



<i>Title:</i> AOS Protocol and Procedure: ALG – Periphyton and Phytoplankton Sampling		<i>Date:</i> 05/02/2022
<i>NEON Doc. #:</i> NEON.DOC.003045	<i>Author:</i> S. Parker	<i>Revision:</i> G

## AOS PROTOCOL AND PROCEDURE: ALG – PERIPHYTON AND PHYTOPLANKTON SAMPLING

<b>PREPARED BY</b>	<b>ORGANIZATION</b>	<b>DATE</b>
Stephanie Parker	AQU	11/5/2019

<b>APPROVALS</b>	<b>ORGANIZATION</b>	<b>APPROVAL DATE</b>
Kate Thibault	SCI	05/02/2022

<b>RELEASED BY</b>	<b>ORGANIZATION</b>	<b>RELEASE DATE</b>
Tanisha Waters	CM	05/02/2022

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## Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	01/22/2016	ECO-03470	Initial release, supersedes NEON.DOC.000691 and NEON.DOC.001203 and revised phytoplankton sampling depths in stratified lakes.
B	02/08/2017	ECO-04359	Update NEON template; Add epilithon/epixylon large substrate sampling; Update sample ID template; Clarify ADFM SOP; Remove <sup>34</sup> S filter
C	2/13/2018	ECO-05326	Remove seston sampling in streams, add barcode labels, change chlorophyll shipping to 7 days, dry instead of freeze algal chemistry filters, move datasheets to appendix, update D14 bout dates
D	12/19/2018	ECO-05967	Decrease in algal taxonomy subsamples for lakes/rivers, add contingencies and rules for stream drying, add Van Dorn
E	1/13/2020	ECO-06285	Template update, change to ADFM data error and recording and lab best practices, clarify epipsammon/epipelon lab practices, add cleaning step for filter funnels
F	03/16/2022	ECO-06781	<ul style="list-style-type: none"> <li>Update to reflect change in terminology from relocatable to gradient sites</li> </ul>
G	05/02/2022	ECO-06824	<ul style="list-style-type: none"> <li>Minor formatting update</li> </ul>



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## 1 OVERVIEW

### 1.1 Background

The aquatic primary producer community is dominated by algae in most systems (Lowe and LaLiberte 2006). Algae are photosynthetic organisms that produce energy (fix carbon) from sunlight using chlorophyll  $\alpha$ , 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), benthic microalgae (periphyton growing on submerged surfaces such as sediment or plants), and sestonic algae (microscopic algae inhabiting the water column in wadeable streams) or phytoplankton (microscopic algae inhabiting the water column). Macroalgae will be sampled as part of the Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling Protocol (RD[11]), while benthic microalgae and seston or phytoplankton will be sampled as part of this protocol.

Benthic microalgae, along with a matrix of cyanobacteria, microbes, and detritus combine on surfaces in aquatic systems to form periphyton. The periphyton community includes algae and associated biofilms living attached to substrata. These are usually the most abundant primary producers in wadeable streams (Allan 1995). Most surfaces that are exposed to light (i.e., relatively shallow waters) in freshwater habitats sustain periphyton communities, and light penetration typically limits periphyton growth. In lakes and non-wadeable streams (or rivers), periphyton are restricted to the shallow littoral zone (**Figure 1**). Periphyton communities are also strongly affected by disturbance and scouring of the bottom, water temperature, current, grazing, and substratum type (Hynes 2001). Diatoms comprise the majority of the periphyton community, with growth forms ranging from prostrate (closely adhered to the substratum), stalked, or colonial (often chain-forming, loosely associated with the periphyton mat; Figure 2). Biofilms often also include heterotrophic bacteria, protozoans, prostrate and filamentous Cyanobacteria, and fine particulate organic matter (FPOM). Benthic microalgae communities can be classified based on the substrata that they colonize: **epilithon** colonize cobbles and boulders, **epixylon** colonize woody debris, **epiphyton** colonize plant surfaces, **epipelon** colonize silty sediments, and **epipsammon** colonize sand (Wehr and Sheath 2003). Epilithic communities (growing on rock surfaces) are typically well-studied in aquatic habitats while epiphyton and epipsammon/epipelon tend to be less well-understood.

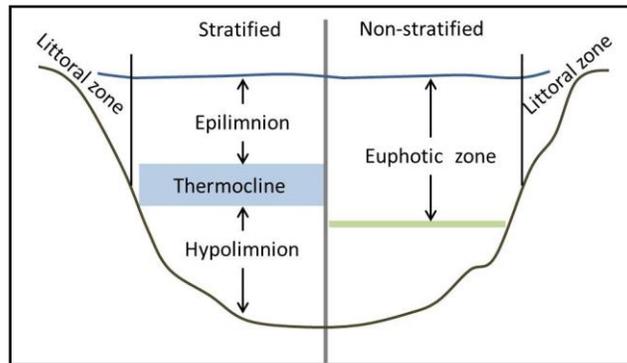


Figure 1. The zones in a stratified and non-stratified lake.

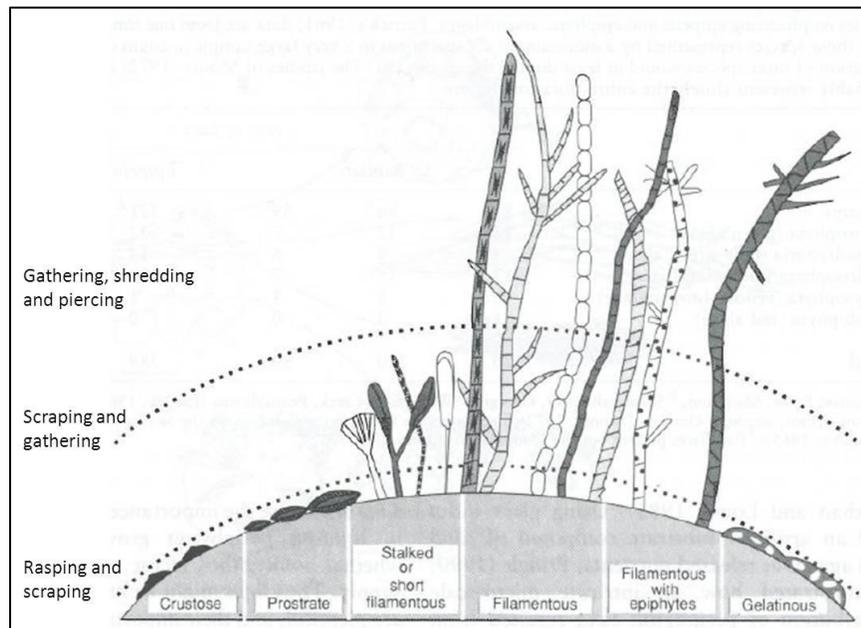


Figure 2. Growth forms of periphyton, ranging from prostrate types to long, filamentous types. Vertical layering differs among growth forms (from Allan 1995).

Periphyton is an important component in the aquatic community as it provides a food source for many consumers. Invertebrate consumers ranging from those with mouthparts adapted for scraping the substrata to those with mouthparts adapted to shredding feed on different forms of periphyton (Figure 2). Periphyton, especially diatoms are abundant in flowing waters and have been shown to be good environmental indicators as they respond quickly to changes in abiotic (e.g., nutrients, disturbance) and biotic factors (e.g., grazing pressure). Environmental factors such as flooding and scouring, water level, light attenuation, and nutrient availability strongly affect aquatic primary producers (Allan 1995, Hynes 2001). Sampling primary producers to determine changes in abundance, biodiversity, community structure, introduction, and spread of invasive species is therefore a crucial component of aquatic ecosystem assessment.



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Free-floating algae (phytoplankton) can exist as single cells or colonies, and can be highly diverse in many freshwater habitats (Wehr and Sheath 2003). Phytoplankton communities are primarily controlled by nutrient supply, light, and consumer grazing pressure. In **lakes and rivers**, phytoplankton are present in higher density above the metalimnion when the system is stratified (the system has a thermocline) and within the euphotic zone (the region through which light penetrates) when the system is non-stratified (Figure 1). Phytoplankton in wadeable streams (also known as seston) are often sparse due to stream flow, and comprised of sloughed benthic algae (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, Charles et al. 2002), the USEPA Sampling Procedures for the Great Lakes (USEPA 2010), the US Environmental Protection Agency (EPA) Rapid Bioassessment Program (RBP; Stevenson and Bahls 1999), the Arctic Streams Long-Term Ecological Research (LTER) program (Slavik et al. 2004) and Methods in Stream Ecology (Lowe and LaLiberte 2006).



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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	EHSS Policy, Program and Management Plan
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.004104	NEON Science Data Quality 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.002652	NEON Level 1, Level 2, Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[06]	NEON.DOC.004257	NEON Standard Operating Procedure (SOP): Decontamination of sensors, field equipment and field vehicles
RD[07]	NEON.DOC.003042	Datasheets for AOS Protocol and Procedure: Periphyton and Phytoplankton Sampling
RD[08]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[09]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[10]	NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[11]	NEON.DOC.003039	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling
RD[12]	NEON.DOC.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD[13]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbial Sampling
RD[14]	NEON.DOC.001196	AOS Protocol and Procedure: Riparian Habitat Assessment in Wadeable Streams
RD[15]	NEON.DOC.001195	AOS Protocol and Procedure: Riparian Habitat Assessment in Lakes and Non-Wadeable Streams
RD[16]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[17]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[18]	NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling



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RD[19]	NEON.DOC.002792	AOS Protocol and Procedure: Secchi Depth and Depth Profile Sampling in Lakes and Non-Wadeable Streams
RD[20]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[21]	NEON.DOC.003282	NEON Protocol and Procedure: Site Management and Disturbance Data Collection
RD[22]	NEON.DOC.003600-NEON.DOC.003618	Aquatic Site Sample Design – NEON Domain ##
RD[23]	NEON.DOC.005224	Shipping Ecological Samples, Sensors and Equipment
RD[24]	NEON.DOC.014048	TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling

### 2.3 Acronyms

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



## 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. Mosses may also have epilithic algae growing on their surface.

**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 on the bottom, geologically defined as 64 to 256 mm diameter. Cobbles are larger than pebbles (4-64 mm), and smaller than boulders (>256 mm).

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

**Epilithon:** Periphyton colonizing rock substrata (**Figure 4**).



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

**Epipelon:** Periphyton colonizing silt substrata.

**Epiphyton:** Periphyton colonizing the surfaces of aquatic plants (**Figure 5**).



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

**Epipsammon:** Periphyton colonizing sand substrata.

**Epixylon:** Periphyton colonizing woody substrata.

**Euphotic zone** (or “Photic zone”): The upper layer of lake water where sunlight penetrates and photosynthesis can occur. Specifically, the depth to which 1% of surface light penetrates.

**Eutrophic:** An ecosystem with high nutrient concentration. In lakes, this often equates to algal proliferation or algal blooms.

**Hypolimnion:** The dense bottom layer of a stratified lake that sits below the thermocline (**Figure 1**). This layer is cooler than the surface water and has less circulation.

**Littoral:** Near-shore area of the lake/river, extends from the high-water mark to the shallow, submerged area of the lake/river; typically the area near shore where sunlight reaches the bottom.

**Macroalgae:** “Large” algae; multicellular, photosynthetic algae visible to the naked eye. In streams, these algae are typically filamentous (**Figure 6**).



**Figure 6.** Macroalgae are large algae that often form blooms in streams and lakes.

**Macrophyte:** Aquatic plant with vascular tissues (**Figure 7**).



**Figure 7.** Aquatic macrophytes are vascular plants that add structure and colonizable area to the stream bottom.

**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:** An ecosystem with 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.

**Pool:** An aquatic habitat unit in a river or stream created by local scour or impoundment and having a structural control. Pool water depth is above average, if all the running water in the stream was shut off, areas in the stream that would still hold water are pools. Pool water velocity is below average for the reach, causing fine sediments to deposit in pools. Pools are generally longer than they are wide (unless they are plunge pools) and are 1.5 x deeper at their maximum depth than they are at their crest (**Figure 8**).



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**Figure 8.** Pools are areas of deep, slow-moving water in the stream.

**Riffle:** Shallow reaches flowing over rough bed material such as boulders and cobbles, creating ripples, waves, and eddies on the water surface (**Figure 9**).



**Figure 9.** Riffles are shallow, fast moving habitats in streams.

**Run:** An aquatic habitat with swiftly flowing water but no surface water agitation, with relatively uniform flow (**Figure 10**).



**Figure 10.** Runs are areas of swiftly moving water, typically deeper than riffles.



**S1 and S2:** Locations of NEON aquatic sensors.

**Sand:** Small sediment particles, 0.25-4 mm diameter.

**Silt:** Very small sediment particles, 3.9-62.5  $\mu\text{m}$  diameter.

**Step pool:** High-gradient streams (steep) where water cascades over a rock or woody snag, dropping into a pool. Due to the gradient and surrounding geology, this pattern continues down the stream: step (drop)—pool—step—pool—etc. (**Figure 11**).



**Figure 11.** Step pools occur in high-gradient streams where there are channel-spanning pools separated by boulder/cobble steps.

**Stratified:** Layers within the system (e.g., warm and cold water layers indicate thermal stratification in a lake).

**Thalweg:** The portion of the stream channel through which the majority of the stream flow is transported. This is typically identified as the deepest portion of the flowing channel.

**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  $^{\circ}\text{C}$  per meter. The denser and cooler layer below the thermocline is the hypolimnion, warmer upper layer is termed the epilimnion.

**Woody snag:** Woody debris that catches on the stream bottom (or stream bank), and collects additional debris from the stream flow (**Figure 12**). Snags often create a refuge for organisms from the stream flow, as well as increased food sources.



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**Figure 12.** Woodysnags are places where pieces of wood fall into the stream and collect other organic matter.



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### 3 METHOD

The goal of the Periphyton and Phytoplankton Sampling Protocol is to determine species diversity, taxa richness, biovolume, biomass, using both chlorophyll *a* as a proxy for biomass as well as ash-free dry mass (AFDM), of periphyton and phytoplankton at aquatic sites. 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. In wadeable streams, periphyton and phytoplankton (seston) samples are collected to fulfill these requirements, while in lakes and non-wadeable streams (or rivers), periphyton and phytoplankton samples are collected.

In wadeable streams, periphyton is sampled using a percent-based macrohabitat approach (after Moulton et al. 2002). Habitats sampled focus on riffles, runs, pools, and step pools depending on the percent cover of habitats present at each NEON Aquatic site. A minimum of three samples per habitat type are taken at each stream. All samples are collected from the surface of the natural substratum present in each macrohabitat. Field protocols differ depending on substrata being sampled. For example, riffles and runs often have cobble/pebble substratum, while pools may have silt or sand substrata. The majority of the periphyton community may be colonizing the leaves of aquatic plants (epiphytes) or woody debris at some sites, thus these substrata are sampled rather than sampling scarcely populated sandy substrata. Appropriate site-specific sampling procedures are determined prior to sampling following NAWQA protocols (Moulton et al. 2002) and presented in site-specific AOS documents as well as in Appendix D.

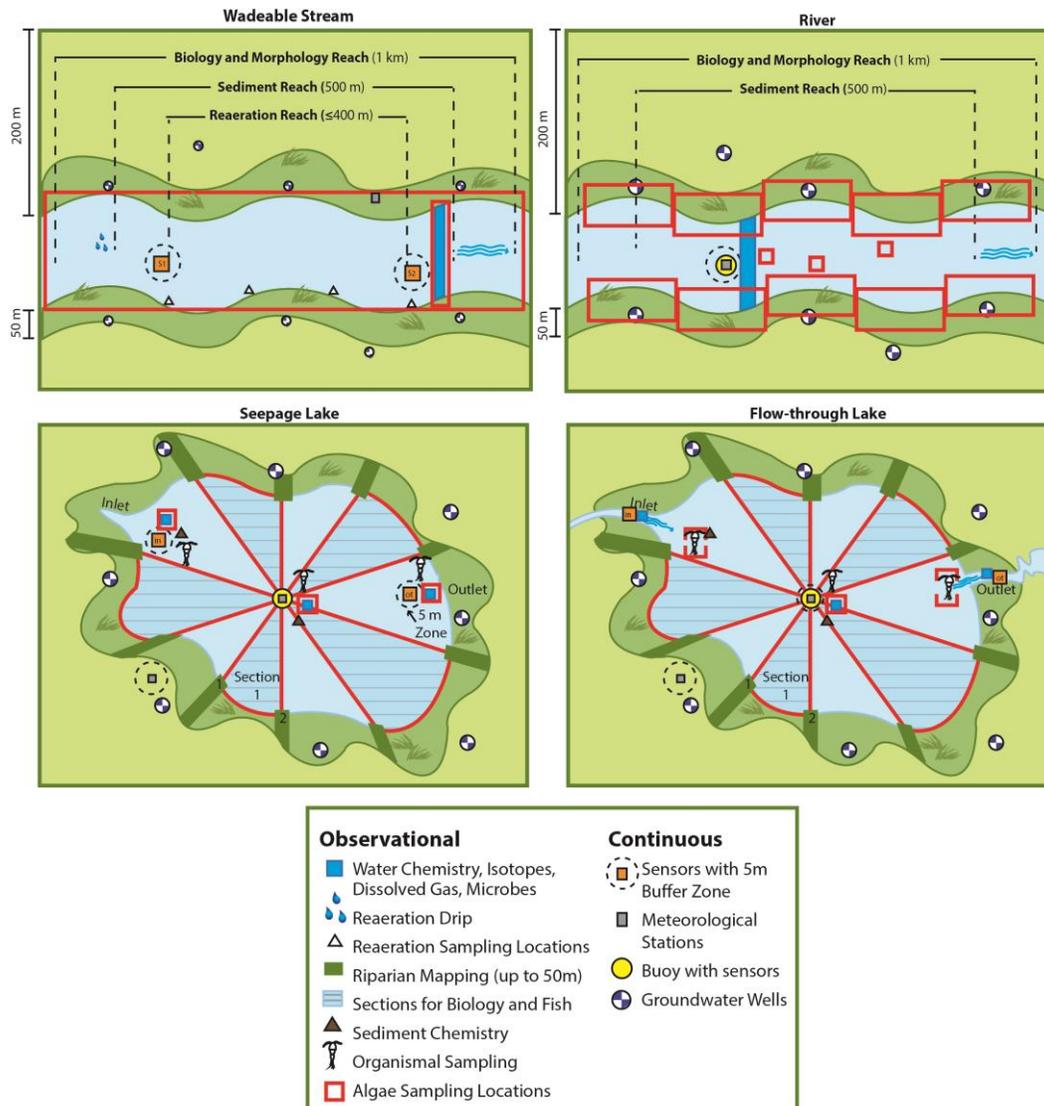
In lakes and rivers, littoral zone periphyton samples are collected following the divisions set forth in the Lake Riparian Habitat Assessment Protocol (RD[15]) and the Stream Riparian Habitat Assessment Protocol (RD[14]) at sites where safety and permitting allows the field staff to step out of the boat and wade. Briefly, one substratum type is chosen in the littoral zone (see Appendix D for site specific recommendations) and composite samples are collected from each of five riparian sections. Field protocols differ depending on substrata being sampled.

