AOS PROTOCOL AND PROCEDURE:
ALG – PERIPHYTON AND PHYTOPLANKTON SAMPLING

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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 AFDM SOP; Remove ^{34}S filter
C	02/13/2018	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	01/13/2020	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	• Update to reflect change in terminology from relocatable to gradient sites
G	05/02/2022	ECO-06824	• Minor formatting update
H	01/04/2024	ECO-07059	• Updated NEON logo • Migrated to protocol template rev L • Lab: Add composite sampling for algal taxonomy and chemistry, change time expected, update total algae sample numbers, add algae metabarcode filter for archiving • Clarify rescheduling, samplingImpractical, and biophysicalCriteria • Add contingent decisions for rivers • Update label format

			• Remove sample timing from appendix • Remove part numbers from equipment list for nonspecific items • Equipment: Require exact brand for GF/F filters

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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 *a*, 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, prominent in lakes and rivers). Macroalgae are sampled as part of the Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling Protocol (RD[11]), while benthic microalgae, seston, and phytoplankton are 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 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.

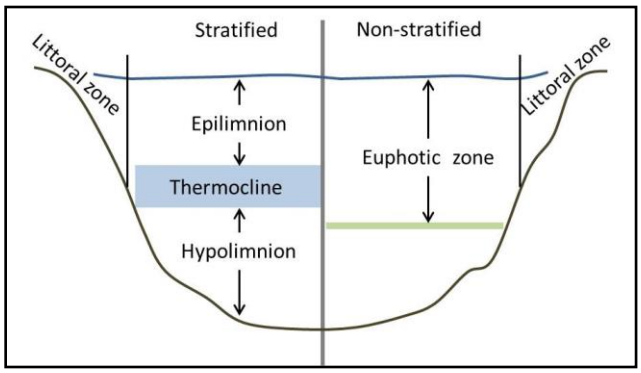

Figure 1. The zones in a stratified and non-stratified lake.

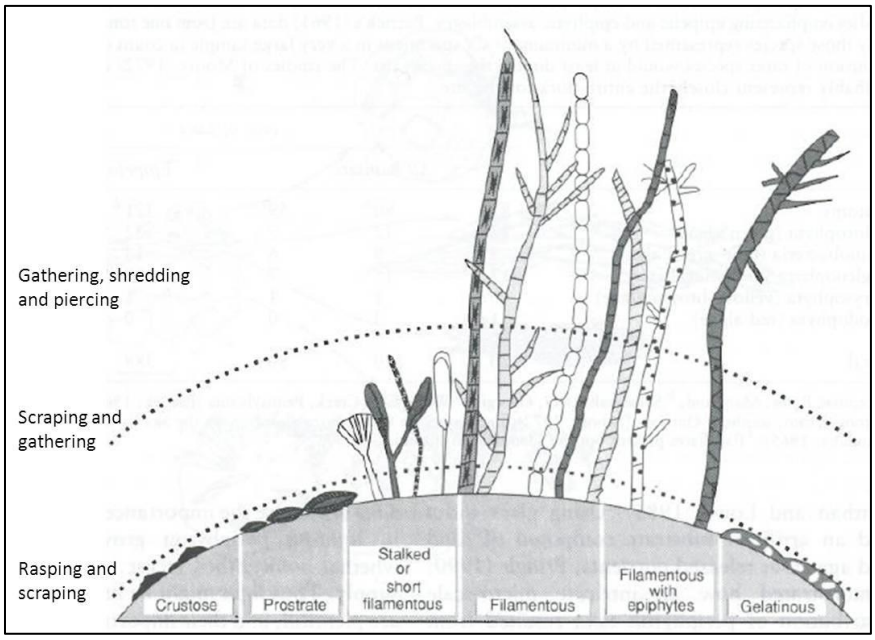

Figure 2. Growth forms of periphyton, ranging from prostrate types to long, filamentous types. Vertical layering differs among growth forms (from Allan 1995).

Periphyton is an important component in the aquatic community, providing 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 (e.g., grazing pressure) factors. 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, and the introduction and spread of invasive species is therefore a crucial component of aquatic ecosystem assessment.

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 (i.e., there is a thermocline present) and within the euphotic zone (i.e., 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 are often 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), National Rivers and Streams Assessment (USEPA 2023), and National Lakes Assessment (USEPA 2022), 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).

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 Data Products Catalog
RD[04]	NEON.DOC.001271	OS Protocol and Procedure: DMP – Data Management
RD[05]	NEON.DOC.001152	NEON Aquatic Sample Strategy
RD[06]	NEON.DOC.004257	Standard Operating Procedure: 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	NEON General AQU & GAG Field Datasheet
RD[09]	NEON.DOC.014037	TOS Protocol and Procedure: HBP – 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: APL – Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling
RD[12]	NEON.DOC.003046	AOS Protocol and Procedure: INV – Aquatic Macroinvertebrate Sampling
RD[13]	NEON.DOC.003044	AOS Protocol and Procedure: AMC – Aquatic Microbial Sampling
RD[14]	NEON.DOC.003826	AOS Protocol and Procedure: RIP – Riparian Habitat Assessment
RD[15]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[16]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[17]	NEON.DOC.002191	Datasheets for AOS Protocol and Procedure: Secchi Disk and Depth Profile Sampling
RD[18]	NEON.DOC.002792	AOS Protocol and Procedure: DEP – Secchi Disk and Depth Profile Sampling in Lakes and Non-Wadeable Streams

RD[19]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[20]	NEON.DOC.003600-NEON.DOC.003618	Aquatic Site Sample Design – NEON Domain ##
RD[21]	NEON.DOC.005224	NEON Protocol and Procedure: SCS – Shipping Ecological Samples and Equipment
RD[22]	NEON.DOC.002905	AOS Protocol and Procedure: SWC – Water Chemistry Sampling in Surface Waters and Groundwater
RD[23]	NEON.DOC.001194	AOS Protocol and Procedure: ZOO – Zooplankton Sampling in Lakes

2.3 Acronyms

Acronym	Definition
AFDM	Ash-free dry mass
C	carbon
°C	degrees Celsius
DSF	Domain Support Facility
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 epiphytic 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 and measured using a Secchi disk.

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 but may also appear as balls or "blobs" (**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 benthos.

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, e.g., open water.

Periphyton: Mixture of algae, cyanobacteria, microbes, and detritus coating submerged surfaces in bodies of water. This term encompasses epilithon, epixylon, epipsammon, epipelon, and epiphyton.

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. When all the running water in the stream are 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**).

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 in wadeable streams.

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

Silt: Very small sediment particles, 3.9-62.5 μm 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 °C per meter. The denser and cooler layer below the thermocline is the hypolimnion, warmer upper layer is 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.

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

3 METHOD

The goal of the Periphyton and Phytoplankton Sampling Protocol is to determine species diversity, taxa richness, and biomass, using both chlorophyll *a* and ash-free dry mass (AFDM) as proxies for biomass of periphyton and phytoplankton at aquatic sites. These variables allow analysis of algal species presence/absence, community structure and function, introduction of invasive species, and chemical analyses. Periphyton and phytoplankton or seston samples are collected to fulfill these requirements in wadeable streams, lakes, and rivers.

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 collected at each stream site if conditions allow sampling (e.g., if the stream has enough water or is not frozen). All samples are collected from the surface of the natural substratum present in each macrohabitat. Collection methods differ depending on the substrata being sampled. For example, riffles and runs often have cobble/pebble substratum, while pools may have silt or sand substrata. Site-specific sampling procedures are determined prior to sampling following NAWQA protocols (Moulton et al. 2002) and presented in site-specific AOS documents (RD[20]) as well as in Appendix D. Starting in 2024, field samples are composited for taxonomic and chemical analyses resulting in one composite sample from the dominant habitat type, and one composite sample from the subdominant habitat type per sampling event.

In lakes and rivers, littoral zone periphyton samples are collected following the divisions set forth in the Riparian Habitat Assessment protocol (RD[14]) at sites where safety and permitting allows the field staff to step out of the boat and wade. 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. Starting in 2024, field samples are composited for taxonomic and chemical analyses resulting in one composite sample from the benthic samples in lakes and rivers per sampling event.

In wadeable streams, rivers, and lakes, phytoplankton and seston are sampled at the water chemistry sampling locations (**Figure 13**, RD[22]). In lakes, phytoplankton is collected at the central location (near the buoy) and the littoral 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 collected near the downstream sensor but is only analyzed for chlorophyll *a* and pheophytin. Starting in 2024, phytoplankton samples are composited resulting in one composite sample per event.

Algae sampling occurs three times per year in order to capture presence and abundance of multiple species across seasons. 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]), NEON site specific sampling designs (RD[20]), and “Temporal design for aquatic organismal sampling across the National Ecological Observatory Network” (Parker and Utz 2022). 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 to ensure that sampling captures the algal community.

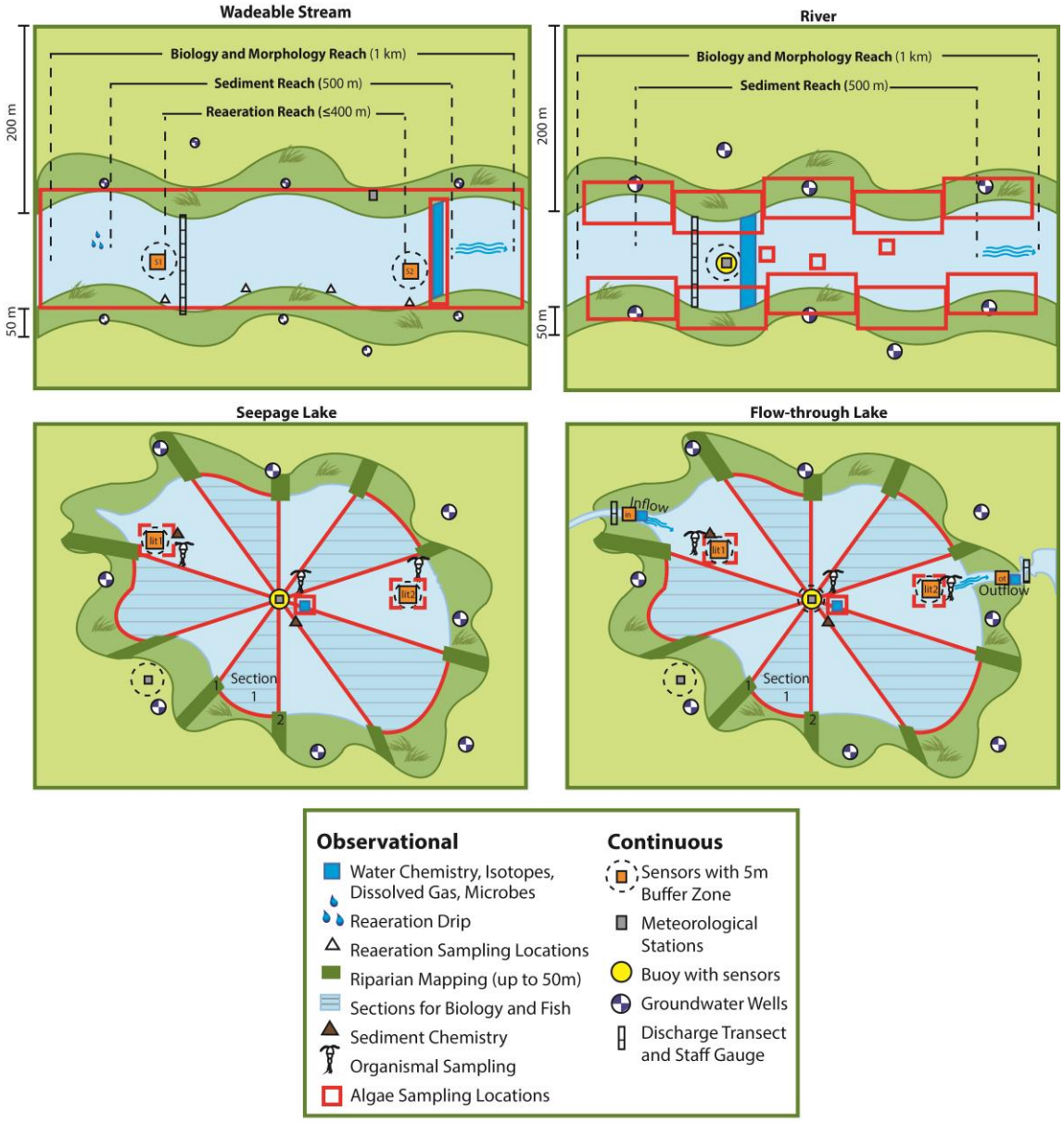

Figure 13. Generic site layouts for lakes, rivers, and wadeable streams with phytoplankton and benthic algae sampling locations. Seepage lakes have no true inlet or outlet stream, while flow-through lakes have inlet and outlet streams.

Standard Operating Procedures (SOPs), in Section 7 of this document, provides 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.

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]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Algal sampling occurs three times per year at each site, roughly spring, summer, and autumn (Parker and Utz 2022, RD[20]). Sampling must be initially scheduled within the first 21 days of the 1 month window specified in RD[20], this helps to allow for rescheduling and weather contingencies. Observe a minimum of two weeks between the sampling date and the start of the next scheduled bout window for Bouts 1 and 2. For Bout 3, allow for 30 days to reschedule missed sampling. 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 Aquatic Site Sampling Design (RD[20) 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[18]).

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.2	lake, river	buoy, littoral1, littoral2, riparian sections, reach	1 day	3	Minimum of 2 weeks between sampling and start of next bout, or 30 days after the end of Bout 3	annual	Uses sampling methods in SOP C
SOP B.3	stream	reach, S2	1 day	3	Minimum of 2 weeks between sampling and start of next bout, or 30 days after the end of Bout 3	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 and must meet the minimum number of 3 samples from a single habitat type. If weather or other conditions force sampling to stop during collection and sampling cannot be rescheduled, contact Science to discuss.
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 from the field to the DSF.
    b. Sample processing must begin within 24 hours of collection.
    c. Chlorophyll/pheophytin filters must be frozen at -20 °C and shipped within 7 days of collection (count the collect date as day 1).

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, Parker and Utz 2022). 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: 1-4 hours for compositing and filtering chemistry samples and preserving taxonomy samples
2. Day 2: 0.5-1 hours for weighing dried filters
3. Day 3: 0.5-1 hours for weighing ashed filters

All filtering should be completed by the end of the day following field sample collection to preserve the integrity of chemistry samples. Chlorophyll *a* samples **must** be filtered and placed in the freezer (-20 ^{o}C) within 24 hours of returning from the field to prevent an increase in chlorophyll *a* concentration 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. For storage and shipping timelines see RD[20] .

4.4 Sample Timing Contingencies

All samples from this protocol must be collected within one day from one sampling site per bout (i.e., all samples per site as detailed in this protocol) because of the fluctuating nature of aquatic habitats and sensitivity to precipitation. Spreading sample collection over multiple days increases the likelihood of variability among samples. Sampling may be rescheduled due to weather or environmental conditions within the bout window (+ 3 days) provided in the Site Sampling Designs (RD[20]). A minimum of 2 weeks between sample collection and the start of the next bout window shall be observed, with the exception of specific domains that have a limited growing season (e.g., D18).

Table 2. Contingent decisions.

