AOS PROTOCOL AND PROCEDURE: PERIPHYTON, SESTON 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.

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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 in **lakes and non-wadeable streams**). Macroalgae will be sampled as part of the Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling Protocol (RD[11]), while benthic microalgae and seston or phytoplankton will be sampled as part of this protocol.

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

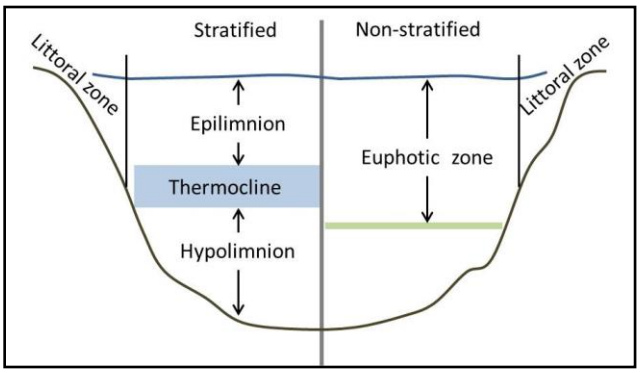

Figure 1. The zones in a stratified and non-stratified lake.

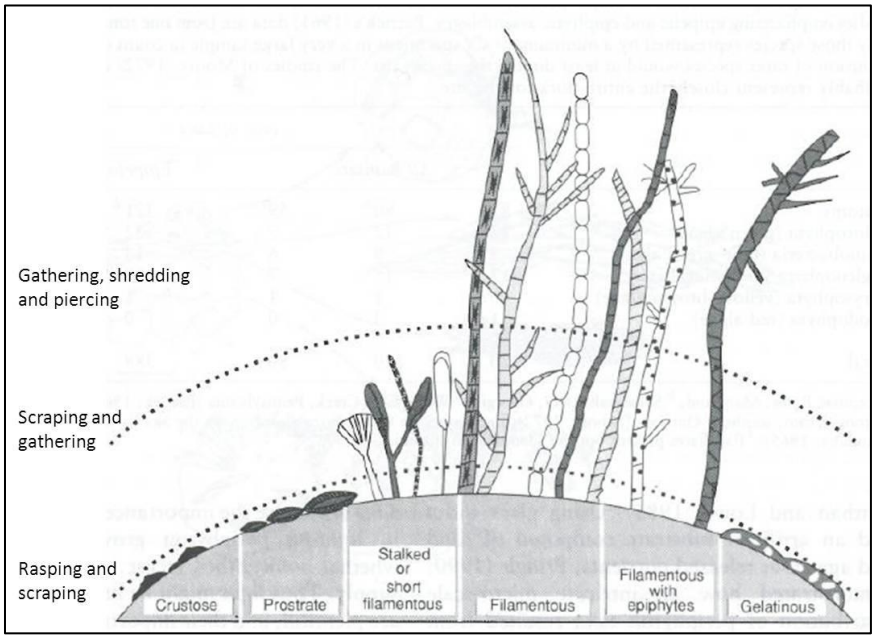

Figure 2. Growth forms of periphyton, ranging from prostrate types to long, filamentous types. Vertical layering differs among growth forms (from Allan 1995).

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

Free floating algae (seston in wadeable streams and phytoplankton in lakes and non-wadeable streams) can exist as single cells or colonies, and can be highly diverse in many freshwater habitats (Wehr and Sheath 2003). Seston and phytoplankton communities are primarily controlled by nutrient supply, light, and consumer grazing pressure. In **lakes and non-wadeable streams**, phytoplankton are present in higher density above the metalimnion when the system is stratified (the system has a thermocline) and within the euphotic zone (the region through which light penetrates) when the system is non-stratified (Figure 1).

1.2 Scope

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

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

This document is based on the protocols of the US Environmental Protection Agency (USEPA) Environmental Monitoring and Assessment (EMAP) program (Baker et al. 1997), the Arctic Long-Term Ecological Research (LTER) Lakes Field Sampling Protocol (Bahr et al. 2002), the Minnesota EPA National Lake Assessment (NLA) protocol (USEPA 2009), the US Geological Survey (USGS) National Field Manual for the Collection of Water Quality Data (NAWQA; Hambrook Berkman and Canova 2007, Charles et al. 2002), the USEPA Sampling Procedures for the Great Lakes (USEPA 2010), the US Environmental Protection Agency (EPA) Rapid Bioassessment Program (RBP; Stevenson and Bahls 1999), the Arctic Streams Long-Term Ecological Research (LTER) program (Slavik et al. 2004) and Methods in Stream Ecology (Lowe and LaLiberte 2006).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1-4 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[06]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[07]	NEON.DOC.003042	Datasheets for AOS Protocol and Procedure: Periphyton, Seston and Phytoplankton Sampling
RD[08]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[09]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[10]	NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[11]	NEON.DOC.003039	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling
RD[12]	NEON.DOC.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD[13]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbial Sampling
RD[14]	NEON.DOC.001196	AOS Protocol and Procedure: Riparian Habitat Assessment in Wadeable Streams
RD[15]	NEON.DOC.001195	AOS Protocol and Procedure: Riparian Habitat Assessment in Lakes and Non-Wadeable Streams
RD[16]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[17]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[18]	NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling
RD[19]	NEON.DOC.002792	AOS Protocol and Procedure: Secchi Depth and Depth Profile Sampling in Lakes and Non-Wadeable Streams
RD[20]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

2.3 Acronyms

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

2.4 Definitions

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

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

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

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

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

Epilithon: Periphyton colonizing rock substrata (Figure 4).

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

Epipelon: Periphyton colonizing silt substrata.

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

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

Epipsammom: Periphyton colonizing sand substrata.

Epixylon: Periphyton colonizing woody substrata.

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

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

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

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

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

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

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

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

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

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

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

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

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

Pool: An aquatic habitat unit in a river or stream created by local scour or impoundment and having a structural control. Pool water depth is above average, if all the running water in the stream was shut off, areas in the stream that would still hold water are pools. Pool water velocity is below average for the reach and because of that fine sediments 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.

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 is a cascade-pool sequence.

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 ^{o}C per meter. The denser and cooler layer below the thermocline is the hypolimnion, warmer upper layer is termed the epilimnion.

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

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, Seston and Phytoplankton Sampling Protocol is to determine species diversity, taxa richness, biovolume, biomass, using both chlorophyll *a* as a proxy for biomass as well as ash-free dry mass (AFDM), of periphyton and seston/phytoplankton at aquatic sites. These variables will be used to build a database over time, so changes can be tracked in algal species presence/absence, community structure and function, and the introduction of invasive species. In , periphyton and seston samples are collected to fulfill these requirements, while in **lakes and non-wadeable streams**, periphyton and phytoplankton samples are collected.

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

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

In wadeable streams, non-wadeable streams, and lakes, seston and phytoplankton are sampled at the water chemistry sampling locations (Figure 13). In wadeable streams, the seston samples are collected near the S2 sensor in the thalweg. In lakes, phytoplankton is collected at the central location (near the buoy) and the inlet and outlet sensor sets. In non-wadeable streams, phytoplankton is sampled near the sensor set, and from the river-left and river-right littoral habitats.

Algae sampling occurs three times per year in order to capture presence and abundance of multiple species. Timing of sampling is site-specific and determined based on historical data. Specific details on sample dates are provided in the NEON Aquatic Sample Strategy Document (RD[05]. Sample bout 1 is an early-season date, representing a period of rapid biomass accumulation after winter, typically after ice-off (where applicable) and prior to leaf out. Sample bout 2 targets low flows and high light (mid-summer) at each site. Sample bout 3 represents the late growing season (typically autumn) at each site during leaf-fall. These dates differ on a site-by-site basis. Sampling should occur at base-flow conditions, and will not occur directly following a flood in the stream (>1.5 x base flow; Biggs et al. 1999)

or under ice in a lake. A period of 14-days will be allowed after a flood event for periphyton to recolonize before sampling occurs.

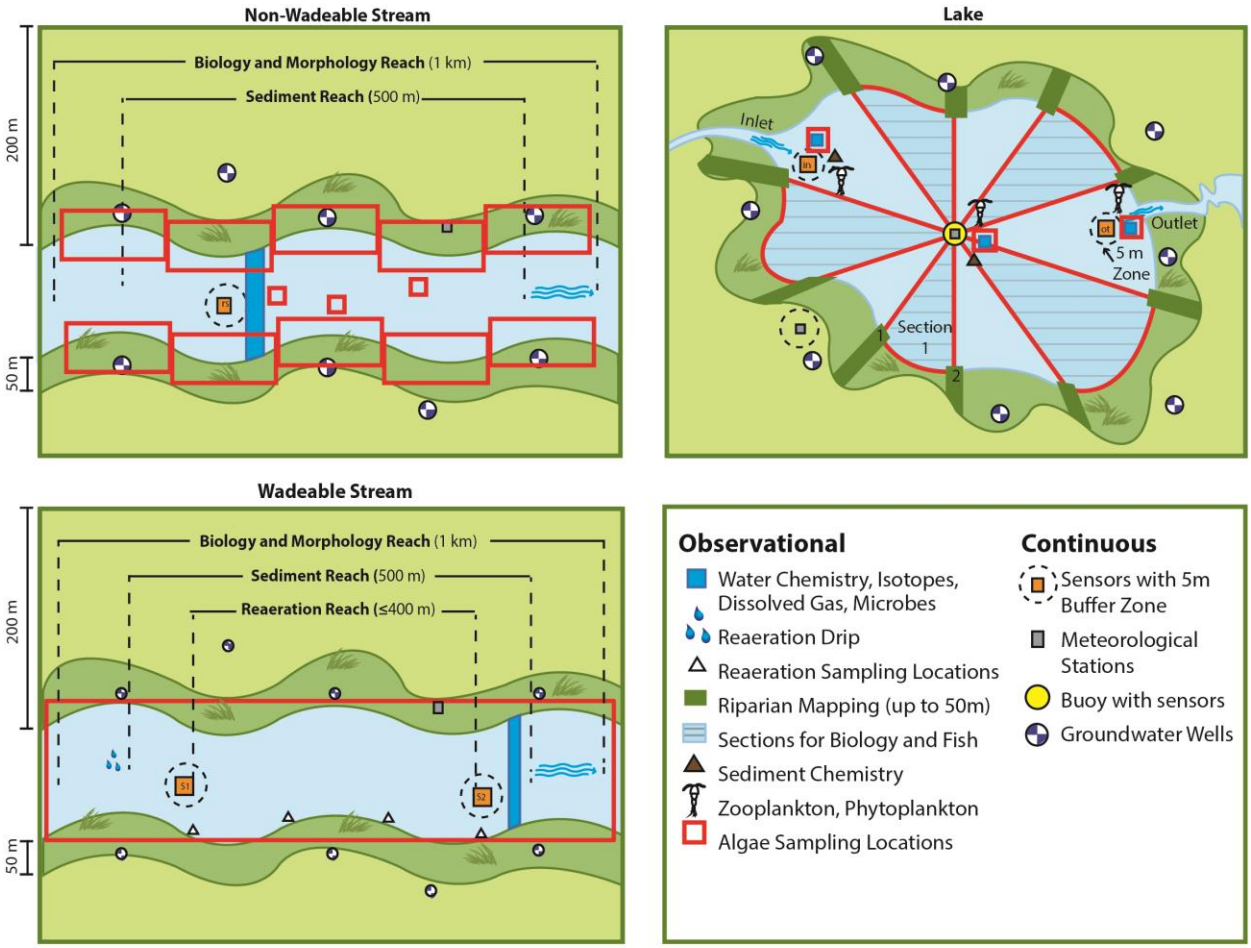

Figure 13. Generic site layouts for lakes, non-wadeable streams, and wadeable streams with phytoplankton, seston, and benthic algae sampling locations.