In wadeable streams, rivers, and lakes, phytoplankton is sampled at the water chemistry sampling locations (**Figure 13**). In lakes, phytoplankton is collected at the central location (near the buoy) and the inlet and outlet sensor sets. In rivers, phytoplankton is sampled near the buoy, and from two other deep-water locations within the 1 km reach. In wadeable streams, phytoplankton (seston) is only analyzed for chlorophyll *a* and pheophytin.

Algae sampling occurs three times per year in order to capture presence and abundance of multiple species. Timing of sampling is site-specific and determined based on historical data. Specific details on sample dates are provided in the NEON Aquatic Sample Strategy Document (RD[05]). 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 low flows and high light (mid-summer) at each site. Sample bout 3 represents the late growing season (typically autumn) at each site during leaf-fall. These dates differ on a site-by-site basis. Sampling should occur at base-flow conditions,



and will not occur directly following a flood in the stream (Biggs et al. 1999) or under ice in a lake. A period of 14-days will be allowed after a flood event for periphyton to recolonize before sampling occurs.



**Figure 13.** Generic site layouts for lakes, rivers, and wadeable streams with phytoplankton and benthic algae sampling locations. Seepage lakes have no true inlet or outlet stream, while flow-through lakes have inlet and outlet streams.

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 ecologists **must** follow the protocol and associated SOPs. Use NEON’s problem reporting system to resolve any field issues associated with implementing this protocol.



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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 field ecologists document the problem and enter it in NEON’s problem tracking system.

Quality assurance will be performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[05]).



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## 4 SAMPLING SCHEDULE

### 4.1 Sampling Frequency and Timing

Algal sampling occurs three times per year at each site, roughly spring, summer, and autumn. Sampling must occur within the first 21 days of the 1 month window specified in Appendix C, with a minimum 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. See the appropriate Aquatic Site Sampling Design for your domain for additional details and scheduling preferences. Use NEON’s problem reporting system to seek guidance and report sampling efforts that take place outside of the defined sampling window.

Phytoplankton sampling takes place on the same day as Secchi Disk and Depth Profile Sampling in Lakes and Non-Wadeable Streams (RD[12]).

**Table 1.** Sampling frequency for algae procedures on a per SOP per site type basis.

SOP	Site Type	Location	Bout Duration	Bouts Per Year	Bout Interval	Yearly Interval	Remarks
SOP B.1	lake, river	buoy, inlet, outlet, riparian sections, reach	1 day	3	Minimum of 2 weeks between bouts	annual	Uses sampling methods in SOP C
SOP B.2	stream	reach, S2	1 day	3	Minimum of 2 weeks between bouts	annual	Uses sampling methods in SOP C

#### **Scheduling Considerations**

1. All samples for a bout at a site must be collected during the same day.
2. **Field Work and Laboratory Processing:** After periphyton, seston, and phytoplankton samples are collected, the following points are critical with respect to timing:
  - a. Keep samples dark and cool (4 °C) during transportation and storage.
  - b. Sample processing must begin within 24 hours of collection.
  - c. Chlorophyll filters must be shipped within 7 days of collection.

### 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 streamflow, ice on/off days, the accumulation of degree days, weather, and riparian phenology (Appendix C). Benthic periphyton in wadeable streams will be collected during periods of stable stream flow (Stevenson and Bahls 1999).

### 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

All filtering should be completed the day after field samples are collected to preserve the integrity of chemistry samples. Chlorophyll *a* samples **must** be filtered and placed in the freezer (-20 °C) within 24 hours of returning from the field to prevent an increase in chlorophyll *a* concentrations in the sample. If unable to maintain this timeline, all remaining filtering must be completed within 48 hours of returning from the field. Preparing labels, aluminum foil, vials, preservative, and aluminum weigh boats prior to lab processing speeds lab processing time. 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. Chlorophyll/pheophytin filters may be stored for 7 days at -20 °C, and algal chemistry filters may be stored at ambient temperature in the desiccator for up to 30 days. For additional storage and shipping timelines see SOP G.

#### 4.4 Sample Timing Contingencies

All samples from this protocol in one sampling site per bout must be collected within one day (i.e., all samples per site as detailed in this protocol) because of the fluctuating nature of aquatic habitats. Spreading sample collection over multiple days increases data variability among samples. Sampling may be rescheduled due to weather or environmental conditions within the 10 days of the scheduled date provided that date is within the bout window (+ 3 days) provided in Appendix A. An incident ticket must be issued if sampling is rescheduled >10 days past the original scheduled date, or >3 days past the end of the bout window in Appendix C. A minimum of 2 weeks between sample bouts at each site shall be observed.

**Table 2.** Contingent decisions.

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and conditions become unsafe (e.g. approaching thunderstorm, rapid increase of water level in the <b>wadeable stream</b> ), or the <b>lake/river</b> becomes too windy (>35 km hr <sup>-1</sup> ) and has unsafe wave heights (>1 m) so that the boat cannot be held stationary over a sampling point while at anchor, return to shore and wait in a safe location for 30 minutes. If conditions improve, resume sampling, if not, discard samples, 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 may be flagged.
14 Days	If flooding occurs in <b>wadeable streams</b> on or <14 days prior to the targeted sampling date (>3x	None as long as samples are collected within the pre-determined sampling



	median discharge for the preceding year) or unsafe wading conditions occur (Lane and Fay 1997) wait a minimum of 14 days after the water level drops below 3x median discharge and is safely wadeable so the periphyton community can recolonize (Clausen and Biggs 1997; maximum wait = 1 month).	window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data may be flagged.
	If a stream has dried and enough water (>100 m length) to sample returns to the channel, wait for the channel to be wet for 14 days of recolonization before sampling.	None as long as samples are collected within the pre-determined sampling window. If waiting for water to return causes sampling to occur outside of the sampling window, data may be flagged.
	Frozen chlorophyll filters have a short shelf life and must be analyzed at the external lab within 14 days of collection. Frozen filters must be shipped within 7 days of collection to give the lab time to receive and analyze. If between the 7 and 14 day windows, contact science to determine whether samples can be shipped late. Samples must be discarded if held longer than 14 days.	Holding samples >7 days cuts into the external lab's timeline to process the samples. Holding samples >14 days affects data quality.
6 Months	Preserved algal taxonomy samples may be held for up to 6 months at 4 °C in the domain lab if circumstances do not allow shipping to the external lab.	Holding samples >30 days affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected.
	Dried algal chemistry filters may be held indefinitely at ambient temperature in the domain lab if circumstances do not allow shipping to the external lab.	Holding samples >30 days affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected.

#### 4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more sampling locations in a given bout. For example:

- Environment – e.g., ice cover, inclement weather, or
- Management activities – e.g., controlled burns, herbicide application

Instances such as those listed above must be documented for scheduling, tracking long-term site suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[21]).

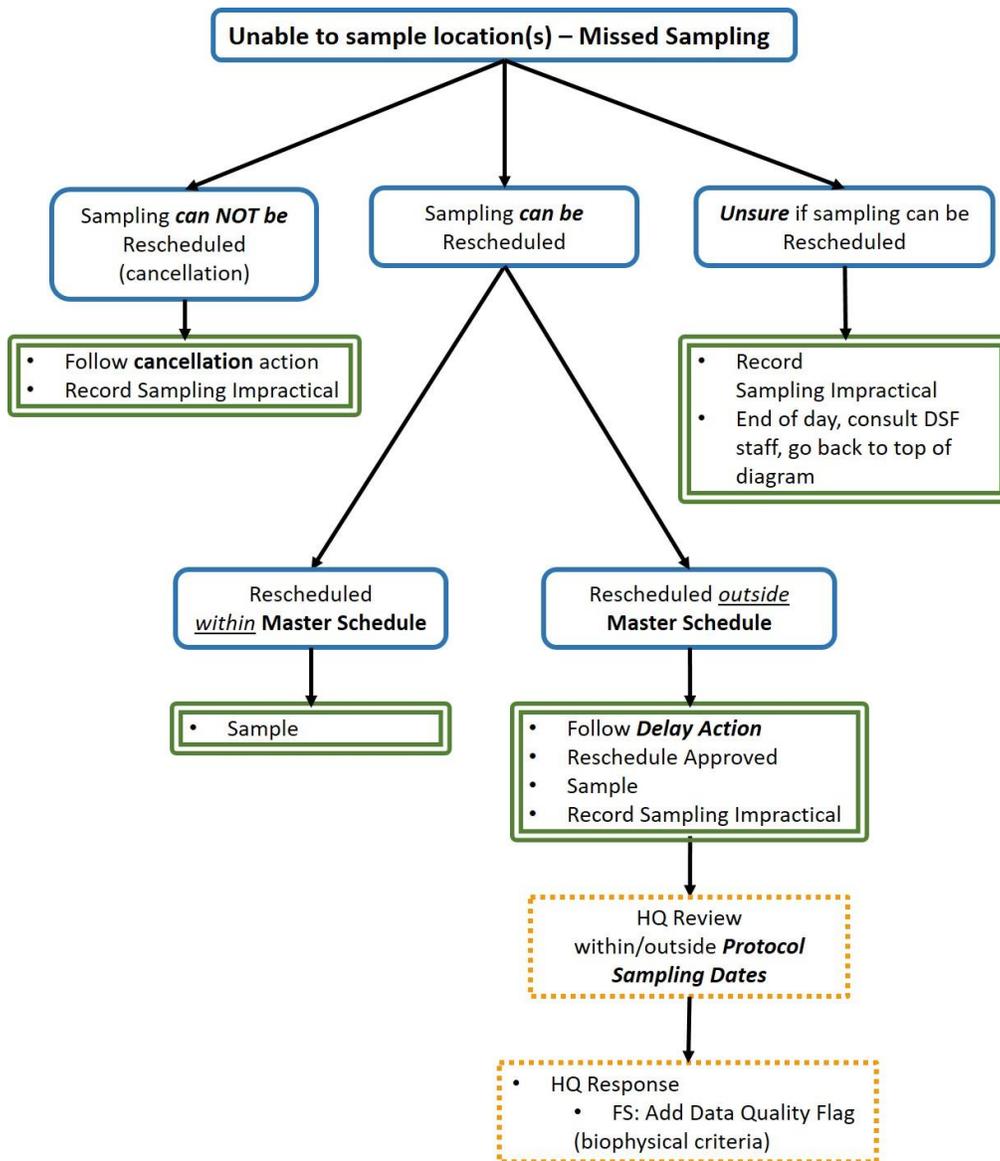


## Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates:** Bout-specific sampling dates (Appendix C).
- **Scheduled Sampling Dates:** Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling:** Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded (e.g., each missed macroinvertebrate sample gets its own record).
- **Sampling Impractical:** The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled:** Missed Sampling is rescheduled for another time within the *protocol sampling dates*, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 14**).



**Figure 14.** The documentation to account for a Missed Sampling event depends on the situation for each plot of each bout that is not sampled. Blue rounded boxes represent contingencies, green double line boxes describe the required actions, orange dotted boxes indicate HQ actions. Required delay and cancellation actions are outlined for each protocol in the ‘Scheduled Field Activities – Delays and Cancellations’ spreadsheet available on the SSL. Missed Sampling events may also require a Data Quality flag and/or creation of a Site Management record.

**To Report Missed or Incomplete Sampling:**

1. Missed or Incomplete Sampling must be communicated to Science by a Service Now Incident.
  - a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 14**).
  - b. The lead Field Ecologist should consult the [Delayed or Cancelled Activities table](#) to best determine when reporting is required.



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2. Create a field record for each **Missed Sampling** event in the field. That is, if data are recorded in the field at the site level, a record must be made.
  - a. Example 1: Lake is ice covered and sampling cannot be attempted.
  - b. Example 2: Stream is dry and cannot be sampled.
3. For each **Missed Sampling** record, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 3**).
  - a. Three algae field samples from the same habitat type should be collected during each sampling event, else sampling should be re-attempted.
4. For **Rescheduled** sampling events that occur outside of the defined Protocol Sampling Dates, a protocol-specific Data Quality Flag must also be recorded (**Figure 14**).
  - a. biophysicalCriteria – An indicator of whether sampling coincided with the intended biophysical conditions
  - b. windAffected – An indicator of whether winds were >35 km hr<sup>-1</sup> at the time of sampling

**Table 3.** Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
Location dry	Location dry
Location frozen	Location frozen
Location snow covered	Location snow covered
Other	Sampling location inaccessible due to other ecological reason described in the remarks

**Table 4.** Contingent decisions for sampling in wadeable streams.

Situation	Action	Outcome for Data Products	Considerations
<u>Wadeable stream</u> site with <500 m aboveground stream length due to stream size or permitting restrictions	Habitat available may be insufficient to accommodate all 8 samples without causing harm to the stream. Reduce sampling by collecting samples only in the dominant habitat type (5 samples total). Enter “sampling impractical” for the missing habitat type.	Lower resolution for diversity metrics.	If the decision is made to decrease the number of samples collected for this protocol, it must also be reflected in the other wadeable stream biology protocols (RD[11], RD[12], RD[13]).
<u>Wadeable stream</u> site with seasonal drying	If the stream experiences seasonal drying such that the chosen habitat types have disappeared, select the next dominant habitat type in B.3, or use the sub-dominant habitat if still present. Reduce sampling by	Less standardization of the dataset.	Habitat types sampled should be present throughout the year. The decision to switch habitat types should only occur during periods of extreme



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	collecting samples only in the dominant habitat type (5 samples total). Enter “sampling impractical” for the missing habitat type.		drying such that typical habitat types are no longer present at the site.
--	--	--	---

#### 4.6 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, use NEON’s problem reporting system to notify Science. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Field sampling requires two field ecologists for four hours per site, plus travel to and from the site. Domain lab processing requires one ecologist for 3-8 hours within 24 hours of field sampling, one ecologist for 1-2 hours the second day, and one ecologist for 1-2 hours the third day.

**Table 5.** Estimated staff and labor hours required for implementation of Periphyton and Phytoplankton Collection.

SOP	Estimated time	Suggested staff	Total person hours
SOP A.1 and A.2: Preparing for data collection and sampling	1 h	1	1 h
SOP B: Determining sampling location and sampler type	1 h first year	2	2 h first year
SOP C: Field sampling methods for each sampler type	4 h	2	8 h
SOP D: Laboratory Sampling and Analysis	3 h	1	3 h
SOP D.6: AFDM	3 h (over multiple days)	1	3 h
SOP E: Data Entry and Verification	1 h	1	1 h
SOP F: Shipping	2 h (multiple shipments)	1	2 h



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## 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 EHSS Policy, Program and Management Plan (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Ecologist 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 made available for all chemicals used in this work (ethanol). Whenever chemicals are used, follow requirements of the site-specific Chemical Hygiene and Biosafety Plan (AD[03]) for laboratory safety and NEON EHSS Policy, Program and Management Plan (AD[01]).

See Section 9 in the NEON Operations Field Safety and Security Plan (AD[02]) for aquatic-specific field safety requirements. In addition, the following safety requirements must be followed:

1. Activities in **wadeable streams** should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is  $\geq 10 \text{ ft}^2/\text{s}$  ( $0.93 \text{ m}^2/\text{s}$ ; Lane and Fay 1997).
2. In **lakes and rivers**, site-specific hazards may be encountered that necessitate sampling from the boat, without dismounting from the vessel. In addition, use extra caution in waters where alligators are present and maintain a safe distance from hazards.
3. All personnel must be wearing a personal flotation device (PFD) prior to entering a boat.
4. All personnel shall have access to a form of communication with other team members such as a two-way radio.

Be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, water release from dams, etc.).



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## 6 PERSONNEL

### 6.1 Training Requirements

Field staff 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 other personnel using a boat shall be aware of boating safety procedures.

Personnel will be trained in field protocols associated with this document, and trained in safe working practices for aquatic field work. Personnel must also be trained in safe handling of glutaraldehyde as documented in the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

### 6.2 Specialized Skills

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



## 7 STANDARD OPERATING PROCEDURES

### SOP Overview

- SOP A: Preparing for Sampling
- SOP B: Determining Sampling Locations and Sampler Type
- SOP C: Field Sampling
- SOP D: Post-Field Sampling Tasks
- SOP E: Laboratory Sampling and Analysis
- SOP F: Data Entry and Verification
- SOP G: Sample Shipment

