top of one another but should not be stacked more than 3 filters deep.
 - b. Place in muffle furnace (500 °C) for 6 hours.
 - c. After 6 hours, remove from furnace, stack filters using filter forceps, and place in original box.
 - d. Label box with permanent marker to read "ASHED, Your Name, Date".
 - e. Place box in sealed zip-top bag.
 - f. Ashed filter may be stored indefinitely, as long as they remain in the box and stay dry.

Table 12. Number of pre-ashed filters required per bout.

Site type	Number of samples (field)	Total number of filters needed per bout
Lake	8	72
Non-wadeable	8	72
Wadeable	9	81

2. Print 1"x2" adhesive labels for 60 mL bottles, chlorophyll *a*/pheophytin filters, and nutrient filters, to be attached to the outside of vials and/or aluminum foil packets (Figure 19, RD[05]).

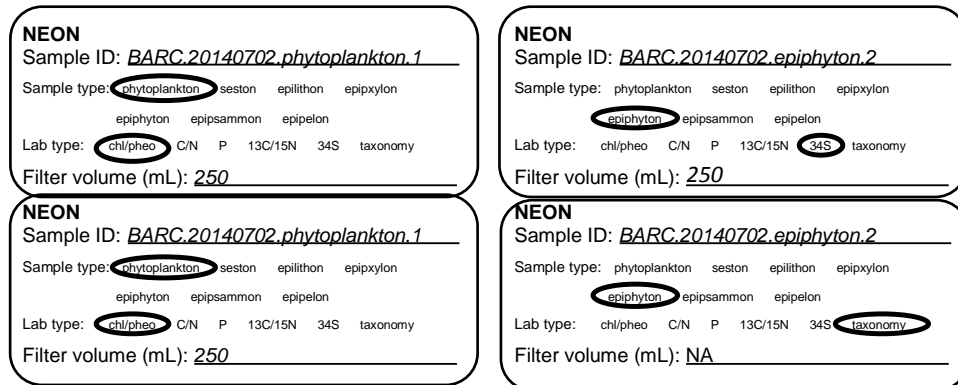


Figure 19. Example of adhesive labels (1" x 2") for periphyton lab samples

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3. If aluminum weigh boats are new, label ~20 boats by inscribing a unique number on the bottom with a pencil (Figure 20). Place boats in the muffle furnace (500 °C) for 6 hours. After 6 hours, carefully remove boats from the furnace using thermal gloves and tongs, and allow to cool.
 - a. This may be done in advance, before field sampling.
 - b. Boats may be reused from previous sampling bouts.



Figure 20. Examples of newly-labeled aluminum weigh boats.

C.3 Sample Processing in the Lab

Samples will be subsampled and filtered in the Domain Support Facility following Figure 21 and Table 13.