Delay/ Situation	Action	Outcome for Data Products
Hours	Stream, lake, river: If weather conditions deteriorate and conditions become unsafe (e.g., approaching thunderstorm, rapid increase of water level in the stream/river) or the lake/river	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur

	becomes too windy (>35 km hr^{-1}) 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 reschedule sampling.	outside of the sampling window, data may be flagged.
14 Days	Stream: If flooding occurs on or <14 days prior to the targeted sampling date (>3x 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).	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data may be flagged.
	River: Use the nearest USGS gauge to determine whether the river is in "flood stage" as determined by USGS or NOAA. Flood stage is typically not safe for boating, let alone sampling. Depending on the site, water level may need to be even lower than flood stage to allow for safe boating and sampling. Allow at least 5 days after water levels drop below flood stage to sample.	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data may be flagged.
	Stream: If the channel 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 (collect date = day 1) 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. Contact science if you exceed the hold time.
6 Months	Preserved algal taxonomy samples may be held for up to 6 months at 4 °C in the domain lab (or longer) if circumstances do not allow shipping to the external lab. If refrigerated storage space is	Holding samples past the scheduled shipping date affects external lab schedules, staffing, and budgets and delays data release on the NEON portal.

	limited, Science may authorize storage at room temperature.	However, sample integrity is not affected.
	Dried algal chemistry filters may be held indefinitely at ambient temperature in the domain lab desiccator 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:

- Logistics – e.g., insufficient staff or equipment
- 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[19]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Canceled Sampling**: Incidence of *scheduled sampling* that did not, and will not, occur. Canceled 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 Canceled Sampling event – i.e., why sampling did not occur. This field is also used to indicate any individually missed samples within an otherwise successful sampling bout, e.g., if a stream is drying and contingent decisions are used.
- **Biophysical Criteria**: This field name associated with a controlled list of values that is included in the data product to explain changes to the schedule.
- **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**).

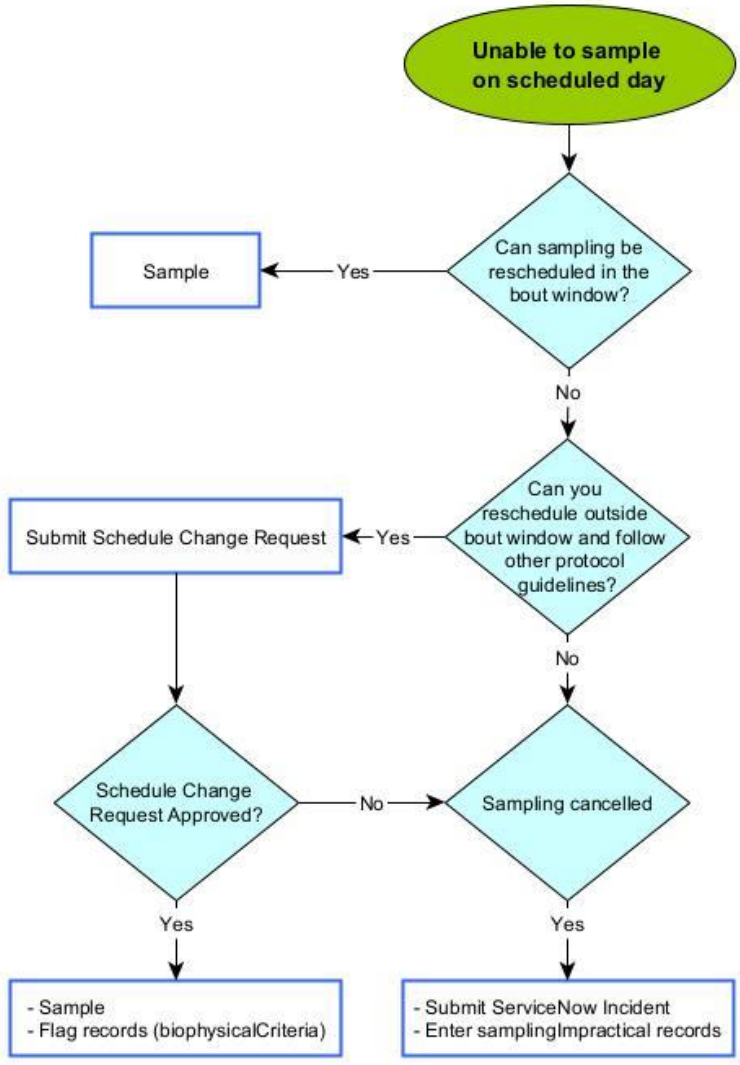

Figure 14. The documentation to account for a Missed Sampling event depends on the situation. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management record, or e) some combination of (a) – (d).

To Report Missed or Incomplete Sampling:

1. Canceled or rescheduled sampling must be communicated to Science by a Service Now Incident or Schedule Change Request.
    a. The lead Field Ecologist should consult the Delayed or Cancelled Activities table to best determine when reporting is required (**Figure 14**).

2. Canceled sampling: For each missed sample, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 3**).
    a. A minimum of three ALG field samples from the same habitat type should be collected during each sampling event if following contingent decisions (**Table 6**), else sampling should be re-attempted or canceled.
    b. Total number of field sampling records expected per event (bout): 8
3. **Rescheduled** sampling events that occur within the biology bout window, sample and record data as normal. Rescheduling within the bout window does not need to be reported to science unless sampling will occur after fish sampling or another disturbance event.
4. **Rescheduled** sampling events that occur outside of the defined AOS biology sampling bout window must be approved by Science in a Schedule Change Request.
5. Sampling events that are rescheduled ±3 days outside the sampling bout require an entry in **Biophysical Criteria**.
    a. biophysicalCriteria – An indicator of whether sampling coincided with the intended biophysical conditions (i.e., within the AOS biology sampling bout window)

Table 3. Guidance for responding to delays and cancellations encountered during implementation of the Periphyton and Phytoplankton Sampling protocol.

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
Periphyton, Seston, and Phytoplankton Sampling	> 3 days outside bio bout window or rescheduling after fish (streams only)	IS/OS Schedule Change Request	Submit incident ticket

Table 4. 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.

Field name	Dropdown list option	Description
Sampling Impractical	Location dry	Location dry
Sampling Impractical	Location frozen	Location frozen
Sampling Impractical	Location snow covered	Location snow covered
Sampling Impractical	High water velocity	Water velocity too high to sample in stream or river
Sampling Impractical	Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)
Biophysical Criteria	OK – within bout window	Sampling occurred within the bout window, no known issues
Biophysical Criteria	conditions not met: sampled after fish	Sampling does not reflect the target biophysical conditions, benthic sampling occurred after benthos was disturbed during seasonal fish sampling
Biophysical Criteria	conditions not met: outside bout window	Sampling was conducted outside of the AOS sampling window

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.

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	4-8 h	2	8-16 h
SOP E: Laboratory Sampling and Analysis	3-4 h	1	3-4 h
SOP E.9: AFDM	1-2 h (over multiple days)	1	1-2 h
SOP G: Data Entry and Verification	1 h	1	1 h
SOP H: Sample Shipment	2-3 h (multiple shipments)	1	2-3 h

4.7 Criteria for Permanent Reallocation of Sampling within a Site

Periphyton, seston, and phytoplankton sampling occurs on the schedule described above at 8 locations per site in two different habitat types in wadeable streams, lakes, and rivers. Ideally, habitats and substrate and surface water sampling locations near infrastructure remain consistent for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (gradient sites). However, circumstances may arise requiring that sampling within a site be moved from one location to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given location becomes compromised, use NEON's problem reporting system to report to Science.

There are two main pathways by which sampling can be compromised. Sampling locations can become inappropriately suited to answer meaningful biological questions (e.g., a stream channel moves after a flood or a habitat type disappears from a site over time). Alternatively, sampling locations may be located in areas that are logistically impossible to sample on a schedule that is biologically meaningful.

A common occurrence in stream sampling is that seasonal drying causes a habitat type to disappear. Contingent decisions in Table 6 should be followed if the wetted area of sampling is decreased. If these sampling reductions occur at a site for three or more consecutive bouts, report to using NEON's problem reporting system to start analysis of rapid habitat data to select different habitat types.

Table 6. Contingent decisions for sampling in wadeable streams.

Situation	Action	Outcome for Data Products	Considerations
Wadeable stream site with <500 m aboveground stream length due to stream size, permitting restrictions, seasonal drying or other environmental factors	Habitat available may be insufficient to accommodate all 8 samples (6 at MCDI) without causing harm to the stream. Reduce sampling using the following options depending on how much habitat is available and enter sampling impractical records for all missing samples: 1. 3 samples dominant, 3 samples subdominant (normal sampling for MCDI due to shortened permitted reach) 2. 5 samples in the 1 remaining habitat type (usually the subdominant habitat) – not applicable to MCDI 3. 3 samples in the 1 remaining habitat type (usually the subdominant habitat).	Lower resolution for diversity metrics, less standardization of the dataset	If the decision is made to decrease the number of samples collected for this protocol, it should also be reflected in the other wadeable stream biology protocols from the same bout (RD[11], RD[12], RD[13]). The remaining habitat type should also be one of the regularly sampled habitats at the site. Contact Science with questions.
Wadeable stream site with seasonal drying such that there is <100 m of wetted channel	If the stream dries such that is <100 m of wetted channel, there is not enough habitat left to sample and sampling is considered impractical. Wait for water to return or contact Science.	Missing data points	Mark as "sampling impractical" for each sample missed in the field data.

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 8 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 ≥ 10 ft^2/s (0.93 m^2/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.
5. 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.).

6 PERSONNEL

6.1 Training Requirements

All technicians must complete protocol-specific training as required in the Field Operations Job Instruction Training Plan (AD[04]). Additional protocol-specific required skills and safety training are described here.

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

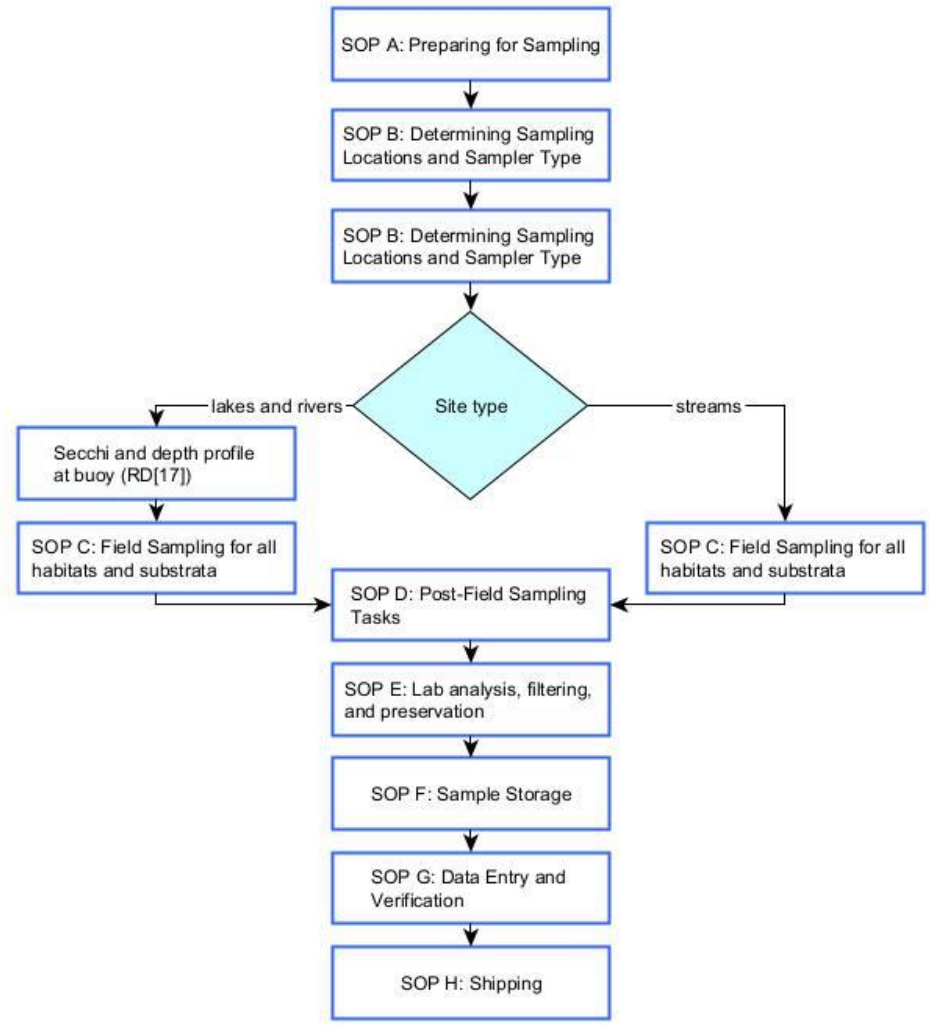

Figure 15. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.

- **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**: Sample Storage
- **SOP G**: Data Entry and Verification

- **SOP H**: Sample Shipment

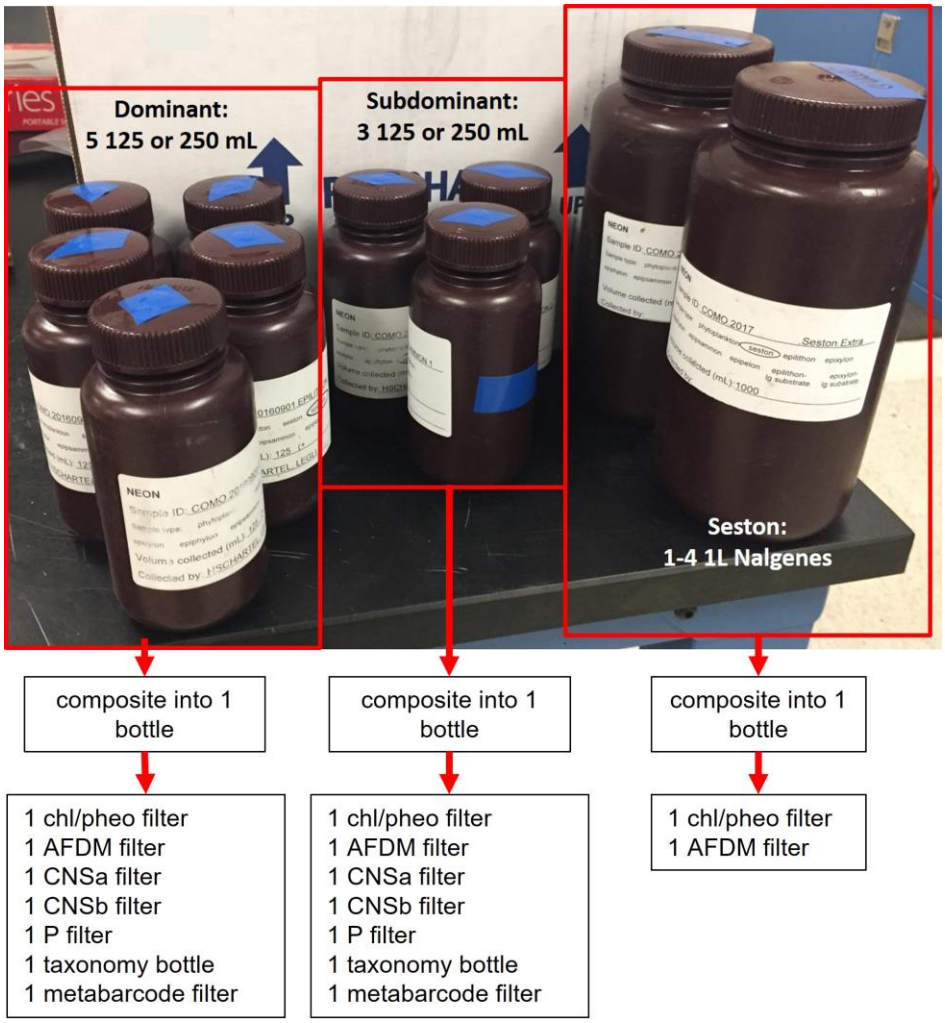

Figure 16. Stream algae field sample suite, including 5 dominant periphyton samples, 3 subdominant periphyton samples, and 1 seston sample (multiple bottles, depending on site water clarity). A total of 8 periphyton samples and 1 seston sample are collected per site per bout.