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

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Algal sampling occurs three times per year at each site, roughly spring, summer, and autumn. Sampling must occur within the first 21 days of the 1 month window specified in Appendix D, with a minimum of two weeks between sampling dates. Accommodations for local weather conditions (e.g., late ice-off) may be made that cause the sample date to fall outside of the pre-determined window. Use NEON's problem reporting system to report sampling efforts that take place outside of the defined sampling window.

4.2 Criteria for Determining Onset and Cessation of Sampling

A range of dates for each site were determined *a priori*, based on historical data including streamflow, ice on/off days, the accumulation of degree days, weather, and riparian phenology (Appendix D). Benthic periphyton in wadeable streams will be collected during periods of stable stream flow (Stevenson and Bahls 1999).

4.3 Timing for Laboratory Processing and Analysis

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

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

All filtering should be completed the day after field samples are collected to preserve the integrity of chemistry samples. Chlorophyll *a* samples **must** be filtered and placed in the freezer (-20 ^{o}C) within 24 hours of returning from the field to prevent an increase in chlorophyll *a* concentrations in the sample. If unable to maintain this timeline, all remaining filtering must be completed within 48 hours of returning from the field. Preparing labels, aluminum foil, vials, preservative, and aluminum weigh boats prior to lab processing speeds lab processing time. Dried samples may be stored between each lab processing day if necessary, days do not need to be consecutive. Taxonomy samples may be stored at 4 ^{o}C for up to 30 days. Filters may be stored at -20 ^{o}C for up to 14 days. For additional storage and shipping timelines see SOP F.

4.4 Sample Timing Contingencies

All samples from one sampling site per bout must be collected within one day. A minimum of 2 weeks between sample bouts at each site shall be observed.

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and conditions become unsafe (e.g. approaching thunderstorm, rapid increase of water level in the **wadeable stream**), or the **lake/non-wadeable stream** becomes too windy (>32 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, return to the Domain Support Facility and sample at another time.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the streamflow or weather requirements, continue to collect samples after the delay. If conditions do not improve, discard any previously collected samples at the site or at the Domain Support Facility and start over on the next appropriate sampling day.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
14 Days	If flooding occurs in **wadeable streams** on or 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; maximum wait = 1 month).	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 must be flagged.
6 Months	Preserved algal taxonomy samples may be held for up to 6 months at 4 ^{o}C in the domain lab if circumstances do not allow shipping to the external lab.	Holding samples >30 days affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected

		and samples do not need to be flagged if held for ≤6 months.
	Frozen algal chemistry filters may be held for up to 6 months at -20 °C in the domain lab if circumstances do not allow shipping to the external lab. Chlorophyll *a* filters in particular go bad after 6 months.	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 and samples do not need to be flagged if held for ≤6 months.

4.5 Sampling-specific Concerns

1. Including bryophyte or aquatic plant leaves in a benthic sample artificially increases chlorophyll concentration. Take care not to scrub substrata with attached leaves.
2. Take care to keep track of the volume of water used rinse the sample in the field and the volume of water used for filtering in the lab, these data are very important for conversion to higher data products.
3. Failure to completely mix a sample before filtering can result in skewed results. All subsamples (filters) are meant to be representative of one-another, so careful mixing is a necessity.
4. Chlorophyll/pheophytin samples must be filtered and frozen in the first 24 hours following sampling.

5 SAFETY

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

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

Safety Data Sheets (SDS) shall be 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 EHS Safety Policy and Program Manual (AD[01]), Section HC-03, Hazard Communication.

See Section 10 in the NEON Operations Field Safety and Security Plan (AD[02]) for aquatic-specific field safety requirements. In addition, the following safety requirements 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 non-wadeable streams**, site-specific hazards may be encountered that cause technicians to conduct sampling from the boat, without dismounting from the vessel. In addition, technicians are required to use extra caution in waters where alligators are present and to make sure a safe distance from hazards is maintained
3. All personnel must be wearing a personal flotation device (PFD) prior to entering a boat.
4. All employees shall have access to a form of communication with other team members such as a two-way radio.
5. Technicians should 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 AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as 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 2. Equipment list – General equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
RD[16] or RD[17]	R	Site-specific Stream Morphology, rapid habitat assessment, or Bathymetry Map	Determining sampling locations	1	N
	R	Handheld GPS unit (with batteries, <4 m accuracy) or Hummingbird	Navigating to sampling location	1	N
	R	Clipboard	Recording data	1	N
	R	Cooler (9-28 qt)	Field sample storage; use size appropriate to samples being collected	1	N
	R	Waders or knee boots	Boating or wading	1 pair per person	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Consumable items					
RD[08]	R	AQU Field Metadata Sheet	Recording metadata	1	N
RD[07]	R	Field data sheets (print on all-weather paper, write in pencil)	Recording data	2	N
	R	Pre-printed adhesive labels (all-weather, 2”x4”)	Labeling samples	15	N
	R	Pre-printed paper labels (all-weather copier paper, write in pencil)	Labeling samples	1 sheet	N
	R	Pencils	Recording data	1	N
	R	Permanent markers	Labeling samples	1	N
	R	Ice Packs	Keeping samples cool	2	N

R/S=Required/Suggested

Table 3. Equipment list – Sampling equipment

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
	R	Template (35 mm plastic slide cassette)	Sampling area for rock and wood scrubs	Epilithon, epixylon	5	N
	R	HDPE bottles with lids, amber wide-mouth (125 or 250 mL)	Sample container; container size is selected by technicians (either 125 mL or 250 mL)	All periphyton (bottles are reused but are site-specific)	8	N
	R	Wire-bristle brush (brass), toothbrush-size	Scrubbing epilithon	Epilithon	2	N
	R	Nylon-bristle toothbrush	Scrubbing epixylon	Epixylon	2	N
	R	Larval insect tray, plastic	Scrubbing container in which sample is collected	All	1	N
	R	125 mL unitary wash bottle	Rinsing substrate and larval tray into sample bottles	All	2	N
MX100379	S	25 mL graduated cylinder, plastic	Measuring extra rinse water	All	1	N
	R	Petri dish lid, plastic, 47 mm diameter	Sample collection	Epipsammon, epipelon	4	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Spatula (metal, offset)	Sample collection	Epipsammon, epipelon	1	N
	R	Scissors	Sample collection	Epiphyton	1	N
	R	Metric ruler	Sample collection	Epiphyton	1	N
MX100393	R	Kemmerer sampler with rope and messenger	Sample collection	Phytoplankton	1	N
	R	4 L HDPE jug	Integrating phytoplankton samples	Phytoplankton	1	N
	R	Amber HDPE wide-mouth sample bottles with caps, 1 L	Sample collection	Phytoplankton, Seston	4-15	N
Consumable items						
	S	Razor blade	Chunky samples	Epilithon	2	N
	R	Whirl-paks®, 24 oz.	Sample container	Epiphyton	20	N
	R	Resealable bags, gallon	Organizing samples	Epiphyton	20	N
	R	DI water	Rinsing substrata	All	1 L	N

R/S=Required/Suggested

Table 4. Equipment list – General laboratory equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Wash bottle, unitary, 125 mL	Rinsing the filter funnel	1	N
	R	Waste container (for non-hazardous liquids)	For rinse water and unused sample	1	N
Consumable items					
	R	Lab data sheets (RD[07])	Recording data	2	N
	R	Adhesive weatherproof labels (~1”x2”)	Labeling samples and filters	1 per sample	N
	R	Aluminum foil	Wrapping GF/F filters for shipment	1	N
	R	Zip-top resealable bags, quart size	Organizing and storing filters	2	N
	R	DI water	Rinsing the filter funnel	1 L	N

R/S=Required/Suggested

Table 5. Equipment list –Epiphyte Processing

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

R/S=Required/Suggested

Table 6. Equipment list – Filtering

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
GB08760000		Vacuum pump filter manifold assembly	Filtering samples	1	N
MX100386 (part of	R	Filter funnel (25 mm diameter, 200 mL)	Filtering samples, part of manifold assembly	1-	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
GB08760000				3	
MX100388 (part of GB08760000)	R	Vacuum filter flask, polypropylene (1L)	Filtering samples, part of manifold assembly	1-3	N
MX100389		Vacuum hand pump	Filtering samples, backup to filter manifold		
	R	Filter forceps (flat ends)	Handling filters	1	N
	S	Hand-held stirrer (periphyton homogenizer)	Homogenizing periphyton and breaking up clumps of algae	1	N
	R	Graduated cylinder, 250 mL	Measuring and adding aliquots of sample to the filter funnel	1	N
	R	Adjustable pipette, 10 mL	Measuring and adding aliquots of sample to the filter funnel	1	N
Consumable items					
	R	Pipette tips, 10 mL	Measuring and adding the volume of sample into the filter funnel	1	N
MX106350	R	GF/F filters (25 mm diameter, pre-ashed)	Filters for AFDM and chemistry samples	90	N

R/S=Required/Suggested

Table 7. Equipment list – Ash-free dry mass

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

R/S=Required/Suggested

Table 8. Equipment list – Algae preservation

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Freezer (-20 °C)	Sample storage	1	N
Consumable items					
	R	Glutaraldehyde, Grade II, 25% in H_2O	Preserving periphyton samples	1 L	Y
	R	High-iodine Lugol's solution	Preserving seston/phytoplankton samples	1 L	Y
MX101218	R	HDPE bottles with lids, wide-mouth (60 mL)	Periphyton taxonomy sample container	8	N
MX100345	R	HDPE bottles with lids, wide-mouth (1 L)	Seston/phytoplankton taxonomy sample container	1	N

R/S=Required/Suggested

Table 9. Equipment list – Shipping supplies

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Dry ice shipping container	Shipping frozen filters	1	N
	R	Non-dry ice shipping container (e.g., 9 qt cooler or cardboard box)	Shipping taxonomy samples	1	N
Consumable items					
	R	Dry ice	Shipping filters	1 lb	Y
	R	Vermiculite, Grade 2	Shipping taxonomy samples - absorbing liquid leaks and cushioning shipment	TBD	N
	S	Cardboard box (~9”x7”x7”)	Shipping taxonomy samples	1	N
RD[20]	R	Shipping inventory	Provides sample information to external lab	1	N

R/S=Required/Suggested

6.2 Training Requirements

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

All personnel required to operate a boat shall be trained through an approved program. All 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. Technicians must also be trained in safe handling of glutaraldehyde (AD[03]).

6.3 Specialized Skills

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

6.4 Estimated Time

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

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

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

1. Collect and prepare all equipment, including sample bottles and labels.
2. Prepare sample bottles.
   a. **NOTE:** Bottles may be rinsed with DI and reused from the last sampling bout (Table 10).

Table 10. Number of field sample labels and bottle/bags required per site type.

	Sample type	Number of labels/containers	Sample container
Lakes/Non-wadeable streams	Phytoplankton	15 (5 per station)	1 L amber bottle
	Periphyton	5	125-250 mL amber bottle **OR** Whirl-pak®/zip-top bag
Wadeable streams	Seston	5 per composite sample	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

3. Print all-weather adhesive 2”x4” labels and adhere to sample bottles (Figure 14, RD[07]).
   a. Adhesive labels/bottles are used for the following sample types:
      i. Phytoplankton
      ii. Seston
      iii. Epilithon/epixylon
      iv. Epipsammon/epipelon
   b. Fill out adhesive labels in permanent marker.
   c. Adhere to sample bottles before bottles get wet.