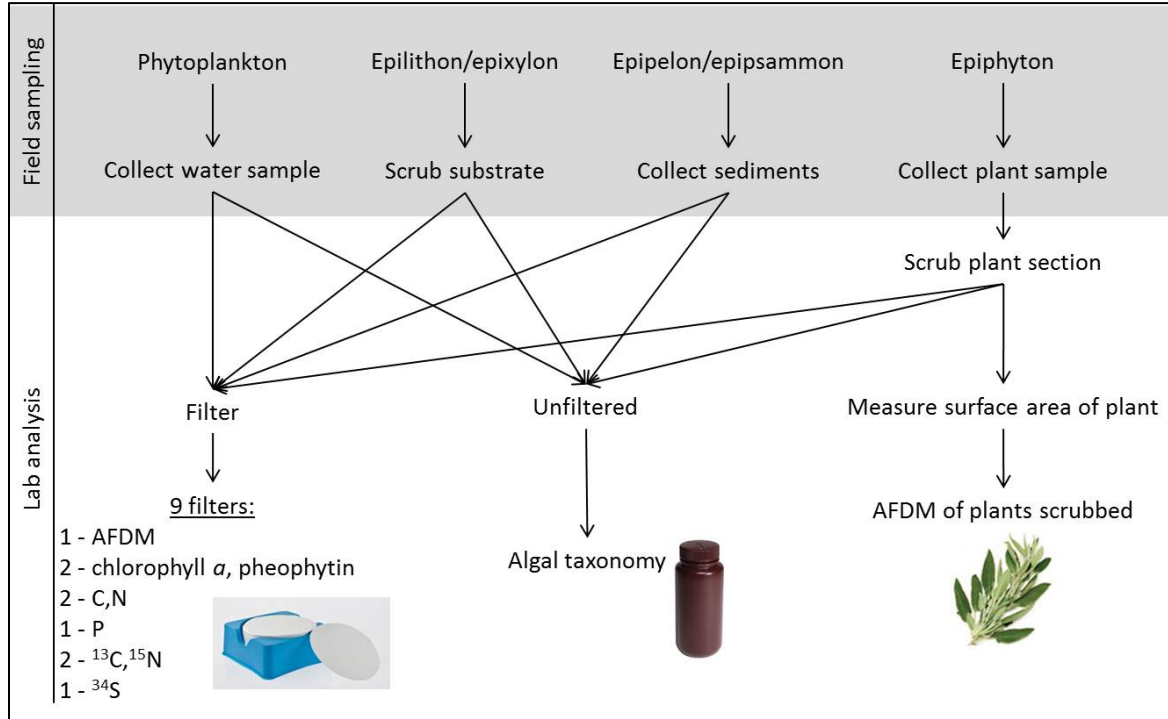


Figure 21. Lab schematic diagram

Table 13. Filtering amounts for chemistry. If unable to push the minimum recommended volume through a filter, then filter as much sample as possible and record on lab data sheet. Follow priority column if not enough sample is available to collect all filter types requested.

Priority	Sample	Parameter	Type	Recommended sample volume
1	Periphyton	Taxonomy	Liquid, preserved in glutaraldehyde	60 mL
2	Periphyton	Chlorophyll <i>a</i> /pheophytin	Filters (2)	5-10 mL (until visible color on filter)
3	Periphyton	AFDM	Filter (1)	5-10 mL (until visible color on filter)
4	Periphyton	C, N	Filters (2)	5-10 mL (until visible color on filter)
5	Periphyton	P	Filter (1)	5-10 mL (until visible color on filter)
6	Periphyton	$\delta^{13}\text{C}$, $\delta^{15}\text{N}$	Filters (2)	As much samples as possible, >10 mL
7	Periphyton	$\delta^{34}\text{S}$	Filter (1)	As much samples as possible, >10 mL


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1	Phytoplankton	Taxonomy	Liquid, preserved in Lugol's	1 L
2	Phytoplankton	Chlorophyll <i>a</i> /pheophytin	Filters (2)	≥250 mL (until visible color on filter)
3	Phytoplankton	AFDM	Filter (1)	≥250 mL (until visible color on filter)
4	Phytoplankton	C, N	Filters (2)	≥500 mL (until visible color on filter)
5	Phytoplankton	P	Filter (1)	≥500 mL (until visible color on filter)
6	Phytoplankton	$\delta^{13}\text{C}$, ^{15}N	Filters (2)	As much samples as possible, >500 mL
7	Phytoplankton	$\delta^{34}\text{S}$	Filter (1)	As much samples as possible, >500 mL

C.4 Epiphyte Samples

1. If there are no epiphyte samples, skip this section and proceed to SOP C.5.
2. **Day 1:** Using forceps, remove the plant sample from plastic sample bag. Place in clean, dry white plastic sample tray (used in field protocol).
3. Fill 125 mL wash bottle to fill line with DI water.
 - a. Make sure that bottom of the meniscus lines up with the “fill line”.
 - b. Since this is a volume-based sampling technique, it is important to keep the sample volume consistent and record the volume of water used.
 - c. Depending on the productivity of the site, >125 mL may be necessary to meet the external lab filtering requirements. See Table 13 for recommended filter volume and adjust water volume and HDPE bottle volume as necessary.
4. Gently scrub surface of sample with a toothbrush. You may have collected leaves and/or stalks, scrub these gently. Take care not to lyse plant cells, these would bias the chlorophyll results.
5. Use DI water periodically to rinse the scrubbed material into the tray.
6. When scrubbing is finished, rinse scrubbed plant and toothbrush thoroughly into the white tray.
7. Remove plant sample and measure the approximate dimensions of the surface area scrubbed (e.g., stalk length and/or leaf length + width, top + bottom) and record the sum in the lab data sheet.
 - a. Measure using calipers or a metric ruler.
 - b. Measure a subset of 5 plants. If <5 plants were collected, measure all.
8. Place plant material in a labeled paper lunch bag.
9. Pour remainder (if any) of 125 mL wash bottle into white tray.
10. Carefully pour the scrubbed material into a 125 mL amber wide-mouth HDPE sample bottle. Proceed to Filtering Protocol, SOP C.5.
11. Place paper bags containing samples in the drying oven for a minimum of 12 hours at 60 °C or until constant weight is achieved (i.e., mass varies by <2% over a one-hour period; RD[14]).