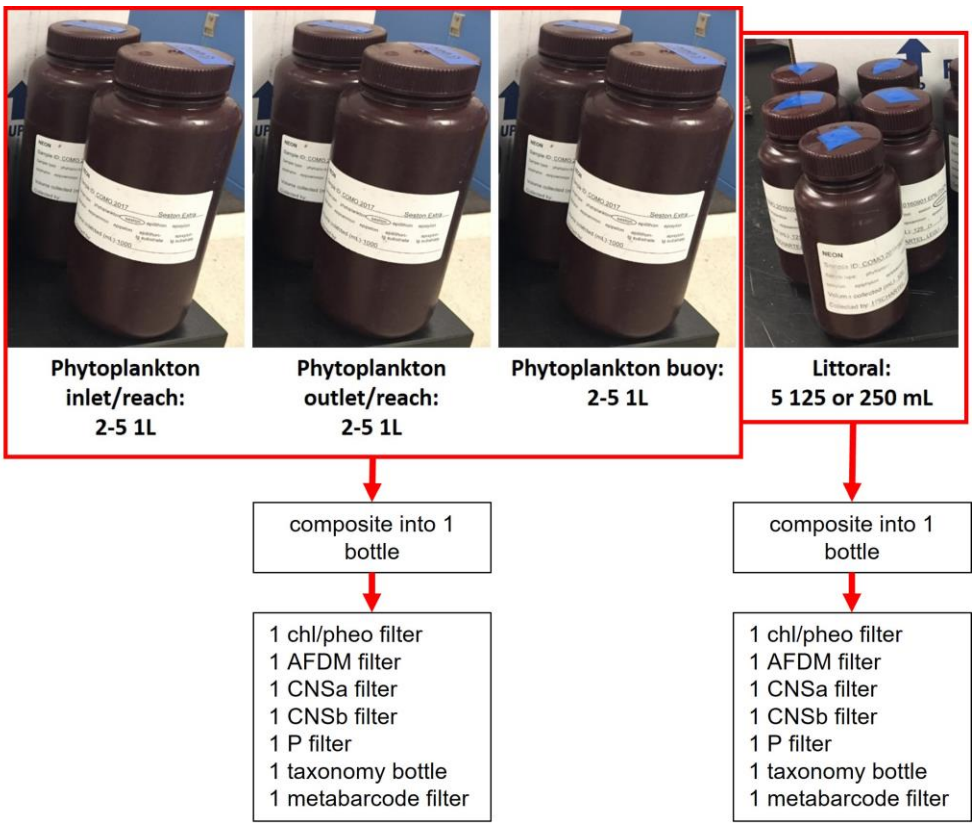

Figure 17. Lake and river algae field sample suite, 3 phytoplankton samples (at least 2 bottles per sample) and 5 littoral periphyton samples. Phytoplankton samples in rivers are collected in the reach rather than at a littoral sensor. A total of 3 phytoplankton and 5 periphyton samples are collected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

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 Preparing for Field Sampling

1. Plan and save sampling routes for field teams using standard site navigation procedures (RD[07]). Route planning enhances sampling efficiency and helps avoid accidental foot traffic at NEON sites. Load GPS sampling coordinates on handheld GPS unit (±4 m accuracy) for lakes/rivers.
2. Collect and prepare all equipment, including sample bottles, sample bags, and pre-printed labels. Know which habitats and substrata are typically sampled at the site.
3. Have ice or ice packs frozen and ready for transportation cooler.
4. Collect and prepare all equipment and consumables required for sampling.
5. Because one of the lab subsamples is a filter for algal metabarcode archive, sampling equipment must be sterilized in the lab similar to macroinvertebrate (RD[12]) and zooplankton (RD[23]) metabarcode collection. Any equipment used for multiple composite samples (across multiple habitats) must be field-sterilized during sampling (SOP C).
    a. Filtered DI is not necessary for this protocol
    b. Wearing nitrile gloves, clean all equipment that will reused and comes in contact with samples using a 10% bleach solution. Follow with a DI rinse to remove the residual bleach (Jane et al. 2014, Laramie et al. 2015). Note that this is a higher concentration than is used in the Aquatic Decontamination Protocol (RD[06]).
    c. Equipment to decontaminate:
        i. Re-used field bottles – may be re-used from bout to bout within a single site
        ii. Slide template (epilithon and epixylon)
        iii. Scissors (epiphyton)
        iv. 50 mm petri dish (epipelon and epipsammon)
        v. Brass-bristle brushes
        vi. Graduated cylinder

vii. White trays

viii. Waders and boots if you plan to wade near the sampling location

Table 7. Number of field sample labels and bottle/bags required per site type.

Site type	Sample type	Number of labels/containers	Sample container
Lakes/Rivers	Phytoplankton	10 (2 per station)	1 L amber bottle
	Periphyton	5	125-250 mL amber bottle **OR** Whirl-Pak/zip-top bag
Wadeable streams	Seston	1 (may need >1 if water is clear)	1 L amber bottle
	Periphyton, dominant habitat	5	125-250 mL amber bottle **OR** Whirl-Pak/zip-top bag
	Periphyton, secondary habitat	3	125-250 mL amber bottle **OR** Whirl-Pak/zip-top bag

6. Human-readable sample IDs will be generated by the mobile app (**Table 8**). Sample IDs written on the physical sample label must match the sample ID generated by the mobile app.

7. If collecting epilithon/epixylon samples, open 35 mm slide template (like a book) and separate into two halves (**Figure 18**). 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.

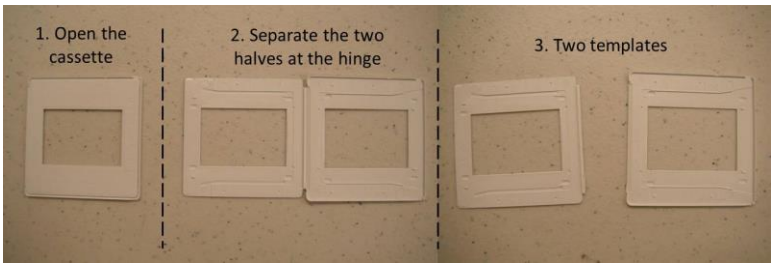

Figure 18. Separating the two halves of the 35 mm slide template.

8. 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”.

    a. Mark the bottle as “verified” (**Figure 19**).

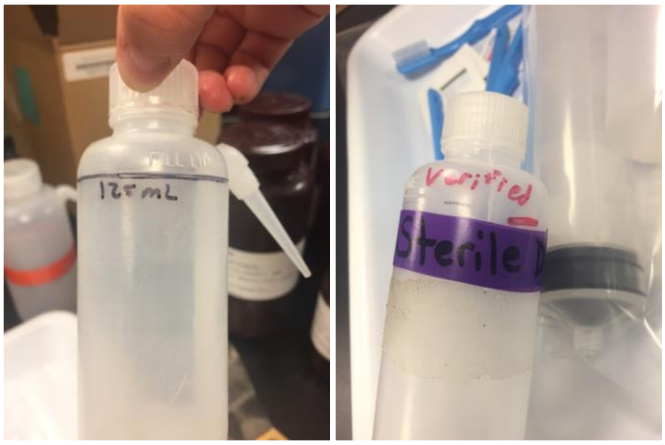

Figure 19. Verified 125 mL bottle examples.

9. At lake and river sites, pre-measure the 4 L jug and mark 1, 2, and 3 liters on the side for easier compositing in the field (**Figure 33**).
10. Check that all equipment is clean, sterile if needed, and in good condition and all electronics are charged.
11. See Laboratory Preparation Procedures in Section E.3 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.**
12. At the site, enter general aquatic field metadata on the mobile app (RD[08]). General field metadata only need to be filled out once per site per day, even if multiple protocols are implemented. At lake and river sites, also enter data in the Secchi app to inform phytoplankton sampling. If other protocols are done in the same day, one record for field metadata and one record for Secchi and depth profile are sufficient (RD[18]).

A.3 Labels and Identifiers

Barcode labels are recommended, but not required, on algae field samples for improved sample tracking. Barcodes on field samples cannot be reused on lab subsamples. 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 have a weather-resistant, adhesive, human readable label on the outside of the bottle (**Figure 20**). This label may be reused along with the cleaned, rinsed bottle from bout to bout within a site.
    a. Add adhesive labels to sample bottles prior to going in the field and getting the bottle wet.
    b. Keep a human-readable label on each bottle with a minimum of the sample ID printed to assist with organization and shipping.

2. For epiphyte samples only: Print all-weather paper labels (**Figure 21**). Cut labels apart using scissors. One label will be placed inside each Whirl-Pak with the field sample.

    a. All-weather paper labels should be filled out using pencil.

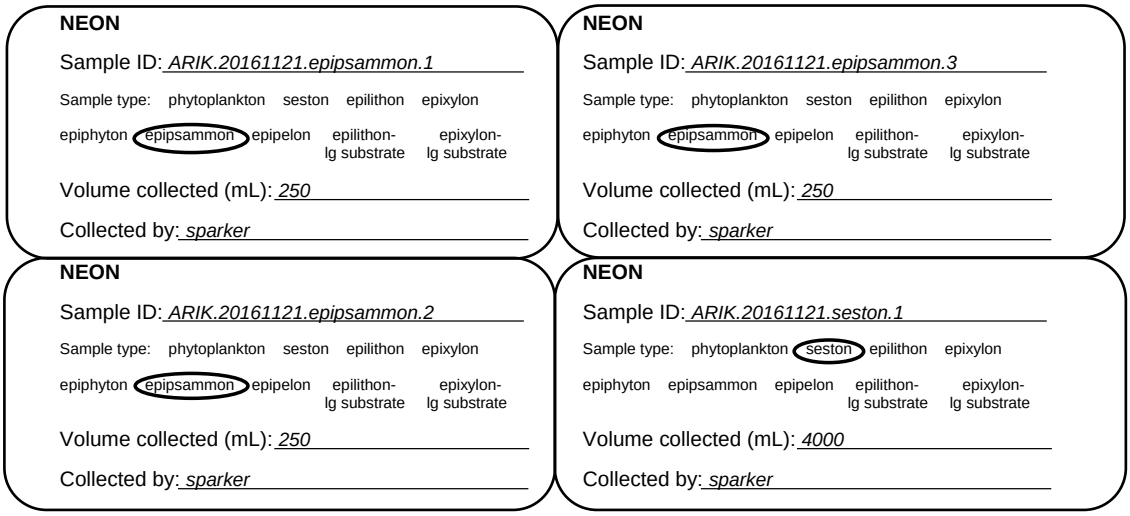

Figure 20. Example adhesive field labels (2” x 4”) for field algae samples. These labels should be filled out with permanent marker.

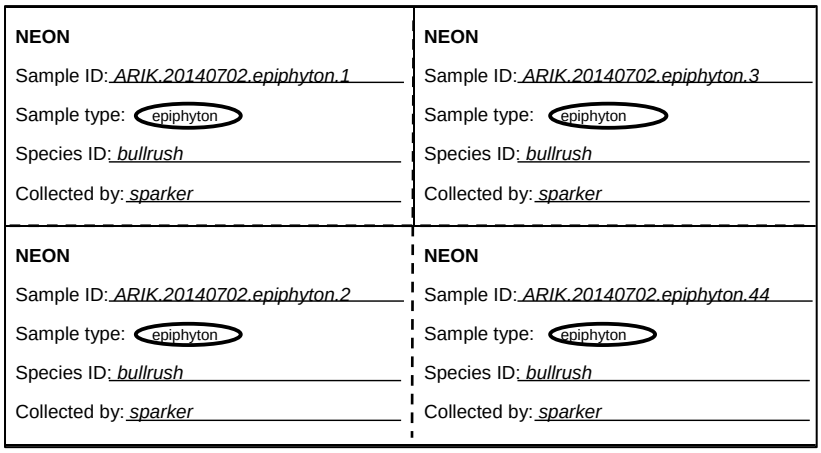

Figure 21. Example of field labels to be printed on all-weather paper. These labels should be filled out with pencil.

3. Suggestions for sample numbering are listed below. It is **required** that the sample numbers and IDs match between the mobile app and the physical label and that the sampleIDs generated are unique.

    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 (1st sampler type) samples are typically numbered 1-5

ii. Subdominant habitat type (2nd 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.

Table 8. Sample ID and barcode requirements for field samples generated by the ALG-Periphyton and Phytoplankton protocol. Barcodes are only required for subsamples sent to external facilities. Samples from the containers below will be combined at the DSF to create composite samples.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required?	Barcode Qty
Phytoplankton	Field sample in lake or river	CRAM.20161027.phytoplankton.1	(AOS) Algae - Field	1 L amber Nalgene	none	No	0
Benthic algae: epilithon or epilithon_large Substrate	Field sample in any aquatic site	MAYF.20161027.epilithon.1	(AOS) Algae - Field	125-250 mL amber Nalgene	none	No	0
Benthic algae: epixylon or epixylon_largeSubstrate	Field sample in any aquatic site	MAYF.20161027.epixylon.1	(AOS) Algae - Field	125-250 mL amber Nalgene	none	No	0
Benthic algae: epipsammon	Field sample in any aquatic site	MAYF.20161027.epipsammon.1	(AOS) Algae - Field	125-250 mL amber Nalgene	none	No	0
Benthic algae: epipelon	Field sample in any aquatic site	MAYF.20161027.epipelon.1	(AOS) Algae - Field	125-250 mL amber Nalgene	none	No	0
Benthic algae: epiphyton	Field sample in any aquatic site	MAYF.20161027.epiphyton.1	(AOS) Algae - Field	Whirl-Pak	none	No	0

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]".

Habitats and sampler types only need to be determined in the first year of sampling and remain the sample from bout to bout and year to year. If you are unable to use the sample samplers used previously at your site, submit an incident ticket to Science to discuss.