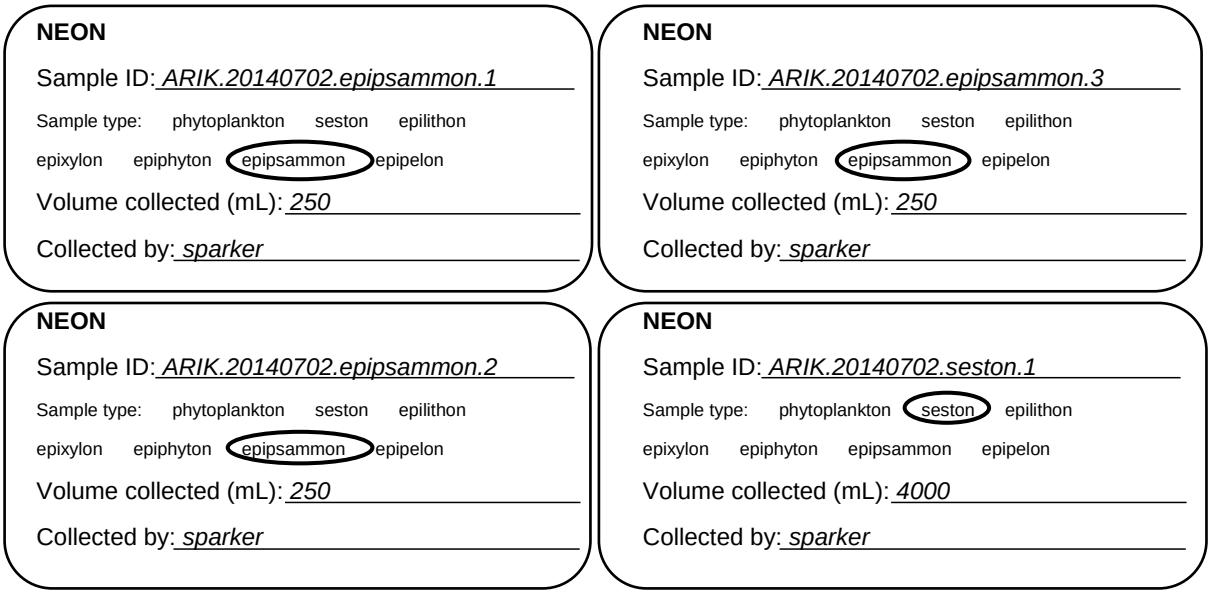

Figure 14. Example field labels (2" x 4") for periphyton samples. These labels should be filled out with permanent marker.

4. For epiphyte samples only: Print all-weather paper labels (Figure 15). Cut labels apart using scissors. One label will be placed inside each Whirl-pak®.
   a. All-weather paper labels should be filled out using pencil.

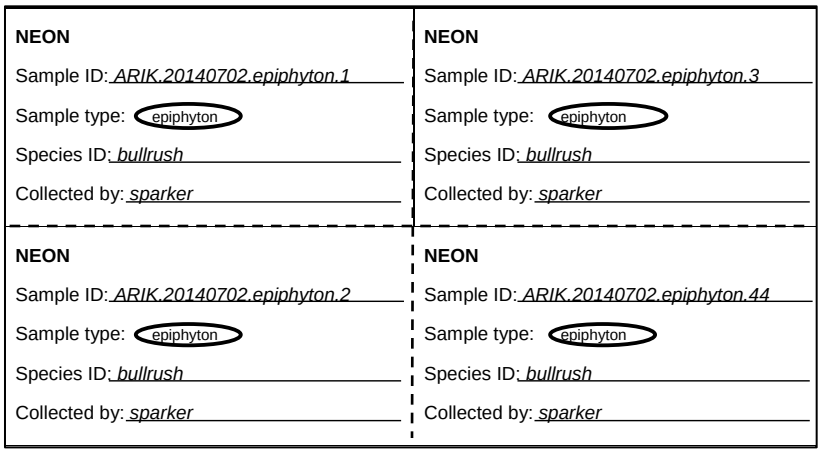

Figure 15. Example of field labels to be printed on all-weather paper. These labels should be filled out with pencil.

5. If collecting epilithon/epixylon samples, open 35 mm slide template (like a book) and separate into two halves (Figure 16). You will get two rectangular templates from each slide cassette.

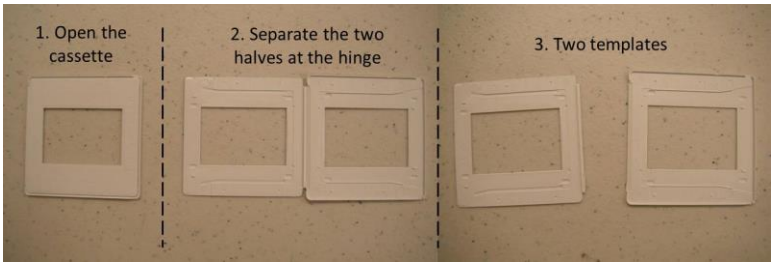

Figure 16. Separating the two halves of the 35 mm slide template.

6. Have ice or ice packs frozen and ready for cooler.
7. Check that all equipment is clean and in good condition and all batteries are charged.
8. See Laboratory Preparation procedures in Section D.1 for additional pre-sampling activities (e.g., filter and weigh boat preparation). **Ensure that enough filters are available and pre-ashed for the full sampling suite.**
9. Fill out general aquatic field metadata sheet (RD[08]) upon every field sampling visit, and Secchi and depth profile data sheet (RD[18]) in **lakes and non-wadeable streams**.

SOP B Determining Sampling Locations and Sampler Type

B.1 Lakes and Non-wadeable Streams

1. Pelagic sampling for Phytoplankton
   a. **Lakes**: Phytoplankton samples are collected at the center (buoy), inlet, and outlet.
   b. **Non-wadeable streams**: Phytoplankton samples are collected at the sensor set/buoy and two other locations 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. **Lakes**: Periphyton samples are collected in five of the ten littoral sections (refer to the site-specific riparian vegetation map, RD[15], Figure 17). Collect 1 sample per riparian section and evenly partition the five samples around the Riparian Sections. If there is a section of the lake where the substrata changes, move to the next section where your chosen dominant substratum can be found.

Figure 17. Example of lake perimeter subdivisions from the Lake Riparian Habitat Assessment Protocol (RD[15]).

   c. **Non-wadeable streams**: Periphyton samples are collected in the littoral areas within five of the ten riparian transects. Replicates may be taken on either river right or river left (or a combination of the two) within the riparian transects.
   d. Determine which periphyton substrata to sample in the littoral areas (see Appendix E for habitat and substrata sampling suggestions at NEON sites). Substrata must account for >20% of littoral habitat.
      i. The habitat type chosen should be present during all sampling bouts.

ii. All 5 replicate 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.

B.2 Wadeable Streams

1. Determine percent cover of habitat types throughout the sampling reach using the Stream Morphology Map (RD[16]) or rapid habitat assessment. See Appendix E for habitat and substrata sampling suggestions at NEON sites.
   a. The habitat type chosen should be present during all sampling bouts.
   b. All replicate 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
   d. Only sample habitat types that account for >20% of the reach throughout the year.
2. Determine dominant substratum type within the two habitats chosen (i.e., highest percent cover of habitat and/or where visible algal community is attached), based on Stream Morphology Maps (RD[10]) or rapid habitat assessment.
   a. If there is only one clear habitat at the site, sample two different types of substrata using the sampling methods below (e.g., in a slow-moving run, take 5 epiphyton samples and 3 epipsammon samples).
   b. If the same substratum type is sampled in both the dominant and secondary habitats (i.e., epiphytes in both runs and pools), then Sample IDs need to be adjusted accordingly. Use replicate numbers 1-8 (i.e., 1-5 for dominant habitat and 6-8 for secondary habitat; SITE.DATE.sample type.replicate).
   c. Spread samples out along the reach. For example, do not collect all replicates for the same substratum in the same riffle, collect replicate samples from 5 different riffles along the reach.

B.3 All Aquatic Sites

1. Target substratum types in order of sampling preference (see Definitions, 2.4). If the preferred substratum is present in high enough density, that takes precedence over a less preferred, more dominant substratum type (e.g., if silt is the dominant substratum type but cobbles are present in high enough density to sample consistently, cobble sampling takes priority).
   a. Cobble (epilithon) → follow sampling procedure SOP C.1
   b. Woody snag (epixylon) → follow sampling procedure SOP C.1
   c. Plant surface (epiphyton) → follow sampling procedure SOP C.2
   d. Sand (epipsammon) → follow sampling procedure SOP C.3

e. Silt (epipelon) → follow sampling procedure SOP C.3

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 technicians have walked or locations that appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).
6. Avoid substrata that are close to the stream/river bank or lake shore and may be exposed to frequent drying.
7. Choose sampling locations that are exposed to ambient light (e.g., not under a log or cut bank).
8. Unless sampling epiphytes, avoid substrata that are heavily colonized with aquatic plants, bryophytes, invertebrates, or have leaf litter clinging to the surface. You may brush some invertebrates off the surface, but the presence of aquatic plants and bryophytes may skew the chlorophyll results.
9. Avoid substrata that have noticeably tumbled or been recently disturbed.
10. Do not collect all replicate samples from the same location.
    a. In **wadeable streams**, collect composite samples from several different runs/riffles/pools, or if that is not possible, from different portions of the same run/riffle/pool.
    b. In **lakes and non-wadeable streams**, collect composite samples from different riparian sections or transects.
11. 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.

B.4 Contingent decisions

Table 11. Contingent decisions for sampling in **wadeable streams**.

Situation	Action	Outcome for Data Products	Considerations
Wadeable stream site with <200 m aboveground stream length due to stream size	Habitat available may be insufficient to accommodate all 8 replicate samples without causing harm to the stream. Reduce sampling by collecting samples only in the dominant habitat type (5 samples total).	Lower resolution for diversity metrics.	If the decision is made to decrease the number of samples collected for this protocol, it must also be reflected in the other wadeable stream biology protocols (RD[11], RD[12], RD[13]).
Wadeable stream	If the stream experiences	Less standardization	Habitat types sampled

site with seasonal drying	seasonal drying such that the chosen habitat types have disappeared, select the next dominant habitat type in B.2, or use the sub-dominant habitat if still present.	of the dataset.	should be present throughout the year. The decision to switch habitat types should only occur during periods of extreme drying such that typical habitat types are no longer present at the site.

SOP C Field Sampling

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

1. Select three cobbles or pieces of woody debris for each composite sample (e.g., 15 total cobbles for 5 composite samples) that meet the requirements in SOP B.3 AND the following [after Richest Targeted Habitat (RTH) requirements; Porter et al. 1993 and Moulton et al. 2002]:
   a. Stable in the stream/river/lake bed (i.e., have not recently tumbled).
   b. Larger than the scrubbing template (i.e., > 2 inches in diameter).
   c. Woody debris pieces may be cut to a manageable size with a bow saw for sampling.
2. Note the dominant substratum size class at the sampling location (record main substratum size within the habitat unit under wood sampled for epixylon) on the field data sheet (Figure 20, RD[07]).
3. Place cobble/woody debris right-side up (the side exposed to the sun) in the white larval tray. Take care to keep the cobble right-side up, this is the surface that has been exposed to sunlight, and will be the portion of the cobble that you sample.
4. Be sure to keep cobbles/woody debris moist with native water until scrubbing.
5. Proceed to a location on the stream bank or boat to process the sample. This location should be out of direct sunlight if possible (Figure 18).