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- a. Use TOS “Lab Drying QC Datasheet” in Measurement of Herbaceous Biomass datasheets (RD[15]).
12. **Day 2:** When dry, remove all bags+samples from drying oven and let cool to room temperature in a plastic bag or desiccator.
 - a. Placing samples in a bag or desiccator is important because samples absorb water quickly from the air as they cool. Samples may be left in desiccator or plastic bags for up to 30 days before proceeding to the next step.
13. Place a clean, plastic weigh boat (small sample) or tray (large sample) on analytical balance. Tare (zero) balance.
14. Place dry sample in the plastic weigh boat/tray and record as **Epiphyton: total plant dry weight** on Lab Data Sheet (Figure 23, RD[05]).
 - a. Sample may be crushed or broken up to fit into weigh boat.
 - b. Place sample in a clean, pre-labeled aluminum weigh boat.
 - c. If sample does not fit in one aluminum weigh boat, grind in Wiley mill using 20 mesh (0.85 mm) screen.
 - d. Place a subsample of ground material in aluminum weigh boat.
 - e. Record **Boat ID** on Lab Data Sheet.
 - f. Clean grinding mill thoroughly with compressed air between samples.
15. Weight boat on 6-place balance, and record as **Dry weight + boat** on Lab Data Sheet.
16. Repeat above steps until all specimens have been processed.
- 
 17. Place aluminum boats + specimens in the muffle furnace using oven gloves and tongs. **TAKE CARE NOT TO BURN YOURSELF!**
 - a. Boats may be stacked on top of each other as long as there is space for air flow between them.
 - b. Place boats on an approved muffle furnace pan (if available) before placing in the furnace. This makes it easier and safer to handle samples.
18. Leave samples in the muffle furnace at 500 °C for at least 6 hours.
 - a. Sample may be left in furnace for longer than 6 hours (e.g., overnight) if necessary.
19. **Day 3:** After 6+ hours, remove aluminum boats carefully from the muffle furnace using oven gloves and crucible tongs. Place on trivet or metal cart to cool.
20. Cover pans/aluminum boats to prevent ash from blowing out of boats and aside to cool on a heat-resistant in an area without drafts from windows, doors, or HVAC ventilation.
21. When boats have cooled enough to handle, place in desiccator.
 - a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
22. After cooling to room temperature, weigh boats again on analytical balance, record as **Ash Weight** on Lab Data Sheet.
23. After weighing, dump ash into the trash and clean the boat with a soft brush or paper towel.
 - a. Set clean boats aside to be used again.

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C.5 Filtering (Chlorophyll a + Pheophytin, AFDM, C, N, and P, and Isotopes)

1. Set up filter funnel, filter flask, and vacuum pump (Figure 22).
 - a. Technicians may use the hand vacuum pump (Figure 22a) with one filter flask and funnel attached, or the filter manifold and electric pump (Figure 22b) with multiple filter funnels.
 - b. Attach flexible tubing to from vacuum pump to hose connection on filter flask.
 - c. Make sure filter stem is inserted into the hole in the rubber stopper.
 - d. Insert rubber stopper into the top of the filter flask. Push in tightly.
 - e. Attach top of filter funnel to filter stem. This may be a magnetic connection or a screw-in connection.

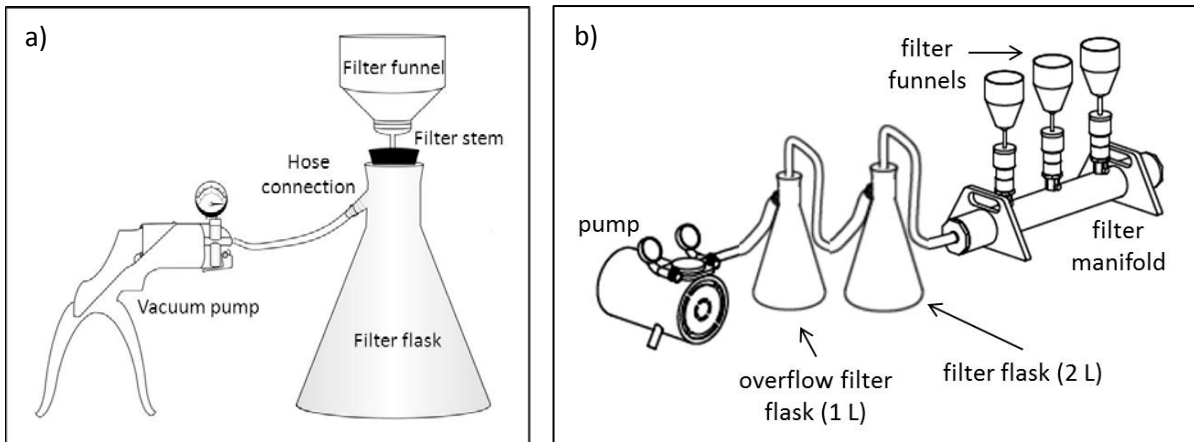


Figure 22. Filter apparatus setup: a) hand pump; b) filter manifold.

