AOS PROTOCOL AND PROCEDURE: PERIPHYTON AND SESTON SAMPLING IN WADEABLE STREAMS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	10/31/2012	ECO-00680	Initial draft release
B	02/07/2014	ECO-01090	Production release
C	08/29/2014	ECO-02210	Minor updates based on feedback from the field
D	11/14/2004	ECO-02439	Migration to new protocol template
E	05/15/2015	ECO-02666	Minor updates including changes to seston sample volume and preservation, addition of sampling contingencies and lab filter manifold, updates to sample shipping and labeling, and the addition of sampling dates to appendix.
F-OBSOLETE	01/22/2016	ECO-03470	OBSOLETE and superseded by NEON.DOC.003045 AOS Protocol and Procedure: Periphyton, Seston, and Phytoplankton Sampling

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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 a diverse group of organisms that are aquatic, photosynthetic, lack a vascular system, and form simple, vegetative structures (Wehr and Sheath 2003). For the purposes of analysis, algae can be broken into three distinct groups: macroalgae, benthic microalgae (periphyton), and sestonic algae (algal cells in the water column). Macroalgae will be sampled as part of the Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling in Wadeable Streams Protocol (RD[09]).

The periphyton community includes algae and associated biofilms living attached to substrata. These are usually the most abundant primary producers in small streams (Allan 1995). Most surfaces that are exposed to light (i.e., relatively shallow waters) in freshwater habitats sustain periphyton communities. Periphyton communities are strongly affected by light availability; disturbance and scouring of the stream 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 1). Biofilms often also includes heterotrophic bacteria, protozoans, prostrate and filamentous Cyanobacteria, and fine particulate organic matter (FPOM).

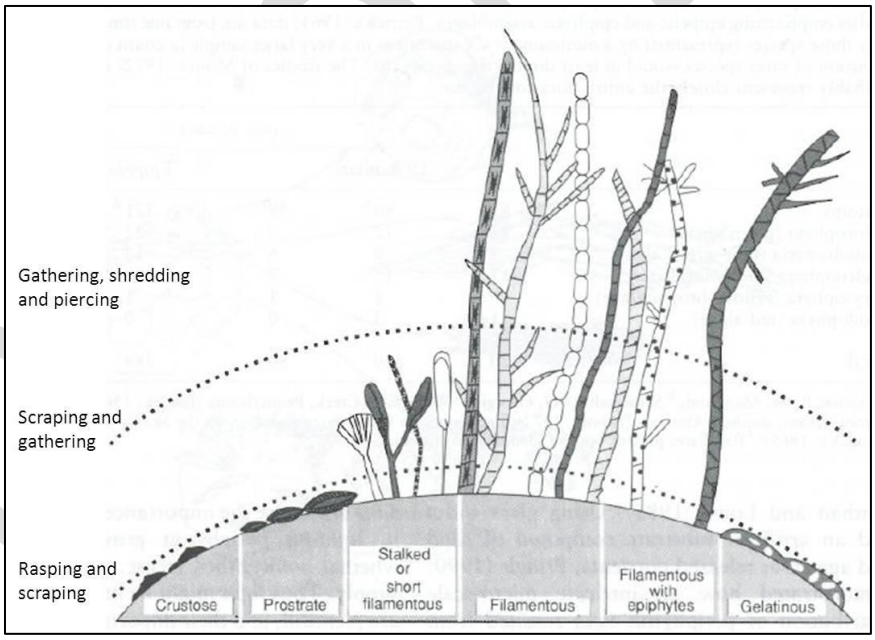

Figure 1. Growth forms of periphyton, ranging from prostrate types to long, filamentous types. Vertical layering differs among growth forms (from Allan 1995).

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 levels of the periphyton (Figure 1). Periphyton, especially diatoms, is 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.

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 protocol is based closely on the protocols of the US Geological Survey (USGS) National Water Quality Assessment (NAWQA; Porter et al. 1993, Charles et al. 2002, and Moulton et al. 2002), the US Environmental Protection Agency (EPA) Rapid Bioassessment Program (RBP; Stevenson and Bahls 1999), 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
AD[05]	NEON.DOC.014051	Field Audit Plan
AD[06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.002199	Datasheets for AOS Protocol and Procedure: Periphyton and Seston Sampling in Wadeable Streams
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[09]	NEON.DOC.000692	AOS Protocol and Procedure: Plant, Bryophyte, Lichen and Macroalgae Sampling in Wadeable Streams
RD[10]	NEON.DOC.001153	AOS Protocol and Procedure: Wadeable Stream Morphology Mapping
RD[11]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[12]	NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[13]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[14]	NEON.DOC.000690	AOS Protocol and Procedure: Macroinvertebrate Sampling in Wadeable streams
RD[15]	NEON.DOC.000692	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling in Wadeable Streams
RD[16]	NEON.DOC.001201	AOS Protocol and Procedure: Microbes in Wadeable Streams

2.3 Acronyms

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

2.4 Definitions

Bryophyte: Aquatic moss, liverworts, or hornworts lacking true vascular tissues (Figure 2).

Figure 2. Aquatic mosses (bryophytes) may grow on rocks in streams. Bryophytes may also have epilithic algae growing on its 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 in the stream bottom, geologically defined as 64 to 256 mm diameter. Cobbles are larger than pebbles (4-64 mm), and smaller than boulders (>256 mm).

Epixylon: Algae colonizing woody substrata.

Epilithon: Algae colonizing rock substrata (Figure 3).

Figure 3. Algal epilithon colonizing the surface of a cobble.

Epipelon: Algae colonizing silt substrata.

Epiphyton : Algae colonizing the surfaces of aquatic plants (Figure 4).

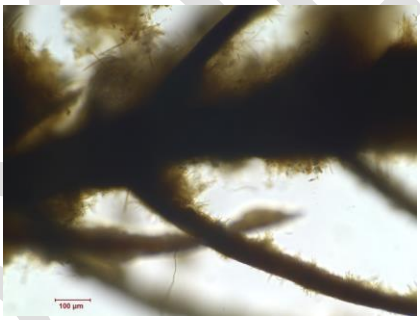

Figure 4. Algal epiphytes growing on the surface of an aquatic moss.

Epipsammom: Algae colonizing sand substrata.

Macroalgae: "Large" algae; multicellular, photosynthetic algae visible to the naked eye. In streams, these algae are typically filamentous (Figure 5).

Figure 5. Macroalgae are large algae that often form blooms in streams and lakes.

Macrophyte: Aquatic plant with vascular tissues (Figure 6).

Figure 6. Aquatic macrophytes are vascular plants that add structure and colonizable area to the stream bottom.

Pool: Areas of slow moving, deep water relative to rest of channel (Figure 7).

Figure 7. Pools are areas of deep, slow-moving water in the stream.

Riffle: Shallow, swiftly moving water, characterized by choppy surface water (Figure 8).

Figure 8. Riffles are shallow, fast moving habitats in streams.

Run: Flowing water, typically deeper than riffles, water surface remains smooth due to relatively uniform flow (Figure 9).

Figure 9. 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 10).

Figure 10. Step pools occur in high-gradient streams where there is a cascade-pool sequence.

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

Woody snag: Woody debris that catches on the stream bottom (or stream bank), and collects additional debris from the stream flow (Figure 11). Snags often create a refuge for organisms from the stream flow, as well as increased food sources.

Figure 11. Woody snags are places where pieces of wood fall into the stream and collect other organic matter.

3 METHOD

The goal of periphyton and associated biofilm sampling is to determine species diversity, taxa richness, biomass, using both chlorophyll *a* as a proxy for biomass as well as ash-free dry mass (AFDM), of periphyton and seston at wadeable stream sites. These variables will be tracked over time and analyzed for changes in relation to species loss or gain, invasive species, and responses to environmental variables.

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. Field

protocols differ depending on habitats being sampled. However, all samples are collected from the surface of the natural substratum present in each macrohabitat. 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.

Periphyton and seston sampling occurs three times per year. 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[07]) and Appendix D. Sample bout 1 is an early-season date, representing a period of rapid biomass accumulation after winter. 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. 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). A period of 14-days will be allowed after a flood event for periphyton to recolonize before sampling occurs.