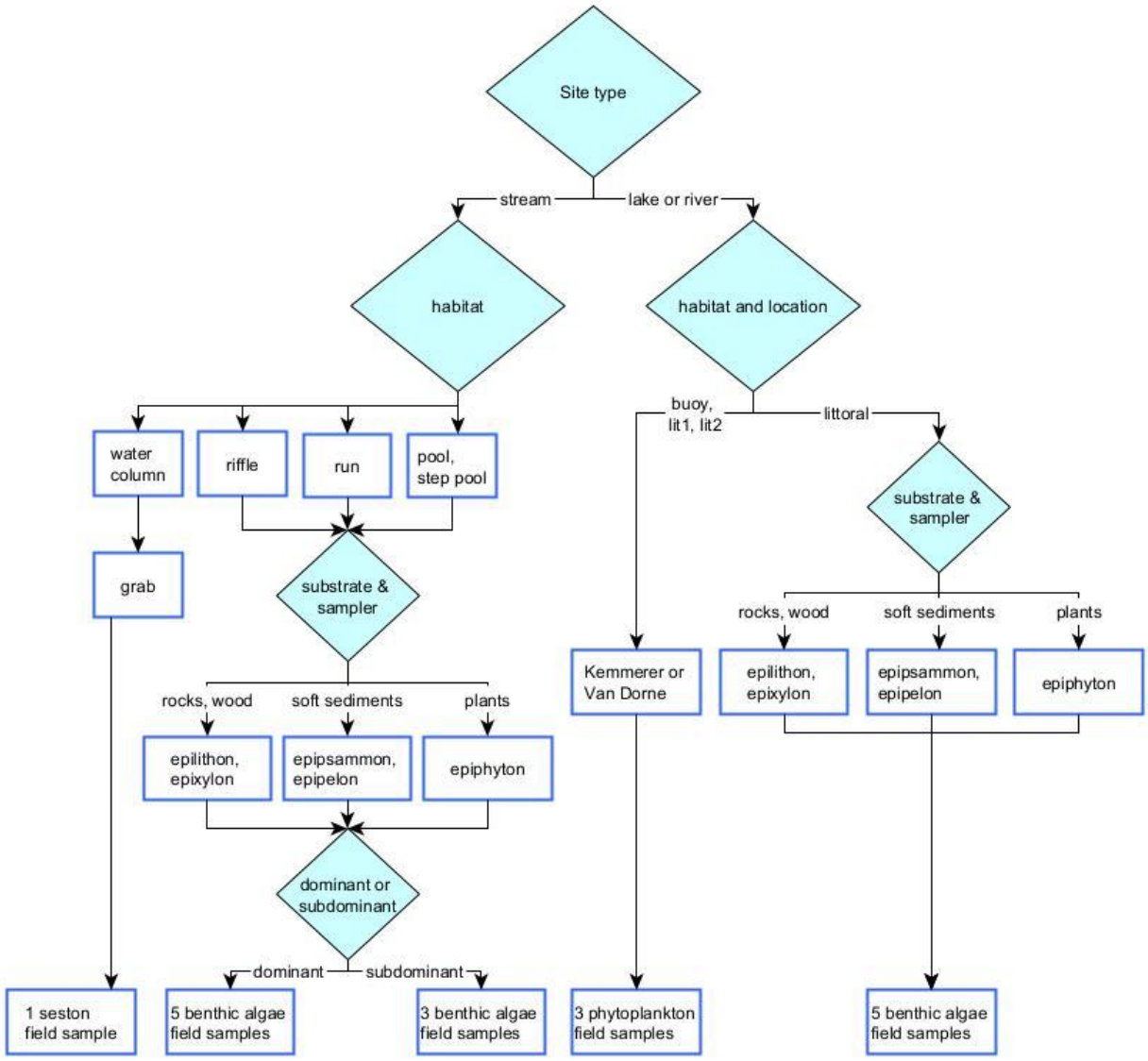

Figure 22. Workflow for field sampling SOPs for periphyton and phytoplankton collection.

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[18]). Euphotic zone and stratification depths are required for determining phytoplankton sampling depths.

B.2 Lake and River Sampling Locations

1. Pelagic sampling for phytoplankton
    a. Lakes: Phytoplankton samples are collected at the center (buoy), littoral1, and littoral2.
    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. First year of sampling: 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. Subsequent years of sampling: Use substrata sampled in previous years and bouts, check that the substrata still meet the >20% requirement. See Appendix D for typical habitat and substrata sampled at NEON sites by a quick visual scan.
        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 (see **Table 6**).
        iii. If appropriate substrata are not available and samples cannot be collected, select "Sampling Impractical" for each missed sample 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[14], **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.

Figure 23. Example of lake perimeter subdivisions from the Riparian Habitat Assessment protocol (RD[14]).

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 Stream Sampling Locations

1. First year of sampling: Determine percent cover of habitat types throughout the sampling reach using the Stream Morphology Map (RD[15]) or rapid habitat assessment. Subsequent years of sampling: Use substrata sampled in previous years and bouts, check that the substrata still meet >20% requirement by a quick visual scan of the reach. See Appendix D for typical habitat and substrata sampled 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 or 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. Year 1 of sampling: 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. This step is only done in year 1 of sampling, after year 1, the same 2 habitat and substratum types should be used for each bout. If the substrate or habitat type is not present and sampling cannot be completed for 3 bouts in a row, submit an incident ticket to Science to reassess habitat and substrata.

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 each sample was missed. 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 – Substrate Selection

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). Substratum type should stay consistent for each habitat type over time, contact Science if this seems to be changing at your site.

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/riverbank or lake shore and may be exposed to frequent drying.

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, macroalgae filaments or colonies, 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 field 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 field composite samples from different riparian sections or transects.

   c. Within the riparian section or habitat unit, there are no restrictions on spacing for the 3 parts of the field composite. As long as they are not all from the same piece of wood, for example, they can all be collected near each other.

d. If contingent decisions are being followed such that there are not 3 or more distinct habitat units (e.g., 3 separate riffles), you may collect samples within the same unit but samples must be > 10 m apart.

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

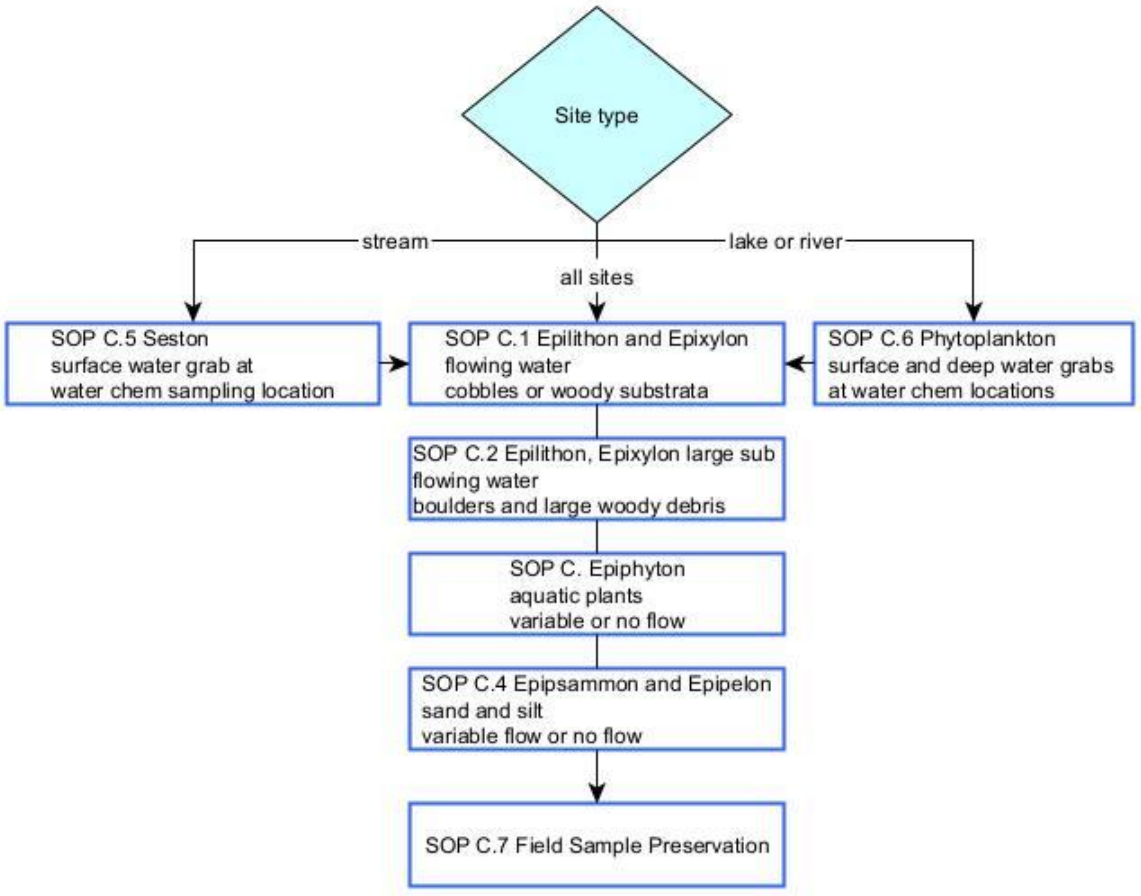

Figure 24. Expanded diagram of the workflow for field sampling SOP C.

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

1. Use field-sterile procedures if a subsample is collected for metabarcoding.
    a. Wear clean nitrile gloves for sampling (ETOH wipe and rinse with DI).
    b. Wipe white tray and slide template with an ETOH wipe and rinse with DI. Rinse reused brushed with ETOH followed by DI.
    c. Because field samples from the same habitat type will be composited at the DSF, you do not need to re-sterilize between field samples that will be in the same composite if you have equipment dedicated to each habitat type.
    d. It is best practice to field-sterilize gloves between all samples.

2. Select three cobbles or pieces of woody debris for each field composite sample (e.g., 15 total cobbles for 5 field 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/lakebed (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 hand saw for sampling.
    e. When possible, avoid woody debris that seem soft and partially decomposed to minimize the amount of wood splinters that enter the algae sample.
3. 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
4. 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.
5. Be sure to keep cobbles/woody debris moist with native water until scrubbing.
6. 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 25**).

Figure 25. Process samples on the stream bank, out of direct sunlight.

7. 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.
8. 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 11 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.
9. 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.

10. 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.

11. 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. Some trays have a pouring lip built into one corner of the tray, which may help when transferring the sample to the bottle.

12. Place white slide template on top of cobble/woody debris (surface that was exposed to light at the stream bottom; **Figure 26**). Check cobble/woody debris again for colonization of invertebrates, bryophytes, or plants.

Figure 26. Template placement for epilithon protocol. (Note: Samples should not be processed in direct sunlight)

13. 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 26**).

d. Scrubbing should be similar to brushing your teeth. Avoid scrubbing so hard that epilithon spatters out of the tray or that wood splinters are added to the sample.

e. Ensure that leaves and grasses from the bank do not enter the sample.

14. 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.
15. Continue scrubbing until the inside of the template is clean.
16. Remove template. There should be a clean rectangle left on the substratum from scrubbing. This is harder to see on woody substrates.
17. Using the wash bottle, rinse the template (front and back) and the cobble/woody debris into the tray.
18. Repeat Steps 11-17 until all 3 cobbles/woody debris collected for the field composite sample have been scrubbed and rinsed.
19. Rinse scrub brush, template, and fingers over the tray. If there is any remaining water in the 125 mL wash bottle after all three cobbles have been scrubbed and rinsed, you may use it to rinse the sample into the amber sample bottle.

    a. If there is any water left in the 125 mL wash bottle after the tray is empty, use for rinsing then pour remaining DI into the sample bottle to maintain a constant volume.

    b. If additional water is needed, measure using the graduated cylinder and record total rinse volume on in the mobile app.

    c. All rinse-water should now be in the white tray and should total 125 mL (or other volume you have determined), **do not discard as this is your sample**.

20. Discard scrubbed cobble/woody debris on the bank or back in the water before you leave the site.
21. Carefully swirl contents of tray (scrubbed material+ rinse-water) to re-suspend the sample.
22. 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. Data processing depends 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 if you need new substrate to scrub or Step 11 if there is enough space left to scrub on the existing substrate.

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.

e. For epixylon, if there are a significant amount of wood particles in the sample, discard and start over.

23. 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 21-22.

24. 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 the amount of water added to the sample using the 25 mL graduated cylinder, and add to the field sample volume in the mobile application. **It is very important to keep track of any changes to the field sample volume.**

    a. Additional water will also require that you use a larger sample bottle (i.e., 250 mL HDPE amber wide mouth).

25. 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.

       NOTE: it is not recommended to submerge samples in rivers as they will be difficult to secure. Please place samples directly in the cooler.

26. Enter data in the mobile app. In the case of app failure, fill out a field datasheet.

    a. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.

27. Rinse tray, brush, and template with native water before starting next sample.

28. Repeat above steps until all locations have been sampled.

a. Wadeable streams, dominant habitat: 5 composite field samples

b. Wadeable streams, subdominant habitat: 3 composite field samples

c. Lakes: 5 composite field samples, 1 from each of 5 riparian sections

d. Rivers: 5 composite field samples, 1 from each of 5 riparian sections

29. 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 field composite of three scrubs.

1. Fill out labels (all-weather adhesive labels, **Figure 20**, RD[07]).
    a. Wadeable streams, dominant habitat: 5 labels
    b. Wadeable streams, secondary habitat: 3 labels
    c. Lakes/Rivers: 5 labels
2. Use the large substrate sampler and modified wire brush and/or modified toothbrush for sampling.
    a. Create a long-handled t-shaped brush by cutting the brush head off a brass-bristle brush (rock scrub) or toothbrush (wood scrub). Make sure the handle is longer than the PVC tube of the sampler.
    b. There are multiple ways to make the t-shaped brush. Recommended materials include a wooden dowel for the handle and an aquarium epoxy stick to adhere the brush head to the dowel.
3. Use field-sterile procedures if a subsample is collected for metabarcoding.
    a. Wear clean nitrile gloves for sampling (ETOH wipe and rinse with DI).
    b. Wipe white tray and slide template with an ETOH wipe and rinse with DI. Rinse reused brushed with ETOH followed by DI.
    c. Because field samples from the same habitat type will be composited at the DSF, you do not need to re-sterilize between field samples that will be in the same composite if you have equipment dedicated to each habitat type.
    d. It is best practice to field-sterilize gloves between all samples.
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 of the sampler. Choose a substratum that is free of plants/bryophytes and minimal macroinvertebrate colonization and is relatively smooth (this will help with sealing the sampler).
6. Fill the 125 mL wash bottle to the fill line with DI.
    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 and allowing water into the PVC will affect the sample volume. You may need to push firmly down onto the substrate.
8. Use the turkey baster to remove existing water from the PVC column prior to scrubbing. Ensure that you have a good seal on the substrate.
9. Using a modified brush (**Figure 27A**), 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.

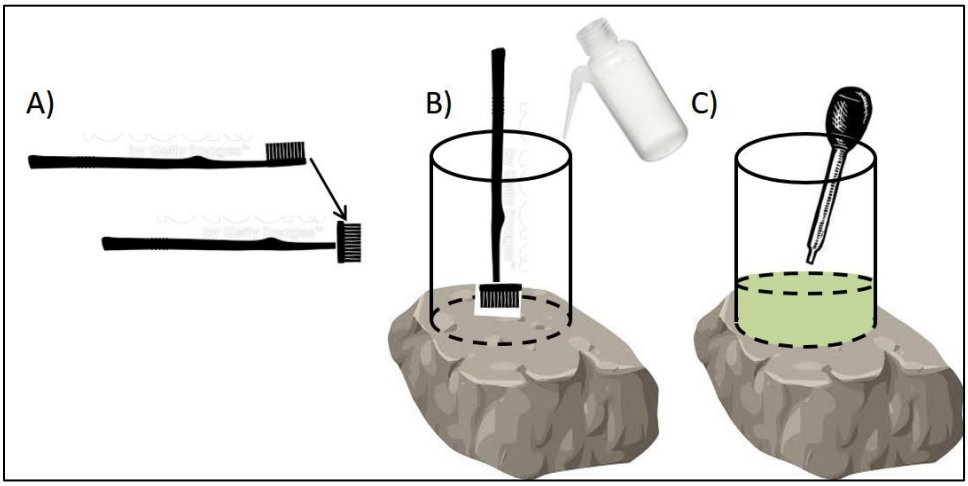

Figure 27. Schematic of PVC periphyton sampler. A) Cut off the head of a brush, and adhere it horizontally to the handle using epoxy. B) Set the PVC sampler on the substrate, and scrub the substrate surface using the modified toothbrush. Rinse rock surface. C) Remove water + algae sample with a turkey baster or disposable pipet.

10. When finished scrubbing, use the turkey baster to pipet the sample out of the PVC tube. Place the sample directly into the sample bottle. It's helpful to have a second person to hold materials.
11. 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.
12. When finished removing the sample from the PVC tube, remove the tube from the stream.