2. Remove the top of the filter funnel from apparatus, rinse with DI water.
3. Place pre-ashed GF/F filter on top of the filter stem (Figure 22), replace top of funnel.
4. Shake sample bottle vigorously for ~30 seconds to mix sample.
 - a. If sample has large chunks of algae, homogenize using clean, hand-held battery operate stirrer for ~30 seconds. Take care that samples does not spill over the top of the sample bottle while stirring.
 - b. Rinse stirrer thoroughly with DI between samples.
5. Filter known volume of sample.
 - a. **Phytoplankton samples:** Measure using a graduated cylinder. Pour sample slowly in ≤ 100 mL increments into filter funnel.
 - 1) Do not pour more sample into funnel than will pass through the filter. All water in the filter funnel must pass through the filter, if filter becomes clogged while there is still sample water in the funnel, discard sample and filter and start again.
 - b. **Episammon/epipelon samples:** Carefully pour entire sample (including sediment) into 250 mL graduated cylinder to measure the total volume of sample. Record sample volume on Lab Data Sheet.

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- 1) You may pipette directly from the graduated cylinder for the following steps, or pour the contents back into the sample bottle to facilitate sample mixing.
- c. **All benthic samples:** Using a clean pipette tip, carefully pipette the desired volume into the filter funnel.
 - 1) Do not aim pipette tip directly at the filter, aim at the side of the funnel. Take care not to puncture filter.
 - 2) Change pipette tip between samples.
- d. **Keep track of the volume of sample filtered on the Lab Data Sheet (RD[05]).**
- e. If sample is too thick for the pipette tips, you may use a graduated cylinder or cut the end of the pipette tip and test that the volume is still accurate using a graduated cylinder.
6. Draw suction on filter apparatus using the hand vacuum pump (or vacuum line or manifold, if available). Do not exceed 15 in. Hg vacuum. High pressure ruptures cells and causes chlorophyll and other compounds to dissolve and pass through the filter.
 - a. If you have added too much sample and the filter appears to be clogged, you may discard the contents of the filter funnel and the filter and start over. **If you decant water from the filter funnel, you must discard the filter and start over.**
7. Check the filter, if it still appears white, repeat filter more sample. If the filter appears green or yellow tinged, proceed to next step.
 - a. **Record the volume of sample filtered on the Lab Data Sheet (RD[05]).**
 - b. Filter the minimum amount suggested in Table 13. If unable to filter the minimum amount, filter as much sample as possible and record volume.
 - c. Periodically discard the water in the filter flasks. If the flasks overflow, they will back up into the pump and potentially cause damage.
8. Rinse inside walls of funnel using wash bottle of DI.
 - a. Do not include DI rinse water in the volume of sample filtered.
9. Continue to draw suction on the filter until there is no water left in the funnel and there is no excess water on top of the filter.
10. Remove top of filter funnel, release suction using the release valve on the hand pump or the valves below the filter funnel on the manifold.
11. Carefully remove the filter from the stem using filter forceps (forceps with flat ends). Take care not to touch the filter with your fingers.
 - a. **AFDM filters:** Place filter in a labeled aluminum weigh boat, record sample information on Lab Data Sheet (RD[05]). And proceed to SOP C.7.
 - b. **Chlorophyll/peophytin, C/N, P, and isotope filters:** Fold the filter in half and place on a clean square of aluminum foil (~4x4 inches). Fold foil securely around the filter to form a packet.
 - c. Label foil packet with adhesive sample label (~1" x 2") (Figure 19).
12. Repeat above steps until you have 9 filters total from the same sample.
 - a. 1 AFDM filter (proceed to SOP C.7)



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- b. 2 chlorophyll *a*/pheophytin filters
 - c. 2 C, N filters
 - d. 1 P filter
 - e. 2 ¹³C, ¹⁵N isotope filters
 - f. 1 ³⁴S isotope filter
13. Place all foil packets from one site inside a resealable bag. Using a permanent marker, label the outside of the resealable bag with Domain, Site, Date, and the “lab type” (i.e., chl/pheo, C/N, P, 13C/15N, or 34S).
- a. Place all filters in -20 °C freezer.
14. Cap amber sample bottle and proceed to SOP C.6.

C.6 Algal Taxonomy Samples (Unfiltered)

1. Label 60 mL (periphyton) of 1 L (phytoplankton) HDPE bottles with 1”x2” adhesive labels (Figure 19, RD[05]). “lab type” is **taxonomy**.
2. Cap and shake amber HDPE sample bottle for 30 seconds to mix sample evenly.
3. Pipette 60 mL periphyton into the appropriately labeled bottle, or measure 1 L phytoplankton using a graduated cylinder and transfer to labeled 1 L bottles.
 - a. If the sample is too thick for the pipette, you may measure 60 mL using a clean (rinsed in DI) graduated cylinder.
4. Cap bottle and proceed to Sample Preservation SOP C.9.