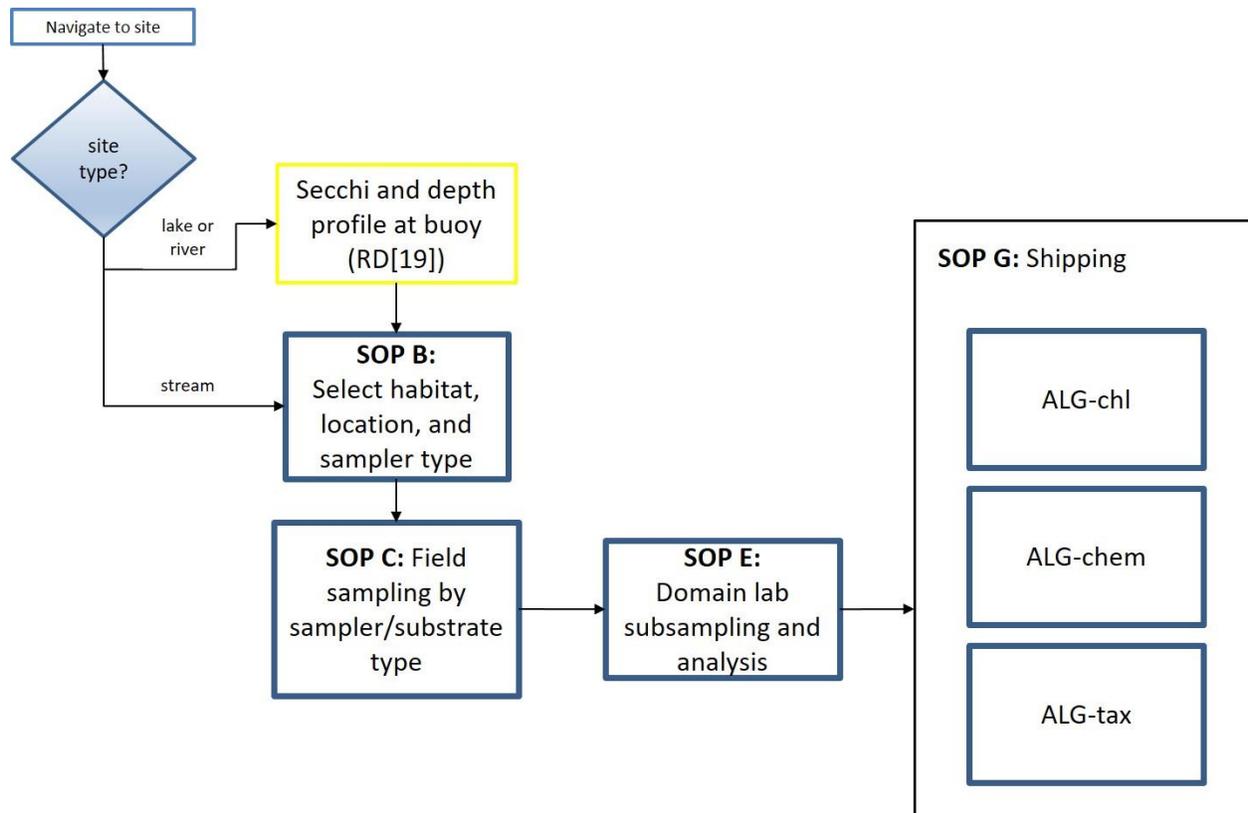
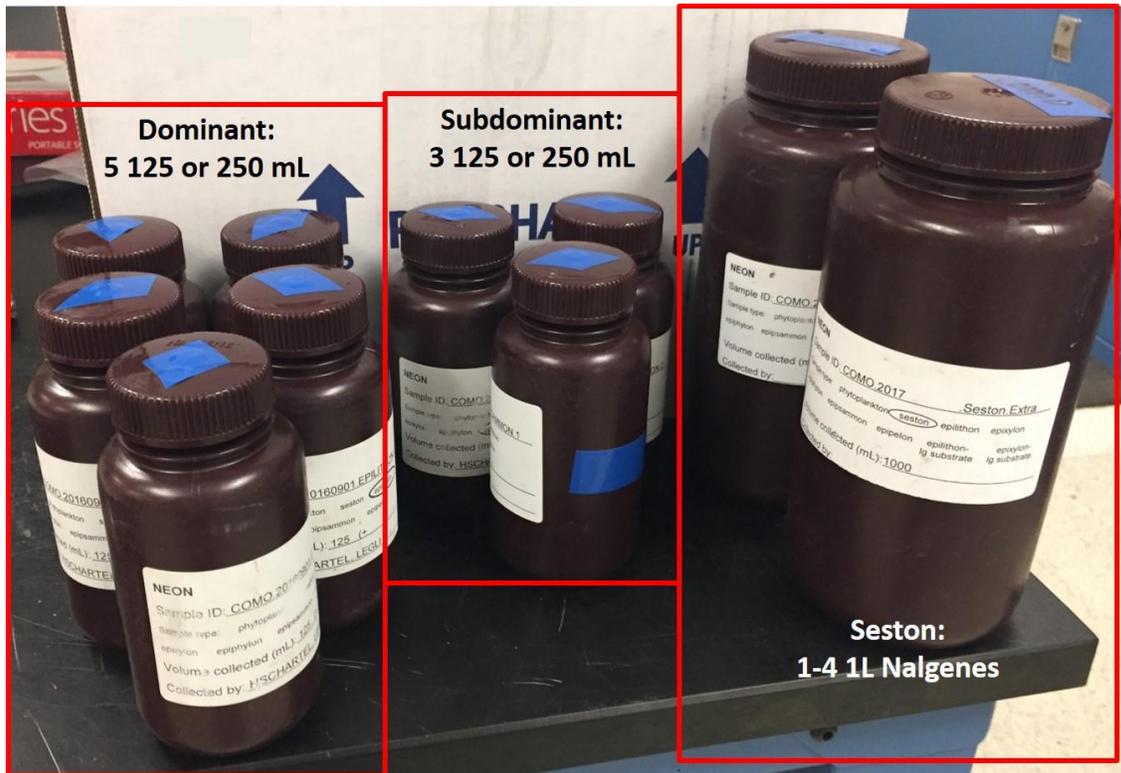
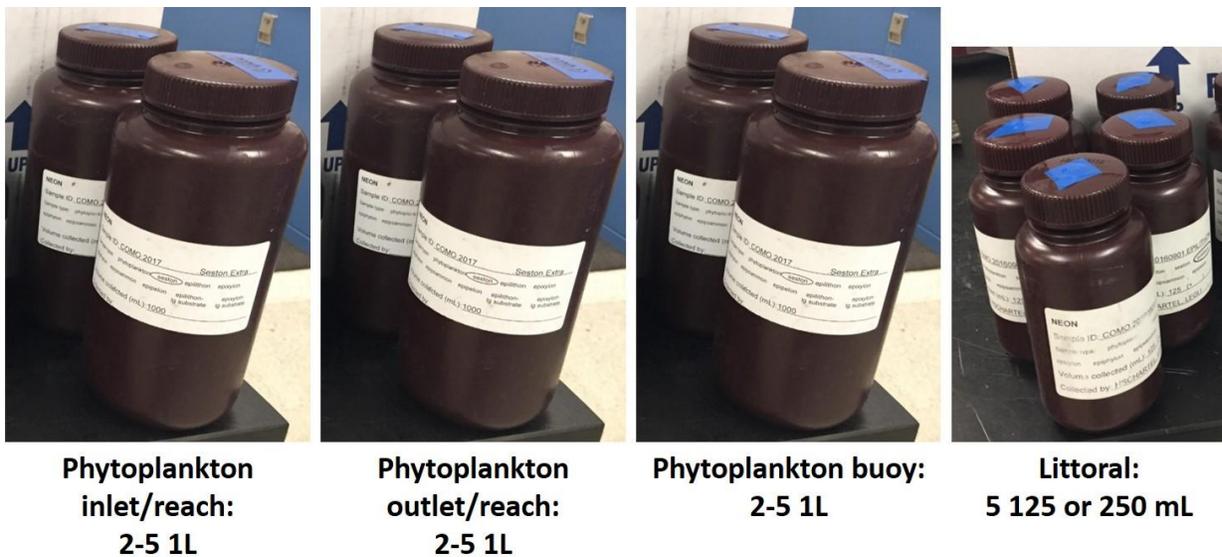


Figure 15. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.



**Figure 16.** Stream algae field sample suite, including 5 dominant periphyton samples, 3 subdominant periphyton samples, and 1 seston sample (multiple bottles, depending on site water clarity). A total of 8 periphyton samples and 1 seston sample are collected per site per bout.



**Figure 17.** Lake and river algae field sample suite, 3 phytoplankton samples (at least 2 bottles per sample) and 5 littoral periphyton samples. Phytoplankton samples in rivers are collected in the reach rather than at an inlet or outlet. A total of 3 phytoplankton and 5 periphyton samples are collected per site per bout.



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## SOP A Preparing for Sampling

### A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged and synced at the beginning of each field day, whenever possible.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

### A.2 Labels and Identifiers

Barcode labels are recommended on algae field samples for improved sample tracking. Barcodes need to be applied to dry containers for 30 minutes before use. Periphyton and phytoplankton field collection use Type I barcodes (prefix A, plus 11 numbers).

1. All periphyton and phytoplankton field samples will have a weather-resistant, adhesive, human readable label on the outside of the bottle (**Figure 18**). This label may be reused along with the cleaned, rinsed bottle from bout to bout within a site.
2. For epiphyte samples only: Print all-weather paper labels (**Figure 19**). Cut labels apart using scissors. One label will be placed inside each Whirl-pak®.
  - a. All-weather paper labels should be filled out using pencil.
3. Add adhesive labels to sample bottles prior to going in the field and getting the bottle wet.
  - a. Keep a human-readable label on each bottle with a minimum of the sample ID printed to assist with organization and shipping.

<p><b>NEON</b></p> <p>Sample ID: <u>ARIK.20161121.epipsammon.1</u></p> <p>Sample type: phytoplankton    seston    epilithon    epixylon</p> <p>epiphyton <u>epipsammon</u> epipelon    epilithon- lg substrate    epixylon- lg substrate</p> <p>Volume collected (mL): <u>250</u></p> <p>Collected by: <u>sparker</u></p>	<p><b>NEON</b></p> <p>Sample ID: <u>ARIK.20161121.epipsammon.3</u></p> <p>Sample type: phytoplankton    seston    epilithon    epixylon</p> <p>epiphyton <u>epipsammon</u> epipelon    epilithon- lg substrate    epixylon- lg substrate</p> <p>Volume collected (mL): <u>250</u></p> <p>Collected by: <u>sparker</u></p>
<p><b>NEON</b></p> <p>Sample ID: <u>ARIK.20161121.epipsammon.2</u></p> <p>Sample type: phytoplankton    seston    epilithon    epixylon</p> <p>epiphyton <u>epipsammon</u> epipelon    epilithon- lg substrate    epixylon- lg substrate</p> <p>Volume collected (mL): <u>250</u></p> <p>Collected by: <u>sparker</u></p>	<p><b>NEON</b></p> <p>Sample ID: <u>ARIK.20161121.seston.1</u></p> <p>Sample type: phytoplankton    <u>seston</u>    epilithon    epixylon</p> <p>epiphyton    epipsammon    epipelon    epilithon- lg substrate    epixylon- lg substrate</p> <p>Volume collected (mL): <u>4000</u></p> <p>Collected by: <u>sparker</u></p>

**Figure 18.** Example adhesive field labels (2" x 4") for field algae samples. These labels should be filled out with permanent marker.



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<p><b>NEON</b></p> <p>Sample ID: <u>ARIK.20140702.epiphyton.1</u></p> <p>Sample type: <u>epiphyton</u></p> <p>Species ID: <u>bullrush</u></p> <p>Collected by: <u>sparker</u></p>	<p><b>NEON</b></p> <p>Sample ID: <u>ARIK.20140702.epiphyton.3</u></p> <p>Sample type: <u>epiphyton</u></p> <p>Species ID: <u>bullrush</u></p> <p>Collected by: <u>sparker</u></p>
<p><b>NEON</b></p> <p>Sample ID: <u>ARIK.20140702.epiphyton.2</u></p> <p>Sample type: <u>epiphyton</u></p> <p>Species ID: <u>bullrush</u></p> <p>Collected by: <u>sparker</u></p>	<p><b>NEON</b></p> <p>Sample ID: <u>ARIK.20140702.epiphyton.44</u></p> <p>Sample type: <u>epiphyton</u></p> <p>Species ID: <u>bullrush</u></p> <p>Collected by: <u>sparker</u></p>

**Figure 19.** Example of field labels to be printed on all-weather paper. These labels should be filled out with pencil.

4. Sample numbering points below are suggestions. It is **required** that the sample numbers and IDs between the mobile app and the physical label match.
  - a. Sample numbers are used to make the sample IDs unique. Numbers are selected by the field technician in the mobile app, and automatically populate into the sample ID (e.g., ARIK.20190827.EPIPHYTON.1, with “1” being the sample number).
  - b. Lakes/rivers
    - i. Phytoplankton samples should be numbered 1-3
    - ii. Littoral samples should be numbered using the riparian section number (1,3,5,7,9 or 2,4,6,8,10)
  - c. Streams
    - i. Dominant habitat type (1<sup>st</sup> sampler type) samples are typically numbered 1-5
    - ii. Subdominant habitat type (2<sup>nd</sup> sampler type) samples are typically numbered 1-3
    - iii. If all samples at the site are collected using the same sampler type (for example, riffles and runs both collect epilithon), number samples 1-8.

### A.3 Periphyton and Phytoplankton Sampling Prep

1. Collect and prepare all equipment and consumables required for sampling.
2. Prepare sample bottles.
  - a. **NOTE:** Bottles may be rinsed with DI and reused from the last sampling bout (**Table 6**).





**Table 6.** Number of field sample labels and bottle/bags required per site type.

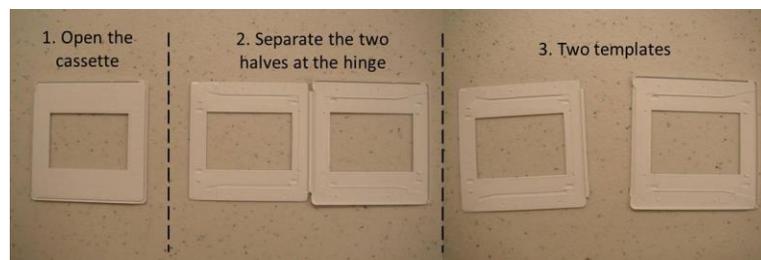
	Sample type	Number of labels/containers	Sample container
<b>Lakes/Rivers</b>	Phytoplankton	15 (5 per station)	1 L amber bottle
	Periphyton	5	125-250 mL amber bottle <b>OR</b> Whirl-pak®/zip-top bag
<b>Wadeable streams</b>	Seston	1 (may need >1 if water is clear)	1 L amber bottle
	Periphyton, dominant habitat	5	125-250 mL amber bottle <b>OR</b> Whirl-pak®/zip-top bag
	Periphyton, secondary habitat	3	125-250 mL amber bottle <b>OR</b> Whirl-pak®/zip-top bag

- Human-readable sample IDs will be generated by the mobile app as follows (**Table 7**). Sample IDs written on the physical sample label must match the sample ID generated by the mobile app.

**Table 7.** Examples of sample IDs generated by the mobile app.

Sample type	Site type	Example sample ID
epilithon	stream, lake, river	REDB.20181123.epilithon.1
epixylon	stream, lake, river	REDB.20181123.epixylon.1
epipsammon	stream, lake, river	REDB.20181123.epipsammon.1
epipelon	stream, lake, river	REDB.20181123.epipelon.1
epiphyton	stream, lake, river	REDB.20181123.epiphyton.1
epilithon_largeSubstrate	stream, lake, river	REDB.20181123.epilithon_largeSubstrate.1
epixylon_largeSubstrate	stream, lake, river	REDB.20181123.epixylon_largeSubstrate.1
seston	stream	REDB.20181123.seston.1
phytoplankton	lake, river	REDB.20181123.phytoplankton.1

- If collecting epilithon/epixylon samples, open 35 mm slide template (like a book) and separate into two halves (**Figure 20**). You will get two rectangular templates from each slide cassette. If the cassette is not split in two, algae can become trapped between the two halves.



**Figure 20.** Separating the two halves of the 35 mm slide template.

- Verify the volume of the 125 mL wash bottle using a graduated cylinder. If it is within 5 mL, use the “fill line”. If the difference is >5 mL, create a new “fill line”.
  - Mark the bottle as “verified” (**Figure 21**).

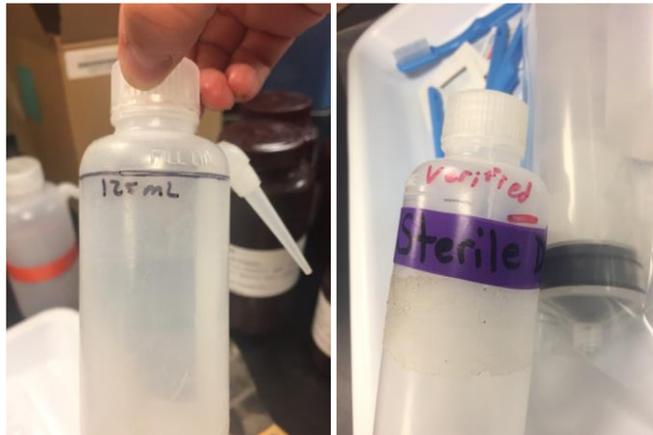


Figure 21. Verified 125 mL bottle examples.

6. Have ice or ice packs frozen and ready for cooler.
7. Check that all equipment is clean and in good condition and all batteries are charged.
8. See Laboratory Preparation procedures in Section E.2 for additional pre-sampling activities (e.g., filter and weigh boat preparation). **Ensure that enough filters are available and pre-ashed for the full sampling suite.**
9. Enter out general aquatic field metadata (RD[08]) in the mobile application once per day upon every field visit, and Secchi and depth profile app in lakes and rivers. If other protocols are done in the same day, one record for field metadata and one record for Secchi and depth profile are sufficient.



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## SOP B Determining Sampling Locations and Sampler Type

Data for field sampling are entered in the (AOS) Algae – Field [PROD] mobile application. Instructions for the use of this application can be found in the Sampling Support Library in the document “Manual for Fulcrum Application: (AOS) Algae – Field [PROD]”.

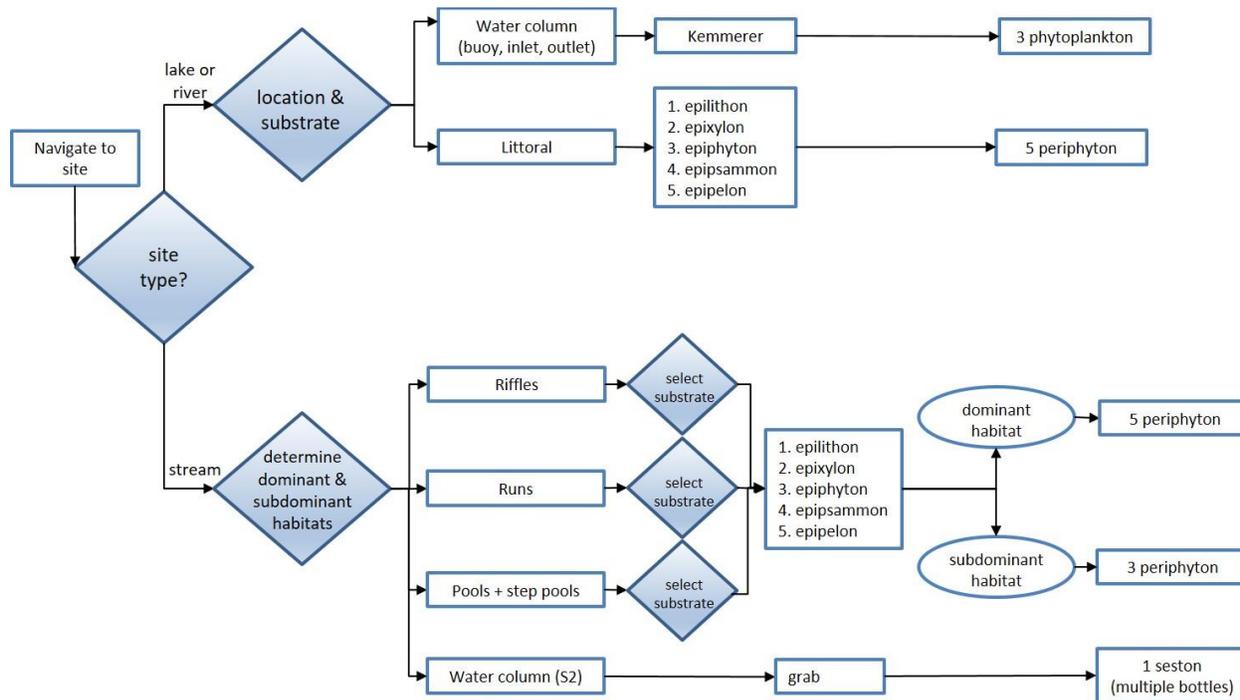


Figure 22. Workflow for field sampling SOPs for periphyton and phytoplankton collection.

### B.1 Spatially and Temporally Linked Protocols

#### Field Metadata and Gauge Height

- Data are entered into the (AOS) Field Metadata and Gauge Height [PROD] app at the start and end of every aquatic field day.

#### Secchi Depth and Depth Profile Sampling

- Secchi and depth profile measurements must be recorded with every protocol that samples the water column, such as phytoplankton (RD[19]) Euphotic zone and stratification depths are required for determining phytoplankton sampling depths.

### B.2 Lakes and Rivers

- Pelagic sampling for Phytoplankton
  - Lakes:** Phytoplankton samples are collected at the center (buoy), inlet, and outlet.