Figure 18. A field technician processes an epilithon sample on the stream bank, out of direct sunlight.

6. Rinse the inside of the 125 mL wash bottle 3 times with stream water. Discard water into the stream.
   a. You only need to rinse the wash bottle if this is your first sample for the day at that stream. No need to re-rinse the wash bottle between samples at the same stream.
7. Fill the 125 mL wash bottle with DI water to "fill line" (as marked on bottle).
   a. Make sure that bottom of the meniscus lines up with the "fill line".
   b. **Since this is a volume-based sampling technique, it is important to keep the sample volume consistent and record the volume of water used.**
   c. Depending on the productivity of the site, >125 mL may be necessary to meet the external lab filtering requirements. See Table 13 for recommended filter volume and adjust water volume and amber HDPE bottle size as necessary. Be sure to record any changes in sample volume.
8. 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.
   a. Samples do not need to be numbered in any particular order (i.e., you do not have to fill bottles in order from 1-5), however for lakes and non-wadeable streams the Riparian Section numbers must match the replicate number (e.g., 1-10).
   b. You can rinse all sample bottles for that site at the same time and set aside, or rinse them separately.
9. Holding cobble/woody debris underwater, briefly 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).
   a. Recheck cobble, if there are more than 10 invertebrates attached to the substratum within your template, discard and choose a new cobble/piece of woody debris.

b. If there is growth of aquatic plants or bryophytes that falls within your template, discard and choose a new cobble.

10. Place cobble/woody debris right-side up (the side exposed to the sun) in white tray and pour any excess water out of the tray.
11. Place white slide template on top of cobble/woody debris (surface that was exposed to light at the stream bottom; Figure 19). Check cobble/woody debris again for colonization of invertebrates, bryophytes, or plants.

Figure 19. Template placement for epilithon protocol.
(Note: Samples should not be processed in direct sunlight.)

12. Holding the template firmly in place on the cobble/woody debris, begin scrubbing inside the template.
    a. Use the wire-bristled brush for cobbles, use the toothbrush for woody debris. The wire-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 19).
    d. Scrubbing should be similar to brushing your teeth.
13. Periodically rinse the inside area of the template using the 125 mL wash bottle **while holding the template in place**. Allow water to run into the white tray – **DO NOT DISCARD** rinse-water.
14. Continue scrubbing until the inside of the template is clean.
15. Remove template. There should be a clean rectangle left on the substratum from scrubbing. This is harder to see on woody substrates.
16. Using the wash bottle, rinse the template (front and back) and the cobble/woody debris into the tray.

17. Repeat Steps 10-16 until all 3 cobbles/woody debris collected for the composite sample have been scrubbed and rinsed.
18. Rinse scrub brush and fingers over the tray. If there is any remaining water in the 125 mL wash bottle after all three cobbles have been scrubbed, dump this into the tray. All rinse-water should now be in the white tray and should total 125 mL, **do not discard as this is your sample**.
19. Discard cobble/woody debris on the stream bank.
    a. If you are finished at this location, you may discard the cobble/woody debris.
    b. If you are not done at this location, hold the cobble/woody debris on the bank and discard in water when you leave the site.
20. Carefully swirl contents of tray (scrubbed material+ rinse-water) to re-suspend the sample.
21. Carefully pour the suspended sample and water into 125 mL or 250 mL amber wide-mouth HDPE bottle. Take care not to spill any of the sample.
    a. If you do spill a small amount (i.e., <10 mL) of the sample at this point, it is ok because this is a volume-based approach.
    b. If you spill a significant amount (i.e., >10 mL) of sample), dump the entire sample and start over at Step 1.
    c. There may be some sand left in the bottom of the tray after swirling and pouring out the sample. This is ok.
22. If you feel that your sample was not properly mixed and some has remained in the tray, you may pour the sample back in the tray and repeat Steps 20-21.
23. If you need more water to rinse with, refill the 125 mL wash bottle with DI water and use for rinsing. If you do this, be sure to use the entire 125 mL or measure using the 25 mL graduated cylinder, and record the total volume used for rinsing on the sample label and on the field data sheet. **It is very important to keep track of any changes in sample volume.**
    a. Additional water will also require that you use a larger sample bottle (i.e., 250 mL HDPE amber widemouth).
24. 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 non-wadeable streams. Please place samples directly in the cooler.
25. Fill out field datasheet in pencil (Figure 20).

NEON Periphyton, Seston and Phytoplankton Collection

Site (4-letter code): *BAR(* **Recorded by:** *sparker@neoninc.org*

Date (YYYY-MM-DD): *2014-07-02* **Collected by:** *jstewart@Field-ops.org*

Local time (HH:MM): *13:3* **Sampling protocol:** *NEON.DOC.003045* **Rev:**

PHYTOPLANKTON

Location ID	Phyto depth 1 (m)	Phyto depth 2 (m)	Sample volume (mL)	Sample type	Replicate	Sample ID
c0	*1.0*	*3.2*	*4000*	*phytoplankton*	*1*	*BARC.20140702.phytoplankton.1*
in	*1.0*	*1.0*	*4000*	*phytoplankton*	*2*	*BARC.20140702.phytoplankton.2*
ot	*1.0*	*1.0*	*4000*	*phytoplankton*	*3*	*BARC.20140702.phytoplankton.3*

PERIPHYTON & SESTON

Location ID	Habitat	Substratum size class	Sample volume (mL)	Sample type	Replicate	Sample ID
s1	*littoral*	*sand*	*NA*	*epiphyton*	*1*	*BARC.20140702.epiphyte.1*
s3	*littoral*	*sand*	*NA*	*epiphyton*	*3*	*BARC.20140702.epiphyte.3*
s5	*littoral*	*sand*	*NA*	*epiphyton*	*5*	*BARC.20140702.epiphyte.5*
s7	*littoral*	*sand*	*NA*	*epiphyton*	*7*	*BARC.20140702.epiphyte.7*
s9	*littoral*	*sand*	*NA*	*epiphyton*	*9*	*BARC.20140702.epiphyte.9*

Figure 20. Example field datasheet. This shows example data from a lake site, a wadeable stream site would not have the top "Phytoplankton" section filled out, and the 'Habitat' would be riffle/run/pool. Blank field datasheets can be found in RD[07].

26. Rinse tray, brush, and template with native water before starting next sample.
27. Repeat above steps until all locations have been sampled.
    a. **Wadeable streams**, dominant habitat: 5 composite samples
    b. **Wadeable streams**, secondary habitat: 3 composite samples
    c. **Lakes**: 5 composite samples, 1 from each of 5 riparian sections
    d. **Non-wadeable streams**: 5 composite samples, 1 from each of 5 riparian sections
28. Return samples to the Domain Support Facility for further processing within 24 hours (SOP D).

C.2 Epiphyton (Aquatic Plant Surfaces)

1. Fill out labels in pencil (all-weather paper labels, Figure 15, RD[07])
   a. **Wadeable streams**, dominant habitat: 5 labels
   b. **Wadeable streams**, secondary habitat: 3 labels
   c. **Lakes/Non-Wadeable streams**: 5 labels
2. Select plants for sampling that are well-colonized with epiphytes (Figure 21) and that meet the following requirements:
   a. Sample from only 1 plant species to standardize sampling.
   b. The plant species should be common (i.e., accounts for >50% of the aquatic plants) in the reach.
   c. Plants should not be covered by sediments.

Figure 21. 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.
   d. 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.
   e. Use a metric ruler to estimate sample area.
   f. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 20; RD[07]).
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®.

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 22), 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.

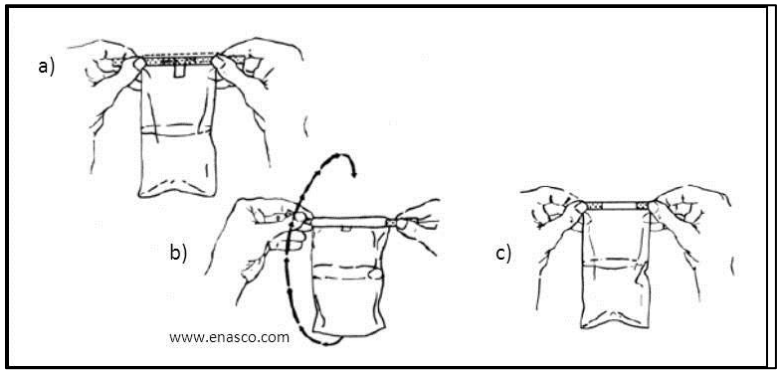

Figure 22. How to close a Whirl-pak® bag: a) hold the wire tabs; b) whirl the bag 3 complete revolutions (or fold the top over); and c) fold the wire ends over to close.

7. Fill out field data sheet in pencil (Figure 20).
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. **Non-wadeable streams**: 1 sample from each of 5 riparian transects
9. Return samples to the Domain Support Facility for further processing within 24 hours (SOP D).

C.3 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 14).
   a. **Lakes/Non-wadeable streams**: 5 sample bottles
   b. **Wadeable streams**, dominant habitat: 5 sample bottles
   c. **Wadeable streams**, secondary habitat: 3 sample bottles
2. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 20; RD[07]).
3. Rinse the inside of the 250 mL amber HDPE sample bottle with stream water. Fill bottle ~1/4 full, cap, and shake vigorously. Discard rinse water into stream away from the location where you intend to sample. Rinse 3 times. Recap bottle and set aside.
   a. Samples do not need to be numbered in any particular order (i.e., you do not have to fill bottles in order from 1-5), however for lakes and non-wadeable streams the Riparian Section numbers must match the replicate number (e.g., 1-10).
   b. You can rinse all sample bottles for that site at the same time and set aside, or rinse them separately.
4. Rinse white plastic sampling tray with stream water.
5. Rinse the lid (larger half) of a 47 mm plastic petri dish in native water. Holding lid upside-down underwater, rub the inside of the lid with your fingers to remove air bubbles.
6. Lightly press the lid into the substratum to be sampled (like a cookie cutter). Take care not to disturb the substratum before placing lid on bottom. If substratum is disturbed, find a new sampling location.
7. Slide spatula under lid to enclose the sample. Holding the petri lid tightly to the spatula, lift out of water (Figure 23). 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 final sample volume.
   a. If sample leaks out of the petri lid when lifting the spatula out of the water, discard and resample.

Figure 23. An epipelon sample collected with the Petri dish lid and metal spatula.

8. Place spatula + sample + petri lid in white sampling tray and invert lid.
9. Repeat Steps 5-8 until you have collected 3 petri lid samples to combine into one composite sample.
    a. 3 petri lid samples = 1 composite sample
10. Fill 125 mL wash bottle with DI water to "fill line" (as marked on bottle).
    a. Make sure that bottom of the meniscus lines up with the "fill line".
    b. **Since this is a volume-based sampling technique, it is important to keep the sample volume consistent and record the volume of water used.**
    c. Depending on the productivity of the site, >125 mL may be necessary to meet the external lab filtering requirements. See Table 13 for recommended filter volume and adjust water volume and amber HDPE bottle size as necessary. Be sure to record any changes in sample volume.
11. Using the 125 mL DI wash bottle, rinse petri lid and spatula into tray. **DO NOT DISCARD RINSE WATER.**
12. Carefully pour sample into 250 mL amber HDPE bottle. Take care not to spill sample.
    a. Use your fingers and the remaining water from the 125 mL wash bottle to help get the sample, including sand/silt, into the bottle.
    b. If there is any water left in the 125 mL wash bottle after the tray is empty, pour this into the sample bottle to maintain a constant volume.
    c. If additional water is needed, measure using the graduated cylinder and record total rinse volume on the field datasheet.
13. Cap bottle tightly, double check labels, and place in a cool storage location out of direct sunlight until sampling is finished.
    a. Place sample bottles in the cooler or submerge the bottles in water near the bank to keep them cool. If submerged, be sure that they will not float downstream. Place bottles in the cooler as soon as possible.
        i. NOTE: it is not recommended to submerge samples in non-wadeable streams. Please place samples directly in the cooler.