13. Move sampler to a second and third location in the same area to create a field composite sample consisting of 3 scrubs.
14. 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.
15. Record data in the mobile app.
    a. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
16. Rinse all equipment well with stream water prior to moving to the next sampling location.
17. Repeat above steps until all locations have been sampled.
    a. Wadeable streams, dominant habitat: 5 composite field samples
    b. Wadeable streams, subdominant habitat: 3 composite field samples
18. 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, **Figure 20**, RD[07])
    a. Wadeable streams, dominant habitat: 5 labels
    b. Wadeable streams, secondary habitat: 3 labels
    c. Lakes/Rivers: 5 labels
2. Use field-sterile procedures if a subsample is collected for metabarcoding.
    a. Wear clean nitrile gloves for sampling (ETOH wipe and rinse with DI).
    b. Wipe white tray and slide template with an ETOH wipe and rinse with DI. Rinse reused brushed with ETOH followed by DI.
    c. Because field samples from the same habitat type will be composited at the DSF, you do not need to re-sterilize between field samples that will be in the same composite if you have equipment dedicated to each habitat type.
    d. It is best practice to field-sterilize gloves between all samples.
3. Select plants for sampling that are well-colonized with epiphytes (**Figure 28**) 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.

d. Plants should be in water that is shallow enough to reach the bottom.

Figure 28. 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 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.

    d. Do not add water to the Whirl-Pak.

    e. Options for delineating the sampling areas include a 10 cm square quadrat or a folding meter stick.

6. Close Whirl-Pak or gallon resealable bag and place in a cool, dark location (e.g., cooler).

    a. Close the Whirl-Pak by holding the wire tabs at either side of the bag (**Figure 29**), 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.

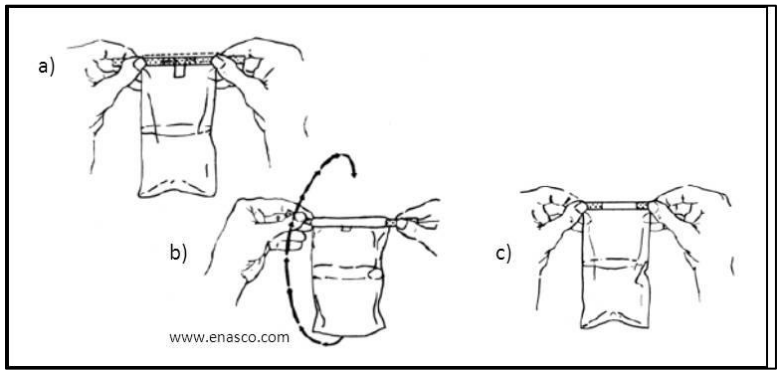

Figure 29. How to close a Whirl-Pak bag: a) hold the wire tabs; b) whirl the bag 3 complete revolutions (or fold the top over); and c) fold the wire ends over to close.

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.
    b. Identify and record the plant taxon that was collected in this sample.
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”x4” all-weather adhesive labels) and adhere to 250 mL wide-mouth amber HDPE bottles (**Figure 20**).
    a. Lakes/Rivers: 5 sample bottles
    b. Wadeable streams, dominant habitat: 5 sample bottles
    c. Wadeable streams, secondary habitat: 3 sample bottles
2. Use field-sterile procedures if a subsample is collected for metabarcoding.
    a. Wear clean nitrile gloves for sampling (ETOH wipe and rinse with DI).

b. Wipe white tray and slide template with an ETOH wipe and rinse with DI. Rinse reused brushed with ETOH followed by DI.

c. Because field samples from the same habitat type will be composited at the DSF, you do not need to re-sterilize between field samples that will be in the same composite if you have equipment dedicated to each habitat type.

d. It is best practice to field-sterilize gloves between all samples.

3. Note the dominant substratum size class at the sampling location in the mobile application.

4. 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.

5. Rinse white plastic sampling tray with stream water.

6. Triple 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.

7. Lightly press the lid into the substratum to be sampled (like a cookie cutter). Take care not to disturb the substratum before placing lid on bottom. If substratum is disturbed, find a new sampling location.

8. Slide spatula under lid to enclose the sample. Holding the petri lid tightly to the spatula, lift out of water (**Figure 30**). 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 30. An epipelon sample collected with the Petri dish lid and metal spatula.

9. Place spatula + sample + petri lid in white sampling tray and invert lid.
10. Repeat Steps 6-9 until you have collected 3 petri lid samples to combine into one field composite sample.
    a. 3 petri lid samples = 1 field composite sample
11. 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 11** 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.

12. Using the 125 mL DI wash bottle, rinse petri lid and spatula into tray. **DO NOT DISCARD RINSE WATER.**
13. 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, use for rinsing then pour remaining DI 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.

14. Cap bottle tightly, double check labels, and place in a cool storage location out of direct sunlight until sampling is finished.
    a. Place sample bottles in the cooler or submerge the bottles in 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.
15. 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.
16. Rinse tray, petri dish, and spatula with stream water before starting next sample.
17. Repeat above steps until all locations have been sampled.
    a. Wadeable streams, dominant habitat: 5 composite field samples
    b. Wadeable streams, secondary habitat: 3 composite field samples
    c. Lakes: 1 field composite sample from each of 5 riparian sections
    d. Rivers: 1 field composite sample from each of 5 riparian transects
18. 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 20**).
    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 a field 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 20**).
    a. One to two 1 L amber HDPE bottles equal 1 field composite sample.
    b. Three field 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[16]) and preloaded GPS coordinates (near the buoy infrastructure)
        ii. Near the lake littoral1 infrastructure
        iii. Near the lake littoral2 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). Locations should be ±10 m of the river depth at the buoy and a minimum of 50 m apart from each other.
3. Using the meter tape, measure out 1 m increments on the Kemmerer rope and mark using electrical tape to more easily read depths during sampling.
4. Navigate the boat to the sampling location.

5. 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.
6. Always sample near the bow of the boat to minimize the effects of the motor on the water column.
7. Use field-sterile procedures if a subsample is collected for metabarcoding.
    a. Wear clean nitrile gloves for sampling (ETOH wipe and rinse with DI).
    b. Wipe white tray and slide template with an ETOH wipe and rinse with DI. Rinse reused brushed with ETOH followed by DI.
    c. Because field samples from the same habitat type will be composited at the DSF, you do not need to re-sterilize between field samples that will be in the same composite if you have equipment dedicated to each habitat type.
    d. It is best practice to field-sterilize gloves between all samples.
8. 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[18]). Euphotic and thermocline depths determined at the buoy will be applied to the littoral sensor locations.
    a. Determine the depth of the euphotic at the buoy zone by multiplying the mean Secchi depth by 2.5. The Secchi data collection app will do this calculation.
        i. If the euphotic depth > maximum depth at the sample location, sample at 0.5 m above the maximum depth. The mobile app will calculate this for you.
    b. Measure to the middle of the Kemmerer or Van Dorn, similar to the water chemistry protocol (RD[22]). Sampling depths are calculated in the data collection app, but it is always a good idea to check those calculations for accuracy.
    c. If thermal stratification is present, integrate the following (Figure 31):
        i. Surface: 0.5 m (±0.5 cm) below water surface → 1 sampler
        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.

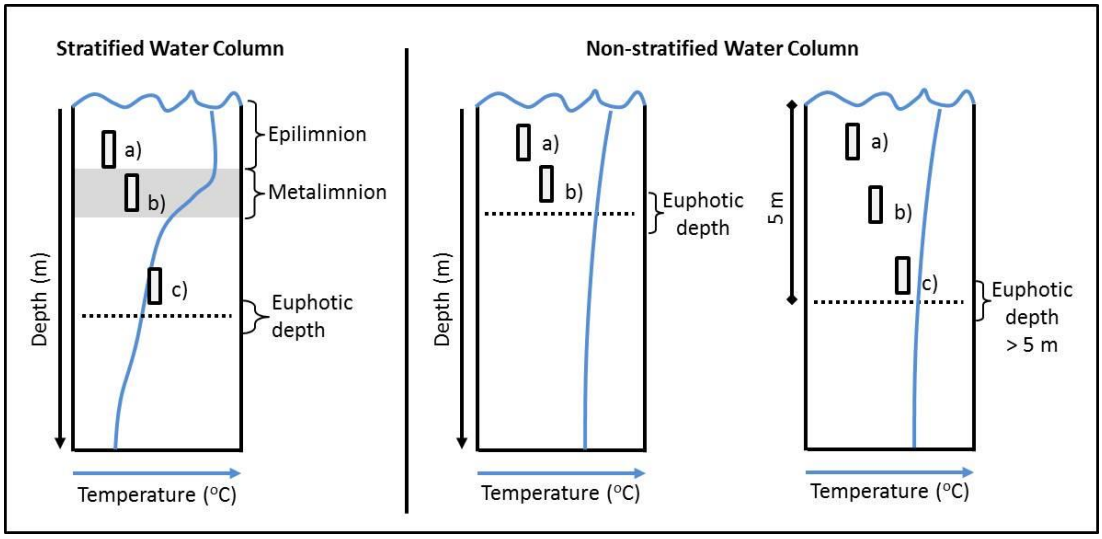

Figure 31. Sample depth selection in a stratified water column. The blue line is a temperature profile indicating the presence or absence of a thermocline. If stratification is present, integrate 3 samplers: a) 0.5 m below the water surface, b) middle of the metalimnion, and c) 0.5 m above bottom of 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.

9. 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.

10. 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 32**). 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.

b. Adding a small piece of flexible tubing to the Kemmerer spigot may help empty the contents of the sampler into the sample bottle (**Figure 33**).

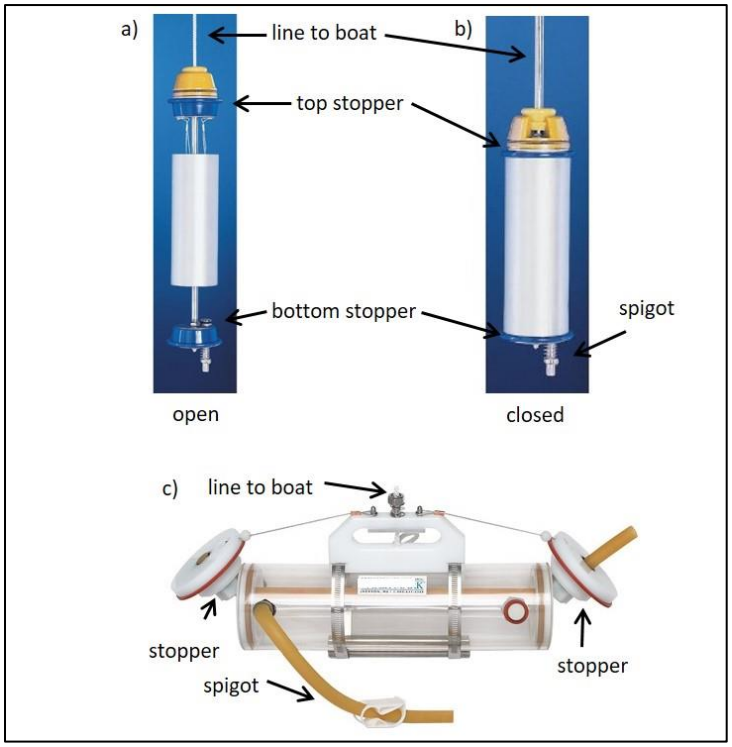

Figure 32. Vertical Kemmerer water sampler in the a) open or cocked position and b) closed (after dropping the messenger) position. c) Horizontal Van Dorn in the open position

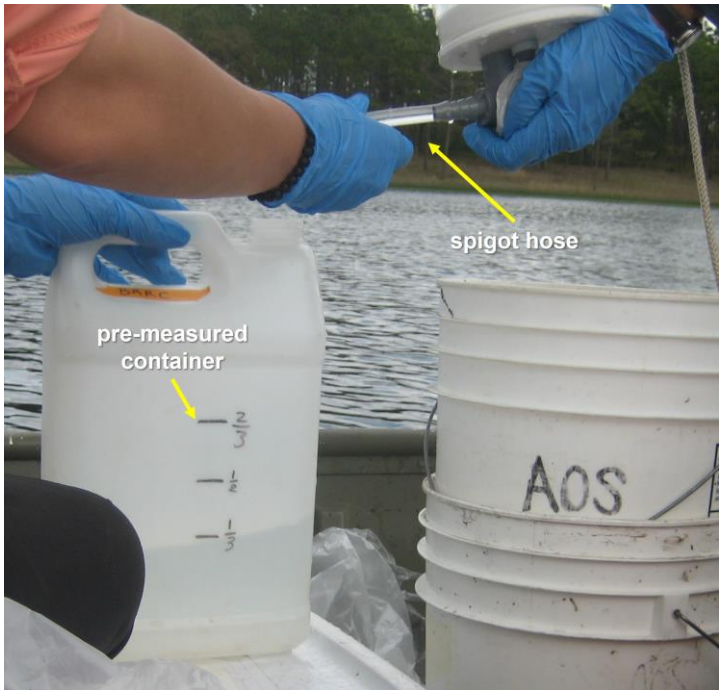

Figure 33. A spigot hose and measurements on the container can be helpful for Kemmerer sampling.

11. Tie the free end of the sampler line to a cleat on the boat to prevent losing the sampler.
12. Always start with the shallowest sample first to avoid disturbing the water column (i.e., sample surface water first and bottom of thermocline last).
13. 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.
14. 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.
15. 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 32b**).
16. 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.
17. Uncap the 4 L HDPE jug.
18. Carefully open the spigot (**Figure 32b**) on the bottom of the sampler, allowing the contents of the cylinder to flow into 4 L jug.

a. Repeat Steps 10- 18 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.

ii. To make integrating the sample easier, mark 1/3 increments on the side of the container with a permanent marker (**Figure 33**).

19. Rinse pre-labeled sample bottles three times with lake/river water, discard water back into the lake/river.

20. Mix integrated sample gently in 4 L jug, then fill 2 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 location where they will not float away.

b. The 1-2 1L bottles = 1 field composite sample.

c. Samples must be kept dark and cool until filtering.

d. Record estimated field sample volume = volume of sample in all field composite bottles. You do not need to get an exact volume here.

21. Record data in the mobile app.

a. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.

22. Proceed to the next sample location and repeat above steps.

C.7 Field Sample Preservation

1. Samples must remain dark (in amber HDPE bottles) and cool (4 °C) until they are processed in the domain lab. Do not allow samples to freeze.

2. Samples must be processed in lab within 24 hours of field sampling; see lab protocol (SOP E).

SOP D Post-Field Sampling Tasks

1. Refresh the sampling kit.
    a. Replace sample jars and resealable bags, 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.
    c. Print new field labels and field data sheets. Make sure barcode labels are available.
2. Equipment, maintenance, cleaning and storage
    a. Decontaminate all equipment that has come in contact with site water according to the Decontamination Protocol (RD[06]) within 48 hours of returning from the field. If sampling occurs on Friday, decontamination can occur the following Monday.
    b. Only reuse amber HDPE bottles at the same site. Keep a separate set of bottles dedicated to each site.
    c. 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.
    d. Dry all items completely between sites and before storage.
    e. 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

SOP E Laboratory Sampling and Analysis

At the Domain Support Facility, periphyton, seston and phytoplankton samples are processed for shipping to analytical facilities for the following parameters: chlorophyll *a* and pheophytin concentration, ash-free dry mass (AFDM), C, N, P, and S content isotopes, algal taxonomic identification, and metabarcode archiving.