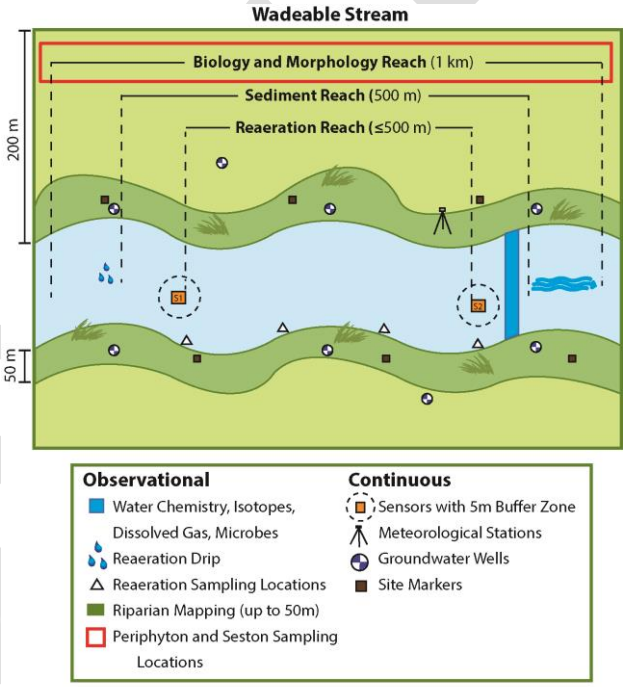

Figure 12. A generic site layout for wadeable streams with periphyton and seston sampling locations.

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

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

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Benthic periphyton and seston sampling in wadeable streams occurs three times per year at each site, roughly spring, summer, and autumn. Sampling must occur within the 1 month window specified in Appendix D, with a minimum of two weeks between sampling dates. Accommodations for local weather conditions (e.g., late ice-off) may be made that cause the sample date to fall outside of the pre-determined window.

4.2 Criteria for Determining Onset and Cessation of Sampling

A range of dates for each site were determined a priori, based on historical data including streamflow, the accumulation of degree days, weather, and riparian phenology (**Error! Reference source not found.**). Periphyton 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

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 Sampling Timing Contingencies

All samples from one sampling bout must be collected within one day (i.e., all 9 samples per stream as detailed in this protocol). A minimum of 2 weeks between sample periods shall be observed.

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products

Hours	If circumstances occur that impede sampling (e.g., wildlife, weather), discard samples and start over the next day that conditions permit.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the streamflow requirements below, continue to collect samples.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If flooding (>1.5x above baseflow) or unsafe wading conditions occur (Lane and Fay 1997) occurs before all samples have been collected for the day, return samples already collected to the stream and start over on the next appropriate day (see 14 day rule below).	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.
	In areas where a Surber sampler is used: If water is above the top of the Surber sampler frame, find a shallower place to sample. If high water is caused by high-flow conditions, wait to sample at baseflow conditions.	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.
14 Days	If flooding occurs on or prior to the targeted sampling date (>1.5x above baseflow) or unsafe wading conditions occur (Lane and Fay 1997) wait a minimum of 14 days until the water level drops so the periphyton community can recolonize (Biggs et al. 1999; 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.

4.5 Sampling-specific Concerns

1. Sampling too soon after a disturbance event (e.g., flooding or wildlife crossing the stream) can dramatically decrease biomass and diversity. Be sure to wait at least 14 days for recolonization to occur.
2. Including bryophyte or aquatic plant leaves in the sample artificially increases chlorophyll concentration. Take care not to scrub substrata with attached leaves.
3. Take care to keep track of the volume of water used to scrub the sample in the field and the volume of water used for filtering in the lab, these data are very important for conversion to higher data products.
4. Failure to completely mix sample before filtering can result in skewed results. All subsamples are meant to be representative of one-another, so careful mixing is a necessity.

5 SAFETY

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

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

Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity*depth is ≥ 10 ft^2/s (0.93 m^2/s; Lane and Fay 1997). See Section 10 in the NEON Operations Field Safety and Security Plan (AD 01]).

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

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 2. Equipment list – General equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
RD[10]	R	Site-specific Stream Morphology Map	Determining sampling locations	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 (hip or chest)	Wading	1 pair per person	N
Consumable items					
RD[06]	R	General AQU Field Metadata Sheet	Recording metadata	1	N
RD[05]	R	Field data sheets (print on all-weather paper, write in pencil)	Recording data	2	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	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	Cobbles and woody snags	5	N
	R	HDPE bottles with lids, amber wide-mouth (125 mL)	Sample container; container size is selected by technicians (either 125 mL or 250 mL)	All	5	N
	R	HDPE bottles with lids, amber wide-mouth (250 mL)	Sample container; container size is selected by technicians (either 125 mL or 250 mL)	All	10	N
	R	HDPE bottles with lids, amber wide-mouth (1 L)	Seston collection	All	4	N
	R	Wire-bristle brush (brass), toothbrush-size	Epilithon scrubbing	Cobbles	2	N
	R	Nylon-bristle toothbrush	Epixylon scrubbing	Woody snags	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
	S	25 mL graduated cylinder, plastic	Measuring extra rinse water	All	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Petri dish lid, plastic, 47 mm diameter	Epipsammon and Epipelon collection	Sand and silt	4	N
	R	Spatula (metal, offset)	Epipsammon and Epipelon collection	Sand and silt	1	N
	R	Scissors	Epiphyton collection	Plant surfaces	1	N
	R	Metric ruler	Epiphyton collection	Plant surfaces	1	N
Consumable items						
	S	Razor blade	Epilithon scraping	Cobbles	2	N
	R	Whirl-paks®, 24 oz.	Epiphyton container	Plant surfaces	20	N
	R	Resealable bags, gallon	Organizing samples, Epiphyton container	Plant surfaces	20	N

R/S=Required/Suggested

Table 4. Equipment list – General laboratory equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	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[05])	Recording data	2	N
	R	Adhesive weatherproof labels (~1”x2”)	Labeling samples and filters	1 sheet	N
	R	Aluminum foil	Wrapping GF/F filters for shipment	1	N
	R	Zip-top resealable bags, quart size	Organizing and storing filters	2	N
	R	DI water	Rinsing the filter funnel	1 L	N

R/S=Required/Suggested

Table 5. Equipment list –Epiphyte Processing

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Soft toothbrush	Scrubbing epiphytes	1	N

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

R/S=Required/Suggested

Table 6. Equipment list – Filtering

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

R/S=Required/Suggested

Table 7. Equipment list – Ash-free dry mass

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

R/S=Required/Suggested

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 samples	1 L	Y
	R	HDPE bottles with lids, wide-mouth (60 mL)	Periphyton sample container	8	N
	R	HDPE bottles with lids, wide-mouth (1 L)	Seston 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 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

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	TBD	N
	S	Cardboard box (~9”x7”x7”)	Shipping taxonomy samples	1	N
	R	Shipping inventory (RD[13])	Provides sample information to external lab	1	N

R/S=Required/Suggested

6.2 Training Requirements

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

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

6.3 Specialized Skills

N/A

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

A.1 Labels

1. Print all-weather adhesive 2”x4” labels for sample bottles (Figure 13, RD[05]).

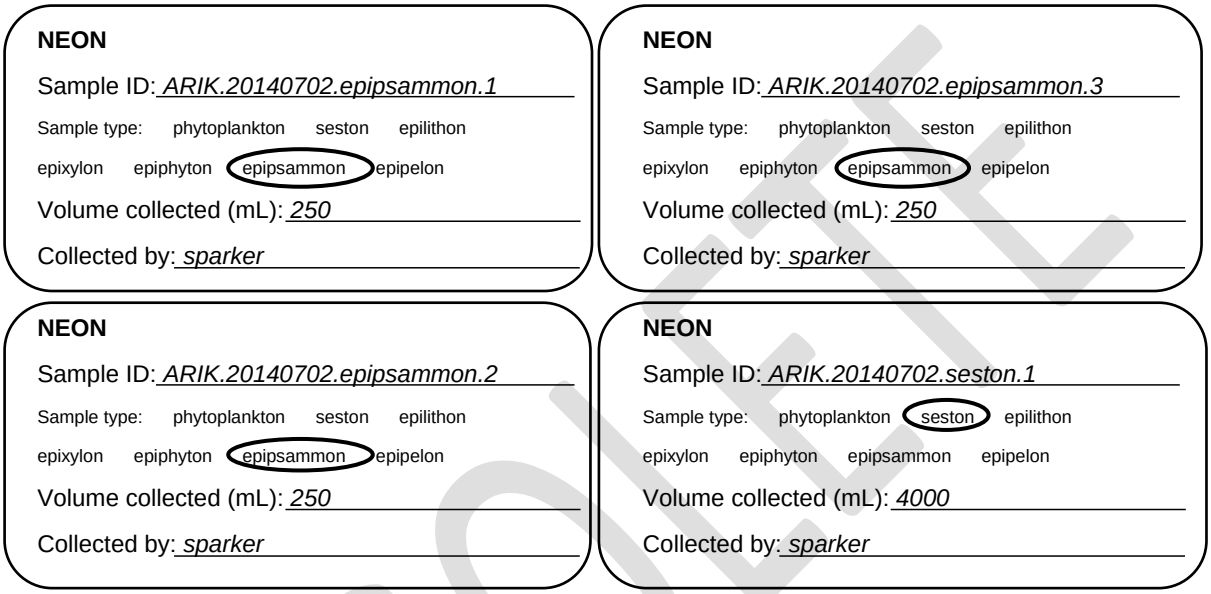

Figure 13. Example field labels (2” x 4”) for periphyton samples. These labels should be filled out with permanent marker.