- b. **Rivers:** Phytoplankton samples are collected at the sensor set/buoy and two other locations within the 1 km reach representative of the open water habitat.
2. Littoral sampling for Periphyton (Benthic Algae)
  - a. 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.
  - b. Determine which periphyton substrata to sample in the littoral areas (see Appendix D for habitat and substrata sampling suggestions at NEON sites). Substrata must account for >20% of littoral habitat.
    - i. The habitat type chosen should be present during all sampling bouts.
    - ii. All 5 samples must be taken from the same substratum type on each sampling bout, unless a major event (i.e., a flood or dewatering of the stream) causes significant changes to the substrata and the habitat type is no longer present.
    - iii. If appropriate substrata are not available and samples cannot be collected, select “Sampling Impractical” in the mobile app to indicate the reason for collected fewer samples than expected. Reasons for selecting sampling impractical are usually related to water level (too high or dry conditions) or snow and ice.
  - c. **Lakes:** Periphyton samples are collected in five of the ten riparian sections (refer to the site-specific riparian map, RD[15], **Figure 23**). Collect 1 sample per riparian section selected. If there is a section of the lake where the substrata change, move to the next section where your chosen dominant substratum can be found.
    - i. Haphazardly choose either even- or odd-numbered sections upon arrival at the site. Look at your watch, if the minute is odd, choose odd sections. If the minute is even, choose even sections.



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**Figure 23.** Example of lake perimeter subdivisions from the Lake Riparian Habitat Assessment Protocol (RD[15]).

- d. **Rivers:** Periphyton samples are collected in the littoral habitats within five of the ten riparian transects.
  - i. Haphazardly choose either even or odd sections upon arrival at the site. Look at your watch, if the minute is odd, choose odd sections. If the minute is even, choose even sections.

### B.3 Wadeable Streams

1. Determine percent cover of habitat types throughout the sampling reach using the Stream Morphology Map (RD[16]) or rapid habitat assessment. See Appendix D for habitat and substrata sampling suggestions at NEON sites.
  - a. The habitat types chosen should be present during all sampling bouts.
  - b. All samples (5 in dominant habitat, 3 in secondary habitat) 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 stream channel.
  - c. Targeted habitat types:
    - i. Riffle
    - ii. Run
    - iii. Pool
    - iv. Step pool
  - d. Only sample habitat types that account for >20% of the reach throughout the year.
  - e. Pools and step pools may be combined to account for 20% in high gradient streams.



2. Determine dominant substratum type within the two habitats chosen (i.e., highest percent cover of habitat and/or where visible algal community is attached), based on Stream Morphology Maps (RD[10]) or rapid habitat assessment.
  - a. If there is only one clear habitat at the site, sample two different types of substrata using the sampling methods below (e.g., in a slow-moving run, take 5 epiphyton samples and 3 epipsammon samples).
  - b. Spread samples out along the reach. For example, if sampling riffle habitat, do not collect all samples for the same substratum in the same riffle, collect samples from 5 different riffles along the reach.
  - c. If appropriate substrata are not available and samples cannot be collected, select “Sampling Impractical” in the mobile app to indicate the reason for collected fewer samples than expected. Reasons for selecting sampling impractical are usually related to water level (too high or dry conditions) or snow and ice.

#### B.4 All Aquatic Sites

1. After selecting the habitat types, target substratum types in order of sampling preference (see Definitions, 2.4). If the preferred substratum is present in high enough density (approximately >25% of the substratum of the location, the preferred substratum 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) → follow sampling procedure SOP C.1
  - b. Woody snag (epixylon) → follow sampling procedure SOP C.1
  - c. Boulder/bedrock (epilithon large substrate) → SOP C.2
  - d. Large woody debris (epixylon large substrate) → SOP C.2
  - e. Plant surface (epiphyton) → follow sampling procedure SOP C.3
  - f. Sand (epipsammon) → follow sampling procedure SOP C.4
  - g. Silt (epipelon) → follow sampling procedure SOP C.4
2. Choose sampling locations with shallow, flowing 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. Choose sampling locations that are representative of the periphyton cover of the reach (i.e., not extremely dense or extremely sparse cover relative to nearby substrata).
4. Do not collect samples within a 5 m radius of the aquatic instrumentation.
5. Do not sample substrata where you or other field people/animals have walked or locations that appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).
6. Avoid substrata that are close to the stream/river bank or lake shore and may be exposed to frequent drying.



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7. Choose sampling locations that are exposed to ambient light (e.g., not under a log or cut bank) and representative of the light regime within the reach (i.e. do not sample in the one sunny patch of a heavily canopied stream).
8. Unless sampling epiphytes, avoid substrata that are heavily colonized with aquatic plants, bryophytes, invertebrates, or have leaf litter clinging to the surface. You may brush some invertebrates off the surface, but the presence of aquatic plants and bryophytes may skew the chlorophyll results.
9. Do not collect all samples from the same location.
  - a. In **wadeable streams**, collect each composite sample from a different habitat unit (i.e., 5 riffle samples should be collected from 5 separate riffles).
  - b. In **lakes and rivers**, collect composite samples from different riparian sections or transects.
10. Start sampling at the bottom of the reach, working upstream so as not to stir up sediments in the water column which can decrease visibility.





## SOP C Field Sampling

### C.1 Epilithon (Rock Scrubs) and Epixylon (Wood Scrubs)

1. Select three cobbles or pieces of woody debris for each composite sample (e.g., 15 total cobbles for 5 composite samples) that meet the requirements in SOP B.4 AND the following [after Richest Targeted Habitat (RTH) requirements; Porter et al. 1993 and Moulton et al. 2002]:
  - a. Stable in the stream/river/lake bed (i.e., have not recently tumbled).
  - b. Larger than the scrubbing template (i.e., > 2 inches in diameter).
  - c. Avoid substrata that are heavily colonized with macroinvertebrates or plants/mosses.
  - d. Woody debris pieces may be cut to a manageable size with a bow saw for sampling.
2. Note the dominant substratum size class at the sampling location (record main substratum size within the habitat unit under wood sampled for epixylon) on the mobile application.
  - a. Size class options:
    - i. Clay (0.001-0.004 mm)
    - ii. Silt (0.004-0.062 mm)
    - iii. Sand (0.062-2.0 mm)
    - iv. Pebble (2.0-64.0 mm)
    - v. Cobble (64.0-256 mm)
    - vi. Boulder (>256 mm)
    - vii. Bedrock
3. Place cobble/woody debris 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 has been exposed to sunlight, and will be the portion of the cobble that you sample.
  - a. You may clean the bottom of the cobbles with your hands to remove excess material so it doesn't get in the tray and contaminate the sample.
4. Be sure to keep cobbles/woody debris moist with native water until scrubbing.
5. Proceed to a location on the stream bank or boat to process the sample. This location should be out of direct sunlight if possible (**Figure 24**).



**Figure 24.** Process samples on the stream bank, out of direct sunlight.

6. Rinse the inside of the 125 mL wash bottle 3 times with DI. Discard water into the stream.
  - a. You only need to rinse the wash bottle if this is your first sample for the day at that stream. No need to re-rinse the wash bottle between samples at the same stream.
7. Fill the 125 mL wash bottle with DI water 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 field 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 9 in Section E.3 for recommended filter volume and adjust water volume and amber HDPE bottle size as necessary. Be sure to record any changes in field sample volume.
  - d. Use DI to minimize the number of algal cells from the source water that will be introduced to the final sample through the rinse water from the 125 mL bottle that will remain in the sample.
8. Rinse the inside of the amber wide-mouth HDPE sample bottle with source water. Fill bottle ~1/4 full, cap, and shake vigorously. Discard rinse water into stream/lake or onto the bank. Rinse each bottle 3 times. Recap bottle and set aside.
  - a. DI is not necessary for rinsing bottles. Using source water for rinsing the container primes the container and introduces negligible algal cells as it is rinsed and discarded.
  - b. Samples do not need to be numbered in any particular order.
  - c. 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 rinse any leaves and/or large invertebrates from surface using stream/lake water (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). Brush debris from the underside of the cobble with your hand so it doesn't contaminate the scrubbing tray.

- a. Recheck cobble, if there are more than 10 invertebrates attached to the substratum within your template, discard and choose a new cobble/piece of woody debris.
  - b. If there is growth of aquatic plants or bryophytes that falls 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 25**). Check cobble/woody debris again for colonization of invertebrates, bryophytes, or plants.



**Figure 25.** Template placement for epilithon protocol. (Note: Samples should not be processed in direct sunlight)

12. Holding the template firmly in place on the cobble/woody debris, begin scrubbing inside the template.
  - a. Use the brass-bristled brush for cobbles, use the toothbrush for woody debris. The brass-bristled brush is necessary when scrubbing cobbles so that tightly adhered algal taxa are removed from the cobble surface. The toothbrush is used for woody debris to minimize pieces of woody material in the sample. Be sure to use the correct brush for the selected substratum.
  - b. 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 sample bottle with remainder of scrubbed sample.
  - c. Be sure to hold the template in place, as slipping would change the area you are sampling (**Figure 25**).
  - d. Scrubbing should be similar to brushing your teeth. Avoid scrubbing so hard that epilithon spatters out of the tray.
  - e. Ensure that leaves and grasses from the bank do not enter the sample.
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.
15. Remove template. There should be a clean rectangle left on the substratum from scrubbing. This is harder to see on woody substrates.
16. Using the wash bottle, rinse the template (front and back) and the cobble/woody debris into the tray.
17. Repeat Steps 10-16 until all 3 cobbles/woody debris collected for the composite sample have been scrubbed and rinsed.



18. Rinse scrub brush and fingers over the tray. If there is any remaining water in the 125 mL wash bottle after all three cobbles have been scrubbed, 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.**
19. Discard cobble/woody debris on the stream bank.
  - a. If you are finished at this location, you may discard the cobble/woody debris.
  - b. If you are not done at this location, hold the cobble/woody debris on the bank and discard in water when you leave the site.
20. Carefully swirl contents of tray (scrubbed material+ rinse-water) to re-suspend the sample.
21. Carefully pour the suspended sample and water into 125 mL or 250 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, and the sample is well-mixed, you may keep the sample and proceed to the next step.
    - i. The data depend on the volume of water used to suspend the algal material. If the slurry is well-mixed, removing a small amount of sample at this point does not affect the data.
  - b. If you spill a significant amount (i.e., >10 mL) of sample), dump the entire sample and start over at Step 1 as you may not have enough sample to create all of the subsamples in SOP E.
  - c. There may be some sand left in the bottom of the tray after swirling and pouring out the sample. This is ok.
  - d. If sampling epixylon, double check the field sample volume. It should equal the original rinse volume. If it is less than the original rinse volume, submit a ticket through the problem reporting system. This check may be done in the field or domain lab.
22. If you feel that your sample was not properly mixed and some has remained in the tray, you may pour the sample back in the tray and repeat Steps 20-21.
23. If you need more water to rinse with, refill the 125 mL wash bottle with DI water 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 record the total volume used for rinsing on the sample label and on the mobile application. **It is very important to keep track of any changes in field sample volume.**
  - a. Additional water will also require that you use a larger sample bottle (i.e., 250 mL HDPE amber wide mouth).
24. Cap bottle tightly, double check labels, and place in a cool storage location out of direct sunlight until sampling is finished.





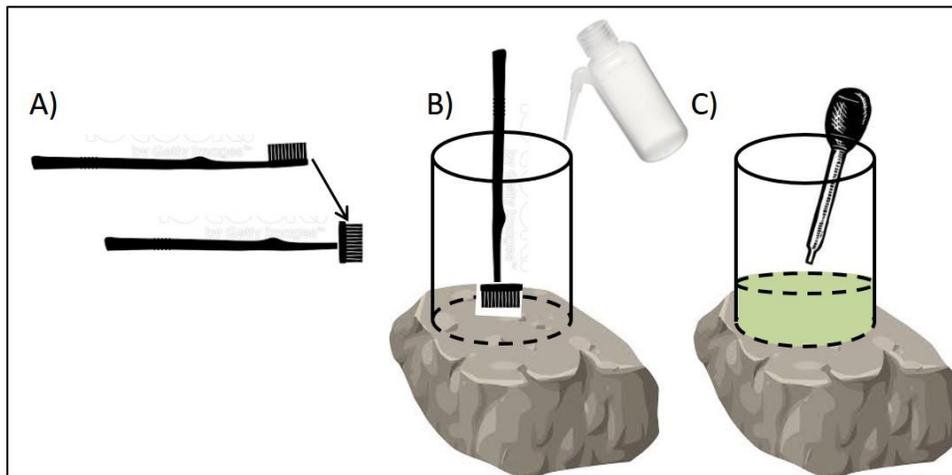
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- a. Place sample bottles in the cooler or submerge the bottles in water near the bank to keep them cool. If submerged, be sure that they will not float downstream. Place bottles in the cooler as soon as possible.
  - i. NOTE: it is not recommended to submerge samples in rivers. Please place samples directly in the cooler.
25. Enter data in the mobile app. In the case of app failure, fill out a field datasheet in pencil.
  - a. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
26. Rinse tray, brush, and template with native water before starting next sample.
27. Repeat above steps until all locations have been sampled.
  - a. **Wadeable streams**, dominant habitat: 5 composite samples
  - b. **Wadeable streams**, secondary habitat: 3 composite samples
  - c. **Lakes**: 5 composite samples, 1 from each of 5 riparian sections
  - d. **Rivers**: 5 composite samples, 1 from each of 5 riparian sections
28. Return samples to the Domain Support Facility to start processing within 24 hours (SOP E).

## C.2 Epilithon (Rock Scrubs) and Epixylon (Wood Scrubs) – Large Substrate

This section is for epilithon and epixylon on substrates that you are not able to pick up to sample, e.g., boulder, bedrock, and large woody debris. Each sample is a composite of three scrubs.

1. Fill out labels (all-weather adhesive labels,
2. Figure 18, RD[07]).
  - a. **Wadeable streams**, dominant habitat: 5 labels
  - b. **Wadeable streams**, secondary habitat: 3 labels
  - c. **Lakes/Rivers**: 5 labels
3. Use the large substrate sampler and modified toothbrush for sampling.
4. Rinse the inside of the amber wide-mouth HDPE sample bottle with native water. Fill bottle ~1/4 full, cap, and shake vigorously. Discard rinse water into stream/lake or onto the bank. Rinse each bottle 3 times. Recap bottle and set aside.
5. Select a sampling location that is shallower than the PVC tube on the sampler. Choose substratum that is free of plants/bryophytes and minimal macroinvertebrate colonization.
6. Fill the 125 mL wash bottle to the fill line with stream water.
  - a. You may use as much rinse water as you need, as long as you keep track of the volume used and record in the mobile application.
7. Place the PVC periphyton sampler tightly on the substratum to be sampled. Hold the sampler as allowing it to slip will affect the benthic area sampled.
8. Using a modified toothbrush (**Figure 26A**), scrub the substratum within the PVC tube. Use the wash bottle to rinse as needed. Bring extra brushes to the field in case they break.
  - a. Use sandpaper to scuff up the ends of the brush before applying adhesive.
  - b. Allow time for epoxy or adhesive to cure when creating the modified brush.



**Figure 26.** Schematic of PVC periphyton sampler. A) Cut off the head of a toothbrush, and re-adhere it horizontally using epoxy. B) Set the PVC sampler on the substrate, and scrub the substrate surface using the modified toothbrush. Rinse rock surface. C) Remove water + algae sample with a turkey baster or disposable pipet.

9. When finished scrubbing, use the turkey baster to pipet the sample out of the PVC tube. Place the sample directly into the sample bottle.
10. Pipet all of the scrubbed sample out of the PVC tube. Before moving the tube, rinse the sides of the tube and the substratum at least once with the wash bottle, and pipet into the sample bottle. Continue to rinse and pipet until it appears that the water is relatively clear and free of scrubbed algae.
11. When finished removing the sample from the PVC tube, remove the tube from the stream.
12. Move sampler to a second and third location in the same area to create a composite samples consisting of 3 scrubs.
13. Ensure that the amount of water in the sample bottle is known. You may add the remainder of the wash bottle directly to the sample bottle if necessary.
14. Record data in the mobile app.
  - a. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
15. Rinse all equipment well with stream water prior to moving to the next sampling location.
16. Repeat above steps until all locations have been sampled.
  - a. **Wadeable streams**, dominant habitat: 5 composite samples
  - b. **Wadeable streams**, secondary habitat: 3 composite samples
17. Return samples to the Domain Support Facility to start processing within 24 hours (SOP E).

### C.3 Epiphyton (Aquatic Plant Surfaces)

1. Fill out labels (all-weather paper labels,
2. Figure 18, RD[07])
  - a. **Wadeable streams**, dominant habitat: 5 labels
  - b. **Wadeable streams**, secondary habitat: 3 labels

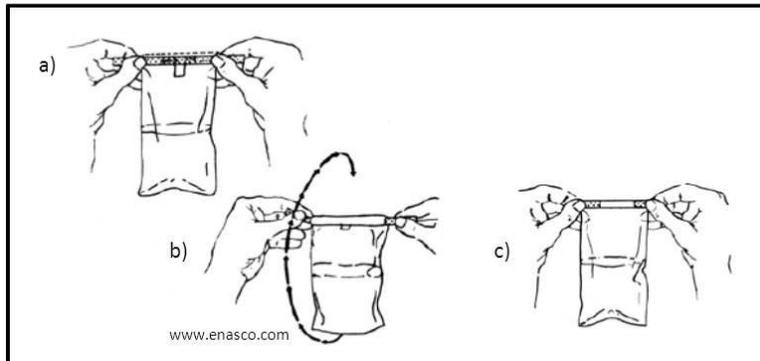


- c. **Lakes/Rivers:** 5 labels
3. Select plants for sampling that are well-colonized with epiphytes (Figure 27) and that meet the following requirements:
  - a. Sample from only 1 plant species to standardize sampling.
  - b. The plant species should be common (i.e., accounts for >50% of the aquatic plants) in the reach.
  - c. Plants should not be covered by sediments.



**Figure 27.** Example of epiphytes growing on rushes in a Colorado stream.

4. Select a 10 x 10 cm area of stream 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 material that is rooted within the selected quadrat.
  - b. Use a metric ruler to estimate sample area.
  - c. Note the dominant substratum size class at the sampling location in the mobile application.
5. Cut all plants rooted within the 10 x 10 cm area at their bases using scissors or clippers and carefully place them inside a Whirl-pak<sup>®</sup> bag or gallon resealable bag. Plants may be folded over to make them fit in the bags.
  - 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. Add the filled-out all-weather paper label to the Whirl-pak<sup>®</sup>.
  - d. Do not add water to the Whirl-pak<sup>®</sup>.
6. Close Whirl-pak<sup>®</sup> or gallon resealable bag and place in a cool, dark location (e.g., cooler).
  - a. Close the Whirl-pak<sup>®</sup> by holding the wire tabs at either side of the bag (**Figure 28**), 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.



**Figure 28.** 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.

7. Enter data into the mobile app. In the case of app failure, fill out a field datasheet in pencil.
  - a. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
8. Repeat above steps until all locations have been sampled
  - a. **Wadeable streams**, dominant habitat: 5 samples
  - b. **Wadeable streams**, secondary habitat: 3 samples
  - c. **Lakes**: 1 sample from each of 5 riparian sections
  - d. **Rivers**: 1 sample from each of 5 riparian sections
9. Return samples to the Domain Support Facility to start processing within 24 hours (SOP E).