14. Fill out field data sheet in pencil (Figure 20).
15. Rinse tray, petri dish, and spatula with stream water before starting next sample.
16. Repeat above steps until all locations have been sampled
    a. **Wadeable streams**, dominant habitat: 5 composite samples
    b. **Wadeable streams**, secondary habitat: 3 composite samples
    c. **Lakes**: 1 composite sample from each of 5 riparian sections
    d. **Non-wadeable streams**: 1 composite sample from each of 5 riparian transects
17. Return samples to the Domain Support Facility for further processing within 24 hours (SOP D).

C.4 Seston: Wadeable Streams ONLY

1. Label 4-5 1 L amber HDPE bottles with domain, date, site, sample number, location, habitat type, type of sample (i.e., seston), and collector's name (Figure 14).
    a. The four 1 L amber HDPE bottles equal 1 composite sample.
2. Sample at the water chemistry sampling location.
    a. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 20; RD[07]).
3. Make sure no one is wading upstream of you.
4. Rinse amber 1 L HDPE bottles 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. Fill additional bottles according to Steps 4-6.
9. Fill out field data sheet in pencil (Figure 20).
10. Double check label and place sample bottles in cooler with ice packs (do not freeze).
11. Return samples to the Domain Support Facility for further processing within 24 hours (SOP D).

C.5 Phytoplankton Sampling- Lake and non-wadeable streams ONLY

1. Label 12-15 1- L amber HDPE bottles with domain, date, site, sample number, location, habitat type, type of sample (i.e., seston), and collector's name (Figure 14).
    a. Four to five 1 L amber HDPE bottles equal 1 composite sample.
2. Sample in three locations per lake or non-wadeable stream.
    a. Lakes (near water chemistry sampling locations, Figure 13):
        i. Deepest point in the lake, determine by bathymetric site map (RD[17]) and preloaded GPS coordinates
        ii. Near the lake inlet
        iii. Near the lake outlet
    b. Non-wadeable streams (5-10 m downstream of sensor set):

i. Thalweg (based on the latest bathymetry map)
ii. Half the distance between the thalweg and the right bank
iii. Half the distance between the thalweg and the left bank

3. Navigate the boat to the sampling location.
4. Gently lower an anchor at the bow and allow boat to float back with wind or current to sampling location. Drop a second anchor at the stern to hold boat in place.
   a. Allow ~5 minutes for sediments to settle after lowering the anchor, you can use this time to prepare the sampling equipment.
   b. Using a bow anchor rope 2 times the water depth will minimize disturbance of the sediment at the sampling location.
   c. Sample on the side of the boat, away from the motor and anchor, so as not to interfere with the Kemmerer or disturb the sediments
5. Always sample near the bow of the boat to minimize the effects of the motor on the water column.
6. Determine the depths of the thermocline and euphotic zone according to the AOS Protocol and Procedure: Secchi Disk and Depth Profile Sampling in Lakes and Non-wadeable Streams (RD[19]).
   a. Determine the depth of the euphotic zone by multiplying the mean Secchi depth by 2.5.
   b. Measure to the bottom of the Kemmerer.
   c. If thermal stratification is present, integrate the following (Figure 24):
      i. Surface: 0.5 m (±0.5 cm) below water surface → 1 Kemmerer sample
      ii. Metalimnion: middle of metalimnion (defined by 1^{o} per m change in water temperature) → 1 Kemmerer sample
      iii. Hypolimnion/Euphotic: 0.5 m (±0.5 cm) above euphotic depth → 1 Kemmerer sample
   d. If the **lake/non-wadeable stream** is not stratified, determine sample depths from the Secchi calculations. Integrate 2 Kemmerers if the euphotic depth is < 5m, integrate 3 Kemmerers if the euphotic depth is > 5m.
      i. Surface: 0.5 m (±0.5 cm) below water surface → 1 Kemmerer sample
      ii. **If euphotic depth is >5 m**, Euphotic midpoint: half the depth of the euphotic zone → 1 Kemmerer sample
      iii. Euphotic bottom: 0.5 m (±0.5 cm) above euphotic depth → 1 Kemmerer sample

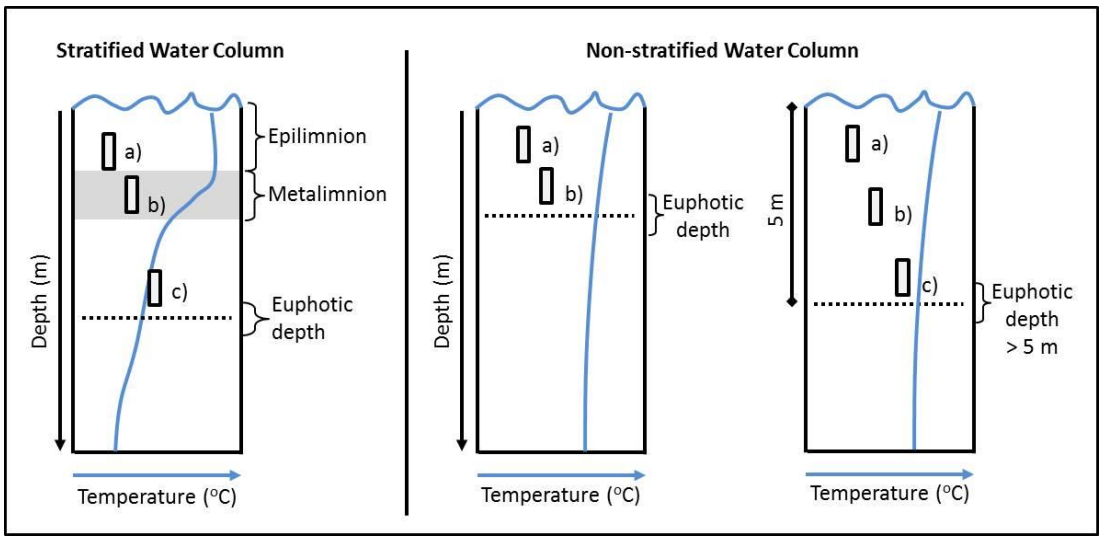

Figure 24. Sample depth selection in a stratified water column. The blue line is a temperature profile indicating the presence or absence of a thermocline. If stratification is present, integrate 3 Kemmerer samples: a) 0.5 m below the water surface, b) middle of the metalimnion, and c) 0.5 m above bottom of euphotic zone. If stratification is not present and the euphotic depth is < 5m, integrate 2 Kemmerer samples: 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 Kemmerer samples: a) 0.5 m below the water surface and b) midpoint of the euphotic depth, and c) 0.5 m above bottom of euphotic zone.

7. Rinse the 4 L HDPE jug and Kemmerer with lake/non-wadeable stream water 3 times over the opposite side of the boat from where you plan to sample. Discard rinse water into the lake/non-wadeable stream. Set 4 L jug aside.
8. Cock the Kemmerer sampler by pulling the trip head into the trip plate by holding the top and bottom stopper and giving a short, hard pull to the bottom stopper (Figure 25).
   a. NOTE: A short, hard pull is important to keep the stoppers open. If the stoppers don't stay open, pull harder.

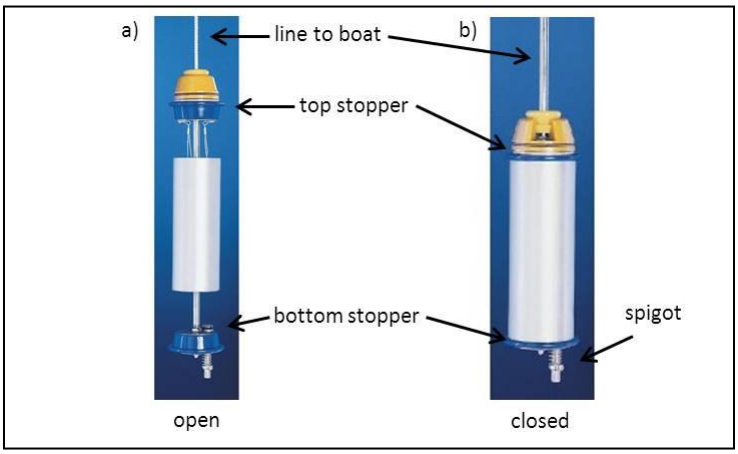

Figure 25. Vertical Kemmerer water sampler in the a) open or cocked position and b) closed (after dropping the messenger) position.

9. Tie the free end of the Kemmerer line to a cleat on the boat to prevent losing the sampler.

10. Always start with the shallowest sample first to avoid disturbing the water column (i.e., sample surface water first and bottom of thermocline last).
11. Carefully lower the Kemmerer sampler over the side of the boat into the water. Keep the messenger on the portion of the rope that remains in the boat and hold the messenger in one hand. Ensure it is secured properly to the line.
12. Continue to lower the sampler until it reaches the desired depth by using the depth markings on the line attached to sampler.
    a. Measure to the bottom of the Kemmerer.
13. When the sampler has reached the desired depth, drop the messenger down the line to the sampler. The messenger will trigger the stoppers to close (Figure 25b).
14. Pull the sampler up by pulling the line into the boat, coiling it neatly.
    a. Note: If the Kemmerer does not close properly when you pull it up, resample in the same location.
15. Uncap the 4 L HDPE jug.
16. Carefully open the spigot (Figure 25b) on the bottom of the Kemmerer sampler, allowing the contents of the cylinder to flow into 4 L jug.
    a. Repeat Steps 8- 16 and integrate samples in the 4 L jug.
        i. If integrating 3 Kemmerer samples, add only 1.3 L from each Kemmerer to the 4 L jug.
17. Rinse pre-labeled (Figure 14) sample bottles three times with lake/non-wadeable stream water, discard water back into the lake/non-wadeable stream.
18. Mix integrated sample gently in 4 L jug, then fill 4 1L amber HDPE bottles. Cap each bottle once filled.
    a. Immediately place all sample bottles in cooler with ice packs until they can be refrigerated in the lab.
    b. The 4-5 1L bottles = 1 composite sample.
    c. Samples must be kept dark and cool until filtering.
19. Proceed to the next sample location and repeat above steps.

C.6 Sample Preservation

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

C.7 Ending the Sampling Day

1. Refresh the sampling kit
    a. Remove old labels from HDPE bottles that will be reused.
    b. Print and fill out new adhesive labels (RD[07]). Attach labels to bottles before going out in the field.
2. Equipment, maintenance, cleaning and storage

a. Wash all equipment that has come in contact with site water according to the NEON Aquatic Decontamination Protocol (RD[06]).
b. Clean boat and motor; remove aquatic plants by hand to prevent spread of invasive taxa. Allow boat and motor to dry completely.
c. Only reuse Amber HDPE bottles at the same site. Keep a separate set of bottles dedicated to each site.
d. Rinse amber HDPE bottles with DI, these will be used and rinsed again in the field during the next field collection. Since each site has a set of bottles, they do not need to be decontaminated between bouts.
e. Dry all items completely between sites and before storage.
f. Discard and replace any broken templates, petri dishes, or worn brushes.