2. Cut labels apart using scissors. One label will be placed inside each Whirl-pak®.

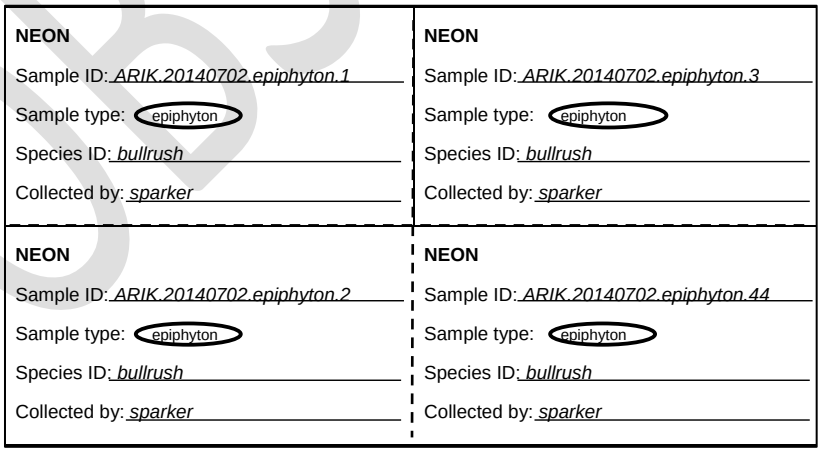

Figure 14. Example of field labels to be printed on all-weather paper. These labels should be filled out with pencil.

A.2 Equipment

1. Collect and prepare all equipment, including sample bottles, filters, and labels.
2. Open 35 mm slide template (like a book) and separate into two halves (Figure 15). You will get two rectangular templates from each slide cassette.

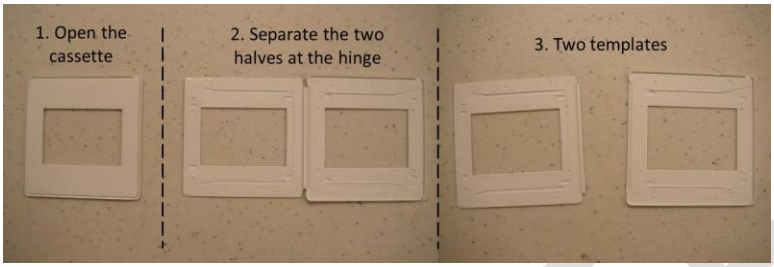

Figure 15. Separating the two halves of the 35 mm slide template.

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

SOP B Determining Habitat and Sampler Type

B.1 Decision Tree: Determining Habitat to Sample

1. Determine percent cover of habitat types throughout the sampling reach using the Stream Morphology Map (RD[10]) and go to Step 2 (see Appendix E for habitat and substrata sampling suggestions at NEON sites).
    a. The habitat type chosen should be present during all sampling bouts. If the site is a STREON site, the same habitat types should be sampled in both the Aquatic and the STREON reaches.
    b. All 5 (or 3) replicate samples must be taken from the same habitat type on each sampling bout, unless a major event (i.e., a flood) causes significant changes to the stream channel.
    c. Targeted habitat types (see Definitions, Section 0):
        1) Riffle
        2) Run
        3) Pool
2. Does habitat type account for >20% of the reach throughout the year?
    a. If YES, go to Step 3.
    b. If NO, ignore this habitat type.
3. Determine dominant substratum type in habitat (i.e., highest percent cover of habitat and/or where visible algal community is attached) and secondary habitat (i.e., second-highest percent cover and/or second-most heavily colonized) and proceed to Step 4.
    a. Targeted substratum types, in order of sampling preference (see Definitions, Section 0:
        1) Cobble (epilithon)
        2) Woody snag (epixylon)
        3) Plant surface (epiphyton)
        4) Sand (epipsammon)
        5) Silt (epipelon)
    b. Dominant habitat/substratum type = 5 replicate samples
    c. Secondary habitat/substratum type = 3 replicate samples
4. Is dominant/secondary substratum cobble?
    a. If YES, follow sampling procedure for epilithon (Section C.1).
    b. If NO got to Step 5.
5. Is dominant/secondary substratum sand or silt?
    a. If YES, follow sampling procedure for epipsammon and epipelon (Section C.3).
    b. If NO, go to Step 6.
6. Is dominant/secondary substratum type woody debris?
    a. If YES, follow sampling procedure for epixylon (Section C.1).
    b. If NO, go to Step 7.
7. Is dominant/secondary substratum type aquatic plants?
    a. If YES, follow sampling procedure for epiphyton (Section C.2).
    b. If NO, return to Step 3 and reassess substratum types.

B.2 Habitat and Sampler Selection

1. Do not sample within a 5 m radius of the aquatic instrumentation or STREON baskets.
2. Determine dominant habitat and substratum types available within the sampling reach based on Stream Morphology Maps (RD 09]).
    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 working at a STREON site, use the same habitat types and sampling methods in both the aquatic reach and the STREON reach.
3. 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.
4. 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.
    a. If the site is too small to use a separate habitat unit for each sample, you may collect samples from two different substratum types within the same habitat unit (e.g., epipelon and epixylon samples).
    b. If working in a STREON reach, all sample replicates must be collected between the STREON sensor sets.
5. Do not sample anywhere you or other field technicians have walked in the reach or locations that appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).
6. The order of preference for sampling substrata is as follows (Porter et al. 1993):
    a. Epilithon (rock substrata)
    b. Epixylon (wood substrata)
    c. Epiphyton (plant substrata)
    d. Epipsammon (sand substrata)
    e. Epipelon (silt substrata)

B.3 Contingent decisions

Table 10. Contingent decisions for sampling.

Situation	Action	Outcome for Data Products	Considerations
Wadeable stream site with <200 m aboveground stream length due to stream size or seasonal drying	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[14], RD[15], RD[16]).

SOP C Field Sampling

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

1. Label (2"x4" all-weather adhesive labels) three (if this is the second-most dominant habitat) or five (if this is the dominant habitat) 125 mL wide-mouth amber HDPE bottles with domain, date, site, sample number, location, habitat type, type of sample (e.g., rock scrub or epilithon), and collector's name (Figure 13).
   a. Write in permanent marker on all-weather adhesive labels.
   b. Adhere labels to bottles before bottles get wet.
   c. **NOTE:** Sample bottles may be rinsed with DI and reused from the last sampling bout.
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. Select three cobbles or pieces of woody debris for each composite sample that meet the following requirements [after Richest Targeted Habitat (RTH) requirements; Porter et al. 1993 and Moulton et al. 2002]:
   a. Located in flowing water. Avoid cobbles that are directly downstream of large boulders (i.e., not in flowing water because of the effects of the boulder). Also avoid cobbles that are close to the stream bank.
   b. Representative of the periphyton cover of the reach (i.e., not extremely dense or extremely sparse cover relative to nearby substrata).
   c. Stable in the stream bed (i.e., have not recently tumbled).
   d. Larger than the scrubbing template (i.e., > 2 inches in diameter).
   e. Avoid cobbles/pieces of woody debris that are heavily colonized with aquatic plants, bryophytes, invertebrates, or have leaf litter clinging to the surface (see Definitions, Section 0 for details on identifying aquatic plants and bryophytes). You may brush some invertebrates off the surface, but the presence of aquatic plants and bryophytes may skew the chlorophyll results.
   f. Avoid cobbles/woody debris that have noticeably tumbled or been recently disturbed.
   g. Avoid cobbles/woody debris that you or other observers may have recently stepped on.
   h. Do not choose all of the cobbles/woody debris from the same location. Collect samples from several different riffles (if possible), or from different portions of the same riffle.
4. Place right-side up (the side exposed to the sun) in the white larval tray. Take care to keep the cobble right-side up, this is the surface that has been exposed to sunlight, and will be the portion of the cobble that you sample.
   a. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 18, RD[05]).
   b. Record the distance downstream from the STREON dripper if sampling at a STREON site (RD[05]).
5. Be sure to keep cobbles/woody debris moist with stream water until scrubbing.