#### C.4 Epipsammon (Sand) and Epipelon (Silt)

1. Fill out adhesive labels in permanent marker (2" x 4" all-weather adhesive labels) and adhere to 250 mL wide-mouth amber HDPE bottles (**Figure 18**)
  - a. **Lakes/Rivers**: 5 sample bottles
  - b. **Wadeable streams**, dominant habitat: 5 sample bottles
  - c. **Wadeable streams**, secondary habitat: 3 sample bottles
2. Note the dominant substratum size class at the sampling location in the mobile application.
3. Rinse the inside of the 250 mL amber HDPE sample bottle with stream 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.
  - b. You can rinse all sample bottles for that site at the same time and set aside, or rinse them separately.
4. Rinse white plastic sampling tray with stream water.
5. Rinse the lid (larger half) of a 47 mm plastic petri dish in native water. Holding lid upside-down underwater, rub the inside of the lid with your fingers to remove air bubbles.
6. 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.





7. Slide spatula under lid to enclose the sample. Holding the petri lid tightly to the spatula, lift out of water (**Figure 29**). Make sure that water and sediment do not leak out. Gently rinse excess silt not enclosed by petri lid from spatula with stream/lake water. Do not include this water in the sample, or count this rinse water in the field sample volume.
  - a. If sample leaks out of the petri lid when lifting the spatula out of the water, discard and resample.



**Figure 29.** An epipelon sample collected with the Petri dish lid and metal spatula.

8. Place spatula + sample + petri lid in white sampling tray and invert lid.
9. Repeat Steps 5-8 until you have collected 3 petri lid samples to combine into one composite sample.
  - a. 3 petri lid samples = 1 composite sample
10. Fill 125 mL wash bottle with DI water 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 field 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 9** in Section E.3 for recommended filter volume and adjust water volume and amber HDPE bottle size as necessary. Be sure to record any changes in field sample volume.
-  11. Using the 125 mL DI wash bottle, rinse petri lid and spatula into tray. **DO NOT DISCARD RINSE WATER.**
12. Carefully pour sample into 250 mL amber HDPE bottle. Take care not to spill sample.
  - a. Use your fingers and the remaining water from the 125 mL wash bottle 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 in the mobile app.
13. 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 water near the bank to keep them cool. If submerged, be sure that they will not float downstream. Place bottles in the cooler as soon as possible.
    - i. NOTE: it is not recommended to submerge samples in rivers. Please place samples directly in the cooler.
14. Enter data into the mobile application. In the case of app failure, fill out afield datasheet in pencil.
  - a. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
  - b. If a sample has mixed substrate sizes, epipsammon + epipelon, record as the larger substrate type.
15. Rinse tray, petri dish, and spatula with stream water before starting next sample.
16. Repeat above steps until all locations have been sampled
  - a. **Wadeable streams**, dominant habitat: 5 composite samples
  - b. **Wadeable streams**, secondary habitat: 3 composite samples
  - c. **Lakes**: 1 composite sample from each of 5 riparian sections
  - d. **Rivers**: 1 composite sample from each of 5 riparian transects
17. Return samples to the Domain Support Facility to start processing within 24 hours (SOP E).

#### C.5 Seston: Wadeable Streams ONLY

1. Label a 1 L amber HDPE bottle with domain, date, site, sample number, location, habitat type, type of sample (i.e., seston), and collector's name (**Figure 18**).
  - a. Wadeable stream seston/phytoplankton will only be used for chlorophyll/pheophytin and AFDM.
  - b. At sites with very clear water, >1 L of water may need to be collected to filter enough material for chlorophyll and AFDM.
2. Sample at the water chemistry sampling location near sensor set S2 (**Figure 13**).
  - a. Note the dominant substratum size class at the sampling location in the mobile application.
3. Make sure no one is wading upstream of you.
4. Rinse amber 1 L HDPE bottle 3 times with stream water, shake vigorously. Discard rinse water into stream. Recap bottle.
5. Standing in the thalweg facing upstream, hold HDPE bottle ~10 cm below the water surface with the top pointed upstream.
6. Tilt bottle slightly underwater to allow stream water to fill bottle.
7. When HDPE bottle is full, cap tightly and place in the cooler.
8. Enter data into the mobile application. In the case of app failure, fill out afield datasheet in pencil.
9. Double check bottle labels and place sample bottles in cooler with ice packs (do not freeze) or stream water is cool, you may place bottles in a shady location where they will not float away.



10. Return samples to the Domain Support Facility to start processing within 24 hours (SOP E).

### C.6 Phytoplankton Sampling- Lakes and Rivers ONLY

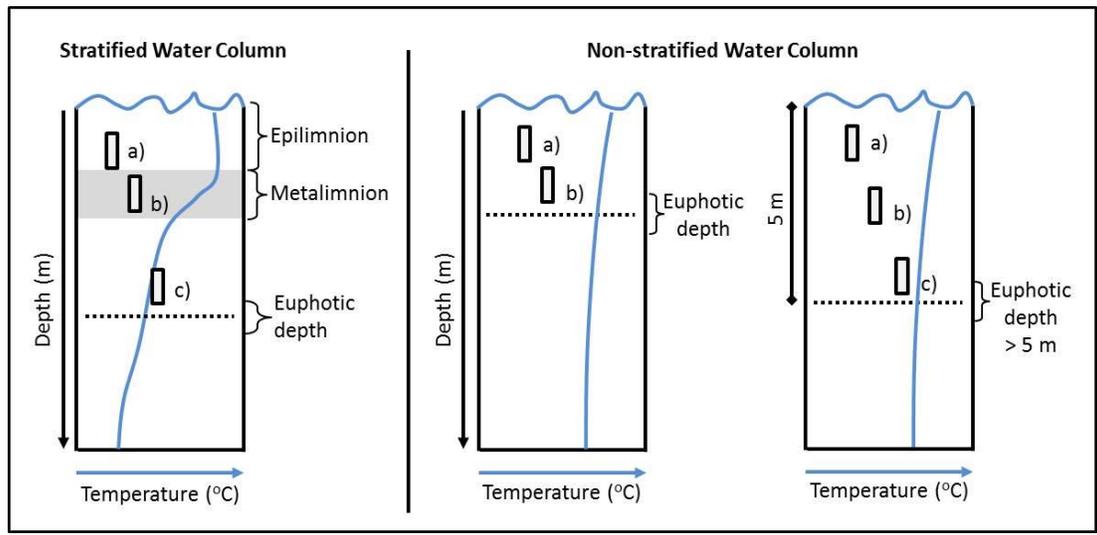
The Kemmerer is the accepted sampler for phytoplankton sampling. At select river sites with fast flow, a Van Dorn may be used in place of the Kemmerer.

1. Label 12-15 1-L amber HDPE bottles with domain, date, site, sample number, location, habitat type, type of sample (i.e., epilithon), and collector's name (**Figure 18**).
  - a. Four to five 1 L amber HDPE bottles equal 1 composite sample.
  - b. Three composite samples total are collected in lakes and rivers.
2. Sample in three locations per lake or river.
  - a. Lakes (near water chemistry sampling locations, **Figure 13**):
    - i. Deepest point in the lake, determine by bathymetric site map (RD[17]) and preloaded GPS coordinates (near the buoy infrastructure)
    - ii. Near the lake inlet infrastructure
    - iii. Near the lake outlet infrastructure
  - b. Rivers:
    - i. Near the buoy infrastructure, 5-10 m downstream of buoy
    - ii. Two other locations within the 1 km reach that are representative of the deeper-water habitat (similar to the buoy location)
3. Navigate the boat to the sampling location.
4. Gently lower an anchor at the bow and allow boat to float back with wind or current to sampling location. Drop a second anchor at the stern to hold boat in place.
  - a. Allow ~5 minutes for sediments to settle after lowering the anchor, you can use this time to prepare the sampling equipment.
  - b. Using a bow anchor rope 2 times the water depth will minimize disturbance of the sediment at the sampling location.
  - c. Sample on the side of the boat, away from the motor and anchor, so as not to interfere with the sampler or disturb the sediments
5. Always sample near the bow of the boat to minimize the effects of the motor on the water column.
6. At the buoy location, determine the depths of the thermocline and euphotic zone according to the AOS Protocol and Procedure: Secchi Disk and Depth Profile Sampling in Lakes and Non-wadeable Streams (RD[19]). Euphotic and thermocline depths determined at the buoy will be applied to the inlet and outlet locations.
  - a. Determine the depth of the euphotic at the buoy zone by multiplying the mean Secchi depth by 2.5.
  - b. Measure to the bottom of the Kemmerer or Van Dorn.
  - c. If thermal stratification is present, integrate the following (**Figure 30**):
    - i. Surface: 0.5 m ( $\pm 0.5$  cm) below water surface  $\rightarrow$  1 sampler



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- ii. Metalimnion: middle of metalimnion (defined by 1° per m change in water temperature) → 1 sampler
- iii. Hypolimnion/Euphotic: 0.5 m (±0.5 cm) above euphotic depth → 1 sampler
- d. If the lake/river is not stratified, determine sample depths from the Secchi calculations. Integrate 2 samplers if the euphotic depth is < 5m, integrate 3 samplers if the euphotic depth is > 5m.
  - i. Surface: 0.5 m (±0.5 cm) below water surface → 1 sampler
  - ii. **If euphotic depth is >5 m**, Euphotic midpoint: half the depth of the euphotic zone → 1 sampler
  - iii. Euphotic bottom: 0.5 m (±0.5 cm) above euphotic depth → 1 sampler
- e. It is possible that the metalimnion sample may sometimes be below the euphotic depth. The metalimnion represents a barrier that captures sinking cells, so a metalimnion sample provides good insight into the community from a few hours to days before the sample was taken.

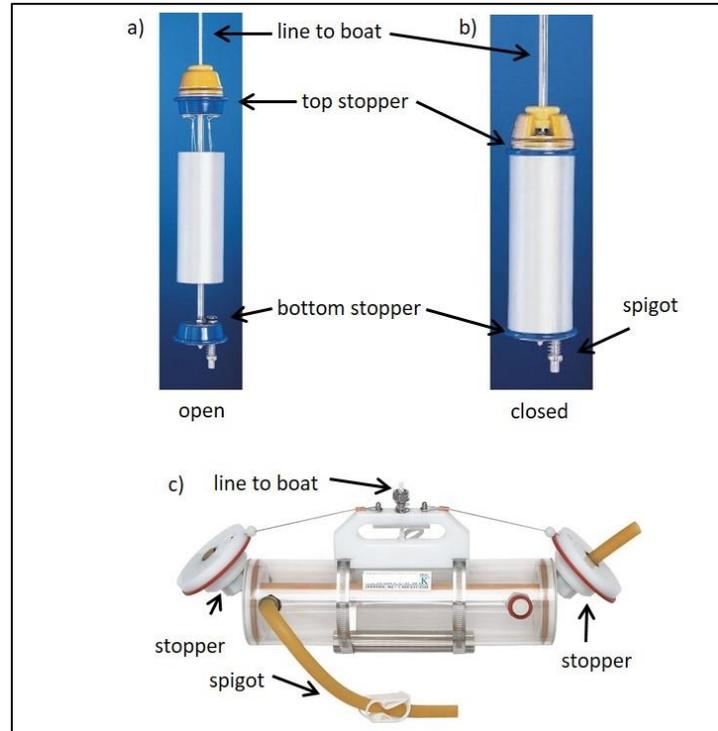


**Figure 30.** Sample depth selection in a stratified water column. The blue line is a temperature profile indicating the presence or absence of a thermocline. If stratification is present, integrate 3 samplers: a) 0.5 m below the water surface, b) middle of the metalimnion, and c) 0.5 m above bottom of euphotic zone. If stratification is not present and the euphotic depth is < 5m, integrate 2 samplers: a) 0.5 m below the water surface and b) 0.5 m above bottom of euphotic zone. If stratification is not present and the euphotic depth is > 5m, integrate 3 samplers: a) 0.5 m below the water surface and b) midpoint of the euphotic depth, and c) 0.5 m above bottom of euphotic zone.

- 7. Rinse the 4 L HDPE jug and Kemmerer or Van Dorn with lake/river water 3 times over the opposite side of the boat from where you plan to sample. Discard rinse water into the lake/river. Set 4 L jug aside. You may use surface water to rinse.
- 8. 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 31**). If using a Van Dorn, open and secure the stopper ends of the sampler.



- a. NOTE: A short, hard pull is important to keep the stoppers open. If the stoppers don't stay open, pull harder.



**Figure 31.** Vertical Kemmerer water sampler in the a) open or cocked position and b) closed (after dropping the messenger) position. c) Horizontal Van Dorn in the open position

9. Tie the free end of the sampler line to a cleat on the boat to prevent losing the sampler.
10. Always start with the shallowest sample first to avoid disturbing the water column (i.e., sample surface water first and bottom of thermocline last).
11. 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 and hold the messenger in one hand. Ensure it is secured properly to the line.
12. Continue to lower the sampler until it reaches the desired depth by using the depth markings on the line attached to sampler.
  - a. Measure to the bottom of the sampler.
13. 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 31b**).
14. Pull the sampler up by pulling the line into the boat, coiling it neatly.
  - a. Note: If the sampler does not close properly when you pull it up, resample in the same location.
15. Uncap the 4 L HDPE jug.



16. Carefully open the spigot (**Figure 31b**) on the bottom of the sampler, allowing the contents of the cylinder to flow into 4 L jug.
  - a. Repeat Steps 8- 16 and integrate samples in the 4 L jug.
    - i. If integrating 3 samplers, add only 1.3 L from each sampler grab to the 4 L jug.
17. Rinse pre-labeled sample bottles three times with lake/river water, discard water back into the lake/river.
18. Mix integrated sample gently in 4 L jug, then 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. If stream water is cool, you may place bottles in a shady locations where they will not float away.
  - b. The 4-5 1L bottles = 1 composite sample.
  - c. Samples must be kept dark and cool until filtering.
  - d. Record estimated field sample volume = volume of sample in all composite bottles. You do not need to get an exact volume here.
19. Record data in the mobile app.
  - a. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
20. Proceed to the next sample location and repeat above steps.

### C.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 E).



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## SOP D Post-Field Sampling Tasks

1. Refresh the sampling kit
  - a. Remove old labels from HDPE bottles that will be reused.
  - b. Print and fill out new adhesive labels (RD[07]). Attach labels to bottles before going out in the field.
2. Equipment, maintenance, cleaning and storage
  - a. Wash all equipment that has come in contact with site water according to the NEON Aquatic Decontamination Protocol (RD[06]).
  - b. Clean boat and motor; remove aquatic plants by hand to prevent spread of invasive taxa. Allow boat and motor to dry completely.
  - c. Only reuse Amber HDPE bottles at the same site. Keep a separate set of bottles dedicated to each site.
  - d. Rinse amber HDPE bottles with DI, these will be used and rinsed again in the field during the next field collection. Since each site has a set of bottles, they do not need to be decontaminated between bouts.
  - e. Dry all items completely between sites and before storage.
  - f. Discard and replace any broken templates, petri dishes, or worn brushes.
3. Data QA/QC
  - a. Required checks
    - i. Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample bottles.
  - b. Nice to check
    - i. Site ID, collect date, sampling protocol version
    - ii. Sampling depths

### D.1 Document Incomplete Sampling Within a Site

Periphyton and phytoplankton (algae) sampling is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in Section 4.1. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (gradient sites). However, sampling may be shifted from one location to another when sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced.

There are two main pathways by which sampling can be compromised. First, sampling locations can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot is compromised after road-building activities, or a stream moves after a flood and the location is no longer within the stream channel). Second, sampling locations may be located in areas that are logistically impossible to sample on a schedule that is biologically meaningful.

Algae sampling locations may become compromised if a stream dries or is snow or ice covered, or if the water level of the lake drops too low to sample near the inlet and outlet infrastructure. If sampling at a



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given location or habitat type is not possible during a given bout a problem ticket should be submitted by Field Science staff.

Please note that 3 periphyton or 3 phytoplankton samples are the minimum that can be collected during a bout to ensure scientifically useful data. Therefore, it is preferable to collect a sample in a different location rather than not collect a sample at all.

To document locations not sampled during the current bout:

1. Review Fulcrum records to determine which locations were scheduled for sampling but were not sampled.
2. Create an incident with the following naming convention to document the missed sampling: ‘AOS Sampling Incomplete: ALG – [Root Cause Description]’
  - a. Example: ‘AOS Sampling Incomplete: ALG – Could not access inlet and outlet due to cows’
3. Staff scientists review incident tickets periodically to determine whether a sampling location is compromised.



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## SOP E Laboratory Sampling and Analysis

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

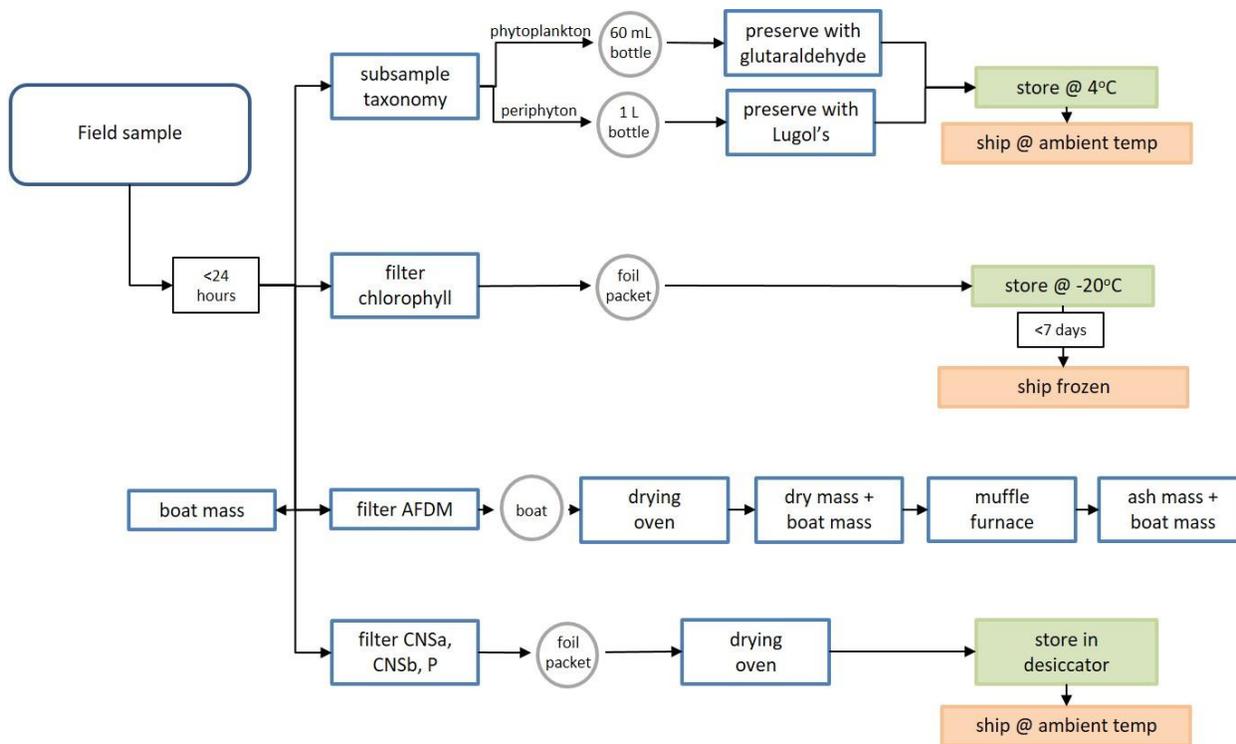


Figure 32. An expanded diagram of the lab workflow for the algae lab SOP.