E.1 General Lab Preparation

1. Create labels and have barcodes and aluminum foil squares ready prior to processing to make processing time more efficient (SOP E.2).
2. Have enough GF/F filters pre-ashed for filtering (SOP E.3).
3. Be sure to have weigh boats pre-ashed, clean, and ready for use (SOP E.3).
4. Gather clean taxonomy bottles and ensure you have enough glutaraldehyde on hand for preservation.
5. If you have epipsammon or epipelon samples, measure the alternate field sample volume following SOP E.4.
6. If you have epiphyton samples, follow instructions for scrubbing and mass measurements in SOP E.4.
7. Have clean, sterilized composite containers ready (SOP E.5).

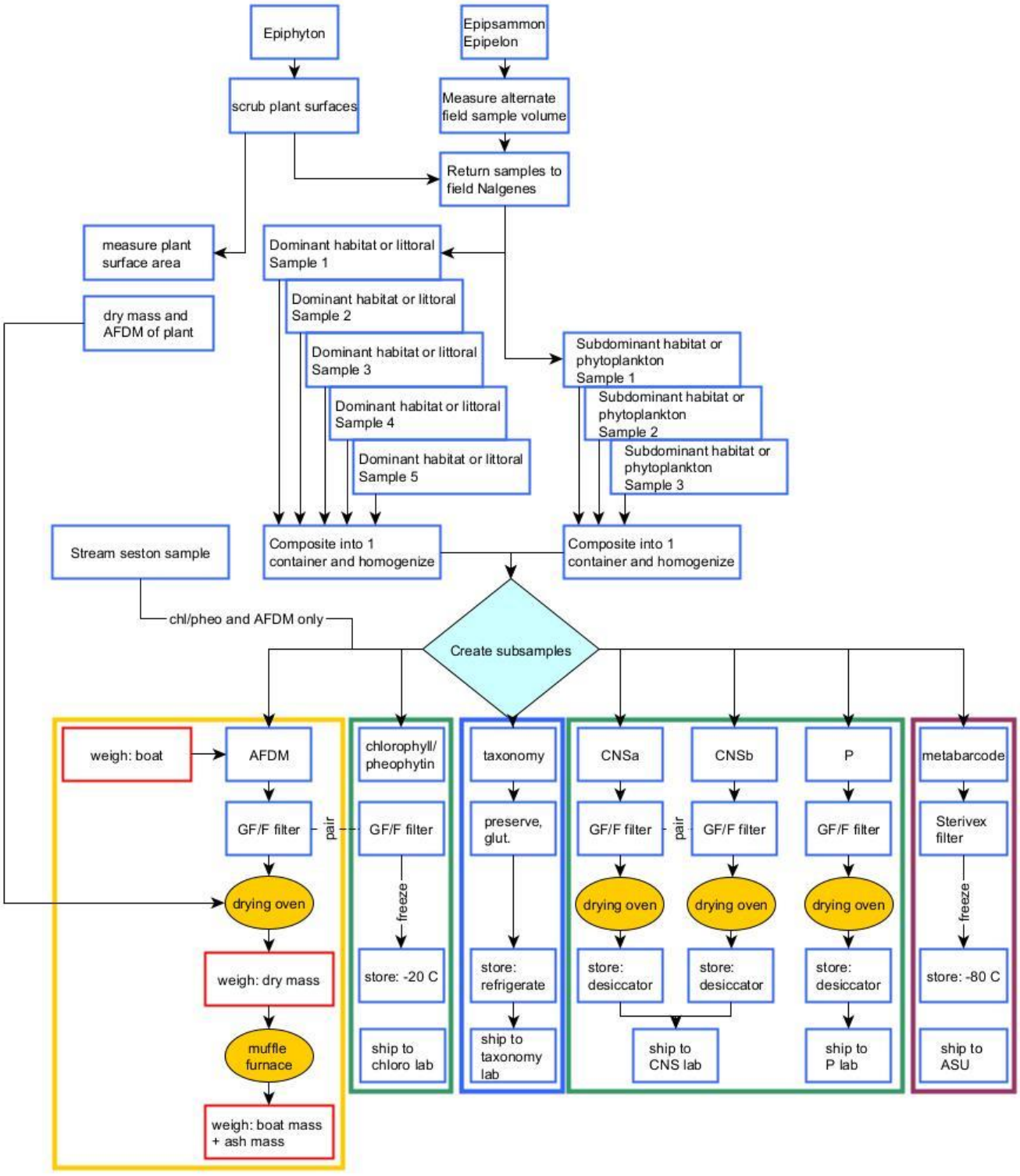

Figure 34. An expanded diagram of the lab workflow for the algae lab SOP. Note the epiphyton and epipsammon/epipelon have addition lab steps that epilithon/epixylon do not have.

E.2 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 at -20 °C and use Type II barcodes. AFDM samples do not need a barcode.

1. All subsamples have a weather-resistant, adhesive, human readable label on the outside of the bottle or packet (**Figure 35**). AFDM samples do not have a human readable label as they are processed completely at the DSF. See **Table 9** for subsample IDs.

Figure 35. Example of algal chemistry packets with human readable label and barcode label.

Table 9. Subsample IDs generates during algal processing. Dominant habitat types use "comp1" and the subdominant uses "comp2" in the subsample ID.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required?	Barcode Qty
Taxonomy	Preserved taxonomy lab subsamples	CRAM.20161027.phytoplankton.comp1.tax.1	(AOS) Algae - Lab	60 mL clear Nalgene	Type I	Yes	2

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required?	Barcode Qty
Chlorophyll/ pheophytin	Frozen chlorophyll filter	MAYF.20161027.epilithon.comp1.1	(AOS) Algae - Lab	Foil packet - frozen	Type II	Yes	2-3
Ash-free dry mass	AFDM filter processed in DSF	MAYF.20161027.epixylon.comp1.AFDM.1	(AOS) Algae - Lab	Aluminum weigh boat	none	No	0
Algal chem - CNS	Dried CNS filters	MAYF.20161027.epipsammon.comp1.CNSa.1 MAYF.20161027.epipsammon.comp1.CNSb.1	(AOS) Algae - Lab	Foil packet - dried	Type I	Yes	4-6
Algal chem - P	Dried P filter	MAYF.20161027.epipelon.comp1.P.1	(AOS) Algae - Lab	Foil packet - dried	Type I	Yes	2-3
Metabarcode	Flash-frozen metabarcode filter	MAYF.20161027.epiphyton.comp1.ARC.1	(AOS) Algae - Lab	Sterivex filter in Whirl-Pak	Type II	Yes	2

2. Adhesive barcode labels should be applied to dry, room temperature bottles, packets, and Sterivex filters (**Figure 36**).

    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. Metabarcode barcode labels should be inside the Whirl-Pak, with a human readable label applied to the Sterivex filter.

    c. 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 36. Cryogenic storage box and Whirl-Paks for ARC samples. Apply human readable labels to the filter capsule and the barcode inside the Whirl-Pak. Use these boxes rather than zip-top bags for shipping to the Biorepository.

E.3 Filter and Weigh Boat Preparation

1. Pre-ash GF/F filters (**Table 10**)
    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 10. Minimum number of pre-ashed filters and taxonomy samples required per bout. *In addition, you will need to create 2 blank filters per domain, and reps for CNSa, CNSb, P, and chl/pheo per site in Bout 1. If Bout 1 is sampling impractical, create reps during Bout 2.

Site type	Number of samples (field)	Total number of filters needed per site per bout	Additional filters for reps, 1 bout per year*	Number of taxonomy subsamples per site per bout
Lake	8	12	4	2
River	8	12	4	2
Wadeable	9	14	4	2

2. If aluminum weigh boats are new and unlabeled:
    a. Label ~10 boats by inscribing a unique number on the bottom with a pencil (e.g., A1, A2, A3, etc.; **Figure 37**).
        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.

Figure 37. Examples of newly labeled aluminum weigh boats.

3. If aluminum weigh boats have been previously labeled and used in the muffle furnace, 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 that have been ashed and stored

in a closed container (e.g., bag, box, or plastic container) to minimize dust contamination for up to 30 days may be used for sampling.

E.4 Processing Epiphyte Samples: Scrubbing and Ash-Free Dry Mass (AFDM)

Epiphyton is scrubbed from plant surfaces at the DSF. Once the material is removed, the plant substrate material is measured for both surface area, dry mass, and ash-free dry mass.

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 volume of water used for each sample consistent.
    c. Depending on the productivity of the site, >125 mL may be necessary to meet the external lab filtering requirements. See **Table 11** 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.
    a. If vegetation has started to senesce, it may break apart more easily. Use extra care when brushing. If large plant pieces break off, pick out of the algae sample and rinse the surface into the algae sample.
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.
    b. Keep volume consistent across samples within a bout.
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 estimated sum of all surfaces that were scrubbed in the **Plant Surface Area** field in 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. Pour remainder (if any) of 125 mL wash bottle into white tray.
9. Carefully pour the scrubbed material into a 125 mL amber wide-mouth HDPE sample bottle. Proceed to Filtering Protocol, Section E.8.
10. Place all plant material scrubbed in a labeled paper lunch bag. Do not subsample scrubbed plant material.

    a. Because there is no subsampling, you do not need to collect Wet Mass or Wet Mass Subsample data for this SOP as is required in the plants protocol (RD[11]). Those data are only used to as a multiplier when subsampling occurs so final data can be extrapolated back to the size of the entire sample.

11. Place paper bags containing scrubbed plant material 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], RD[22]).

12. **Day 2**: When dry, remove all bags+samples from drying oven and let cool to room temperature in a plastic bag or desiccator.

    a. Placing samples in a bag or desiccator is important because samples absorb water quickly from the air as they cool. Samples may be left in desiccator or plastic bags for up to 30 days before proceeding to the next step.

13. Place a clean, plastic weigh boat (small sample) or tray (large sample) on analytical balance. Tare (zero) balance.
14. Place dry sample in the plastic weigh boat/tray and record as **Plant Dry Mass** in the mobile app.

    a. Sample may be crushed or broken up to fit into weigh boat.

15. 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.

16. Weigh boat on an analytical balance (0.0001 g), and record as **Dry Mass Plus Boat Mass** in the mobile app.
17. Repeat above steps until all specimens have been processed.
18. 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.
19. 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 workflow.
20. **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.
21. 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.
22. 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.
23. 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 **Ash Mass Plus Boat Mass**, as the muffle furnace burns off organic material.
24. 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.

25. Set clean boats aside to be used again.

E.5 Compositing Samples

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 and their counterpart AFDM filters. Other filter types are not affected significantly by light.
2. Composite field benthic and phytoplankton samples by habitat/substrate type into a larger HDPE container. Seston samples are not composited.
    a. The container must be larger than the summed volume of all field samples from the habitat type. It's preferred that the container is amber to block light during processing, and have a wide mouth for easy pouring.
    b. Keep separate composite containers dedicated to each site and habitat/substrate type to reuse from bout to bout.
    c. Phytoplankton: Use the 4 L jug used in the field for compositing. Note that this container is likely not amber and extra care should be taken to keep this out of the light.
    d. Benthic algae: Use an amber Nalgene large enough to fit all field samples, either a 1 L amber wide mouth bottle or 2 L amber wide mouth rectangular bottle.
3. Triple rinse composite containers with DI and discard rinse water.
4. Carefully pour all field samples from the dominant (5 samples) habitat/substrate type into one composite container, and all field samples from the subdominant or phytoplankton (3 samples) habitat into a second composite container.
    a. If a field sample has large chunks of algae, homogenize using clean, hand-held battery operated stirrer in the field sample bottle for ~30 seconds. Take care that samples do not spill over the top of the sample bottle while stirring. Add to composite after removing the chunks.
    b. Rinse stirrer thoroughly with DI between samples.
5. Cap composite container and homogenize, shake for at least 30 seconds to mix samples evenly.
6. Proceed to following sections for taxonomy subsampling and filtering.

Table 11. Subsample volume and priority for lab processing. "Periphyton" indicates benthic samples scrubbed from surfaces or collected as grabs. "Phytoplankton" indicates water column samples in lakes and rivers. 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. Note that chlorophyll and AFDM filters must be filtered together as they a both proxies for algal biomass.

Priority	Sample	Parameter	Type	Recommended subsample volume
1	Periphyton	Taxonomy	Liquid, preserved in glutaraldehyde	60 mL
2	Periphyton	Chlorophyll *a*/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 (CNSa, CNSb, P)	Filters (3)	As much sample as possible, 5-10 mL
5	Periphyton	Metabarcode archive	Sterivex filter (1)	As much sample as possible, >500-1000 mL
1	Phytoplankton	Taxonomy	Liquid, preserved in glutaraldehyde	1 L
2	Phytoplankton	Chlorophyll *a*/pheophytin	GF/F filter (1)	≥250 mL (until visible color on filter)
3	Phytoplankton	AFDM	GF/F filter (1)	≥250 mL (until visible color on filter)
4	Phytoplankton	Algal chemistry (C, N, P, and C, N, S isotopes)	GF/F filters (3)	As much sample as possible, >500 mL
5	Phytoplankton	Metabarcode archive	Sterivex filter (1)	As much sample as possible, >500-1000 mL
1	Seston (wadeable streams)	Chlorophyll *a*/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.6 Processing Epipsammon and Epipelon Samples: Alternate Field Sample Volume

Epipsammon and epipelon samples have sand and silt in the bottle in addition to the sample water added in the field. The field sample volume (volume of water in the sample) is measured at the DSF to get a more accurate estimate to correct the field estimate.

1. After compositing, thoroughly mix (shake) composite sample bottle prior to removing any sample material.

2. 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. There may still be fine particulate matter in the upper layer.

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 in that it is the step that dislodges microalgae from the particles.

g. Enter volume from graduated cylinder as “Alternate Field Sample Volume” in the mobile app.

E.7 Algal Taxonomy Samples (Unfiltered)

1. Label 60 mL (periphyton, clear HDPE) or 1 L (phytoplankton, amber or clear HDPE) HDPE bottles with 1”x2” adhesive labels (**Figure 38**, RD[07]). Use Type I barcode labels.

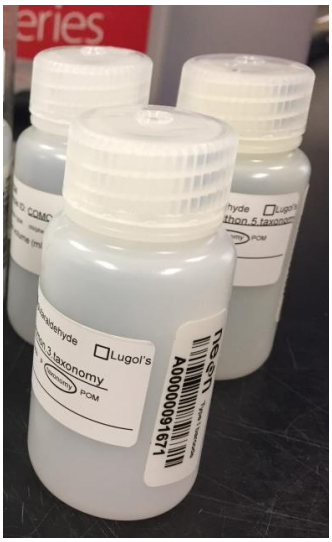

Figure 38. Algal taxonomy bottles and labels.

2. Cap and shake the lab composite container for 30 seconds to mix sample evenly.
3. 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 the labeled 1 L bottle.
    a. If the sample is too thick for the pipette, you may measure 60 mL using a clean (triple 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.

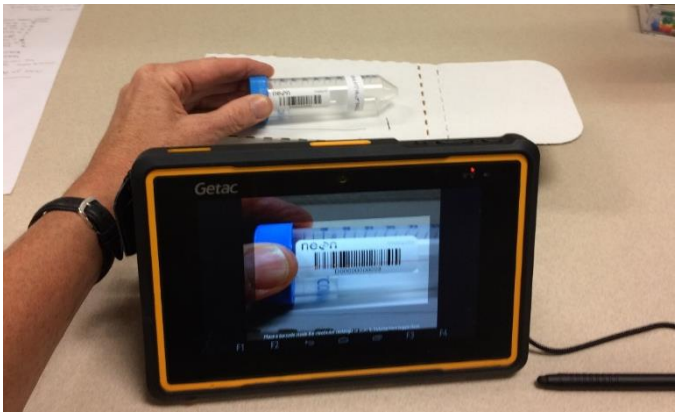

Figure 39. Barcode label scanning.