6. Proceed to a location on the stream bank to process the sample. This location should be out of direct sunlight if possible (Figure 16).

Figure 16. A field technician processes an epilithon sample on the stream bank, out of direct sunlight.

7. 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.
8. Fill 125 mL wash bottle with stream 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 11 for recommended filter volume and adjust water volume and amber HDPE bottle size as necessary.
9. Rinse the inside of the amber wide-mouth HDPE sample bottle with stream water – fill bottle ~1/4 full, cap, and shake vigorously. Discard rinse water into stream or on stream 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).
    b. You can rinse all sample bottles for that stream at the same time and set aside, or rinse them separately.

10. Holding cobble/woody debris underwater, briefly rinse any leaves and/or large invertebrates from surface using stream 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 within your template, discard and choose a new cobble/piece of woody debris.
    b. If there is growth of aquatic plants or bryophytes that falls within your template, discard and choose a new cobble.
11. Place cobble/woody debris right-side up (the side exposed to the sun) in white tray and pour any excess water out of the tray.
12. Place white slide template on top of cobble/woody debris (surface that was exposed to light at the stream bottom; Figure 17). Check cobble/woody debris again for colonization of invertebrates, bryophytes, or plants.
13. Holding the template firmly in place on the cobble/woody debris, begin scrubbing inside the template (scrub gently if woody debris).
    a. If the substratum is colonized by a thick mat of algal material (e.g., *Didymosphenia geminata*), first scrape the inside of the template with a razor blade before scrubbing with a brush. Place scraped material in sample bottle with remainder of scrubbed sample.
    b. Use the wire-bristled brush for cobbles, use the toothbrush for woody debris.
    c. Be sure to hold the template in place, as slipping would change the area you are sampling (Figure 17).
    d. Scrubbing should be similar to brushing your teeth.

Figure 17. Template placement for epilithon protocol.
(Note: Samples should not be processed in direct sunlight.)

14. Periodically rinse the inside area of the template using the 125 mL wash bottle **while holding the template in place**. Allow water to run into the white tray – **DO NOT DISCARD** rinse-water.
15. Continue scrubbing until the inside of the template is clean (scrub cobble longer than woody debris).
16. Remove template. There should be a clean rectangle left on the substratum from scrubbing.
17. Using the wash bottle, rinse the template (front and back) and the cobble/woody debris.

18. Discard cobble/woody debris in the stream if you are finished collecting substrata at that location.
19. Repeat Steps 12-18 until all 3 cobbles/woody debris collected for the composite sample have been scrubbed and rinsed.
20. Rinse scrub brush and fingers over the tray. If there is any remaining water in the 125 mL wash bottle, dump this into the tray. All rinse-water should now be in the white tray and should total 125 mL, **do not discard as this is your sample**.
21. Discard cobble/woody debris.
    a. If you are finished at that location, you may discard the cobble/woody debris in the stream.
    b. If you are not done at that location, hold the cobble/woody debris on the bank and discard in stream when you leave the site.
22. Carefully swirl contents of tray (scrubbed material+ rinse-water) to re-suspend the sample.
23. Carefully pour the suspended sample and water into 125 mL amber wide-mouth HDPE bottle. Take care not to spill any of the sample.
    a. If you do spill a small amount (i.e., <10 mL) of the sample at this point, it is ok because this is a volume-based approach.
    b. If you spill a significant amount (i.e., >10 mL of sample), dump the entire sample and start over at Step 1.
24. There may be some sand left in the bottom of the tray after swirling and pouring out the sample. This is ok.
25. If you feel that your sample wasn't properly mixed and some has remained in the tray, you may pour the sample back in the tray and repeat Steps 22-24.
26. If you need more water to rinse with, refill the 125 mL wash bottle and use for rinsing. If you do this, be sure to use the entire 125 mL or measure using the 25 mL graduated cylinder, and record this change in volume on the sample label and on the field data sheet. **It is very important to keep track of any changes in sample volume.**
    a. Additional water will also require that you use a larger sample bottle.
27. 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 stream water to keep them cool. If submerged, be sure that they will not float downstream. Place bottles in the cooler upon returning to the car.
28. Fill out field data sheet in pencil (Figure 18).

NEON Aquatic Periphyton and Seston Collection
Wadeable Streams

Site (4-letter code): *ARIK* | **Recorded by:** *sparker*
Date (YYYYMMDD): *20140702* | **Collected by:** *kgoodman*
Local time (HH:MM): *0930* | **Sampling protocol & Rev:** *NEON.DOC.000691vB*

Location ID	Habitat	Sample type	Replicate	Sample ID	Habitat percent	Substratum size class	Sample volume (mL)
DS sensors	run	epiphyton	1	ARIK.20140702.epiphyton.1	75%	sand	250
DS sensors	run	epiphyton	2	ARIK.20140702.epiphyton.2	75%	sand	250
sensor reach	run	epiphyton	3	ARIK.20140702.epiphyton.3	75%	sand	250
US sensors	run	epiphyton	4	ARIK.20140702.epiphyton.4	75%	sand	250
US sensors	run	epiphyton	5	ARIK.20140702.epiphyton.5	75%	sand	250
DS sensors	pool	epipsammon	1	ARIK.20140702.epipsammon.1	20%	sand	NA
DS sensors	pool	epipsammon	2	ARIK.20140702.epipsammon.2	20%	sand	NA
US sensors	pool	epipsammon	3	ARIK.20140702.epipsammon.3	20%	sand	NA
sesnor reach	run	seston	1	ARIK.20140702.seston.1	NA	NA	4000

Figure 18. Example of field data sheet for periphyton sampling.

29. Rinse tray, brush, and template with stream water before starting next sample.
30. Repeat above steps until 5 composite samples (if dominant habitat) or 3 composite samples (if second-most dominant habitat) have been sampled.

C.2 Epiphyton (Aquatic Plant Surfaces)

1. Label three (if this is the second-most dominant habitat) or five (if this is the dominant habitat) all-weather paper labels (Figure 14, RD[05]).
2. Select plants for sampling that are well-colonized with epiphytes (Figure 19) and that meet the following requirements:
   a. The plant species should be common (i.e., accounts for >50% of the aquatic plants) in the reach.
   b. Plants should be exposed to ambient light (e.g., not under a log or cut bank).
   c. Plants should not be covered by sediments.
   d. Have not been recently disturbed or trampled.
   e. Do not take all of the samples from the same location. Collect samples from several different runs/riffles/pools (if possible) or from different sections of the same run/riffle/pool.

Figure 19. Example of epiphytes growing on rushes in a Colorado stream.

3. Select a 10 x 10 cm area of stream bottom to sample where plants are rooted.
    a. NOTE: If plant cover is thick, plants may be growing across the area and not necessarily rooted. Collect all rooted material within the selected quadrat.
    b. Use a metric ruler to estimate sample area.
    c. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 18; RD[05]).
    d. Record the distance downstream from the STREON dripper if sampling at a STREON site (RD[05]).
4. Cut all plants within the 10 x 10 cm area at their bases using scissors or clippers and carefully place them inside a Whirl-pak® bag 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. Do not add water to the Whirl-pak®.
5. Close Whirl-pak® bag and place in a cool, dark location (e.g., cooler).
    a. Close the Whirl-pak® by holding the wire tabs at either side of the bag (Figure 20), 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.