SOP D Laboratory Sampling and Analysis

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

D.1 Preparation

1. Pre-ash GF/F filters (Table 12)
   a. Place layers of 25 mm GF/F filters on aluminum foil using filter forceps or while wearing nitrile gloves. Use multiple layers of foil if needed, filters can be touching and placed on top of one another but should not be stacked more than 3 filters deep.
   b. Place in muffle furnace (500 °C) for 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 filter may be stored indefinitely, as long as they remain in the box and stay dry.

Table 12. Minimum number of pre-ashed filters required per bout.

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

2. Print 1"x2" adhesive labels for 60 mL taxonomy bottles, chlorophyll *a*/pheophytin filters, and nutrient filters, to be attached to the outside of bottles and/or aluminum foil packets (Figure 26, RD[07]).

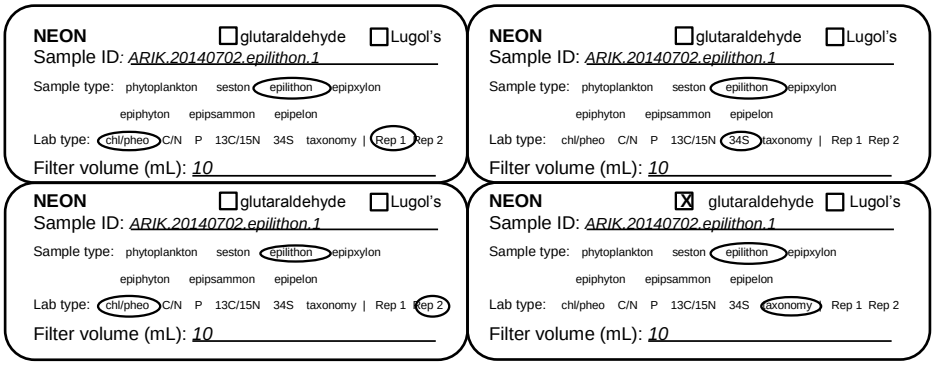

Figure 26. Example adhesive labels (1" x 2") for periphyton, seston or phytoplankton lab samples.

3. If aluminum weigh boats are new and unlabeled:

a. Label ~20 boats by inscribing a unique number on the bottom with a pencil (e.g., A1, A2, A3, etc.; Figure 27).
   i. Note: It doesn't matter what the labels are as long as they are unique and easy to read.
b. Place new empty labeled boats in the muffle furnace (500 ^{o}C) for 6 hours to burn off any residue.
c. After 6 hours, carefully remove boats from the furnace and allow to cool 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) on lab data sheet (RD[07]).
d. This may be done in advance, before field sampling.
e. Boats may be reused from previous sampling bouts.

Figure 27. Examples of newly labeled aluminum weigh boats.

4. If aluminum weigh boats have been previously labeled and used, clean with a soft brush to remove any residual ash.
   a. Boats must be re-weighed upon every use, but do not need to be re-ashed.

D.2 Processing Samples

Samples will be subsampled and filtered in the Domain Support Facility following Figure 28 and Table 13.

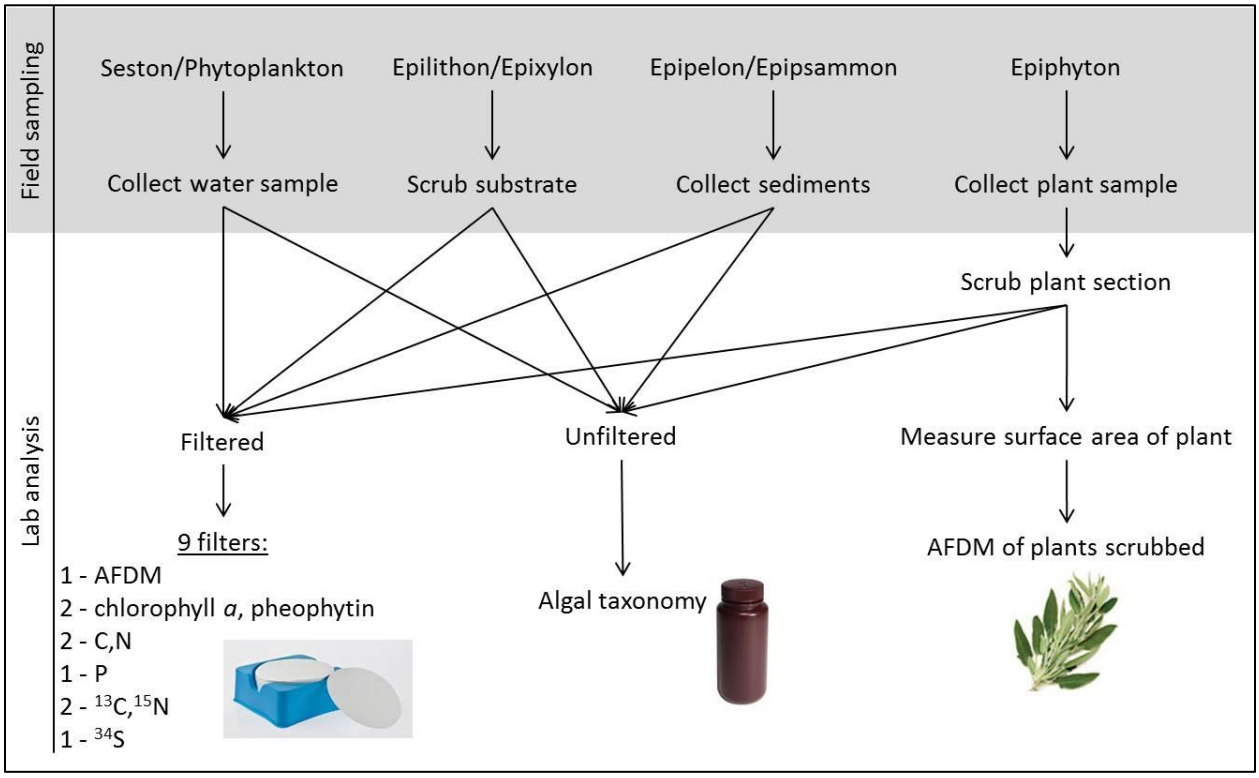

Figure 28. Lab schematic diagram for lakes and non-wadeable streams

Table 13. Subsample volume and priority for lab processing. If unable to push the minimum recommended volume through a filter, then filter as much sample as possible and record volume on lab data sheet. Please note, if filtering the maximum amount, a 250 mL parent sample is required.

Priority	Sample	Parameter	Type	Recommended subsample volume
1	Periphyton	Taxonomy	Liquid, preserved in glutaraldehyde	60 mL
2	Periphyton	Chlorophyll *a*/pheophytin	Filters (2)	2-10 mL (until visible color on filter)
3	Periphyton	AFDM	Filter (1)	2-10 mL mL (until visible color on filter)
4	Periphyton	C, N	Filters (2)	2-10 mL (until visible color on filter)
5	Periphyton	P	Filter (1)	2-10 mL (until visible color

				on filter)
6	Periphyton	$\delta^{13}C$, ^{15}N	Filters (2)	As much sample as possible, 5-10 mL
7	Periphyton	$\delta^{34}S$	Filter (1)	As much sample as possible, 5-10 mL
1	Seston or Phytoplankton	Taxonomy	Liquid, preserved in Lugol's	1 L
2	Seston or Phytoplankton	Chlorophyll *a*/pheophytin	Filters (2)	≥250 mL (until visible color on filter)
3	Seston or Phytoplankton	AFDM	Filter (1)	≥250 mL (until visible color on filter)
4	Seston or Phytoplankton	C, N	Filters (2)	≥500 mL (until visible color on filter)
5	Seston or Phytoplankton	P	Filter (1)	≥500 mL (until visible color on filter)
6	Seston or Phytoplankton	$\delta^{13}C$, ^{15}N	Filters (2)	As much sample as possible, >500 mL
7	Seston or Phytoplankton	$\delta^{34}S$	Filter (1)	As much sample as possible, >500 mL

D.3 Algal Taxonomy Samples (Unfiltered)

1. Label 60 mL (periphyton) or 1 L (seston or phytoplankton) HDPE bottles with 1"x2" adhesive labels (Figure 26, RD[07]). Lab type is "**taxonomy**".
2. Cap and shake amber HDPE sample bottle for 30 seconds to mix sample evenly.
3. Pipette 60 mL periphyton into the appropriately labeled 60 mL bottle, or measure 1 L seston or phytoplankton using a graduated cylinder and transfer to labeled 1 L bottle.
   a. If the sample is too thick for the pipette, you may measure 60 mL using a clean (rinsed in DI) graduated cylinder.
4. Cap bottles and proceed to Sample Preservation Section D.8.

D.4 Epiphyte Samples

1. **Day 1**: Using forceps, remove the plant sample from Whirl-pak®. Place in clean, dry white plastic sample tray (used in field protocol).
2. Fill 125 mL wash bottle to fill line with DI water.
   a. Make sure that bottom of the meniscus lines up with the "fill line".
      i. Check volume with a graduated cylinder. If volume is off by >5 mL, use graduated cylinder to measure water that goes into the wash bottle.
   b. Since this is a volume-based sampling technique, it is important to keep the sample volume consistent and record the volume of water used.

c. Depending on the productivity of the site, >125 mL may be necessary to meet the external lab filtering requirements. See Table 13 for recommended filter volume and adjust water volume and HDPE bottle volume as necessary.

3. Gently scrub surface of sample with a toothbrush. You may have collected leaves and/or stalks, scrub these gently. Take care not to lyse plant cells, these would bias the chlorophyll results.
4. Use DI water periodically to rinse the scrubbed material into the tray.
5. When scrubbing is finished, rinse scrubbed plant and toothbrush thoroughly into the white tray.
6. Remove plant sample and measure the approximate dimensions of the surface area scrubbed (e.g., stalk length and/or leaf length + width, top + bottom) and record the estimate sum of all surface are scrubbed in the **Epiphyton: Plant Surface Area** column of the lab data sheet.
   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.
7. Place plant material in a labeled paper lunch bag.
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 D.5.
10. Place paper bags containing samples in the drying oven for a minimum of 12 hours at 60 ^{o}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]).
11. **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.
12. Place a clean, plastic weigh boat (small sample) or tray (large sample) on analytical balance. Tare (zero) balance.
13. Place dry sample in the plastic weigh boat/tray and record as **Epiphyton: total plant dry weight** on Lab Data Sheet (Figure 29, RD[07]).
    a. Sample may be crushed or broken up to fit into weigh boat.
14. Place sample in a clean, pre-labeled aluminum weigh boat.
    a. If sample does not fit in one aluminum weigh boat, grind in Wiley mill using 20 mesh (0.85 mm) screen.
    b. Place a subsample of ground material in aluminum weigh boat.
    c. Record **Boat ID** on Lab Data Sheet.
    d. Clean grinding mill thoroughly with compressed air between samples.
15. Weigh boat on 6-place balance, and record as **Dry weight + boat** on Lab Data Sheet.
16. Repeat above steps until all specimens have been processed.

NEON Aquatics Periphyton/Seston/Phytoplankton Lab Datasheet

Site (4-letter code): *BARC* | **Date analysis finished (YYYY-MM-DD):** *2014-07-07*

Date collected (YYYY-MM-DD): *2014-07-02* | **Recorded by:** *sparker@neoninc.org*

Date analysis started (YYYY-MM-DD): *2014-07-03* | **Sampling protocol:** *NEON.DOC.003044* **Rev:** *A*

Filters, page 1

PERIODICALLY CHECK THAT BALANCE IS ZEROED!