5. Create one taxonomy sample from the dominant lab composite container and 1 from the subdominant lab container. If you were able to samples all habitats in the field, you should have two taxonomy samples total per site.
6. Proceed to Sample Preservation Section E.12.

E.8 Filtering (Chlorophyll *a* + Pheophytin, AFDM, C, N, and P and Isotopes)

All composite samples are subsampled for the full suite of filters. Seston samples are subsampled only for chlorophyll/pheophytin and AFDM filters.

1. Set up filter funnel, filter flask, and vacuum pump (**Figure 40**).
    a. Either the hand vacuum pump (**Figure 40a**) with one filter flask and funnel attached or the filter manifold and electric pump (**Figure 40b**) 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.

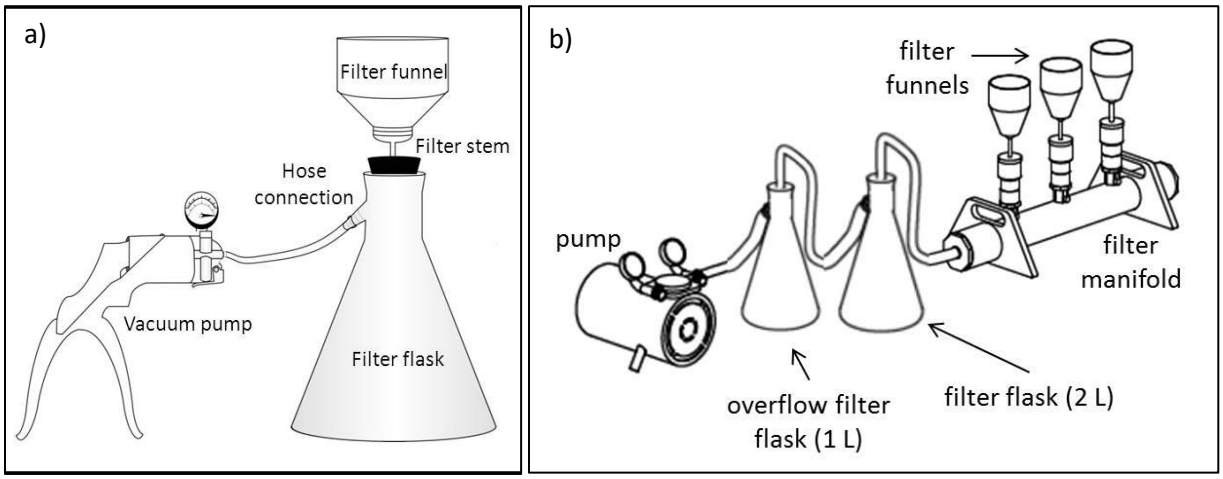

Figure 40. Filter apparatus setup: a) hand pump; b) filter manifold.

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 40), replace top of funnel.

4. Shake sample bottle vigorously for ~30 seconds to homogenize sample. This must be done anytime the sample bottle has been allowed to sit and the sample starts to settle.

    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. **Homogenize sample by gently shaking the capped bottle prior to pipetting on each filter.** Replicate filters should be as similar to each other as possible, consistent homogenization helps ensure this.

5. Filters intended to be replicates of each other should be treated in the same way throughout the domain lab process. This includes volume filtered, roughly similar volumes of rinsewater used (no need to measure, just use good judgement), exposure to light, and homogenization. The following filters are replicates of each other:

    a. chl/pheo and AFDM: chl/pheo and AFDM filters are two different ways to approximate algal biomass in periphyton/phytoplankton samples.

b. CNSa and CNSb: The external lab uses the filters interchangeably. They usually use just 1 of the filters, but about 25% of the time they lose a filter on the instrument and use the backup filter.

c. During Bout 1, randomly select one of the field samples per site for replicates of chl/pheo, CNSa, CNSb, and P. Replicates should be grouped on the filter manifold as follows (assuming the manifold has 3 towers):

   i. chl/pheo.rep1, AFDM, chl/pheo.rep2

   ii. CNSa.rep1, CNSb.rep1, P.rep1

   iii. CNSa.rep2, CNSb.rep2, P.rep2

   iv. Create replicates during Bout 1. If sampling was impractical during Bout 1, create replicates during the next bout sampled.

6. Filter a known volume of sample.

   a. **Phytoplankton samples**: Measure using a graduated cylinder. Pour sample slowly in ≤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 acurate using a graduated cylinder.

      iv. NOTE: Pipette tips may be reused for all of the subsamples and replicates for a given lab composite sample, but may not be reused from composite to composite or bout to bout. Use a separate tip for preservative.

      v. 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.

7. 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.**

8. Check the filter, if it still appears white, filter more sample. If the filter appears green or yellow tinged, proceed to next step. Use similar filter volumes as in previous years and bouts. Contact Science to discuss existing data if you would like to change that volume.

    a. **Record the volume of sample filtered in the mobile lab app.**

    b. Filter the minimum amount suggested in Table 11. 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.

9. 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.

10. 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.

11. 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.

12. If any insects or plant pieces are on the filter, remove them carefully using forceps.

13. 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. On an ETOH-cleaned lab bench, cut several aluminum foil squares (~4x4 inches). Heavy-duty aluminum foil may help with folding.

    b. Label square with adhesive sample label (~1” x 2”) and barcode label (Figure 41**)**. Place label on the top of the packet, not over the folded edges of the foil.

    c. **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.9.

    d. **Chlorophyll/pheophytin filters**: Fold filter <u>exactly in half</u> 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. Freeze at -20 °C.

e. CNSa, CNSb, P, and blank filters: Using filter forceps, place the wet filter in the middle of the foil square.

i. Create a crease in the filter by partially folding within the foil square (see attached photo: filter_crease.png). Do not fold the filter completely or let the two sides of the filter touch or smear the sample.

ii. The wet filter will stick to the aluminum foil, creasing with relative ease as the foil is folded in on itself. If the filter is not folding easily within the foil pack, use a pair of filter forceps to hold the filter in place, then fold the foil.

iii. Leave the filter unfolded, but creased, in the foil pack. It will partially unfold on its own if the two sides are not touching.

iv. Enclose the sample in the pre-labeled foil pack by folding one edge of the foil pack in, then folding the top down (Figure 42). Leave one end open to allow drying in the drying oven.

v. Place open packets in the drying oven tray in a single layer to promote drying, do not stack packets.

vi. After the filter is dried, remove the foil pack from the drying oven and fold the remaining end closed. Ensure the label and barcode are clearly visible.

vii. Store in the desiccator until shipping.

f. Scan the barcode label with the tablet.

g. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.

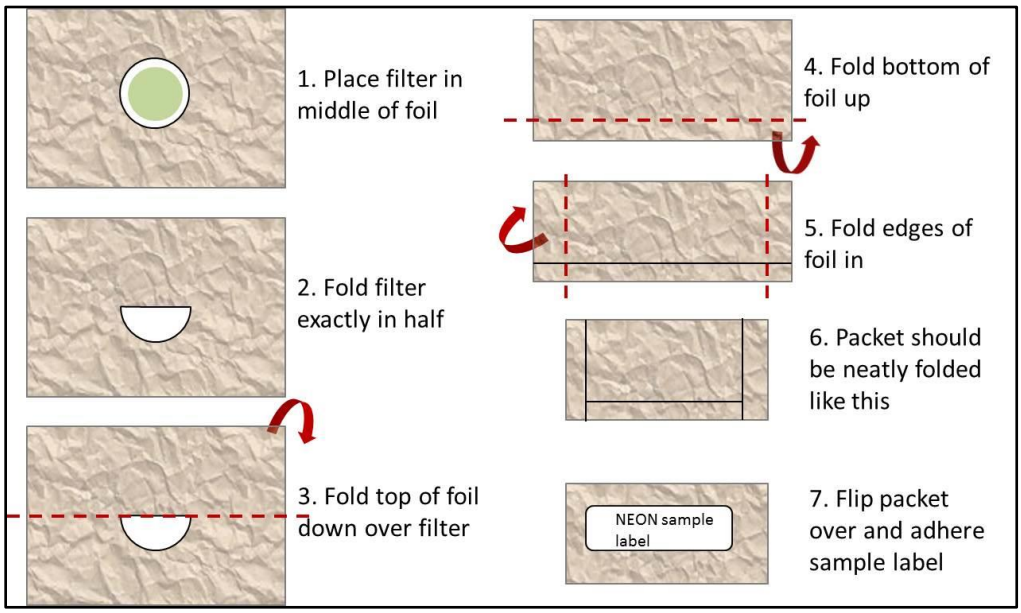

Figure 41. Correct filter packet folding procedure. Make sure packet can be easily opened at the external lab without destroying the NEON sample label or barcode label. Do not use excess foil.

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

15. Repeat above steps until you have 5 filters total from the same composite sample. During Bout 1 (or the next bout sampled during the year if sampling was impractical during Bout 1), randomly select 1 composite sample (approximately 10% of samples over the year) to create a full set of replicate filters (do not create replicates for taxonomy or AFDM). The goal is to create replicates for approximately 10% of samples per year. 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.9) – never create replicates

    b. 1 chlorophyll *a*/pheophytin filter

    c. 2 CN +^{13}C, ^{15}N, ^{34}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.

16. 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. Create blanks along with the first site that is scheduled and processed in the DSF for the bout. Then select these filters as a reference for the second and third (if applicable) sites.

c. If CNS and P samples are split to different labs, send one blank with CNS and one blank with P. Check the shipping protocol and KBAs for the most up to date information on shipping (RD[21]).

17. Separate all foil packets for chlorophyll/pheophytin from the remaining algal chemistry packets (CNSa, CNSb, P, and blanks). See Section E.13 for packet organization.

18. **Place all chlorophyll/pheophytin filters in -20 °C freezer until shipping** (Section H.1).

19. Place all algal chemistry foil packets (CNSa, CNSb, and P) and blank packets in the drying oven at 65 °C with packets slightly open (**Figure 42**). **Do not place chlorophyll filters in the drying oven.** 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.

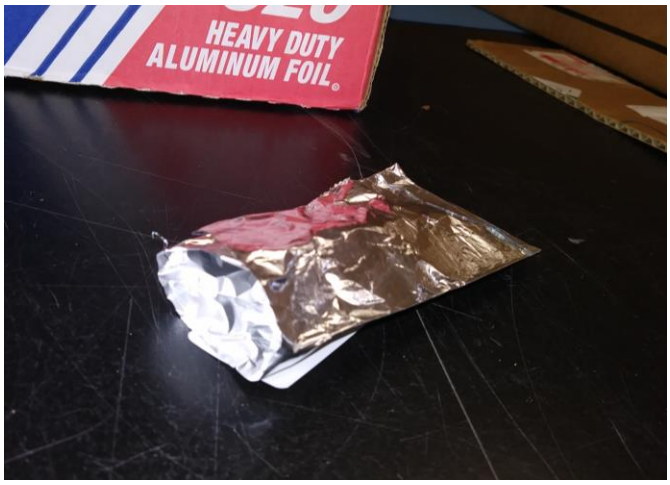

Figure 42. Example of a filter packet left slightly open for drying algal chemistry filters in the drying oven.

20. Cap composite container and store in 4 °C until all samples are complete.

21. Completely clean filter funnels used for the next site or processing day.

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.9 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 on different filter runs).

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** and **Boat Mass** 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 ^{o}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 then place in the desiccator for at least 20 minutes. 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 **Dry Mass Plus Boat 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 ^{o}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 **Ash Mass Plus Boat 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 **Ash Mass Plus Boat Mass** is not smaller than **Dry Mass Plus Boat Mass**, this indicates that an error occurred in this process. Common errors include not taring the balance consistently (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.
    d. If the difference is within 0.0005 g, this is considered a below detection. The mobile app will select the appropriate data quality flag in **ashMassdataQF**.

e. If **Ash Mass Plus Boat Mass** is not smaller than **Dry Mass Plus Boat Mass**, indicate in the mobile app the appropriate data quality flag (**ashMassdataQF)** describing 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 and corresponding boats in the desiccator until you have quality checked the data.
13. Set clean boats aside to be used again.

E.10 Metabarcode Archive Filters

Metabarcode archive filters will be frozen at -80 °C and stored at the Biorepository for future barcoding technology. Filters must be created using filed and lab sterile methods.

1. Wear ETOH cleaned nitrile gloves.
2. Depending on how much volume you are filtering, you may choose to use the peristaltic pump or a 100-200 mL syringe for filtering.
    a. Rinse any equipment that is not in sealed sterile packaging from the manufacturer that comes in direct contact with the sample with 10% bleach followed by a DI rinse to sterilize prior to filters. Sterilized equipment may be stored for up to 30 days in a clean zip-top bag until next processing. Equipment to sterilize:
        i. 100-200 mL syringe with Luer-lok tip
        ii. Peristaltic pump tubing and modified 3 mL syringe adapter
3. Keep the 0.22 um Sterivex filter in the sterile packaging until ready to use.
4. Open package and adhere human readable label to the filter capsule before the Sterivex filter gets wet.
5. Shake composite sample to homogenize prior to filtering.
6. Select filtering method:
    a. Peristaltic pump (works best for large sample volume, e.g. phytoplankton)
        i. Set peristaltic pump speed below manufacturer specifications (45 psi for Millipore Sterivex SVGP® filter). If pump speed is set too high, the filter can rupture.
        ii. Check that the 3 mL syringe adapter is in place to connect pump tubing to the capsule filter (Figure 4).
        iii. Attach 3/8” inner diameter C-flex tubing to the peristaltic pump. Use a zip tie, small hose clamp, or adhesive to firmly attach the tubing to the hose connector

iv. Rinse tubing by pumping ~100 mL of sample water completely through the tube.

v. After tubing is flushed with sample water, open Sterivex® filter and attach to the luer lock end of 3 mL syringe/filter adapter.

vi. Begin pumping water through the filter using peristaltic pump. Make sure the tube is filled with water to reduce air and reduce the potential to blow a hole in the filter.

b. Syringe method

i. Rinse the sterilized syringe with sample water.

ii. Attach Sterivex filter directly to the Luer-lok end of the syringe.

7. Capture filtered water in a graduated cylinder. Use this to measure the volume filtered for each sample.
8. Filter as much sample as possible before filter clogs. You may need to refill the syringe with sample multiple times. Because this is an archived sample and we are not sure how it will be used in the future, filtering as much material as possible will ensure that future researchers have options when using the filters.
9. Record the total volume of sample filtered in the mobile app.
10. After filtering, gently push air through the Sterivex filter to remove water from the capsule. Water left in the capsule will crack the filter upon freezing.
11. Cap both ends of the Sterivex with male and female Luer-lok caps.
12. Place the capped, labeled Sterivex filter in a small Whir-Pak. Place barcode label inside Whirl-Pak so that when the Whirl-Pak is folded, the barcode can still be scanned.
13. Place Sterivex filters in a cryobox at -80 ^{o}C until shipping (**Figure 36**).

E.11 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 (+glutaraldehyde) 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. 1 Sterivex filter

h. One full set of replicate filters for algal chemistry (chl/pheo, CNSa, CNSb, P only) from one bout per year

i. 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 and composite containers 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.12 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*V1=C2*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 glutaraldehyde to reach a final concentration of 2% (20 mL glutaraldehyde 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.