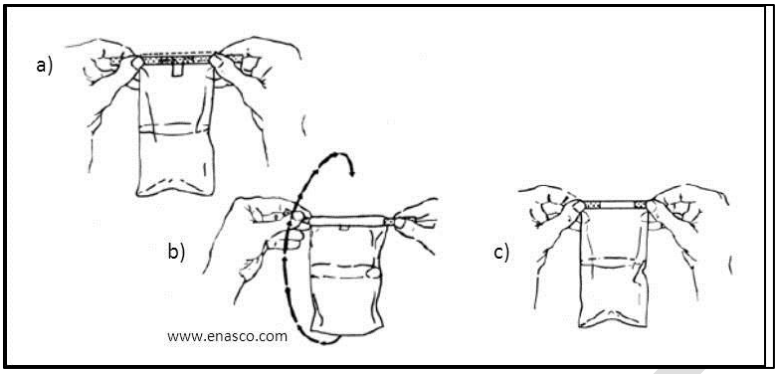

Figure 20. How to close a Whirl-pak® bag: a) hold the wire tabs; b) whirl the bag 3 complete revolutions (or fold the top over); and c) fold the wire ends over to close.

6. Fill out field data sheet in pencil (Figure 18).
7. Repeat above steps until 5 (if this is the dominant habitat) or 3 (if this is the second-most dominant habitat) samples have been collected.
8. Place samples in a cool, dark location (i.e., cooler).
9. Return samples to the Domain Support Facility for further processing within 24 hours (SOP D).

C.3 Epipsammon (Sand) and Epipelon (Silt)

1. Label (2”x4” all-weather adhesive labels) three (if this is the second-most dominant habitat) or five (if this is the dominant habitat) 250 mL amber HDPE (Figure 13).
   a. Write in permanent marker on all-weather adhesive labels.
   b. Adhere labels to bottles before bottles get wet.
   c. **NOTE:** Sample bottles may be rinsed with DI and reused from the last sampling trip.
2. Choose sampling locations with relatively shallow (<1 m) water that appear to be historically wetted (i.e., are usually underwater). Avoid areas that have been recently dried (e.g., extremely shallow areas).
3. Select locations to sample that meet the following requirements [after Richest Targeted Habitat (RTH) requirements; Porter et al. 1993 and Moulton et al. 2002]:
   a. Representative of the epipsammon/epipelon habitat and periphyton cover of the reach (i.e., not extremely dense or extremely sparse periphyton cover).
   b. Exposed to ambient light (e.g., not under a log or under a cut bank).
   c. Avoid areas that are heavily colonized with aquatic plants, invertebrates, or have leaf litter covering the surface.
   d. Have been recently disturbed (e.g., stepped on or sampled for another protocol).
   e. Do not take all of the samples from the same location. Collect samples from several different runs/riffles/pools (if possible), or from different sections of the same run/riffle/pool.
4. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 18; RD[05]).

a. Record the distance downstream from the STREON dripper if sampling at a STREON site (RD[05]).

5. Rinse the inside of the 125 mL wash bottle three times with stream water. Discard water into the stream away from where you intend to sample so as not to disturb the sediments.
   a. You only need to rinse the wash bottle if this is your first sample for the day at that stream.
6. 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).
7. Rinse white plastic sampling tray with stream water.
8. Rinse the lid of a 47 mm plastic petri dish in stream water. Holding lid upside-down underwater, rub the inside of the lid with your fingers to remove air bubbles.
9. 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.
10. Slide spatula under lid to enclose the sample. Holding the petri lid tightly to the spatula, lift out of water. Make sure that water and sediment do not leak out. Gently rinse excess silt not enclosed by petri lid from spatula with stream water in a wash bottle. Do not count this rinse water in the final sample volume.
    a. If sample leaks out of the petri lid when lifting the spatula out of the water, discard and resample.
11. Place spatula + sample + petri lid in white sampling tray and invert lid.
12. Repeat Steps 9-11 until you have collected 3 petri lid samples to combine into one composite sample.
13. Fill 125 mL wash bottle with stream 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. If additional water is necessary for rinsing, use 25 mL graduated cylinder to add in increments. Keep track of volume used on field data sheet.
    c. Depending on the productivity of the site, >125 mL may be necessary to meet the external lab filtering requirements. See Table 11 for recommended filter volume and adjust water volume and amber HDPE bottle size as necessary.
14. Using the 125 mL wash bottle, rinse petri lid and spatula into tray. **DO NOT DISCARD RINSE WATER.**
15. 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.

16. 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 stream water to keep them cool. If submerged, be sure that they will not float downstream. Place bottles in the cooler upon returning to the car.
17. Fill out field data sheet in pencil (Figure 18).
18. Rinse tray, petri dish, and spatula with stream water before starting next sample.
19. Repeat above steps until 5 (if this is the dominant habitat) or 3 (if this is the second-most dominant habitat) composite samples have been collected.

C.4 Seston (Phytoplankton, Water Column)

1. Label four 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 13).
    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 18; RD[05]).
    b. Record the distance downstream from the STREON dripper if sampling at a STREON site (RD[05]).
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 second and third bottles according to Steps 5-7.
9. Fill out field data sheet in pencil (Figure 18).
10. Double check label and place sample bottles in cooler with ice packs (do not freeze).

C.5 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.6 Ending the Sampling Day

1. Refreshing the sampling kit
    a. Remove old labels from HDPE bottles that will be reused.

b. Print and fill out new adhesive labels (RD[05]). Attach labels to bottles before going out in the field.

2. Equipment, Maintenance, Cleaning and Storage
   a. Wash all equipment that has come in contact with stream water according to the NEON Aquatic Decontamination Protocol (RD[08]).
   b. Rinse amber HDPE bottles with DI, these will be rinsed again in the field during the next field collection. A set of HDPE bottles should be dedicated to each stream and do not need to be decontaminated between bouts.
   c. Dry all items (except amber HDPE bottles) completely between sites and before storage.
   d. Discard and replace any broken templates, petri dishes, or worn brushes.

SOP D Laboratory Sampling and Analysis

At the Domain Support Facility, periphyton and seston 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 Sample Processing Timing

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

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

D.2 Preparation

1. Pre-ash GF/F filters:
   a. Place layers of 25 mm GF/F filters on aluminum foil using filter forceps or while wearing nitrile gloves. Use multiple layers of foil if needed, filters can be touching and placed on top of one another but should not be stacked more than 3 filters deep.
   b. Place in muffle furnace (500 °C) for 6 hours.
   c. After 6 hours, remove from furnace, stack filters using filter forceps, and place in original box.
   d. Label box with permanent marker to read "ASHED, Your Name, Date".
   e. Place box in in sealed zip-top bag.
   f. Ashed filter may be stored indefinitely, as long as they remain in the box and stay dry.
2. Print 1"x2" adhesive labels for 60 mL bottles, chlorophyll *a*/pheophytin filters, and nutrient filters, to be attached to the outside of vials and/or aluminum foil packets (Figure 21, RD[05]).

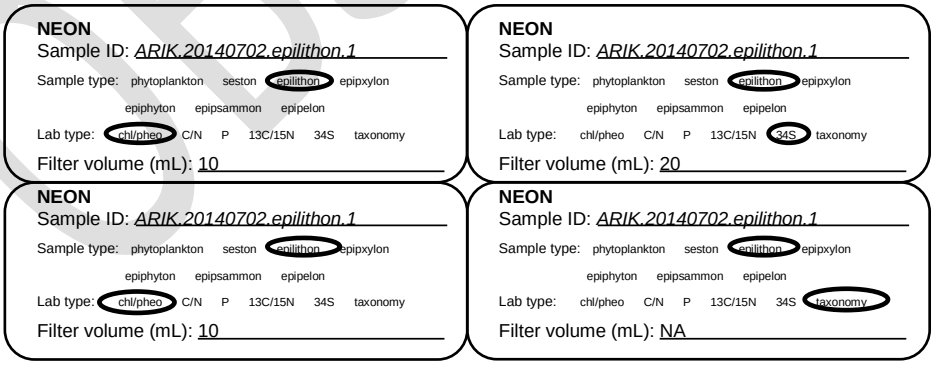

Figure 21. Example adhesive labels (1" x 2") for periphyton lab samples.

3. If aluminum weigh boats are new, label ~20 boats by inscribing a unique number on the bottom with a pencil (Figure 22). Place boats in the muffle furnace (500 °C) for 6 hours. After 6 hours, carefully remove boats from the furnace using thermal gloves and tongs, and allow to cool.
   a. This may be done in advance, before field sampling.

b. Boats may be reused from previous sampling bouts.

Figure 22. Examples of newly labeled aluminum weigh boats.