C.7 Ash-free Dry Mass (AFDM) of Filters

1. **Day 1:** Remove filter from filter apparatus using filter forceps, place filter in aluminum weigh boat, and copy sample information onto lab data sheet with appropriate **Boat ID** (RD[05]).
2. Place all boats containing filters in the drying oven for a minimum of 12 hours at 60 °C or until constant weight is achieved (i.e., mass varies by <2% over a one-hour period).
 - a. You may place several boats in a shallow cardboard box for easier loading and unloading in the drying oven.
 - b. Cover boats with paper or cardboard to prevent air circulation in the drying oven from blowing the filters out of the boats.
3. **Day 2:** Remove boats+filters from the drying oven and allow to cool to room temperature.
 - a. If the boats+filters will not be weighed right away, place them in a desiccator. Filters can be left in the desiccator for up to 30 days if necessary.
4. Place boat + filter on tared (zeroed) analytical balance. Record on **Lab Data Sheet** as **Dry weight + boat**.
5. Place aluminum boats + filters in the muffle furnace using oven gloves and tongs. **TAKE CARE NOT TO BURN YOURSELF!**
 - a. Boats may be stacked on top of each other as long as there is space for air flow between them.



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- b. Place boats on an approved muffle furnace tray (if available) before placing in the furnace. This makes it easier and safer to handle samples.
6. Leave samples in the muffle furnace at 500 °C for at least 6 hours.
 - a. Samples may be left in furnace for longer than 6 hours (e.g., overnight) if necessary.
7. After 6+ hours, remove boats carefully from the muffle furnace using oven gloves and crucible tongs.
8. Cover pans/aluminum boats to prevent ash from blowing out of boats and aside to cool on a heat-resistant in an area without drafts from windows, doors, or HVAC ventilation.
9. When boats have cooled to room temperature, place in desiccator.
 - a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
10. After cooling to room temperature, weigh boats again on analytical balance (0.0001 g) and record as **Ash weight** on Lab Data Sheet.
11. After weighing, discard ashed filters into the trash and clean the boat with a soft brush or paper towel.
12. Set clean boats aside to be used again.

NEON Aquatics Periphyton/Seston/Phytoplankton Lab Data Sheet									
Site (4-letter code): <i>BARC</i>					Date analysis finished (YYYYMMDD): <i>20140706</i>				
Date collected (YYYYMMDD): <i>20140702</i>					Recorded by: <i>sparker</i>				
Date analysis started (YYYYMMDD): <i>20140703</i>					Sampling protocol & Rev.: <i>NEON.DOC.001203vB</i>				
PERIODICALLY CHECK THAT BALANCE IS ZEROED!									
<i>from field datasheet</i>									
Sample type	Lab type	Sample ID	Sample volume (mL)	Volume filtered (mL)	Epiphyton: total surface area scrubbed (cm ³)	Epiphyton: total plant dry weight (g)	Boat ID	Dry weight + boat (g)	Ash weight (g)
phytoplankton	AFDM	BARC.20140702.phytoplankton.1	4000	250		NA	B1	2.1342	2.5460
phytoplankton	chl/pheo	BARC.20140702.phytoplankton.1	4000	250		NA	NA		
phytoplankton	chl/pheo	BARC.20140702.phytoplankton.1	4000	250		NA	NA		
phytoplankton	CN	BARC.20140702.phytoplankton.1	4000	250		NA	NA		
phytoplankton	CN	BARC.20140702.phytoplankton.1	4000	250		NA	NA		
phytoplankton	P	BARC.20140702.phytoplankton.1	4000	250		NA	NA		
phytoplankton	13C15N	BARC.20140702.phytoplankton.1	4000	250		NA	NA		
phytoplankton	13C15N	BARC.20140702.phytoplankton.1	4000	250		NA	NA		
phytoplankton	34S	BARC.20140702.phytoplankton.1	4000	250		NA	NA		
epiphyton	AFDM	BARC.20140702.epiphyton.1	250	5	15.71	12.24	B1	4.1561	3.0072

Figure 23. Example of Lab Data Sheet for phytoplankton and periphyton samples.

C.8 Sample Disposal

1. Double check that all components have been completed. You should have the following products for each field sample:
 - a. 1 AFDM filter (processed at Domain Support Facility)

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- b. 2 chlorophyll/pheophytin filters
 - c. 2 C,N filters
 - d. 1 P filter
 - e. 2 $\delta^{13}\text{C}$, ^{15}N isotope filters
 - f. 1 $\delta^{34}\text{S}$ isotope filter
 - g. 60 mL periphyton (+ glutaraldehyde) or 1 L phytoplankton (+ Lugol's) sample for taxonomy lab
2. After all components have been filtered and bottled, you may discard the remaining sample material from the field sample bottles.
 3. Rinse the field sample bottles with DI water, discard rinse water and recap bottles to be reused.
 - a. Bottles may be dried or recapped and stored while still damp inside.