### E.1 Labels and Identifiers

Barcodes must be applied to any sample that is shipped to an external facility for analysis. All barcodes need to be applied to dry containers 30 minutes before use. Algal taxonomy subsamples and algae chemistry (CNSa, CNSb, P) foil packets use Type I barcodes (prefix A, plus 11 numbers). Chlorophyll/pheophytin packets are frozen and use Type II barcodes. AFDM samples will not have a barcode.

1. All subsamples will have a weather-resistant, adhesive, human readable label on the outside of the bottle or packet (Figure 33). AFDM samples will not have a human readable label.



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**Figure 33.** Example of algal chemistry packets with human readable label and barcode label.

2. Adhesive barcode labels should be applied to dry, room temperature bottle and packets (**Figure 34**).
  - a. Algal taxonomy: barcode labels should be aligned lengthwise along the bottle as the scanner will not work on a curved surface (i.e. horizontally wrapped around the bottle; **Figure 16**).
  - b. Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.



**Figure 34.** Example of adhesive barcode labels, Type I. These large-size, field-tolerant barcodes have a prefix of 'A' followed by 11 numbers.



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## E.2 Preparation

1. Pre-ash GF/F filters (**Table 8**)
  - 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 at least 6 hours.
  - c. After furnace has cooled, remove filters 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 in sealed zip-top bag.
  - f. Ashed filters may be stored indefinitely, as long as they remain in the box and stay dry.

**Table 8.** Minimum number of pre-ashed filters and taxonomy samples required per bout. In addition, you will need to create 2 blank filters per domain per bout.

Site type	Number of samples (field)	Total number of filters needed per site per bout	Number of taxonomy subsamples per site per bout
Lake	8	46	6
River	8	46	6
Wadeable	9	48	8 (send 6 to lab, hold 2 at DSF)

2. If aluminum weigh boats are new and unlabeled:
  - a. Label ~20 boats by inscribing a unique number on the bottom with a pencil (e.g., A1, A2, A3, etc.; **Figure 35**).
    - i. Note: It does not matter what the labels are as long as they are unique from each other and easy to read.
    - ii. If the boat has a tab on the side, you can inscribe the number on the tab in addition to the boat bottom for easier reading during analysis. Do not rely on the tab alone as they tend to fall off with use.
  - b. Place new empty labeled boats in the muffle furnace (500 °C) for 6 hours to burn off any residue.
  - c. After 6 hours, carefully remove boats from the furnace and allow to cool completely (>20 minutes) to room temperature.
    - i. Use thermal gloves and tongs when handling hot material
    - ii. Set boats aside in a safe, heatproof location
    - iii. After cooling, weigh boats on analytical balance (0.0001 g) and record boat number and weight (g) in mobile app. You may weigh directly prior to use, or up to 2 days prior to algal sample processing to prevent debris from entering the boats prior to weighing.
  - d. This may be done in advance, before field sampling.
  - e. Boats may be reused from previous sampling bouts.



**Figure 35.** Examples of newly labeled aluminum weigh boats.

3. If aluminum weigh boats have been previously labeled and used, clean with a soft brush to remove any residual ash.
  - a. Boats should be re-ashed to minimize residue between sampling events.
  - b. Boats must be re-weighed prior to every use. Minimize touching the boat with un-gloved hands after weighing. Oils from your hands can skew results.
  - c. Best practice is to re-ash boats prior to use, rather than storing ashed and weighed boats for a long period of time between bouts. Boats may be stored in a closed container (e.g., bag, box, or plastic container) to minimize dust contamination.

### E.3 Processing Samples

Samples will be subsampled and filtered in the Domain Support Facility following **Figure 36** and **Table 9**.



1. Prolonged exposure to light, including overhead lights in the lab, may artificially affect pheophytin data. If samples take longer than 15 minutes to filter, filter in the dark or devise a hood (e.g., aluminum foil cover) to shade the filter manifold while filtering chlorophyll/pheophytin filters. Other filter types are not affected.
2. Thoroughly mix (shake) sample bottles prior to removing any subsamples (includes taxonomy and filtering).
3. Measure alternate field sample volume (amount of water in the sample). Complete this step for **epipsammon/epipelon samples**, and any other samples that appear to have lost or gained water relative to the amount of water added in the field.
  - a. Pour entire sample and sediments into a graduated cylinder (Fetscher et al. 2015). Pour back and forth between the bottle and the graduated cylinder to loosen the sample.
  - b. Wait ~2-5 minutes to allow sediment to settle out.
  - c. When a boundary between sediment and water becomes clear, measure and record the water volume in the mobile app.
  - d. Pour all material back into sample container. Gently pour sample water back and forth between the sample container and the graduated cylinder until all sediment is transferred back to the sample container. It may be difficult to get all sediment back into the bottle, get as much as possible after multiple pours. Do not spend more than 5 minutes on this step, it is ok if some sand/silt is left behind.



- e. If you add additional rinse water to make this transfer from the graduated cylinder to the bottle easier, you must add that to the alternate sample volume.
- f. Shake sample container for 30 seconds to remove periphyton from substrata (**epipsammon/epipelon** samples). This is similar to the scrubbing step for other algal types.
- g. Enter volume from graduated cylinder as “Alternate Field Sample Volume” in the mobile app.

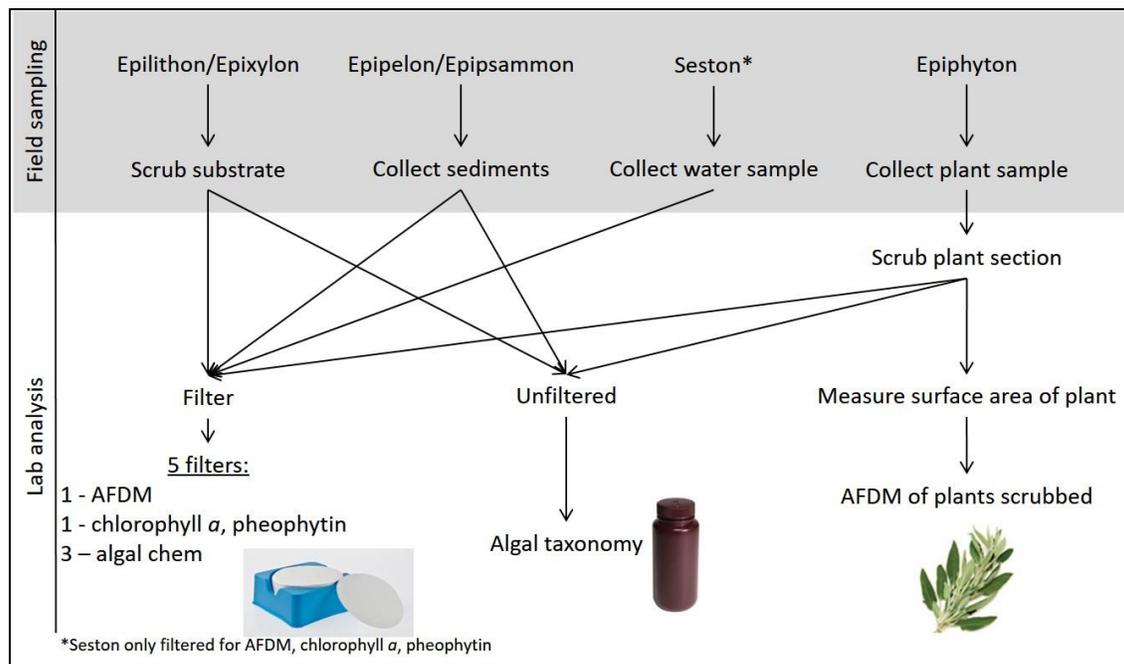


Figure 36. Lab schematic diagram for all aquatic sites.

Table 9. Subsample volume and priority for lab processing. If unable to push the minimum recommended volume through a filter, then filter as much sample as possible and record volume in the mobile app. Please note, if filtering the maximum amount for benthic samples, a 250 mL parent sample is required.

Priority	Sample	Parameter	Type	Recommended subsample volume
1	Periphyton	Taxonomy	Liquid, preserved in glutaraldehyde	60 mL
2	Periphyton	Chlorophyll <i>a</i> /pheophytin	Filter (1)	2-10 mL (until visible color on filter)
3	Periphyton	AFDM	Filter (1)	2-10 mL (until visible color on filter)
4	Periphyton	Algal chemistry (C, N, P, and C, N, S isotopes)	Filters (3)	As much sample as possible, 5-10 mL
1	Phytoplankton	Taxonomy	Liquid, preserved in Lugol's	1 L



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2	Phytoplankton	Chlorophyll <i>a</i> /pheophytin	Filter (1)	≥250 mL (until visible color on filter)
3	Phytoplankton	AFDM	Filter (1)	≥250 mL (until visible color on filter)
4	Phytoplankton	Algal chemistry (C, N, P, and C, N, S isotopes)	Filters (3)	As much sample as possible, >500 mL
1	Seston (wadeable streams)	Chlorophyll <i>a</i> /pheophytin	Filter (1)	≥250 mL (until visible color on filter)
2	Seston (wadeable streams)	AFDM	Filter (1)	≥250 mL (until visible color on filter)

#### E.4 Algal Taxonomy Samples (Unfiltered)

1. PLEASE NOTE that starting in 2019, the number of taxonomy subsamples has been decreased in lakes and rivers after completing an optimization analysis of algal taxonomic richness and cell density.
  - a. Lake and river sites: select 3 of the 5 littoral samples for taxonomy. Using the riparian sections, select 3 sections haphazardly (e.g., if riparian sections 1, 3, 5, 7, and 9 were sampled, select 1, 5, and 7 for taxonomy).
  - b. Stream sites: after lab processing, select 3 of the 5 dominant taxonomy samples to send to the lab, spreading them out along the reach (e.g., if samples are numbered 1 – 5 from downstream to upstream, select sample 1, 3, and 5 to send to the lab). Hold the other 2 taxonomy samples at the DSF to await further optimization analysis.
  - c. 5 algal samples from lakes/rivers will be subsampled for algal chemistry and AFDM
  - d. 3 algal samples from lakes/rivers will be subsampled for algal taxonomy
  - e. 5 algal samples from wadeable streams sites will be subsampled for all products: algal chemistry, AFDM, and taxonomy
2. Label 60 mL (periphyton, clear HDPE) or 1 L (phytoplankton, amber HDPE) HDPE bottles with 1"x2" adhesive labels (**Figure 37**, RD[07]). Lab type is "**taxonomy**". Use Type I barcode labels.



Figure 37. Algal taxonomy bottles and labels.

3. Cap and shake amber HDPE field sample bottle for 30 seconds to mix sample evenly.
4. Pipette or use graduated cylinder to transfer 60 mL periphyton into the appropriately labeled 60 mL bottle, or measure 1 L phytoplankton using a graduated cylinder and transfer to labeled 1 L bottle.
  - a. If the sample is too thick for the pipette, you may measure 60 mL using a clean (rinsed in DI) graduated cylinder.
  - b. If adjustable pipette has not been recently calibrated, use a graduated cylinder to get a more accurate volume measure.
4. Cap bottles and record data in the mobile lab app.
  - a. Scan the barcode label with the tablet.
  - b. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.



Figure 38. Barcode label scanning.

5. Proceed to Sample Preservation Section E.9.



## E.5 Epiphyte Samples

1. **Day 1:** Using forceps, remove the plant sample from Whirl-pak®. Place in clean, dry white plastic sample tray (used in field protocol).
2. 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”.
    - i. Check volume with a graduated cylinder. If volume is off by >5 mL, use graduated cylinder to measure water that goes into the wash bottle.
  - b. Since this is a volume-based sampling technique, it is important to keep the alternate field 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 9** for recommended filter volume and adjust water volume and HDPE bottle volume as necessary.
3. 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.
4. Use DI water periodically to rinse the scrubbed material into the tray.
5. When scrubbing is finished, rinse scrubbed plant and toothbrush thoroughly into the white tray.
6. Record the volume of water used for scrubbing in the **Alternate Field Sample Volume** field.
  - a. Enter the total volume of water added to the sample, you do not need to re-measure after scrubbing
7. Remove plant sample and measure the approximate dimensions of the surface area scrubbed (e.g., stalk length and width to calculate surface area of a cylinder, plus leaf length and width times 2 to account for the top and bottom of the leaf) and record the estimate sum of all surface area scrubbed in the **Epiphyton: Plant Surface Area** form of the mobile app.
  - a. Measure using calipers or a metric ruler.
  - b. Measure a subset of 5 plants and scale up to the total number of plants scrubbed. If <5 plants were collected, measure all.
8. Weigh wet mass of all plant material scrubbed.
9. Place plant material in a labeled paper lunch bag.
10. Pour remainder (if any) of 125 mL wash bottle into white tray.
11. Carefully pour the scrubbed material into a 125 mL amber wide-mouth HDPE sample bottle. Proceed to Filtering Protocol, Section E.6.
12. Place paper bags containing samples in the drying oven for a minimum of 12 hours at 65 °C or until constant weight is achieved (i.e., mass varies by <2% over a one-hour period; RD[09]).
  - a. Use TOS “Lab Drying QC Datasheet” in Measurement of Herbaceous Biomass datasheets (RD[10]).
13. **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.



14. Place a clean, plastic weigh boat (small sample) or tray (large sample) on analytical balance. Tare (zero) balance.
15. Place dry sample in the plastic weigh boat/tray and record as **Epiphyton: total plant dry weight** on mobile app.
  - a. Sample may be crushed or broken up to fit into weigh boat.
16. Place sample in a clean, pre-labeled aluminum weigh boat.
  - a. Handle the weigh boat using tongs or forceps to avoid transferring oils from your hands, or powder or residue from your gloves, to the sample.
  - b. If sample does not fit in one aluminum weigh boat, grind in Wiley mill using 20 mesh (0.85 mm) screen.
  - c. Use a sample splitter to subsample the ground material.
  - d. Place a subsample of ground material in aluminum weigh boat.
  - e. Record **Boat ID** and **Boat Mass** in the mobile app.
  - f. Clean grinding mill thoroughly with compressed air between samples.
17. Weigh boat on an analytical balance (0.0001 g), and record as **Dry weight + boat** in the mobile app.
18. Repeat above steps until all specimens have been processed.
19. Place aluminum boats + specimens in the muffle furnace using oven gloves and tongs. **TAKE CARE**



**NOT TO BURN YOURSELF!**

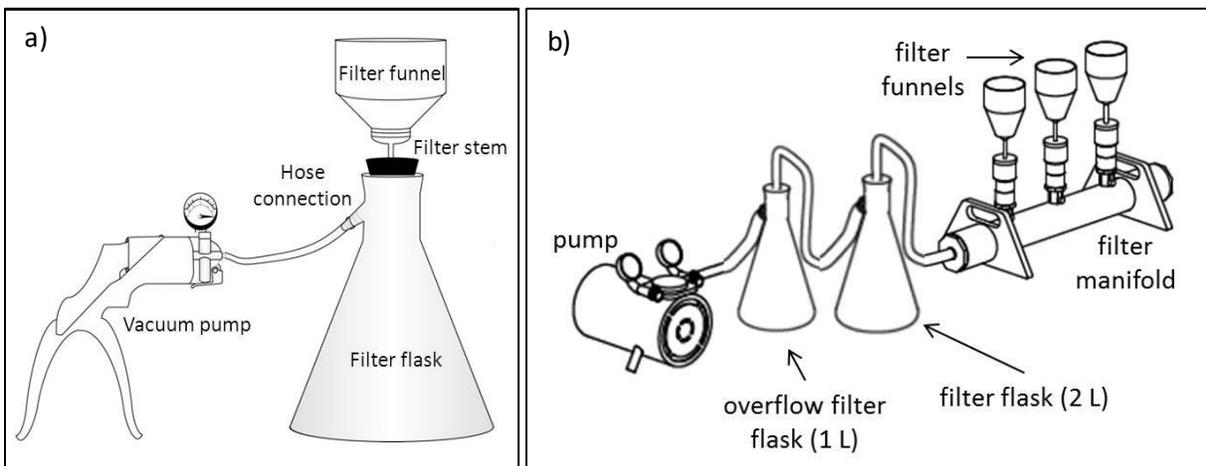
- a. Boats may be stacked on top of each other as long as there is space for air flow between them.
  - b. Place boats on an approved muffle furnace pan (if available) before placing in the furnace. This makes it easier and safer to handle samples.
  - c. If drafts are a problem in the furnace, cover pans/boats with aluminum foil.
20. 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.
    - b. Samples may be allowed to cool in muffle furnace depending on work flow.
  21. **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.
  22. Cover pans/aluminum boats with aluminum foil to prevent ash from blowing out of boats and set aside to cool on a heat-resistant surface in an area without drafts from windows, doors, or HVAC ventilation. Placing warm boats in the desiccator may cause a change in air pressure in the desiccator that causes ash to become airborne.
  23. When boats have cooled enough to handle, place in desiccator. Keep boats covered with aluminum foil. Allow samples to continue to cool to room temperature in the desiccator for at least 20 minutes prior to weighing.
    - a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
    - b. Keep boats covered with aluminum foil while storing in the desiccator.
  24. After cooling to room temperature, weigh boats again on analytical balance (0.0001 g), record as **Boat + Ash Mass** in the mobile app.
    - a. **Boat + ash mass** should be a smaller number than **Boat + dry mass**, as the muffle furnace burns off organic material.



25. After weighing, discard ashed filters into the trash and clean the boat with a soft brush or paper towel.
  - a. If there have been problems with the balance, consider storing the ashed samples in the desiccator until you have quality checked the data.
26. Set clean boats aside to be used again.

#### E.6 Filtering (Chlorophyll *a* + Pheophytin, AFDM, C, N, and P and Isotopes)

1. Set up filter funnel, filter flask, and vacuum pump (**Figure 39**).
  - a. Either the hand vacuum pump (**Figure 39a**) with one filter flask and funnel attached or the filter manifold and electric pump (**Figure 39b**) with multiple filter funnels may be used.
  - b. Attach flexible tubing to from vacuum pump to hose connection on filter flask(s).
  - 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 39.** 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 dampened with DI (**Figure 39**), replace top of funnel.
4. Shake sample bottle vigorously for ~30 seconds to mix sample.
  - a. Allow epipsammon/epipelon samples to settle for ~30 seconds after shaking prior to pipetting to prevent sand/silt from being pipetted onto the filter.
  - b. If sample has large chunks of algae, homogenize using clean, hand-held battery operated stirrer for ~30 seconds. Take care that samples do not spill over the top of the sample bottle while stirring.
    - i. Rinse stirrer thoroughly with DI between samples.