D.3 Processing Samples

Samples will be subsampled and filtered in the Domain Support Facility following Figure 23 and Table 11.

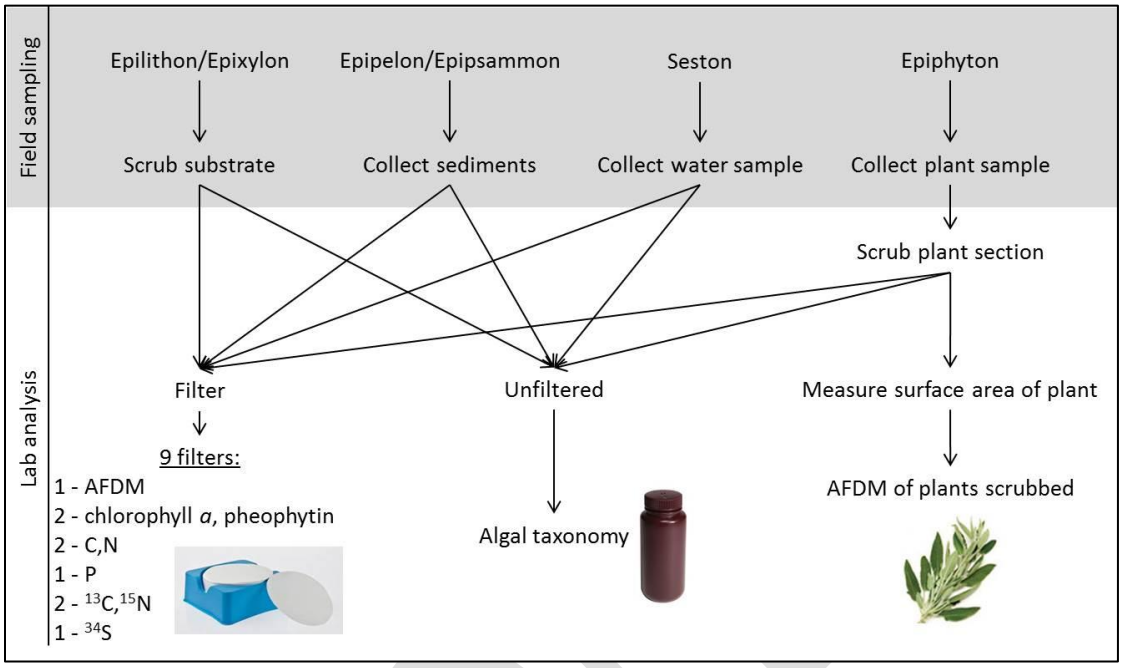

Figure 23. Sampling schematic diagram.

Table 11. Filtering amounts for chemistry. 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. Follow priority column if not enough sample is available to collect all filter types requested.

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

D.4 Epiphyte Samples

1. If there are no epiphyte samples, skip this section and proceed to Section D.5.
2. **Day 1**: Using forceps, remove the plant sample from Whirl-pak®. Place in clean, dry white plastic sample tray (used in field protocol).
3. Fill 125 mL wash bottle to fill line with DI water.
    a. Make sure that bottom of the meniscus lines up with the "fill line".
    b. Since this is a volume-based sampling technique, it is important to keep the sample volume consistent and record the volume of water used.
    c. Depending on the productivity of the site, >125 mL may be necessary to meet the external lab filtering requirements. See Table 11 for recommended filter volume and adjust water volume and HDPE bottle volume as necessary.
4. Gently scrub surface of sample with a toothbrush. You may have collected leaves and/or stalks, scrub these gently. Take care not to lyse plant cells, these would bias the chlorophyll results.
5. Use DI water periodically to rinse the scrubbed material into the tray.
6. When scrubbing is finished, rinse scrubbed plant and toothbrush thoroughly into the white tray.
7. Remove plant sample and measure the approximate dimensions of the surface area scrubbed (e.g., stalk length and/or leaf length + width, top + bottom) and record the sum in the NOTES section of the lab data sheet.
8. Place plant material in a labeled paper lunch bag.
9. Pour remainder (if any) of 125 mL wash bottle into white tray.
10. Carefully pour the scrubbed material into a 125 mL amber wide-mouth HDPE sample bottle. Proceed to Filtering Protocol, Section D.5.
11. 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[11]).
    a. Use TOS "Lab Drying QC Datasheet" in Measurement of Herbaceous Biomass datasheets (RD[12]).
12. **Day 2**: When dry, remove all bags+samples from drying oven and let cool to room temperature in a plastic bag or desiccator.
    a. Placing samples in a bag or desiccator is important because samples absorb water quickly from the air as they cool. Samples may be left in desiccator or plastic bags for up to 30 days before proceeding to the next step.
13. Place a clean, plastic weigh boat (small sample) or tray (large sample) on analytical balance. Tare (zero) balance.
14. Place dry sample in the plastic weigh boat/tray and record as **Epiphyton: total plant dry weight** on Lab Data Sheet (Figure 25, RD[05]).
    a. Sample may be crushed or broken up to fit into weigh boat.
15. Place sample in a clean, pre-labeled aluminum weigh boat.
    a. 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.

16. Weigh boat on analytical place balance, and record as **Dry weight + boat** on Lab Data Sheet.
17. Repeat above steps until all specimens have been processed.
18. Place aluminum boats + specimens in the muffle furnace using oven gloves and tongs. **TAKE CARE NOT TO BURN YOURSELF!**
    a. Boats may be stacked on top of each other as long as there is space for air flow between them.
    b. Place boats on an approved muffle furnace pan (if available) before placing in the furnace. This makes it easier and safer to handle samples.
19. 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.
20. **Day 3**: After 6+ hours, remove aluminum boats carefully from the muffle furnace using oven gloves and crucible tongs. Place on trivet or metal cart to cool.
21. Cover pans/aluminum boats to prevent ash from blowing out of boats and set aside to cool on a heat-resistant in an area without drafts from windows, doors, or HVAC ventilation.
22. 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.
23. After cooling to room temperature, weigh boats again on analytical balance, record as **Ash Weight** on Lab Data Sheet.
24. After weighing, dump ash into the trash and clean the boat with a soft brush or paper towel.
25. 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 24).
    a. Technicians may use the hand vacuum pump (Figure 24a) with one filter flask and funnel attached, or the filter manifold and electric pump (Figure 24b) 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.

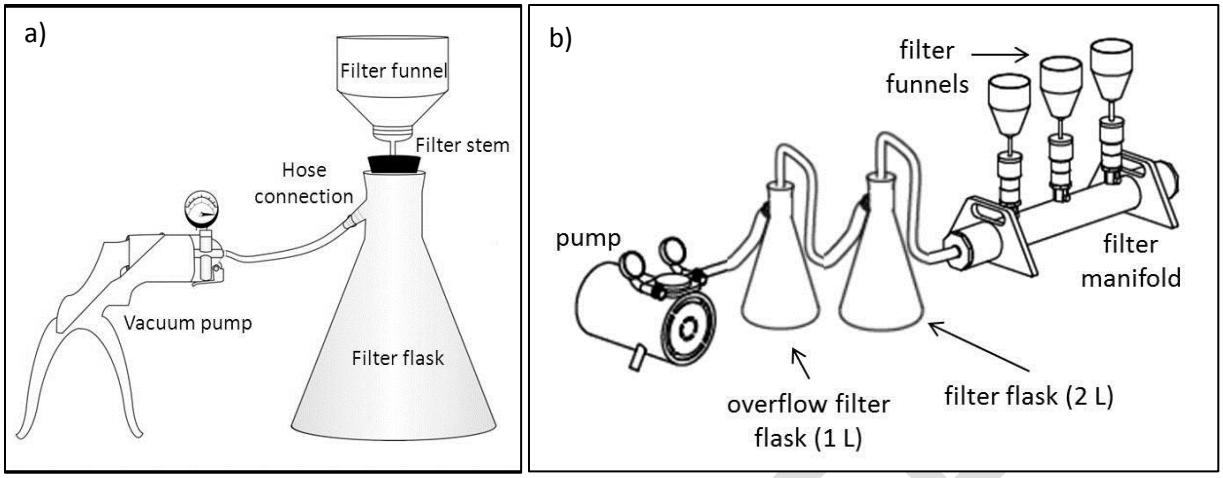

Figure 24. Filter apparatus setup: a) hand pump; b) filter manifold.