C.9 Preserving Samples

1. **Periphyton taxonomy:** Uncap each sample bottle. Using a disposable pipet, preserve each 60 mL sample with glutaraldehyde to reach a final concentration of 0.5% glutaraldehyde in the sample. Record type and volume of preservative used on the AOS Sample Shipping Inventory (RD[16]).
 - a. Use $C_1V_1=C_2V_2$ to calculate preservative volume, where:
 - 1) C_1 =original concentration of preservative
 - 2) V_1 =volume of preservative added to sample
 - 3) C_2 =final concentration of preservative in sample
 - 4) V_2 =final volume of sample
2. **Phytoplankton taxonomy:** Uncap each sample bottle, using a pipette, preserve each 1 L samples with high-iodine Lugol's solution to reach a final concentration of 2% (20 mL Lugol's per L of sample).
3. **Filters (chlorophyll *a*/pheophytin, C/N and P filters, and isotope filters):** Place labeled foil packets in sealed zip-top bags (a separate labeled bag for each parameter). Place in dark -20°C freezer until shipping. Ship on dry ice within 2 weeks.

C.10 Ending the Processing Day

1. Refreshing the laboratory supplies
 - a. Pre-ash GF/F filters in muffle furnace.
 - b. Check the volume of preservative left in the lab, re-order if necessary in preparation for the next sampling date.
2. Equipment maintenance, cleaning and storage
 - a. Clean aluminum weigh boats for reuse.
 - b. Empty all amber HDPE field sample bottles, rinse with DI water. Dry or cap tightly while still wet to use on the next sampling date.
 - c. Clean filter funnels, filter flasks, and filter stems by rinsing well with DI water.

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SOP D Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

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SOP E Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the [CLA shipping document](#) on [CLA's NEON intranet site](#).

Shipments are to include a hardcopy of the “per sample” tab of the shipping inventory as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. The shipment tracking number (shipment ID) must be included in the electronic version of the shipping inventory as well as the email, but is not necessary on the hard copy.

E.1 Algal Taxonomy Sample Shipping

1. Taxonomy samples must not freeze, take care to avoid shipping at times when the samples may be subject to sitting for long periods in a frozen warehouse (e.g., take note of lab weekend and holiday receiving schedule).
2. Place bottles into one or several gallon-size resealable zip-top bags, grouped by site.
3. Line a cardboard box or 9 qt cooler with a trash bag to prevent leaks.
4. Place all bottles right-side up inside the liner bag. Add packing material (Vermiculite or other) to take up excess space in container and cushion samples.
 - a. If using 9 qt coolers, include return shipping label for external lab to send cooler back.
 - b. Combine shipment with macroalgae taxonomy samples if possible.
5. Include shipping inventory/manifest in additional zip-top bag.
6. Ship ground at ambient temperature.

E.2 Algal Filter Sample Shipping

1. Filters must be kept frozen, do not ship on Fridays and ensure that the receiving lab will be open when the shipment arrives (e.g., take note of holiday schedules).
2. Place filters in resealable zip-top bags grouped by site/date.
3. Place inside small dry-ice shipper.
4. Keeps filters/bags from directly touching dry ice using cardboard or additional bags.
5. Include shipping inventory/manifest in additional zip-top bag.
6. Follow instructions for shipping overnight on dry ice AD[03].

E.3 Handling Hazardous Material

Glutaraldehyde and Lugol’s iodine in the concentration and volume shipped by NEON are not considered hazardous.

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E.4 Supplies/Containers and Conditions

See sections E.1, E.2, and Table 11 for specific shipping materials.

E.5 Timelines

1. Taxonomy samples
 - a. Shipping should occur within one week of sampling if possible, but samples may be stored at the domain support facility 4 °C for up to 30 days before shipping if necessary.
2. Filters
 - a. Shipping should occur within one week of sampling if possible, but samples may be stored at -20 °C for up to 14 days if necessary.

E.6 Grouping/Splitting Samples

Group samples by site per bout.

E.7 Return of Materials or Containers

Include return shipping label if any shipping materials need to be returned to the domain support facility (e.g., cooler).

E.8 Shipping Inventory

Shipments are to have a hardcopy of the shipping inventory (RD[16]) sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. Also include the shipment tracking number in the email.

E.9 Laboratory Contact Information and Shipping/Receipt Days

See the [CLA shipping document](#) on [CLA's NEON intranet site](#).

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 14. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002192	Datasheets for AOS Protocol and Procedure: Algae Sampling in Lakes and Non-Wadeable Streams
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling
NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass

These datasheets can be found in Agile or the NEON Document Warehouse.

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APPENDIX B QUICK REFERENCES

B.1 Steps for Algae Sampling

Step 1 – Check the algae field sampling kit to make sure all supplies are packed.

Step 2 – Prepare labels (2" * 4").

NEON

Sample ID: BARC.20140702.phytoplankton.1

Sample type: phytoplankton seston epilithon
epixylon epiphyton epipsammon epipelon

Volume collected (mL): 4000

Collected by: sparker

Step 3 – Ensure the General AQU Field Metadata Sheet (RD[06]) and Secchi and depth datasheets (RD[07]) are completed per field site visit.