Columns A-E can be pre-printed in Excel to save time during filtering

Sample type	Replicate	Sample ID	Lab type	Filter replicate	Filter volume (mL)	Epiphyton: Plant Surface Area (cm^2)	Epiphyton: total plant dry mass (g)	Boat ID	Boat mass (g)	Dry mass + boat (g)	Ash mass + boat (g)
epipsammon	1	BARC.20140702.epipsammon.1	AFDM		10			A1	2.2503	2.8541	2.6340
epipsammon	1	BARC.20140702.epipsammon.1	chl/pheo	1	10						
epipsammon	1	BARC.20140702.epipsammon.1	chl/pheo	2	10						
epipsammon	1	BARC.20140702.epipsammon.1	CN	1	10						
epipsammon	1	BARC.20140702.epipsammon.1	CN	2	10						
epipsammon	1	BARC.20140702.epipsammon.1	P		10						
epipsammon	1	BARC.20140702.epipsammon.1	13C15N	1	10						
epipsammon	1	BARC.20140702.epipsammon.1	13C15N	2	10						
epipsammon	1	BARC.20140702.epipsammon.1	34S		10						

NEON Aquatic Periphyton, Seston and Phytoplankton Lab Datasheet

Taxonomy, page 4

Sample ID	Lab Type	Preservative type	Taxonomy sample volume (mL)	Preservative volume (mL)
BARC.20140702.epipsammon.1	taxonomy	glutaraldehyde	60	1.2
BARC.20140702.epipsammon.3	taxonomy	glutaraldehyde	60	1.2
BARC.20140702.epipsammon.5	taxonomy	glutaraldehyde	60	1.2
BARC.20140702.epipsammon.7	taxonomy	glutaraldehyde	60	1.2
BARC.20140702.epipsammon.9	taxonomy	glutaraldehyde	60	1.2
BARC.20140702.phytoplankton.1	taxonomy	Lugol's	1000	20
BARC.20140702.phytoplankton.2	taxonomy	Lugol's	1000	20
BARC.20140702.phytoplankton.3	taxonomy	Lugol's	1000	20

Figure 29. Example of lab datasheets for periphyton, seston and phytoplankton chemistry and taxonomy samples.

17. Place aluminum boats + specimens in the muffle furnace using oven gloves and tongs. **TAKE CARE NOT TO BURN YOURSELF!**
    a. Boats may be stacked on top of each other as long as there is space for air flow between them.
    b. Place boats on an approved muffle furnace pan (if available) before placing in the furnace. This makes it easier and safer to handle samples.
    c. If drafts are a problem in the furnace, cover pans/boats with aluminum foil.
18. 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. Samples may be allowed to cool in muffle furnace depending on work flow.
19. **Day 3**: After 6+ hours, remove aluminum boats carefully from the muffle furnace using oven gloves and crucible tongs. Place on trivet or metal cart to cool.

20. Cover pans/aluminum boats to prevent ash from blowing out of boats and set aside to cool on a heat-resistant surface in an area without drafts from windows, doors, or HVAC ventilation.
21. When boats have cooled enough to handle, place in desiccator.
    a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
22. After cooling to room temperature, weigh boats again on analytical balance (0.0001 g), record as **Ash Weight + Boat** on Lab Data Sheet.
23. After weighing, discard ashed filters into the trash and clean the boat with a soft brush or paper towel.
24. Set clean boats aside to be used again.

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

1. Set up filter funnel, filter flask, and vacuum pump (Figure 30).
    a. Technicians may use the hand vacuum pump (Figure 30a) with one filter flask and funnel attached, or the filter manifold and electric pump (Figure 30b) with multiple filter funnels.
    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.

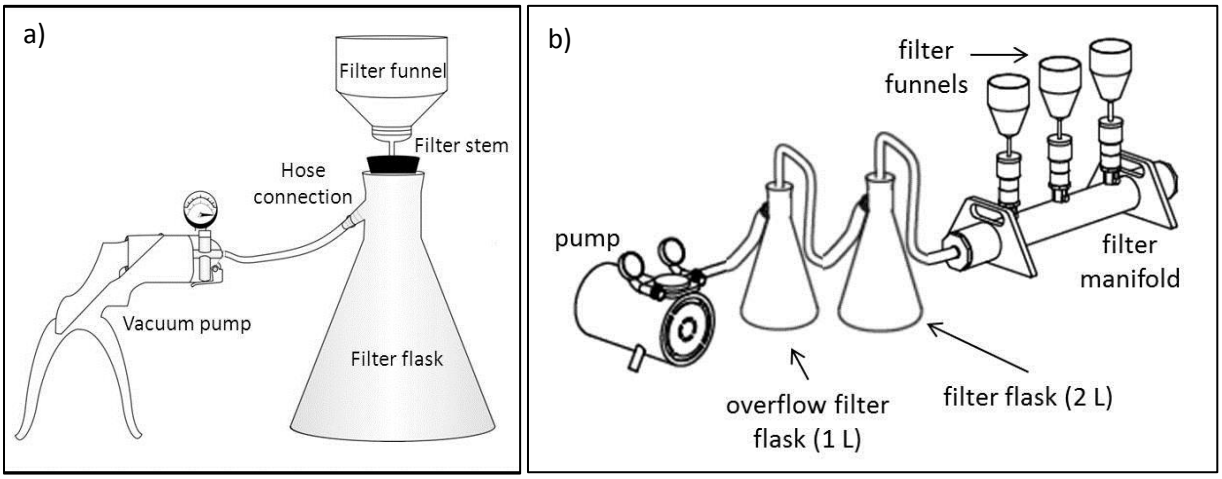

Figure 30. Filter apparatus setup: a) hand pump; b) filter manifold.

2. Remove the top of the filter funnel from apparatus, rinse with DI water.
3. Place pre-ashed GF/F filter on top of the filter stem (Figure 30), replace top of funnel.
4. Shake sample bottle vigorously for ~30 seconds to mix sample.
    a. If sample has large chunks of algae, homogenize using clean, hand-held battery operated stirrer for ~30 seconds. Take care that samples does not spill over the top of the sample bottle while stirring.
        i. Rinse stirrer thoroughly with DI between samples.

5. Filter known volume of sample.
   a. **Seston or 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. **Epipsammon/epipelon samples**: Measure volume of water contained in the sample. Pour entire sample and sediments into graduated cylinder (Fetscher et al. 2015).
      i. Wait ~5 minutes to allow sediment to settle out.
      ii. When a boundary between sediment and water becomes clear, measure and record the water volume on the lab data sheet.
      iii. 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.
      iv. If you add additional rinse water to make this process easier, you must add that to the sample volume.
      v. Shake sample container for 30 seconds to remove periphyton from substrata.
   c. **All benthic samples**: Using a clean 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. Allow epipelon samples to settle 30-60 seconds prior to pipetting. Settling should be long enough to allow sediment to settle, but not so long as to allow algal cells to settle.
   d. **Keep track of the volume of sample filtered** on the Lab Data Sheet (RD[07]).
6. Draw suction on filter apparatus using the hand vacuum pump (or vacuum manifold, if available). Do not exceed 15 in. Hg vacuum. High pressure ruptures cells and causes chlorophyll and other compounds to dissolve and pass through the filter.
   a. If you have added too much sample and the filter appears to be clogged, you may discard the contents of the filter funnel and the filter and start over. **If you decant water from the filter funnel, you must discard the filter and start over.**

7. Check the filter, if it still appears white, filter more sample. If the filter appears green or yellow tinged, proceed to next step.

   a. **Record the volume of sample filtered on the Lab Data Sheet (RD[07]).**
   b. Filter the minimum amount suggested in Table 13. If unable to filter the minimum amount, filter as much sample as possible and record volume.
   c. Periodically discard the water in the filter flasks. If the flasks overflow, they will back up into the pump and potentially cause damage.

8. Rinse inside walls of funnel using wash bottle of DI.
   a. Do not include DI rinse water in the volume of sample filtered.
9. Continue to draw suction on the filter until there is no water left in the funnel and there is no excess water on top of the filter.
10. Remove top of filter funnel, release suction using the release valve on the hand pump or the valves below the filter funnel on the manifold.
11. Carefully remove the filter from the stem using filter forceps (forceps with flat ends). Take care not to touch the filter with your fingers.
    a. **AFDM filters**: Place filter in a labeled aluminum weigh boat, record sample information on Lab Data Sheet (RD[07]) and proceed to Section D.6.
    b. **Chlorophyll/pheophytin, C/N, P, and isotope filters**: Fold filter in half and place on a clean square of aluminum foil (~4x4 inches). Fold foil securely around the filter to form a packet.
    c. Label foil packet with adhesive sample label (~1" x 2") (Figure 26).
12. Repeat above steps until you have 9 filters total from the same sample.
    a. 1 AFDM filter (proceed to Section D.6)
    b. 2 chlorophyll *a*/pheophytin filters
    c. 2 C, N filters
    d. 1 P filter
    e. 2 ^{13}C, ^{15}N isotope filters
    f. 1 ^{34}S isotope filter
13. Place all foil packets for one lab type from the same site inside a resealable bag. Using a permanent marker, label the outside of the resealable bag with Domain, Site, Date, and the "lab type" (i.e., chl/pheo, C/N, P, 13C/15N, or 34S).
    a. Place all filters in -20 ^{o}C freezer.
14. Cap amber sample bottle and store in 4 ^{o}C until all samples are complete.

D.6 Ash-free Dry Mass (AFDM) of Filters

1. **Day 1**: Remove filter from filter apparatus using filter forceps, place filter in labeled aluminum weigh boat, and copy sample information onto lab data sheet with appropriate **Boat ID** (RD[07]).
2. Place all boats containing filters in the drying oven for a minimum of 12 hours at 60 ^{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 for easier loading and unloading in the drying oven.
   b. Cover boats with paper or cardboard to prevent air circulation in the drying oven from blowing the filters out of the boats.
3. **Day 2**: Remove boats+filters from the drying oven and allow to cool to room temperature.
   a. If the boats+filters will not be weighed right away, place them in a desiccator. Filters can be left in the desiccator for up to 30 days if necessary.
4. Place boat + filter on tared (zeroed) analytical balance. Record on **Lab Data Sheet** as **Dry weight + boat**.

5. Place aluminum boats + filters in the muffle furnace using oven gloves and tongs. **TAKE CARE NOT TO BURN YOURSELF!**
   a. Boats may be stacked on top of each other as long as there is space for air flow between them.
   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.
6. 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.
7. **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.
8. Cover pans/aluminum boats to prevent ash from blowing out of boats and aside to cool on a heat-resistant surface in an area without drafts from windows, doors, or HVAC ventilation.
9. When boats have cooled to room temperature, place in desiccator.
   a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
10. After cooling to room temperature, weigh boats again on analytical balance (0.0001 g) and record as **Ash weight + Boat** on Lab Data Sheet.
11. After weighing, discard ashed filters into the trash and clean the boat with a soft brush or paper towel.
12. Set clean boats aside to be used again.

D.7 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 seston/phytoplankton (+ Lugol's) sample for taxonomy lab
   b. 1 AFDM filter (processed at Domain Support Facility)
   c. 2 chlorophyll/pheophytin filters
   d. 2 C,N filters
   e. 1 P filter
   f. 2 δ ^{13}C, ^{15}N isotope filters
   g. 1 δ ^{34}S isotope filter
2. After all components have been filtered and bottled, you may discard the remaining sample material from the field sample bottles.
3. Rinse the field sample bottles with DI water, discard rinse water and recap bottles to be reused.
   a. Bottles may be dried or recapped and stored while still damp inside.