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 24), replace top of funnel.
4. Shake sample bottle vigorously for ~30 seconds to mix sample.
    a. If sample has large chunks of algae, homogenize using clean, hand-held battery operate stirrer for ~30 seconds. Take care that samples does not spill over the top of the sample bottle while stirring.
        1) Rinse stirrer thoroughly with DI between samples.
5. Filter known volume of sample.
    a. **Seston samples**: Measure using a graduated cylinder. Pour sample slowly in ≤100 mL increments into filter funnel.
        1) Do not pour more sample into funnel than will pass through the filter. All water in the filter funnel must pass through the filter, if filter becomes clogged while there is still sample water in the funnel, discard sample and filter and start again.
    b. **Epipsammon/epipelon samples**: Carefully pour entire sample (including sediment) into 250 mL graduated cylinder to measure the total volume of sample. Record sample volume on Lab Data Sheet.
        1) You may pipette directly from the graduated cylinder for the following steps, or pour the contents back into the sample bottle to facilitate sample mixing.
    c. **All non-seston samples**: Using a clean pipette tip, carefully pipette the desired volume into the filter funnel.
        1) Do not aim pipette tip directly at the filter, aim at the side of the funnel. Take care not to puncture filter.
        2) Change pipette tip between samples.

    d. **Keep track of the volume of sample filtered on the Lab Data Sheet (RD[05])**.
    e. If sample is too thick for the pipette tips, you may use a graduated cylinder or cut the end of the pipette tip and test that the volume is still acurate using a graduated cylinder.

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[05]).**
    b. Filter the minimum amount suggested in Table 11. If unable to filter the minimum amount, filter as much sample as possible and record volume.
    c. Periodically discard the water in the filter flasks. If the flasks overflow, they will back up into the pump and potentially cause damage.
8. Rinse inside walls of funnel using wash bottle of DI.
    a. Do not include DI rinse water in the volume of sample filtered.
9. Continue to draw suction on the filter until there is no water left in the funnel and there is no excess water on top of the filter.
10. Remove top of filter funnel, release suction using the release valve on the hand pump or the valves below the filter funnel on the manifold.
11. Carefully remove the filter from the stem using filter forceps (forceps with flat ends). Take care not to touch the filter with your fingers.
    a. **AFDM filters**: Place filter in a labeled aluminum weigh boat, record sample information on Lab Data Sheet (RD[05]) and proceed to Section D.7.
    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 21).
12. Repeat above steps until you have 9 filters total from the same sample.
    a. 1 AFDM filter (proceed to Section D.7)
    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 from one site inside a resealable bag. Using a permanent marker, label the outside of the resealable bag with Domain, Site, Date, and the "lab type" (i.e., chl/pheo, C/N, P, 13C/15N, or 34S).
    a. Place all filters in -20 ^{o}C freezer.
14. Cap amber sample bottle and proceed to Section D.6.

D.6 Algal Taxonomy Samples (Unfiltered)

1. Label 60 mL (periphyton) or 1 L (seston) HDPE bottles with 1"x2" adhesive labels (Figure 21, RD[05]). "lab type" is **taxonomy**.
2. Cap and shake amber HDPE sample bottle for 30 seconds to mix sample evenly.
3. Pipette 60 mL periphyton into the appropriately labeled 60 mL bottle, or measure 1 L seston 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.9.

D.7 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[05]).
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 tray (if available) before placing in the furnace. This makes it easier and safer to handle samples.
6. Leave samples in the muffle furnace at 500 ^{o}C for at least 6 hours.
    a. Sample may be left in furnace for longer than 6 hours (e.g., overnight) if necessary.
7. After 6+ hours, remove boats carefully from the muffle furnace using oven gloves and crucible tongs.
8. Cover pans/aluminum boats to prevent ash from blowing out of boats and aside to cool on a heat-resistant in an area without drafts from windows, doors, or HVAC ventilation.
9. When boats have cooled to room temperature, place in desiccator.
    a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
10. After cooling to room temperature, weigh boats again on analytical balance (0.0001 g) and record as **Ash weight** on Lab Data Sheet.

11. After weighing, discard ashed filters into the trash and clean the boat with a soft brush or paper towel.
12. Set clean boats aside to be used again.

NEON Aquatics Periphyton/Seston/Phytoplankton Lab Data Sheet

Site (4-letter code): *ARIK* | **Date analysis finished (YYYYMMDD):** *20140705*

Date collected (YYYYMMDD): *20140702* | **Recorded by:** *sparker*

Date analysis started (YYYYMMDD): *20140703* | **Sampling protocol & Rev.:** *NEON.DOC.00391vB*

PERIODICALLY CHECK THAT BALANCE IS ZEROED!

		from field label						
Sample type	**Lab type**	**Sample ID**	**Sample volume (mL)**	**Volume filtered (mL)**	**Epiphyton: total plant dry weight (g)**	**Boat ID**	**Dry weight + boat (g)**	**Ash weight (g)**
epipsammon	*AFDM*	*ARIK.20140702.epipsammon.1*	*250*	*10*	*NA*	*B1*	*2.1341*	*2.5460*
epipsammon	*chl/pheo*	*ARIK.20140702.epipsammon.1*	*250*	*10*	*NA*	*NA*		
epipsammon	*chl/pheo*	*ARIK.20140702.epipsammon.1*	*250*	*10*	*NA*	*NA*		
epipsammon	*CN*	*ARIK.20140702.epipsammon.1*	*250*	*10*	*NA*	*NA*		
epipsammon	*CN*	*ARIK.20140702.epipsammon.1*	*250*	*10*	*NA*	*NA*		
epipsammon	*P*	*ARIK.20140702.epipsammon.1*	*250*	*10*	*NA*	*NA*		
epipsammon	*13C15N*	*ARIK.20140702.epipsammon.1*	*250*	*10*	*NA*	*NA*		
epipsammon	*13C15N*	*ARIK.20140702.epipsammon.1*	*250*	*10*	*NA*	*NA*		
epipsammon	*34S*	*ARIK.20140702.epipsammon.1*	*250*	*10*	*NA*	*NA*		

Figure 25. Example of lab data sheet for seston and periphyton samples.

D.8 Sample Disposal

1. Double check that all components have been completed. You should have the following products for each field sample:
   a. 1 AFDM filter (processed at Domain Support Facility)
   b. 2 chlorophyll/pheophytin filters
   c. 2 C,N filters
   d. 1 P filter
   e. 2 δ ^{13}C, ^{15}N isotope filters
   f. 1 δ ^{34}S isotope filter
   g. 60 mL periphyton or 1 L seston sample for taxonomy lab
2. After all components have been filtered and bottled, you may discard the remaining sample material from the field sample bottles.
3. Rinse the field sample bottles with DI water, discard rinse water and recap bottles to be reused.
   a. Bottles may be dried or recapped and stored while still damp inside.

D.9 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 ^{o}C up to 30 days until shipping. Record type and volume of preservative used on the AOS Sample Shipping Inventory (RD[13]).
2. **Seston 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[13]).

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.10 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.

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.

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 CLA's NEON intranet site.

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

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.

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 AD[03].

F.3 Handling Hazardous Material

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

F.4 Supplies/Containers and Conditions

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

F.5 Timelines

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

F.6 Grouping/Splitting Samples

Group samples by site per bout.

F.7 Return of Materials or Containers

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

F.8 Shipping Inventory

Shipments are to have a hardcopy 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 CLA shipping document on CLA's NEON intranet site.

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 12. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002199	Datasheets for AOS Protocol and Procedure: Periphyton and Seston Sampling in Wadeable Streams
NEON.DOC.001646	General AQU Field Metadata Sheet

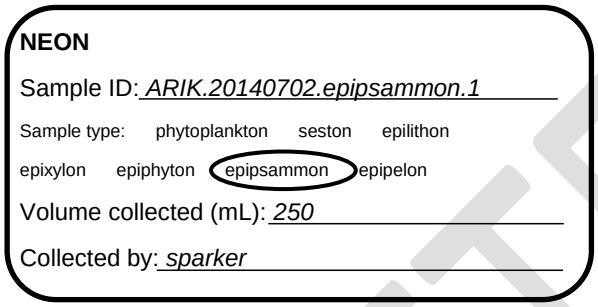
These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

B.1 Steps for Periphyton and Seston Sampling

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

Step 2 – Prepare labels (2" * 4").



Step 3 – Ensure the General AQU Field Metadata Sheet (RD[06]) is completed per field site visit.