Step 4 – Collect phytoplankton samples:

1. Lakes (near water chemistry sampling locations):
 - a. Deepest point in the lake, determine by bathymetric site map (RD[13]) and preloaded GPS coordinates
 - b. Near the lake inlet
 - c. Near the lake outlet
2. Non-wadeable streams (50-100 m downstream of sensor set):
 - a. Thalweg
 - b. Half the distance between the thalweg and the right bank
 - c. Half the distance between the thalweg and the left bank

Step 5 – Collect benthic algae samples:

1. For lakes, refer to the site-specific riparian vegetation map (created in the Riparian Vegetation Mapping Protocol (RD[11]) which divides the lakeshore into 10 sections (Figure 15).
2. For non-wadeable streams (rivers), refer to the site-specific riparian vegetation map which divides the sampling reach into 10 transects.
3. Evenly partition the five samples around the Riparian Sections.

Step 6 – Filter for Chlorophyll *a* + Pheophytin, AFDM, C, N, and P and isotopes in the Domain Support Facility within 24 hours of sampling (lab day 1).

Step 7 – Dry AFDM filters in the drying oven and wrap all other filters in aluminum foil. Store foil packets in sealed zip-top bags and place in dark -20 °C freezer until shipping (lab day 1).

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Step 8 – Transfer unfiltered periphyton taxonomy samples to 60 mL HDPE bottles and add preservative to reach a final concentration of 0.5% glutaraldehyde or phytoplankton samples to 1 L HDPE bottles to reach a final concentration of 2 % Lugol’s within 24 hours of sampling (lab day 1).

Step 9 – Weigh AFDM filters, place in muffle furnace (lab day 2).

Step 10 – Weigh ashed AFDM of filters (lab day 3).

Step 11 – For each field sample you should have the following products:

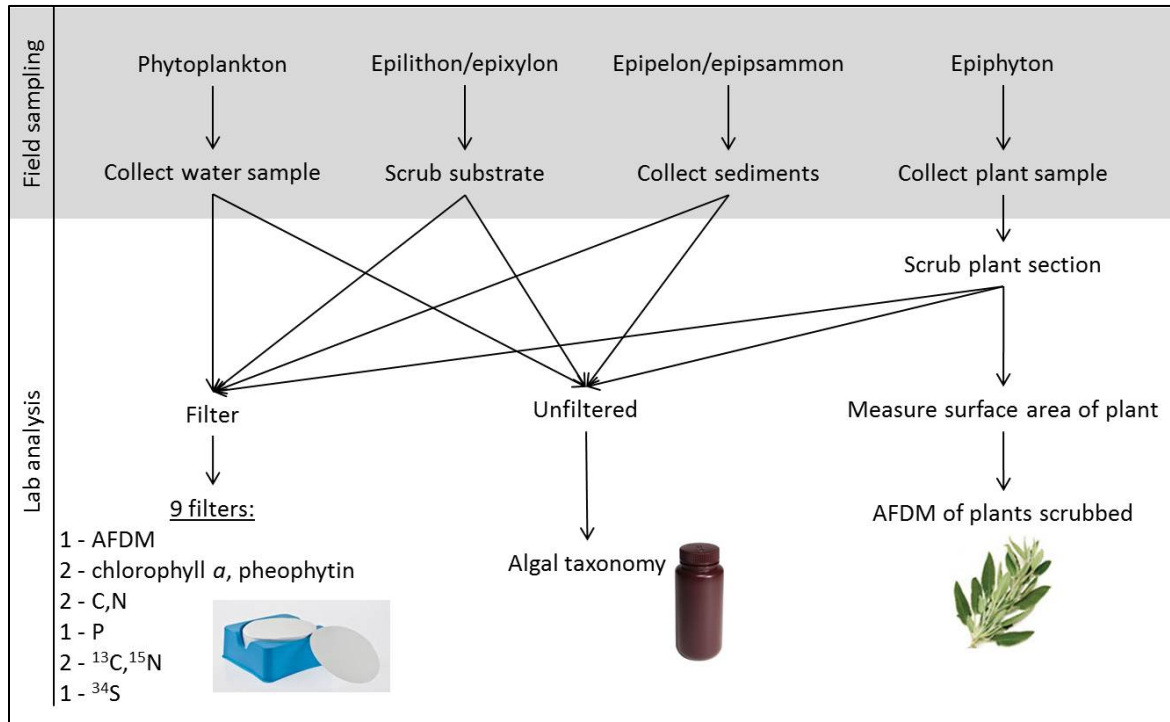
1. 1 AFDM filter (processed at Domain Support Facility)
2. 2 chlorophyll/pheophytin filters
3. 2 C,N filters
4. 1 P filter
5. 2 $\delta^{13}\text{C}$, ^{15}N isotope filters
6. 1 $\delta^{34}\text{S}$ isotope filter
7. 60 mL periphyton or 1 L phytoplankton sample for taxonomy lab

B.2 Order of Preference for Sampling Substrata

Determine which periphyton substrata to sample in the littoral areas (Appendix E). Substrata must account for >20% of littoral habitat.