3. Always work with chemicals in the fume hood with proper PPE. Glutaraldehyde requires careful handling.

E.13 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, this helps the lab check in frozen samples more quickly without thawing.
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).
    a. Organize filters according to the order the shipping manifest generates. This will make it easier for the external lab to check the shipment.
3. **Sterivex filters**: Neatly stack Sterivex filters in Whirl-Paks in the cryobox. Ship overnight on dry ice. Samples may be stored at the DSF for up to 1 year.

E.14 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. Rinse field sample bottles and composite containers with DI, these will be used and rinsed again prior to lab processing. Since each site has a set of bottles, they do not need to be decontaminated between bouts.
    b. Clean aluminum weigh boats for reuse.
    c. 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.
    d. 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 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

SOP F Sample Storage

1. Taxonomy: DO NOT ALLOW TAXONOMY SAMPLES TO FREEZE. Store in a 4 °C refrigerator. Check the refrigerator settings to ensure that bottles do not freeze during storage.
2. Chlorophyll/pheophytin: Store in a -20 °C freezer. Remember to ship samples within 7 days of collection (with the collect day counting as day 1).
3. CNSa, CNSb, P: Store in foil packets in the desiccator.
4. AFDM: No sample storage is necessary as samples are fully analyzed at the DSF.
5. Metabarcode: Store in cryoboxes at -80 °C.

SOP G 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 H Sample Shipment

Information included in this SOP conveys science-based handling and packaging instructions for the sample types included in this protocol. For shipping instructions, see RD[21]. There are 4 groups of samples to ship:

1. Chlorophyll/pheophytin: Frozen filters much be bagged by site/date and organized in the same order as the shipping manifest.
2. CNSa, CNSb: Dried filters must be bagged by site/date and organized in the same order in the shipping container as in the shipping manifest. Include blank number 1 with this shipment.
3. P: Dried filters must be bagged by site/date and organized in the same order in the shipping container as in the shipping manifest. Include blank number 2 with this shipment.
4. Taxonomy: Preserved bottles should be bagged by site and date.
5. Metabarcode: Frozen filters must be shipped on dry ice overnight, in cryoboxes.

H.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 in these concentrations are not considered hazardous. See RD[21] for further shipping instructions.

H.2 Algal Filter Sample Shipping – Chlorophyll/Pheophytin

1. Chlorophyll/pheophytin filters must be shipped within 7 days of collection, with the collect day counting as day 1. 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[21] for further shipping instructions.
4. **Frozen filters must be bagged by site/date and organized in the same order** in the shipping container as in the shipping manifest.

H.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[21] for further shipping instructions.

H.4 Archive Metabarcode Filters - Sterivex

1. Metabarcode archive filters should be shipped within 1 year of collection. They can be stored in the -80 freezer as long as needed prior to shipping.
2. Organize filters within the cryobox in the order of the shipping manifest. This makes receiving samples easier for the Biorepository.

H.5 Handling Hazardous Material

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

H.6 Supplies/Containers

See sections H.1, H.2, H.3, and RD[21] for specific shipping materials.

H.7 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**).
4. **Metabarcode filters**:
    a. Samples may be held for up to a year until shipping.

H.8 Grouping/Splitting Samples

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

H.9 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).

H.10 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.

H.11 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. Samples 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. Littoral1 sensor
   c. Littoral2 sensor
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[15]) 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[14]) 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 – Composite field samples
Step 2 – Filter for Chlorophyll *a* + pheophytin, AFDM, CNS , P, and metabarcode in the Domain Support Facility within 24 hours of sampling (lab day 1).
Step 3 – 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. Store metabarcode filters at -80 °C.
Step 4 – Transfer unfiltered periphyton and phytoplankton taxonomy samples to 60 mL HDPE bottles and add preservative to reach a final concentration of 0.5% glutaraldehyde within 24 hours of sampling (lab day 1).
Step 5 – Weigh dried AFDM filters, place in muffle furnace (lab day 2).
Step 6 – Weigh ashed AFDM filters (lab day 3).
Step 7 – 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 ^{13}C, ^{15}N, and ^{34}S isotopes)

A.3 Order of Preference for Sampling Substrata in the Field

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[15]), rapid habitat assessment, or the site-specific bathymetric map and riparian vegetation map (RD[16], RD[14]).
- **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:

- Composite samples by dominant and subdominant habitat, keep seston sample separate.
- 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 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 are based on the NEON temporal sampling strategy (Parker and Utz 2022). Refer to the Aquatic Site Sampling Design for your domain (RD[20) for bout window start and end dates.

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	HOPB	Riffle (epilithon)	Pool (epipsammon)	ss (S2)
D02	LEWI	Run (epipelon)	Riffle (epilithon)	ss (S2)
D02	POSE	Riffle (epilithon)	Pool (epipelon)	ss (S2)
D03	FLNT*	Littoral (epipsammon)	NA	c0 + 2 locations
D03	BARC	Littoral (epiphyton)	NA	c0, in, ot
D03	SUGG	Littoral (epiphyton)	NA	c0, in, ot
D04	GUIL	Riffle (epilithon)	Pool (epilithon)	ss (S2)
D04	CUPE	Riffle (epilithon)	Run (epilithon)	ss (S2)
D05	CRAM	Littoral (epixylon)	NA	c0, in, ot
D05	LIRO	Littoral (epixylon)	NA	c0, in, ot
D06	KING	Riffle/run (epilithon)	Pool (epilithon)	ss (S2)
D06	MCDI	Riffle (epilithon)	Pool (epilithon)	ss (S2)
D07	LECO	Riffle (epilithon)	Pool (epipsammon)	ss (S2)
D07	WALK	Riffle (epilithon)	Run (epixylon)	ss (S2)
D08	MAYF	Riffle/run (epixylon)	Run/pool (epipsammon)	ss (S2)
D08	BLWA	Littoral (epixylon)	NA	c0 + 2 locations
D08	TOMB	Littoral (epixylon)	NA	c0 + 2 locations
D09	PRLA	Littoral (epilithon)	NA	c0, in, ot
D09	PRPO	Littoral (epilithon)	NA	c0, in, ot
D10	ARIK	Run (epiphyton)	Pool (epipsammon)	ss (S2)
D11	PRIN	Run (epipsammon)	Riffle (epilithon)	ss (S2)
D11	BLUE	Run (epilithon)	Riffle (epilithon)	ss (S2)
D12	BLDE	Riffle (epilithon)	Run (epilithon)	ss (S2)
D13	COMO	Riffle (epilithon)	Run (epipsammon)	ss (S2)
D13	WLOU	Riffle (epilithon)	Pool/step pool (epipsammon)	ss (S2)
D14	SYCA	Run (epipsammon)	Riffle (epilithon)	ss (S2)
D15	REDB	Step pool (epipsammon)	Run (epilithon)	ss (S2)
D16	MCRA	Step pool/riffle (epilithon)	Step pool/pool (epilithon)	ss (S2)
D16	MART	Riffle (epilithon)	Pool (epilithon)	ss (S2)
D17	TECR	Riffle (cobble - epilithon)	Riffle (epilithon large substrate)	ss (S2)
D17	BIGC	Run (epipsammon)	Riffle (epilithon)	ss (S2)
D18	OKSR	Run (epilithon)	Riffle (epilithon)	ss (S2)
D18	TOOK	Littoral (epilithon)	NA	c0, in, ot
D19	CARI	Run (epilithon)	Riffle (epilithon)	ss (S2)

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

APPENDIX E D09 PRLA RIPARIAN SECTION SELECTION FOR LITTORAL BENTHIC SAMPLES

Riparian sections 11 and 12 were added to D09 PRLA in 2020 due to a permitting change. The table below details the 5 riparian sections to select for littoral benthic macroinvertebrate sampling for each bout. After year 4 in the table, start back at the top. Years are noted in the "Sampling date" column as an example.

Site	Year	Bout	Sampling date	Section selection for littoral sampling
PRLA	1	1	2020 & 2024 Bout1 (odd-1)	3,5,7,9,11
PRLA	1	2	2020 & 2024 Bout2 (even-2)	4,6,8,10,12
PRLA	1	3	2020 & 2024 Bout3 (odd-3)	1,5,7,9,11
PRLA	2	1	2021 & 2025 Bout1 (even-4)	2,6,8,10,12
PRLA	2	2	2021 & 2025 Bout2 (odd-5)	1,3,7,9,11
PRLA	2	3	2021 & 2025 Bout3 (even-6)	2,4,8,10,12
PRLA	3	1	2022 & 2026 Bout1 (odd-7)	1,3,5,9,11
PRLA	3	2	2022 & 2026 Bout2 (even-8)	2,4,6,10,12
PRLA	3	3	2022 & 2026 Bout3 (odd-9)	1,3,5,7,11
PRLA	4	1	2023 & 2027 Bout1 (even-10)	2,4,6,8,12
PRLA	4	2	2023 & 2027 Bout2 (odd-11)	1,3,5,7,9
PRLA	4	3	2023 & 2027 Bout3 (even-12)	2,4,6,8,10

APPENDIX F 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 ±4 m for lake navigation.

Table 12. Equipment list – General equipment.

Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
	N	Site-specific Stream Morphology, Rapid Habitat Assessment, or Bathymetry Map	Determining sampling locations	1
	N	Mobile data entry tablet	Field data entry	1
	N	Handheld GPS unit (with batteries, <4 m accuracy) or Hummingbird	Navigating to sampling location	1
	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
Consumable items				
RD[07], RD[08]	N	AQU Field Metadata Sheet, Field data sheets (print on all-weather paper, write in pencil)	Recording metadata in case tablet fails	1
		Mobile device and application	Recording data	1
	N	Pre-printed adhesive labels (all-weather, 2"x4")	Labeling samples	15

Item No.	Exact Brand	Description	Purpose	Quantity
	Y	Adhesive barcode labels (Type-I, Type-II)	Labeling samples	1 sheet
	N	Pencils	Recording data	1
	N	Permanent markers	Labeling samples	1
	N	Ice Packs	Keeping samples cool	2
	N	Chlorine bleach (6%)	Sterilizing metabarcode equipment	< 1 gal

Table 13. Equipment list – Sampling equipment.

Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
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
	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
	N	Brass-bristle brush, toothbrush-size	Scrubbing epilithon	2
	N	Nylon-bristle toothbrush	Scrubbing epixylon	2
	N	Wooden dowel, <12” long	Handle for modified t-shaped brush	1
	N	Aquarium epoxy stick	Adhesive for modified t-shaped brush	1

Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Larval insect tray, plastic	Scrubbing container in which sample is collected	1
	N	125 mL unitary wash bottle	Rinsing substrate and larval tray into sample bottles	2
	N	25 mL graduated cylinder, plastic	Measuring extra rinse water	1
	N	Petri dish lid, plastic, 47 mm diameter	Epipsammon and epipelon sample collection	4
	N	Spatula (metal, offset)	Epipsammon and epipelon sample collection	1
	N	Scissors	Epiphyte sample collection	1
	N	Metric ruler	Epiphyte sample collection	1
	Y	Kemmerer sampler with rope and messenger	Phytoplankton sample collection	1
	Y	Horizontal Van Dorn sampler with rope and messenger	Phytoplankton sample collection in rivers with fast flow	1
	N	4 L HDPE jug	Integrating phytoplankton samples	1
	N	Amber HDPE wide-mouth sample bottles with caps, 1 L	Phytoplankton and seston sample collection	4-15
Consumable items				
	N	Razor blade	Chunky epilithon samples	2
	N	Flat head screwdriver or putty knife	Chunky, calcified epilithon samples	1
	N	Disposable pipets	Sample removal for large substrate epilithon and epixylon	5
	N	Whirl-Paks, 24 oz.	Epiphyton sample container	20
	N	Resealable bags, gallon	Organizing epiphyton samples	20

Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	DI water	Rinsing substrata	1 L
	N	Sterile 70% ethanol wipes (e.g., www.soscleanroom.com item TX3044P pre-wetted wipe OR TX3215 dry wipe)	Field sterilization	10
	N	Nitrile gloves, powderless	Sterile collection methods	2 pair

Table 14. Equipment list – General laboratory equipment.

Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
	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
Consumable items				
	N	Lab data sheets (RD[07])	Recording data if tablet fails	2
	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
	N	Aluminum foil	Wrapping GF/F filters for shipment	1
	N	Zip-top resealable bags, quart size	Organizing and storing filters	2
	N	DI water	Rinsing the filter funnel	1 L

Table 15. Equipment list – Epiphyte Processing.

Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	R	Ruler (metric) or calipers (borrow from TOS flora)	Measuring length of epiphytes	1
	R	Larval tray, plastic (may have pouring lip)	Scrubbing container in which sample is collected	1
Consumable items				
	R	Nylon toothbrush, disposable	Scrubbing epiphytes	1
	R	Paper lunch bags	Drying plants associated with epiphyte samples	20

Table 16. Equipment list – Filtering.

Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Vacuum pump filter manifold assembly	Filtering samples	1
	N	Filter funnel (25 mm diameter, 200 mL)	Filtering samples, part of manifold assembly	1-3
	N	Vacuum filter flask, polypropylene (1L)	Filtering samples, part of manifold assembly	1-3
	N	Vacuum hand pump	Filtering samples, backup to filter manifold	
	N	Vacuum hand pump maintenance kit	Replacement parts for vacuum hand pump	1
	N	Filter forceps (flat ends)	Handling filters	2
	N	Hand-held stirrer (periphyton homogenizer)	Homogenizing periphyton and breaking up clumps of algae	1

Item No.	Exact Brand	Description	Purpose	Quantity
	N	Graduated cylinder, 250 mL	Measuring and adding aliquots of sample to the filter funnel	1
	N	Adjustable pipette, 10 mL	Measuring and adding aliquots of sample to the filter funnel	1
Consumable items				
	N	Pipette tips, 10 mL	Measuring and adding the volume of sample into the filter funnel	3
	Y	Whatman GF/F filters (25 mm diameter, 0.7 um pore size)	Filters for AFDM and chemistry samples	15
	Y	0.22 m Sterivex SVGP® L10RC capsule filter with luer lock outlet	Metabarcode archive filter	2
	N	Luer lock male closures, sterile	Capping the Sterivex® filters	4
	N	Luer lock female closures, sterile	Capping the Sterivex® filters	4
	N	Whirl-Pak, small	Enclosing the capped Sterivex filter	2
	N	Cryovial storage box, 13.2 x 13.2 x 5.1 cm	Box for storing and ARC filters to the Biorepository. Dividers not needed.	1

Table 17. Equipment list – Ash-free dry mass.

Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Aluminum weigh boats	AFDM sample processing	20
	N	Analytical balance (0.0001 g)	Measuring weight	1
	N	Drying oven	Drying samples	1
	N	Muffle furnace	Burning organic matter for ash-free dry mass determination	1

Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Aluminum baking pan	Sample storage in muffle furnace	2
	N	Heat-proof gloves (pair)	Safe handling of equipment in the muffle furnace and drying oven	1
	N	Crucible tongs	Safe handling of equipment in the muffle furnace and drying oven	1
	N	Desiccator (bench top)	Storing dried samples	1
Consumable items				
	N	Desiccant packs	For bench top desiccator	1-2
	N	Plastic weigh boats	Measuring dry weight	20

Table 18. Equipment list – Algae preservation.

Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
	N	Freezer (-20 °C)	Sample storage	1
Consumable items				
	N	Glutaraldehyde, Grade II, 25% in H_2O	Preserving periphyton samples	1 L
	N	HDPE bottles with lids, wide mouth (60 mL)	Periphyton taxonomy sample container	8
	N	Amber HDPE bottles with lids, wide mouth (1 L)	Phytoplankton taxonomy sample container	1