D.8 Preserving Samples

1. **Periphyton taxonomy**: Uncap each sample bottle. Using a disposable pipet, preserve each 60 mL sample with glutaraldehyde to a reach a final concentration of 0.5% glutaraldehyde in the sample. Store at 4 °C up to 30 days until shipping. Record type and volume of preservative used on the AOS Sample Shipping Inventory (RD[20]).
   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.
      i. C1 = 0.25 (25% glutaraldehyde)
      ii. V1 = solve for V1
      iii. C2 = 0.005 (0.5% glutaraldehyde)
      iv. V2 = 60 mL
2. **Seston or phytoplankton taxonomy**: Uncap each sample bottle, using a pipette, preserve each 1 L samples with high-iodine Lugol's solution to reach a final concentration of 2% (20 mL Lugol's per L of sample). Store at 4 °C up to 30 days until shipping. Record type and volume of preservative used on the AOS Sample Shipping Inventory (RD[20]).
3. **Filters (chlorophyll *a*/pheophytin, C/N, P, and isotope filters)**: Place labeled foil packets in sealed zip-top bags (a separate labeled bag for each parameter). Place in dark -20 °C freezer until shipping. Ship on dry ice within 2 weeks.

D.9 Ending the Processing Day

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

SOP E Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the

maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Enter all data from field datasheets into the AOS database, PDA, or Web UI if available.

SOP F Sample Shipment

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

Shipments are to include a hardcopy of the "per sample" tab of the shipping inventory (RD[20]) as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. The shipment tracking number (shipment ID) must be included in the electronic version of the shipping inventory as well as the email, but is not necessary on the hard copy.

F.1 Algal Taxonomy Sample Shipping

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

F.2 Algal Filter Sample Shipping

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

F.3 Handling Hazardous Material

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

F.4 Supplies/Containers

See sections F.1, F.2, and Table 9 for specific shipping materials.

F.5 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 ^{o}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 1).
2. **Filters**: Filters may be stored at -20 ^{o}C for up to 14 days prior to shipping.
   a. Frozen samples may be held for up to 6 months if the lab is not able to receive samples (Table 1).

F.6 Grouping/Splitting Samples

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

F.7 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).

F.8 Shipping Inventory

Shipments are to have a hardcopy of the per sample tab of the shipping inventory (RD[13]) sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. Also include the shipment tracking number in the email.

F.9 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 DATASHEETS

The following datasheets are associated with this protocol:

Table 14. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.003042	Datasheets for AOS Protocol and Procedure: Periphyton, Seston and Phytoplankton Sampling
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling
NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass
NEON.DOC.002494	Datasheets for AOS Shipping Inventory

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

APPENDIX B QUICK REFERENCES

B.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 Sheet (RD[08]) and Secchi and depth datasheets (RD[18]) are completed per field site visit as needed.

Step 4 –Collect seston/phytoplankton samples:

1. Seston: **Wadeable streams**
   a. Sample at the water chemistry sampling location (sensor location S2)
2. Phytoplankton: **Lakes** (near water chemistry sampling locations):
   a. Deepest point in the lake or buoy location
   b. Lake inlet
   c. Lake outlet
3. Phytoplankton: **Non-wadeable streams**:
   a. Thalweg or sensor location
   b. Half the distance between the thalweg and the right bank
   c. Half the distance between the thalweg and the left bank

Step 5 – Collect benthic algae samples:

1. **Wadeable streams**
   a. Determine habitat sampling locations using the Stream Morphology Map (RD[16]) or rapid habitat assessment.
   b. Determine sampler type based on the habitats present and the order of preference for sampling substratum.
   c. Collect samples: 5 per dominant habitat type, 3 per second-most dominant habitat type.
2. **Lakes**
   a. Refer to the site-specific riparian vegetation map (created in the Riparian Habitat Assessment Protocol (RD[15]) which divides the lakeshore into 10 sections (Figure 17).
   b. Evenly partition the five samples around the Riparian Sections.
3. **Non-wadeable streams**
   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.

B.2 Algae Lab Processing

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

Step 2 – Dry AFDM filters in the drying oven and wrap all other filters in aluminum foil. Store foil packets in sealed zip-top bags and place in dark -20 ^{o}C freezer until shipping (lab day 1).

Step 3 – Transfer unfiltered periphyton taxonomy samples to 60 mL HDPE bottles and add preservative to reach a final concentration of 0.5% glutaraldehyde or phytoplankton samples to 1 L HDPE bottles to reach a final concentration of 2 % Lugol's within 24 hours of sampling (lab day 1).

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

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

Step6 – For each field sample 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. 2 chlorophyll/pheophytin filters
4. 2 C,N filters
5. 1 P filter
6. 2 δ ^{13}C, ^{15}N isotope filters
7. 1 δ ^{34}S isotope filter

B.3 Order of Preference for Sampling Substrata

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

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

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

Sample collection: Be sure to…

- **Wadeable streams**: Determine the dominant habitat and second-most dominant habitat based on the Stream Morphology Map (RD[16]), rapid habitat assessment, or the site-specific bathymetric map and riparian vegetation map (RD[17], RD[15])
- **Lakes and Non-wadeable streams**: Determine the dominant habitat in the littoral areas.
- Choose the appropriate sampler.
- **Wadeable and Non-wadeable streams**: Start sampling at the bottom of the reach, working upstream so as not to decrease visibility and disrupt periphyton communities.
- Spread replicate 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 technicians have walked, or locations that appear recently disturbed

Sample processing: Be sure to…

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

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for biological sampling bouts. Also see the Site Specific Sampling Strategy Document on NEON's FOPS intranet site.

Domain	Site	Bout 1	Bout 2	Bout 3
D01	Hop Brook	11Apr-9May	9Jul-6Aug	3Oct-31Oct
D02	Mill Run*	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D02	Posey Creek	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D03	Ichawaynochaway Creek	21Feb-21Mar	27Jun-25Jul	7Oct-4Nov
D03	Lake Barco	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D03	Lake Suggs	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D04	Rio Guilarte	26Jan-23Feb	21Jun-19Jul	9Nov-7Dec
D04	Rio Cupeyes	24Jan-21Feb	21Jun-19Jul	10Nov-8Dec
D05	Crampton Lake	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D05	Round Lake	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D06	Kings Creek	23Mar-20Apr	3Jul-31Jul	3Oct-31Oct
D06	McDowell Creek	20Mar-17Apr	3Jul-31Jul	27Sep-25Oct
D07	Leconte Creek	15Mar-12Apr	30Jun-28Jul	12Oct-9Nov
D07	Walker Branch	9Mar-6Apr	1Jul-29Jul	19Oct-16Nov
D08	Mayfield Creek	5Mar-2Apr	29Jun-27Jul	31Oct-28Nov
D08	Black Warrior River	19Feb-19Mar	27Jun-25Jul	31Oct-28Nov
D08	Tombigbee River	22Feb-22Mar	26Jun-24Jul	2Nov-30Nov
D09	Prairie Lake	18Apr-16May	5Jul-2Aug	11Sep-9Oct
D09	Prairie Pothole	20Apr-18May	5Jul-2Aug	11Sep-9Oct
D10	Arikaree River	21Mar-18Apr	4Jul-1Aug	20Sep-18Oct
D11	Pringle Creek	17Feb-17Mar	29Jun-27Jul	23Oct-20Nov
D11	South Pond at Klemme	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D12	Blacktail Deer Creek	1May-29May	13Jul-10Aug	30Aug-27Sep
D13	Como Creek	20May-17Jun	14Jul-11Aug	30Aug-27Sep
D13	West St. Louis Creek	2May-30May	5Jul-2Aug	3Sep-1Oct
D14	Sycamore Creek	17Feb-17Mar	29Jun-27Jul	21Oct-18Nov
D15	Red Butte Creek	29Mar-26Apr	6Jul-3Aug	29Sep-27Oct
D16	McRae Creek	10Apr-8May	11Jul-8Aug	23Sep-21Oct
D16	Martha Creek	6Apr-4May	5Jul-2Aug	22Sep-20Oct
D17	Convict Creek*	31Mar-29Apr	8Jul-5Aug	15Sep-13Oct
D17	Providence Creek*	19Mar-16Apr	1Jul-29Jul	25Sep-23Oct
D18	Oksrukuyik Creek	21May-18Jun	29Jun-27Jul	7Aug-4Sep
D18	Toolik Lake	21May-18Jun	29Jun-27Jul	6Aug-3Sep
D19	Caribou Creek	2May-30May	26Jun-24Jul	18Aug-15Sep

*soft sites as of November 2015

APPENDIX E 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)	Seston, Phytoplankton
D01	Hop Brook	Riffles (epilithon)	Pools (epipsammon)	ss (S2)
D02	Mill Run*			ss (S2)
D02	Posey Creek	Riffles (epilithon)	Pools (epipelon)	ss (S2)
D03	Ichawaynochaway Creek*	Epilithon	NA	rs + 2 locations
D03	Lake Barco	Epiphyton	NA	c0, in, ot
D03	Lake Suggs	Epiphyton	NA	c0, in, ot
D04	Rio Guilarte	Riffles (epilithon)	Pools (epilithon)	ss (S2)
D04	Rio Cupeyes	Riffles (epilithon)	Runs (epilithon)	ss (S2)
D05	Crampton Lake	Epixylon	NA	c0, in, ot
D05	Round Lake	tbd	NA	c0, in, ot
D06	Kings Creek	Riffle/run	Pools	ss (S2)
D06	McDowell Creek*	tbd	tbd	rs + 2 locations
D07	Leconte Creek	Riffle (epilithon)	Pools (epipsammon)	ss (S2)
D07	Walker Branch	Riffle (epilithon)	Runs (epixylon)	ss (S2)
D08	Mayfield Creek	Runs/riffles (epixylon)	Runs (epipsammon)	ss (S2)
D08	Black Warrior River	tbd	NA	rs + 2 locations
D08	Tombigbee River	tbd	NA	rs + 2 locations
D09	Prairie Lake	Epilithon	NA	c0, in, ot
D09	Prairie Pothole	Epilithon	NA	c0, in, ot
D10	Arikaree River	Runs (epiphyton)	Pools/Runs (epipsammon)	ss (S2)
D11	Pringle Creek	tbd	tbd	ss (S2)
D11	South Pond at Klemme*	Epipelon	NA	c0, in, ot
D12	Blacktail Deer Creek	Riffles (epilithon)	Riffles/Runs (epixylon)	ss (S2)
D13	Como Creek	Riffles (epilithon)	Runs (epipsammon)	ss (S2)
D13	West St. Louis Creek	Riffles (epilithon)	Pools (epipsammon)	ss (S2)
D14	Sycamore Creek	Riffles (epilithon)	Runs/Pools (epipsammon)	ss (S2)
D15	Red Butte Creek	Riffles (epilithon)	Runs (epilithon)	ss (S2)
D16	McRae Creek	Riffles (epilithon)	Step pools (epixylon)	ss (S2)
D16	Martha Creek	Riffles (epilithon)	Pools (epixylon)	ss (S2)
D17	Convict Creek*			ss (S2)
D17	Providence Creek*			ss (S2)
D18	Oksrukuyik Creek	Riffles (epilithon)	Pools (epipelon)	ss (S2)
D18	Toolik Lake	Epilithon	NA	c0, in, ot
D19	Caribou Creek	Riffles (epilithon)	Pools (epipsammon)	ss (S2)

*soft sites as of November 2015