8. Epilithon (rock substrata)
9. Epixylon (wood substrata)
10. Epiphyton (plant substrata)
11. Epipsammon (sand substrata)

B.3 Epilithon (silt substrata) Sampling Schematic Diagram



B.4 Filtering Amounts for Chemistry

Sample	Parameter	Type	Minimum sample volume
Periphyton	AFDM	Filter (1)	5-10 mL (until visible color on filter)
Periphyton	Chlorophyll <i>a</i> /pheophytin	Filters (2)	5-10 mL (until visible color on filter)
Periphyton	C, N	Filters (2)	5-10 mL (until visible color on filter)
Periphyton	P	Filter (1)	5-10 mL (until visible color on filter)
Periphyton	$\delta^{13}\text{C}, \delta^{15}\text{N}$	Filters (2)	As much samples as possible, >10 mL
Periphyton	$\delta^{34}\text{S}$	Filter (1)	As much samples as possible, >10 mL
Periphyton	Taxonomy	Liquid, preserved in glutaraldehyde	60 mL
Phytoplankton	AFDM	Filter (1)	≥250 mL (until visible color on filter)
Phytoplankton	Chlorophyll <i>a</i> /pheophytin	Filters (2)	≥250 mL (until visible color on filter)
Phytoplankton	C, N	Filters (2)	≥500 mL (until visible color on filter)
Phytoplankton	P	Filter (1)	≥500 mL (until visible color on filter)
Phytoplankton	$\delta^{13}\text{C}, \delta^{15}\text{N}$	Filters (2)	As much samples as possible, >500 mL
Phytoplankton	$\delta^{34}\text{S}$	Filter (1)	As much samples as possible, >500 mL
Phytoplankton	Taxonomy	Liquid, preserved in Lugol's	1 L

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APPENDIX C REMINDERS

Before heading into the field: Make sure you...

- Collect and prepare all equipment including labels.
- Pre-print labels on waterproof paper.

Sample collection: Be sure to...

- Determine the dominant habitat based on the site-specific bathymetric map and riparian vegetation map (RD[13], RD[11])
- Choose the appropriate sampler.
- Keep track of the volume of water used to scrub and rinse the sample.
- Do not sample anywhere you or other field technicians have walked, or locations that appear recently disturbed.

Sample processing: Be sure to...

- Completely homogenize sample before filtering.
- Keep track of the volume of sample filtered.
- DO NOT FREEZE taxonomy samples.

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APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for biological sampling bouts in lakes and non-wadeable streams . Also see the Site Specific Sampling Strategy Document on [AQU’s NEON intranet site](#).

Domain	Site	Bout 1	Bout 2	Bout 3
D03	Ichawaynochaway Creek	21Feb-21Mar	27Jun-25Jul	7Oct-4Nov
D03	Lake Barco	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D03	Lake Suggs	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D05	Crampton Lake	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D06	McDowell Creek	20Mar-17Apr	3Jul-31Jul	27Sep-25Oct
D08	Black Warrior River	19Feb-19Mar	27Jun-25Jul	31Oct-28Nov
D08	Tombigbee River	22Feb-22Mar	26Jun-24Jul	2Nov-30Nov
D09	Prairie Lake	18Apr-16May	5Jul-2Aug	11Sep-9Oct
D09	Prairie Pothole	20Apr-18May	5Jul-2Aug	11Sep-9Oct
D11	South Pond at Klemme	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D18	Toolik Lake	21May-18Jun	29Jun-27Jul	6Aug-3Sep

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APPENDIX E SITE-SPECIFIC INFORMATION: HABITAT AND SUBSTRATA RECOMMENDATIONS FOR LAKES AND NON-WADEABLE STREAMS

For more information see the Site Specific Sampling Strategy Document on [AQU's NEON intranet site](#).

Domain	Site	Pelagic	Littoral
D03	Ichawaynochaway Creek	Kemmerer	Epixylon
D03	Lake Barco	Kemmerer	Epiphyton
D03	Lake Suggs	Kemmerer	Epiphyton
D05	Crampton Lake	Kemmerer	*
D05	<i>Site to be determined</i>	Kemmerer	*
D08	Black Warrior River	Kemmerer	Epixylon
D08	Tombigbee River	Kemmerer	Epixylon
D09	Prairie Lake	Kemmerer	Epilithon
D09	Prairie Pothole	Kemmerer	Epilithon
D11	South Pond at Klemme	Kemmerer	Epipelon
D18	Toolik Lake	Kemmerer	Epilithon

* *Sampler to be determined as of May 2014*