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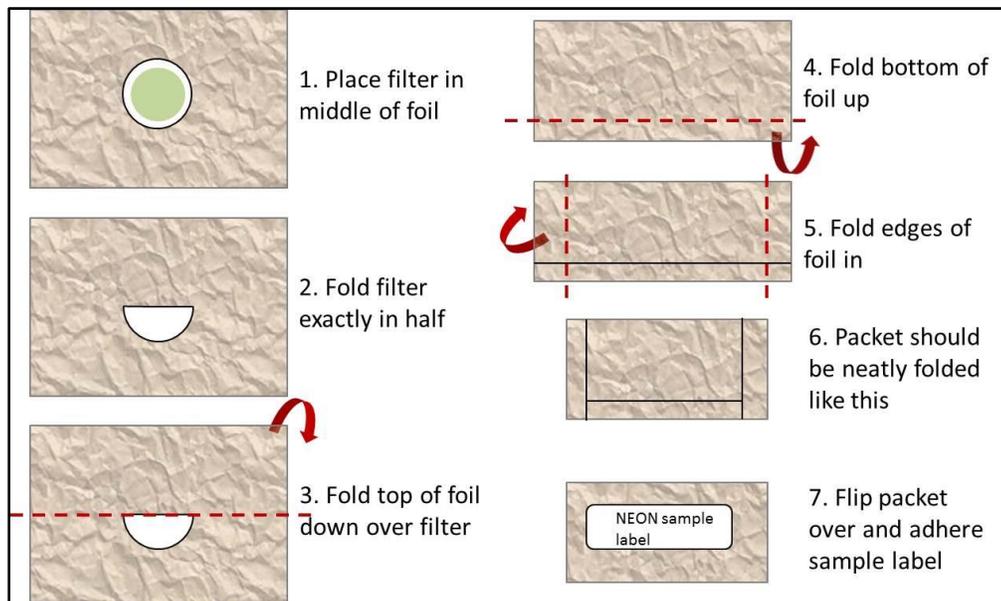
- c. Shake sample prior to pipetting on each filter. Replicate filters should be as similar to each other as possible.
5. Filter known volume of sample.
  - a. **Phytoplankton samples:** Measure using a graduated cylinder. Pour sample slowly in  $\leq 100$  mL increments into filter funnel.
    - i. 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. **All benthic samples:** Using a new pipette tip, carefully pipette the desired volume into the filter funnel.
    - i. Do not aim pipette tip directly at the filter, aim at the side of the funnel. Take care not to puncture filter.
    - ii. Change pipette tip between samples.
    - iii. 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.
    - iv. Allow epipelonal samples to settle 30-60 seconds prior to pipetting. Settling should be long enough to allow most of sediment to settle, but not so long as to allow algal cells to settle.
    - v. NOTE: Pipette tips may be reused for all of the subsamples for a given sample, but may not be reused from sample to samples or bout to bout. Use a separate tip for preservative.
    - vi. Best practice for use of adjustable pipettes is to select a new pipette tip for each sample.
    - c. Adjustable pipettes must stay vertical during use to prevent liquid from entering the pipette mechanism. Use a pipette stand or large graduated cylinder to keep the pipette vertical.
  - d. **Keep track of the volume of sample filtered** on the mobile lab app.
6. Draw suction on filter apparatus using the hand vacuum pump (or vacuum 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, filter more sample. If the filter appears green or yellow tinged, proceed to next step.
  - a. **Record the volume of sample filtered in the mobile lab app.**
  - b. Filter the minimum amount suggested in **Table 9**. 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. After the sample water is passed through the filter, rinse the inside walls of funnel onto the filter using the wash bottle of DI to capture any algae that might adhere to the sides of the funnel.
  - a. Do not include DI rinse water in the volume of sample filtered.





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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. If any insects or plant pieces are on the filter, remove them carefully using forceps.
12. 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:** Weigh aluminum boat and record mass prior to placing sample in the boat. Place filter in a labeled aluminum weigh boat, record sample information in the mobile lab app and proceed to Section E.7.
  - b. **Chlorophyll/pheophytin and algal chemistry filters:** Fold filter exactly in half and place on a clean square of aluminum foil (~4x4 inches). Fold foil securely around the filter to form a packet.
    - i. If filters are not folded exactly in half, there will be loss of sample material.
    - ii. Do not use excess foil as it can be difficult for the external lab to open.
    - iii. For chemistry filters, leave one end of the packet open to facilitate drying.
  - c. Label foil packet with adhesive sample label (~1" x 2") (**Figure 40**). Place label on the top of the packet, not over the folded edges of the foil. Use barcode label on each packet.
  - d. Scan the barcode label with the tablet.
  - e. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.



**Figure 40.** Correct filter packet folding procedure. Make sure packet can be easily opened at the external lab without destroying the NEON sample label or barcode label. Do not use excess foil.

13. Rinse the funnel and stem with DI between filters.



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14. Repeat above steps until you have 5 filters total from the same sample. Randomly select 1 sample per site/bout (approximately 10% of samples over the year) to create a full set of replicate filters (do not create replicates for taxonomy or AFDM). These samples will have a total of 9 filters. The replicate sample will have “.rep2” appended to the end of the sample ID.
  - a. 1 AFDM filter (proceed to Section E.7) – never create replicates
  - b. 1 chlorophyll *a*/pheophytin filter
  - c. 2 CN +<sup>13</sup>C, <sup>15</sup>N, <sup>34</sup>S isotope filters (algal chemistry) – “CNSa” and “CNSb”
    - i. Filter the same volume on both CNSa and CNSb for the same sample to streamline analysis and data collection.
  - d. 1 P filter (algal chemistry)
  - e. NOTE: Wadeable stream seston (phytoplankton) samples are only filtered for chlorophyll *a*/pheophytin and AFDM.
15. Create 2 domain lab blank filters per shipment to be shipped with the **algal chemistry** samples (CNSa, CNSb, P). This should be a pre-ashed filter that has been treated the same way as the filters you used for filtering (e.g., has been carried to the same places, comes from the same box). Filter a similar amount of DI through the filter as you typically use to rinse a sample, and record the volume of DI filtered.
  - a. Create 2 blank filters per shipment, both can be from the same site. You do not need 2 blanks per site.
  - b. If your domain has multiple sites, it works best to create the blanks with the filters created for the first site scheduled in the bout, then you can select those records during data collection for the following site(s).
16. Separate all foil packets for chlorophyll/pheophytin from the remaining algal chemistry packets (CN/CNS and P). See Section E.10 for packet organization.
  - a. Place all chlorophyll/pheophytin filters in -20 °C freezer until shipping (Section G.1).
17. Place all algal chemistry foil packets and blank packets in the drying oven at 65 °C with packets slightly open (**Figure 41**). Leave enough space between packets that air flow will dry the filters. Allow to dry ~12 hours (overnight). Filters can be left in the oven longer, this does not affect quality. After drying, seal packet and place in desiccator until shipping.



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**Figure 41.** Example of a filter packet left slightly open for drying algal chemistry filters in the drying oven.

18. Cap amber sample bottle and store in 4 °C until all samples are complete.
19. Clean filter funnels completely using for the next site or processing day (RD[24]).
  - a. Fill 2 wash basins ~3/4 full with DI.
  - b. Wearing gloves, immerse filter funnel and stem in the first basin and swirl. This is the “dirty” basin.
  - c. Transfer filter funnel and stem to the second basin and swirl. This is the “rinse” basin.
  - d. Rinse filter funnel and stem 3x with house DI water (from wall unit, carboy, or squirt bottle).
  - e. Shake to remove excess water. Allow to dry if not using right away.

#### **E.7 Ash-free Dry Mass (AFDM) of Filters**

Ash-free dry mass is another way of estimating algal biomass, and compliments the chlorophyll/pheophytin data. Filter chlorophyll/pheophytin and AFDM filters at the same time to maintain good data quality (e.g., do not separate chl/pheo and AFDM filters for a given sample across 2 days).

1. Weigh the aluminum boat and record boat mass prior to placing a sample in the boat.
2. **Day 1:** Remove filter from filter apparatus using filter forceps, place filter in labeled, pre-weighed, aluminum weigh boat, and enter appropriate **Boat ID** in mobile app.
  - a. Handle the weigh boat using tongs or forceps to avoid transferring oils from your hands or powder from your gloves to the sample. This can introduce error to mass measurements because the difference between dry mass and AFDM is often very small.
  - b. Do not touch the top of the filter containing the sample with fingers or forceps.
3. Place all boats containing filters in the drying oven for a minimum of 12 hours at 65 °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 or metal tray for easier loading and unloading in the drying oven.



- b. Cover boats loosely with aluminum to prevent air circulation in the drying oven from blowing the filters out of the boats.
4. **Day 2:** Remove boats+filters from the drying oven and allow to cool to room temperature prior to placing in the desiccator. Placing warm boats in the desiccator may cause a change in air pressure in the desiccator that blows material around.
  - a. Allow boats + filters to cool to room temperature and constant mass for at least 20 minutes in the desiccator. Keep covered.
  - b. If the boats+filters will not be weighed right away, place them in a desiccator. Keep covered with aluminum foil. Filters can be left in the desiccator for up to 30 days if necessary.
  - c. Handle with tongs or forceps as mentioned above.
5. Place boat + filter on tared (zeroed) analytical balance. Record in mobile app as **Boat + Dry mass**.
6. 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.
  - 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.
  - c. If drafts are a problem in the furnace, cover pans/boats with aluminum foil.
7. 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.
  - b. If necessary, the muffle furnace may cool down prior to removing the samples.
8. **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.
9. Cover pans/aluminum boats with aluminum foil to prevent ash from blowing out of boats and set aside to cool on a heat-resistant surface in an area without drafts from windows, doors, or HVAC ventilation. Placing warm boats in the desiccator may cause a change in air pressure in the desiccator that causes ash to become airborne.
10. When boats have cooled to room temperature, place in desiccator. Allow samples to cool to room temperature and constant weight in the desiccator for at least 20 minutes prior to weighing.
  - a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
  - b. Keep boats covered with aluminum foil while storing in the desiccator.
11. After cooling to room temperature, weigh boats again on analytical balance (0.0001 g) and record as **Boat + Ash mass** in the mobile app.
  - a. If logistically possible in your lab, the preference is to go directly from the desiccator to the balance without allowing the samples to sit out on the benchtop.
  - b. **Boat + ash mass** must be a smaller number than **Boat + dry mass**, as the muffle furnace burns off organic material.
  - c. If **Boat + ash mass** is not smaller than **Boat + dry mass**, this indicates that an error occurred in this process. Common errors include not taring the balance consistent (instrument drift), transferring oils from hand to the weigh boat, not allowing boats to cool to room temperature prior to weighing, or drafts near the balance in the lab.





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- d. If **Boat + ash mass** is not smaller than **Boat + dry mass**, record in the mobile app with the appropriate sample condition that describes the error.
12. After weighing, discard ashed filters into the trash and clean the boat with a soft brush or paper towel.
  - a. If there have been problems with the balance, consider storing the ashed samples in the desiccator until you have quality checked the data.
13. Set clean boats aside to be used again.

## E.8 Sample Disposal

1. Double check that all components have been completed. You should have the following products for each field sample:
  - a. 60 mL periphyton (+ glutaraldehyde) or 1 L phytoplankton (+ Lugol's) sample for taxonomy lab (create only 3 taxonomy subsamples from littoral samples lakes and rivers)
  - b. 1 AFDM filter (processed at Domain Support Facility)
  - c. 1 chlorophyll/pheophytin filter
  - d. 1 CNSa filter
  - e. 1 CNSb filter
  - f. 1 P filter
  - g. One full set of replicate filters for algal chemistry (chl/pheo, CNSa, CNSb, P only) one sample during each site/bout
  - h. 2 blank algal chemistry filters per shipment
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.

## E.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. Store at 4 °C up to 30 days until shipping.
  - a. Use  $C1 \cdot V1 = C2 \cdot V2$  to calculate preservative volume, where:
    - i. **C1=concentration** of preservative before adding to sample
    - ii. **V1=volume** of preservative before adding to sample
    - iii. **C2=concentration** of preservative in final sample
    - iv. **V2=volume** of final sample (this will be slightly more than 60 mL after adding the preservative, this is ok)
  - b. **Example:** For a final 60 mL sample preserved with 0.5% glutaraldehyde, use 1.2 mL of 25% glutaraldehyde.
    - v.  $C1 = 0.25$  (25% glutaraldehyde)
    - vi.  $V1 =$  solve for  $V1$



- vii. C2 = 0.005 (0.5% glutaraldehyde)
- viii. V2 = 60 mL
- 2. **Phytoplankton taxonomy (lakes and rivers only):** Uncap each sample bottle, using a pipette, preserve each 1 L sample with high-iodine Lugol's solution to reach a final concentration of 2% (20 mL Lugol's per L of sample). Store at 4 °C up to 30 days until shipping.
  - a. Adjustable pipettes must stay vertical during use to prevent liquid from entering the pipette mechanism. Use a pipette stand or large graduated cylinder to keep the pipette vertical.
  - b. Lugol's is light-sensitive. Samples preserved with Lugol's must be in amber bottles.
- 3. Always work with chemicals in the fume hood with proper PPE. Glutaraldehyde requires careful handling.

#### E.10 Organize filters

- 1. **Chlorophyll/pheophytin filters:** Place labeled foil packets in sealed zip-top bags organized by site. Place in dark -20 °C freezer until shipping. Ship on dry ice within 7 days.
  - a. Bundle filters by site. Organize in the same order as presented on the shipping manifest.
- 2. **Algal chemistry filters (CNSa, CNSb, and P filters):** Place labeled foil packets in sealed zip-top bags. Place in desiccator until shipping. Ship ambient, ground within 30 days. May ship with aquatic stable isotope particulate organic matter (POM) filters).

#### E.11 Organize filters according to the order the shipping manifest generates. This will make it easier for the external lab to check the shipment. Ending the Processing Day

- 1. Refreshing the laboratory supplies
  - a. Pre-ash GF/F filters in muffle furnace.
  - b. Check 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. Allow to dry.
- 3. Data QA/QC
  - a. Required checks
    - i. Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample bottles/packets.
    - ii. Check that the barcode labels in the mobile application(s) match the barcode labels adhered to the samples. At a minimum, check the last few numbers of the barcode.
  - b. Nice to check
    - i. Site ID, collect date, sampling protocol version
    - ii. Filter volume, AFDM measurements



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**SOP F      Data Entry and Verification**

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription error and improve data quality. Adhesive barcode labels will be used and scanned into the mobile application. Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL).

Given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. 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.

**Data and sample IDs must be entered digitally and quality checked prior to shipping samples to an external lab.**



## SOP G Sample Shipment

Information included in this SOP conveys science-based handling and packaging instructions for the samples types included in this protocol. For shipping instructions, see RD[23].

### G.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. Ship ground at ambient temperature. Glutaraldehyde and Lugol's in these concentrations are not considered hazardous. See RD[23] for further shipping instructions.

### G.2 Algal Filter Sample Shipping – Chlorophyll/Pheophytin

1. Chlorophyll/pheophytin filters must be shipped within 7 days of collection. Plan ahead to make sure you can meet this requirement as samples can only be shipped Monday – Wednesday.
2. Filters must be kept frozen during shipping, do not ship on Fridays (suggest shipping M-W only), and ensure that the receiving lab will be open when the shipment arrives (e.g., take note of holiday schedules).
3. Follow instructions for shipping overnight on dry ice in AD[03], see RD[23] for further shipping instructions.

### G.3 Algal Filter Sample Shipping – Algal Chemistry Filters (CNSa, CNSb, P, blanks)

1. Algal chemistry filters and blank filters should be shipped within 30 days of collection.
2. Bundle filters in the same order as referenced in the shipping manifest. This makes receiving samples easier for the external lab.
3. Filters may be shipped ambient, ground as they are dry and stable. See RD[23] for further shipping instructions.

### G.4 Handling Hazardous Material

Glutaraldehyde and Lugol's iodine in the concentration and volume shipped by NEON for this protocol are not considered hazardous.

### G.5 Supplies/Containers

See sections G.1, G.2, G.3, and RD[23] for specific shipping materials.

### G.6 Timelines and Conditions

1. **Taxonomy samples:** Shipping should occur within one week of sampling if possible, but samples may be stored at the domain support facility at 4 °C for up to 30 days if necessary.



- a. Preserved samples may be held for up to 6 months if the lab is not able to receive samples (**Table 2**).
2. **Chlorophyll filters:** Filters may be stored at -20 °C for up to 7 days prior to shipping.
  - a. Sampling will be suspended if the lab is not able to receive samples and samples are not able to be frozen and stored (**Table 2**).
3. **Algal chemistry filters:** Filters may be stored in the desiccator for 30 days prior to shipping.
  - a. Samples may be held for up to 12 months if the lab is not able to receive samples, contact Science to resolve space and storage issues (**Table 2**).

#### **G.7 Grouping/Splitting Samples**

Group taxonomy samples by site per bout. Group filters by lab type per site per bout.

#### **G.8 Return of Materials or Containers**

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

#### **G.9 Shipping and Chain of Custody**

The mobile shipping applications are used to create chain of custody documents. Shipments are to include a hardcopy of the shipping manifest (RD[13]) in each box as well as an electronic shipping manifest that is emailed to the receiving laboratory and to NEON Collections and Laboratory Analysis at the time of shipment (created by the shipping application). The shipping manifest must accurately document the physical samples inside the shipping container.

#### **G.10 Laboratory Contact Information and Shipping/Receipt Days**

See the Shipping Information for External Facilities on NEON's CLA intranet site.



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## APPENDIX A QUICK REFERENCES

### A.1 Algae Field Sampling

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

**Step 2** – Prepare field labels (2" x 4").

**Step 3** – Ensure the General AQU Field Metadata app and Secchi and depth data collection are completed per field site visit as needed.

**Step 4** – Collect seston/phytoplankton samples:

1. Seston/Phytoplankton: **Wadeable streams**
  - a. Sample at the water chemistry sampling location (sensor location S2)
  - b. Sample are only processed for chlorophyll *a*/pheophytin and AFDM in the domain support facility.
2. Phytoplankton: **Lakes** (near water chemistry sampling locations):
  - a. Deepest point in the lake or buoy location
  - b. Lake inlet
  - c. Lake outlet
3. Phytoplankton: **Rivers**:
  - a. Thalweg or sensor location
  - b. Two other deep water sampling locations within the 1 km reach

**Step 5** – Collect benthic algae samples:

1. **Wadeable streams**
  - a. Determine habitat sampling locations using the Stream Morphology Map (RD[16]) or rapid habitat assessment.
  - b. Determine sampler type based on the habitats present and the order of preference for sampling substratum.
  - c. Collect samples: 5 per dominant habitat type, 3 per second-most dominant habitat type.
2. **Lakes**
  - a. Refer to the site-specific riparian vegetation map (created in the Riparian Habitat Assessment Protocol (RD[15]) which divides the lakeshore into 10 sections (**Figure 23**).
  - b. Evenly partition the five samples around the Riparian Sections.
3. **Rivers**
  - a. Refer to the site-specific riparian vegetation map which divides the sampling reach into 10 transects.
  - b. Evenly partition the five samples around the Riparian Transects. Move to the next section if the appropriate substratum is not present.



**Step 6** – Take care to note volume of water used for rinsing substrata in the field.

## A.2 Algae Lab Processing

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

**Step 2** – Dry AFDM filters in the drying oven and wrap all other filters in aluminum foil. Store chlorophyll/pheophytin foil packets in sealed zip-top bags and place in dark -20 °C freezer until shipping (lab day 1). Store CNS and P filters, dried, in the desiccator until shipping.

**Step 3** – 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 4** – Weigh dried AFDM filters, place in muffle furnace (lab day 2).

**Step 5** – Weigh ashed AFDM filters (lab day 3).

**Step 6** – For each field sample (except seston) you should have the following products:

1. 60 mL periphyton or 1 L phytoplankton sample for taxonomy lab
2. 1 AFDM filter (processed at Domain Support Facility)
3. 1 chlorophyll/pheophytin filter
4. 3 algal chemistry filters (for C, N, P, and <sup>13</sup>C, <sup>15</sup>N, and <sup>34</sup>S isotopes)

## A.3 Order of Preference for Sampling Substrata

1. Epilithon (rock substrata)
2. Epixylon (wood substrata)
3. Epiphyton (plant substrata)
4. Epipsammon (sand substrata)
5. Epipelon (silt substrata)



## APPENDIX B REMINDERS

### Before heading into the field:

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

### Sample collection:

- **Wadeable streams:** Determine the dominant habitat and second-most dominant habitat based on the Stream Morphology Map (RD[16]), rapid habitat assessment, or the site-specific bathymetric map and riparian vegetation map (RD[17], RD [15]).
- **Lakes and Rivers:** Determine the dominant habitat in the littoral areas.
- Choose the appropriate sampler.
- **Wadeable streams and Rivers:** Start sampling at the bottom of the reach, working upstream so as not to decrease visibility and disrupt periphyton communities.
- Spread samples out along the reach, or in different riparian sections/transects.
- Keep track of the volume of water used to scrub and rinse the sample.
- Do not sample anywhere you or other field people/animals have walked, or locations that appear recently disturbed

### Sample processing:

- Completely homogenize sample before filtering.
- Keep track of the volume of sample filtered.
- DO NOT FREEZE taxonomy samples.
- Ship chlorophyll/pheophytin within 7 days of collection.

### Data QA/QC:

#### *Required checks*

- Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample bottles/packets.
- Check that the barcode labels in the mobile application(s) match the barcode labels adhered to the samples. At a minimum, check the last few numbers of the barcode.

#### *Nice to check*

- Site ID, collect date, sampling protocol version
- Filter volume, AFDM measurements



**APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING**

Preliminary date ranges for biological sampling bouts. General guidelines for developing these dates are presented in the NEON Aquatic Sample Strategy Document (RD[05]). Also see the Site Specific Sampling Strategy Document on NEON’s FOPS intranet site.

Domain	Site	Bout 1	Bout 2	Bout 3
D01	Hop Brook	11Apr-9May	9Jul-6Aug	30Oct-31Oct
D02	Lewis Run	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D02	Posey Creek	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D03	Flint River	21Feb-21Mar	27Jun-25Jul	7Oct-4Nov
D03	Lake Barco	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D03	Lake Suggs	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D04	Rio Guilarte	26Jan-23Feb	21Jun-19Jul	9Nov-7Dec
D04	Rio Cupeyes	24Jan-21Feb	21Jun-19Jul	10Nov-8Dec
D05	Crampton Lake	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D05	Little Rock Lake	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D06	Kings Creek	23Mar-20Apr	3Jul-31Jul	30Oct-31Oct
D06	McDiffett Creek	23Mar-20Apr	3Jul-31Jul	30Oct-31Oct
D07	Leconte Creek	15Mar-12Apr	30Jun-28Jul	12Oct-9Nov
D07	Walker Branch	9Mar-6Apr	1Jul-29Jul	19Oct-16Nov
D08	Mayfield Creek	5Mar-2Apr	29Jun-27Jul	31Oct-28Nov
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
D10	Arikaree River	21Mar-18Apr	4Jul-1Aug	20Sep-18Oct
D11	Pringle Creek	17Feb-17Mar	29Jun-27Jul	23Oct-20Nov
D11	Blue River	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D12	Blacktail Deer Creek	1May-29May	13Jul-10Aug	30Aug-27Sep
D13	Como Creek	20May-17Jun	14Jul-11Aug	30Aug-27Sep
D13	West St. Louis Creek	2May-30May	5Jul-2Aug	3Sep-10Oct
D14	Sycamore Creek	12Jan-11Feb	24Mar-23Apr	3Jun-3Jul
D15	Red Butte Creek	29Mar-26Apr	6Jul-3Aug	29Sep-27Oct
D16	McRae Creek	10Apr-8May	11Jul-8Aug	23Sep-21Oct
D16	Martha Creek	6Apr-4May	5Jul-2Aug	22Sep-20Oct
D17	Teakettle 2 Creek	10Apr-8May	9Jul-6Aug	25Sep-23Oct
D17	Upper Big Creek	2Apr-30May	9Jul-6Aug	28Sep-26Oct
D18	Oksrukuyik Creek	21May-18Jun	29Jun-27Jul	7Aug-4Sep
D18	Toolik Lake	19Jun-17Jul	27Jul-24Aug	3Sep-10Oct
D19	Caribou Creek	2May-30May	26Jun-24Jul	18Aug-15Sep



**APPENDIX D SITE-SPECIFIC INFORMATION: HABITAT AND SUBSTRATA RECOMMENDATIONS**

For more information, see the Site Specific Sampling Strategy Document on NEON’s FOPS intranet site.

Domain	Site	Benthic habitat 1 (5 reps)	Benthic habitat 2 (3 reps)	Phytoplankton
D01	Hop Brook	Riffle (epilithon)	Pool (epipsammon)	ss (S2)
D02	Lewis Run	Run (epipelon)	Riffle (epipelon)	ss (S2)
D02	Posey Creek	Riffle (epilithon)	Pool (epipelon)	ss (S2)
D03	Flint River*	Littoral (epipsammon)	NA	c0 + 2 locations
D03	Lake Barco	Littoral (epiphyton)	NA	c0, in, ot
D03	Lake Suggs	Littoral (epiphyton)	NA	c0, in, ot
D04	Rio Guilarte	Riffle (epilithon)	Pool (epilithon)	ss (S2)
D04	Rio Cupeyes	Riffle (epilithon)	Run (epilithon)	ss (S2)
D05	Crampton Lake	Littoral (epixylon)	NA	c0, in, ot
D05	Little Rock Lake	Littoral (epixylon)	NA	c0, in, ot
D06	Kings Creek	Riffle/run (epilithon)	Pool (epilithon)	ss (S2)
D06	McDiffett Creek	Riffle (epilithon)	Pool (epipsammon)	ss (S2)
D07	Leconte Creek	Riffle (epilithon)	Pool (epipsammon)	ss (S2)
D07	Walker Branch	Riffle (epilithon)	Run (epixylon)	ss (S2)
D08	Mayfield Creek	Riffle/run (epixylon)	Run (epipsammon)	ss (S2)
D08	Black Warrior River	Littoral (epixylon)	NA	c0 + 2 locations
D08	Tombigbee River	Littoral (epixylon)	NA	c0 + 2 locations
D09	Prairie Lake	Littoral (epilithon)	NA	c0, in, ot
D09	Prairie Pothole	Littoral (epilithon)	NA	c0, in, ot
D10	Arikaree River	Run (epiphyton)	Pool (epipsammon)	ss (S2)
D11	Pringle Creek	Run (epipsammon)	Riffle (epilithon)	ss (S2)
D11	Blue River	Run (epilithon)	Riffle (epilithon)	ss (S2)
D12	Blacktail Deer Creek	Riffle (epilithon)	Run (epilithon)	ss (S2)
D13	Como Creek	Riffle (epilithon)	Run (epipsammon)	ss (S2)
D13	West St. Louis Creek	Riffle (epilithon)	Pool (epipsammon)	ss (S2)
D14	Sycamore Creek	Run (epilithon)	Pool (epipsammon)	ss (S2)
D15	Red Butte Creek	Step pool (epipelon)	Run (epilithon)	ss (S2)
D16	McRae Creek	Step pool/riffle (epilithon)	Step pool/pool (epilithon)	ss (S2)
D16	Martha Creek	Riffle (epilithon)	Pool (epixylon)	ss (S2)
D17	Teakettle 2 Creek	Riffle (cobble)	Riffle (bedrock)	ss (S2)
D17	Upper Big Creek	Run (epipsammon)	Riffle (epilithon)	ss (S2)
D18	Oksrukuyik Creek	Run (epilithon)	Riffle (epilithon)	ss (S2)
D18	Toolik Lake	Epilithon	NA	c0, in, ot
D19	Caribou Creek	Run (epilithon)	Riffle (epilithon)	ss (S2)

\*A Van Dorn sampler is used for phytoplankton at D03 FLNT



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**APPENDIX E 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 boats, safety equipment, charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

This protocol suggests the use of a GPS unit with WAAS, specifically a Hummingbird 1198c, used for navigating to lake sampling locations. Any GPS unit can be used as long as the navigation accuracy is  $\pm 4$  m for lake navigation.

**Table 10.** Equipment list – General equipment.

Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable items</b>				
	N	Site-specific Stream Morphology, Rapid Habitat Assessment, or Bathymetry Map	Determining sampling locations	1
CDW-G: 4452963	N	Mobile data entry tablet	Field data entry	1
Forestry Suppliers, Inc.: 39481 Recreational Equipment Inc.: 895022 West Marine Products, Inc.: 15131444	N	Handheld GPS unit (with batteries, <4 m accuracy) or Hummingbird	Navigating to sampling location	1
Forestry Suppliers, Inc.: 53190 Grainger, W.W.: 1GEJ1	N	Clipboard	Recording data	1
	N	Cooler (9-28 qt)	Field sample storage; use size appropriate to samples being collected	1
Grainger, W.W. Forestry Suppliers, Inc. Cabela's, Simm's	N	Waders or knee boots	Boating or wading	1 pair per person
<b>Consumable items</b>				
RD[07], RD[08] Forestry Suppliers, Inc.: 010510-1 49247	N	AQU Field Metadata Sheet, Field data sheets (print on all-weather paper, write in pencil)	Recording metadata in case tablet fails	1
CDW-G: 4452963		Mobile device and application	Recording data	1



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Item No.	Exact Brand	Description	Purpose	Quantity
Amazon Capital Services Inc.: B00006IBUW Forestry Suppliers, Inc.: 010510-1 49247	N	Pre-printed adhesive labels (all-weather, 2"x4")	Labeling samples	15
	Y	Adhesive barcode labels (Type-I, Type-II)	Labeling samples	1 sheet
	N	Pencils	Recording data	1
Grainger, W.W.: 1JU51	N	Permanent markers	Labeling samples	1
VWR: 15715152	N	Ice Packs	Keeping samples cool	2

**Table 11.** Equipment list – Sampling equipment.

Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable items</b>				
B&H Photo: GESM7001	N	Template (35 mm plastic slide cassette)	Sampling area for epilithon and epixylon	5
	N	PVC large substrate sampler: 2" inner diameter PVC rubber seal or O-ring	Sampling area for large substrate epilithon and epixylon	1
	N	Turkey baster	Sample removal for large substrate epilithon and epixylon samples	1
Fisher Scientific Company, LLC: 029253C 02923103	N	HDPE bottles with lids, amber wide-mouth (125 or 250 mL)	Periphyton sample container; container size is selected by field staff (either 125 mL or 250 mL, bottles are reused but are site-specific)	8
Fisher Scientific Company, LLC: NC0836109	N	Brass-bristle brush, toothbrush-size	Scrubbing epilithon	2
Fisher Scientific Company, LLC: 19027438	N	Nylon-bristle toothbrush	Scrubbing epixylon	2



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Item No.	Exact Brand	Description	Purpose	Quantity
BioQuip Products Inc. Fisher Scientific Company, LLC: 1426B NC0095946	N	Larval insect tray, plastic	Scrubbing container in which sample is collected	1
Thomas Scientific, Inc. Fisher Scientific Company, LLC: 1207M51 0340910C	N	125 mL unitary wash bottle	Rinsing substrate and larval tray into sample bottles	2
Thomas Scientific, Inc.: 3571A15 Fisher Scientific Company, LLC: 0300739	N	25 mL graduated cylinder, plastic	Measuring extra rinse water	1
Thomas Scientific, Inc.: 3489B60	N	Petri dish lid, plastic, 47 mm diameter	Epipsammon and epipelon sample collection	4
Grainger, W.W.: 5C936	N	Spatula (metal, offset)	Epipsammon and epipelon sample collection	1
	N	Scissors	Epiphyte sample collection	1
Fisher Scientific Company, LLC: S90532C	N	Metric ruler	Epiphyte sample collection	1
Fisher Scientific Company, LLC: EW0548610	Y	Kemmerer sampler with rope and messenger	Phytoplankton sample collection	1
Forestry Suppliers, Inc.: 77901	Y	Horizontal Van Dorn sampler with rope and messenger	Phytoplankton sample collection in rivers with fast flow	1
Thomas Scientific, Inc.: 1212W94	N	4 L HDPE jug	Integrating phytoplankton samples	1
Thomas Scientific, Inc.: 1185B56	N	Amber HDPE wide-mouth sample bottles with caps, 1 L	Phytoplankton and seston sample collection	4-15
<b>Consumable items</b>				
Grainger, W.W.: 4A807	N	Razor blade	Chunky epilithon samples	2
	N	Flat head screwdriver or putty knife	Chunky, calcified epilithon samples	1
Amazon Capital Services Inc.: B005IQTSE0	N	Disposable pipets	Sample removal for large substrate epilithon and epixylon	5



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Item No.	Exact Brand	Description	Purpose	Quantity
Thomas Scientific, Inc.: 1303N48	N	Whirl-paks®, 24 oz.	Epiphyton sample container	20
Grainger, W.W.: 5LH30	N	Resealable bags, gallon	Organizing epiphyton samples	20
	N	DI water	Rinsing substrata	1 L

**Table 12.** Equipment list – General laboratory equipment.

Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable items</b>				
Thomas Scientific, Inc.: 1207M51 Fisher Scientific Company, LLC: 0340910C	N	Wash bottle, unitary, 125 mL	Rinsing the filter funnel	1
	N	Waste container (for non-hazardous liquids)	For rinse water and unused sample	1
<b>Consumable items</b>				
	N	Lab data sheets (RD[07])	Recording data if tablet fails	2
Grainger, W.W. : 5NHH1 Amazon Capital Services Inc.: B00006IBUV Arrow SameDay: 5520	N	Adhesive weatherproof labels 1"x 2-5/8" (e.g., Avery 5661)	Labeling samples and filters	1 per sample
	N	Adhesive barcode labels	Labeling sample bottles with barcode-readable	1 sheet
Grainger, W.W.: 6CHG5	N	Aluminum foil	Wrapping GF/F filters for shipment	1
Grainger, W.W.: 5CNK1	N	Zip-top resealable bags, quart size	Organizing and storing filters	2
	N	DI water	Rinsing the filter funnel	1 L



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**Table 13.** Equipment list – Epiphyte Processing.

Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable items</b>				
Fisher Scientific Company, LLC: S90532C	R	Ruler (metric) or calipers (borrow from TOS flora)	Measuring length of epiphytes	1
BioQuip Products Inc.: 1426B Fisher Scientific Company, LLC: NC0095946	R	Larval tray, plastic	Scrubbing container in which sample is collected	1
<b>Consumable items</b>				
Fisher Scientific Company, LLC: 19027438	R	Nylon toothbrush, disposable	Scrubbing epiphytes	1
Grainger, W.W.: 12R027	R	Paper lunch bags	Drying plants associated with epiphyte samples	20

**Table 14.** Equipment list – Filtering.

Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable items</b>				
NEON GB08760000	N	Vacuum pump filter manifold assembly	Filtering samples	1
NEON GB08760000	N	Filter funnel (25 mm diameter, 200 mL)	Filtering samples, part of manifold assembly	1-3
NEON GB08760000	N	Vacuum filter flask, polypropylene (1L)	Filtering samples, part of manifold assembly	1-3
Amazon Capital Services Inc.: MV8510	N	Vacuum hand pump	Filtering samples, backup to filter manifold	
Amazon Capital Services Inc.: MVM8900	N	Vacuum hand pump maintenance kit	Replacement parts for vacuum hand pump	1
Fisher Scientific Company, LLC: 975350	N	Filter forceps (flat ends)	Handling filters	2



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Item No.	Exact Brand	Description	Purpose	Quantity
Amazon Capital Services Inc.: B0001RT1OS	N	Hand-held stirrer (periphyton homogenizer)	Homogenizing periphyton and breaking up clumps of algae	1
Fisher Scientific Company, LLC: 300742	N	Graduated cylinder, 250 mL	Measuring and adding aliquots of sample to the filter funnel	1
Fisher Scientific Company, LLC: FBE10000	N	Adjustable pipette, 10 mL	Measuring and adding aliquots of sample to the filter funnel	1
<b>Consumable items</b>				
Fisher Scientific Company, LLC: 2681412	N	Pipette tips, 10 mL	Measuring and adding the volume of sample into the filter funnel	1
Fisher Scientific Company, LLC: 987464	N	GF/F filters (25 mm diameter, pre-ashed)	Filters for AFDM and chemistry samples	90

**Table 15.** Equipment list – Ash-free dry mass.

Item No.	R/S	Description	Purpose	Quantity
<b>Durable items</b>				
Fisher Scientific Company, LLC: 8732100	N	Aluminum weigh boats	AFDM sample processing	20
Fisher Scientific Company, LLC: 1910200	N	Analytical balance (0.0001 g)	Measuring weight	1
Thomas Scientific, Inc.: 1229N43	N	Drying oven	Drying samples	1
Fisher Scientific Company, LLC: 1055423	N	Muffle furnace	Burning organic matter for ash-free dry mass determination	1
Amazon Capital Services Inc.: B000OSNQXQ	N	Aluminum baking pan	Sample storage in muffle furnace	2
Grainger, W.W.: 2EWX3	N	Heat-proof gloves (pair)	Safe handling of equipment in the muffle furnace and drying oven	1
Grainger, W.W.: 5ZPT8 Fisher Scientific Company, LLC: 15207 Thomas Scientific, Inc.: 0241B17	N	Crucible tongs	Safe handling of equipment in the muffle furnace and drying oven	1



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Item No.	R/S	Description	Purpose	Quantity
Fisher Scientific Company, LLC: 0864223B	N	Desiccator (bench top)	Storing dried samples	1
<b>Consumable items</b>				
Fisher Scientific Company, LLC: 075783A	N	Desiccant packs	For bench top desiccator	1-2
Fisher Scientific Company, LLC: 8732115	N	Plastic weigh boats	Measuring dry weight	20

**Table 16.** Equipment list – Algae preservation.

Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable items</b>				
	N	Freezer (-20 °C)	Sample storage	1
<b>Consumable items</b>				
Sigma-Aldrich, Inc.: G62571L	N	Glutaraldehyde, Grade II, 25% in H <sub>2</sub> O	Preserving periphyton samples	1 L
Sigma-Aldrich, Inc.: 626501LF	N	High-iodine Lugol’s solution	Preserving phytoplankton samples	1 L
Fisher Scientific Company, LLC: 0331315B	N	HDPE bottles with lids, wide-mouth (60 mL)	Periphyton taxonomy sample container	8
Thomas Scientific, Inc.: 1185B56	N	Amber HDPE bottles with lids, wide-mouth (1 L)	Phytoplankton taxonomy sample container	1