Step 4 – Determine habitat sampling locations from the Stream Morphology Map (RD[10]).

Step 5 – Determine sampler type based on the habitats present and the order of preference for sampling substratum.

Step 6 – Collect samples: 5 per dominant habitat type, 3 per second-most dominant habitat type.

Step 7 – Take care to note volume of water used for rinsing substrata in the field.

Step 8 – 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 9 – 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 10 – 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 11 – Weigh dried AFDM filters, place in muffle furnace (lab day 2).

Step 12 – Weigh ashed AFDM filters (lab day 3).

Step 13 – For each field sample you should have the following products:

1. 1 AFDM filter (processed at Domain Support Facility)
2. 2 chlorophyll/pheophytin filters
3. 2 C,N filters
4. 1 P filter
5. 2 δ ^{13}C, ^{15}N isotope filters

6. 1 δ ^{34}S isotope filter
7. 60 mL periphyton or 1 L seston sample for taxonomy lab

B.2 Order of Preference for Sampling Substrata

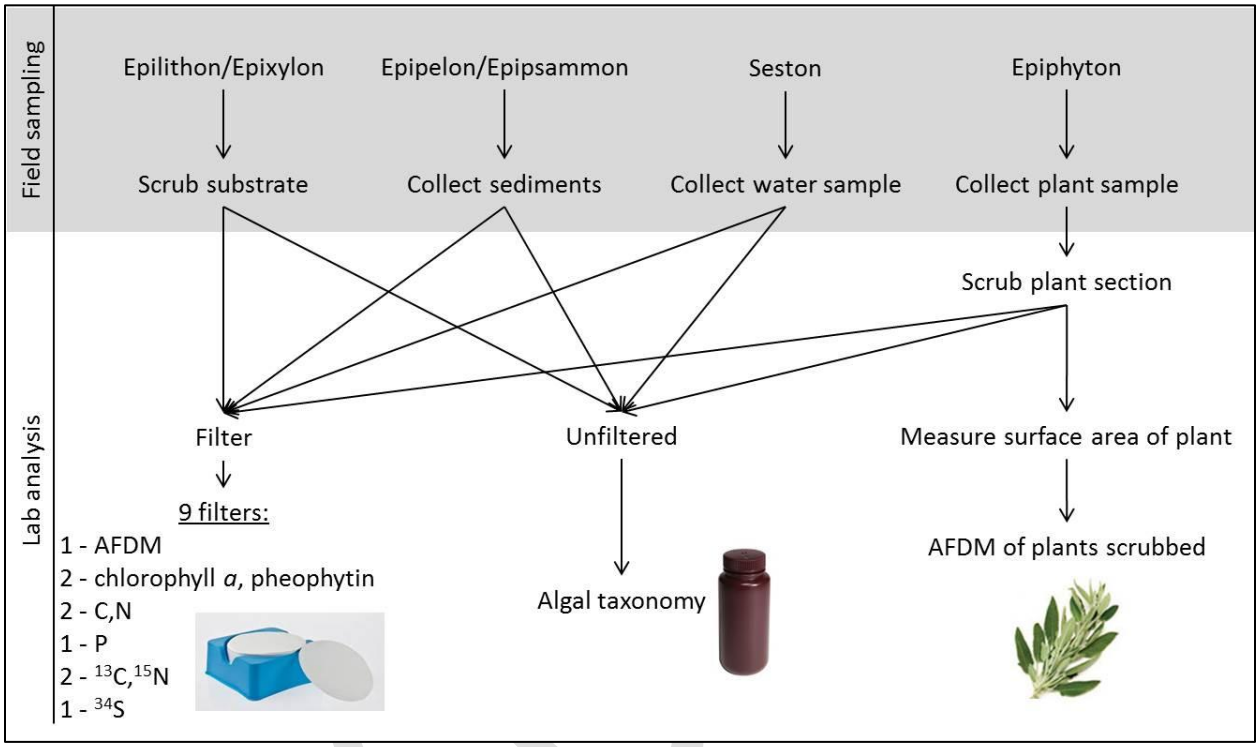
1. Epilithon (rock substrata)
2. Epixylon (wood substrata)
3. Epiphyton (plant substrata)
4. Epipsammon (sand substrata)
5. Epipelon (silt substrata)

B.3 Determining Habitat to Sample

1. Determine percent cover of habitat types throughout the sampling reach using the Stream Morphology Map (RD[10]) and go to Step 2 (see Appendix E for habitat and substrata sampling suggestions at NEON sites).
    a. Targeted habitat types (see Definitions, Section 0):
        1) Riffle
        2) Run
        3) Pool
2. Does habitat type account for >20% of the reach?
    a. If YES, go to Step 3.
    b. If NO, ignore this habitat type.
3. Determine dominant substratum type in habitat (i.e., highest percent cover of habitat and/or where visible algal community is attached) and secondary habitat (i.e., second-highest percent cover and/or second-most heavily colonized) and proceed to Step 4.
    a. Targeted substratum types, in order of sampling preference (see Definitions, Section 0):
        1) Cobble (epilithon)
        2) Woody snag (epixylon)
        3) Plant surface (epiphyton)
        4) Sand (epipsammon)
        5) Silt (epipelon)
    b. Dominant habitat/substratum type = 5 replicate samples
    c. Secondary habitat/substratum type = 3 replicate samples
4. Is dominant/secondary substratum cobble?
    a. If YES, follow sampling procedure for epilithon (Section C.1).
    b. If NO got to Step 5.
5. Is dominant/secondary substratum sand or silt?
    a. If YES, follow sampling procedure for epipsammon and epipelon (Section C.3).
    b. If NO, go to Step 6.
6. Is dominant/secondary substratum type woody debris?
    a. If YES, follow sampling procedure for epixylon (Section C.1).
    b. If NO, go to Step 7.

7. Is dominant/secondary substratum type aquatic plants?
   a. If YES, follow sampling procedure for epiphyton (Section C.2).
   b. If NO, return to Step 3 and reassess substratum types.

B.4 Sampling Schematic Diagram



B.5 Filtering Amounts for Chemistry

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

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

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

Sample collection: Be sure to…

- ☑ Determine the dominant habitat and second-most dominant habitat based on the Stream Morphology Map (RD[10])
- ☑ Choose the appropriate sampler.
- ☑ 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.
- ☑ 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 in the reach, 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 in wadeable streams. Also see the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

Domain	Site	Bout 1	Bout 2	Bout 3
D01	West Branch Bigelow Brook*	11Apr-9May	9Jul-6Aug	3Oct-31Oct
D01	Sawmill Brook	8Apr-6May	9Jul-6Aug	9Oct-6Nov
D02	Mill Run*	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D02	Posey Creek	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D04	Rio Guilarte	26Jan-23Feb	21Jun-19Jul	9Nov-7Dec
D04	Rio Cupeyes	24Jan-21Feb	21Jun-19Jul	10Nov-8Dec
D05	Pickerel Creek*	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D06	Kings Creek	23Mar-20Apr	3Jul-31Jul	3Oct-31Oct
D07	Leconte Creek	15Mar-12Apr	30Jun-28Jul	12Oct-9Nov
D07	Walker Branch	9Mar-6Apr	1Jul-29Jul	19Oct-16Nov
D08	Mayfield Creek	5Mar-2Apr	29Jun-27Jul	31Oct-28Nov
D10	Arikaree River	21Mar-18Apr	4Jul-1Aug	20Sep-18Oct
D11	Pringle Creek	17Feb-17Mar	29Jun-27Jul	23Oct-20Nov
D12	Bozeman Creek	11Apr-9May	7Jul-4Aug	6Sep-4Oct
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	Planting 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
D19	Caribou Creek	2May-30May	26Jun-24Jul	18Aug-15Sep

*soft sites as of November 2014

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

For more information see the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

Domain	Site	Habitat 1	Habitat 2
D01	West Branch Bigelow Brook	Pools (epipsammon)	Pools (epixylon)
D01	Sawmill Brook	*	*
D02	*Site to be determined*	*	*
D02	Posey Creek	Riffles (epilithon)	Pools (epipelon)
D04	Rio Guilarte	Riffles (epilithon)	*
D04	Rio Cupeyes	Riffles (epilithon)	*
D05	*Site to be determined*	*	*
D06	Kings Creek	*	*
D06	McDowell Creek	*	*
D07	Leconte Creek	*	*
D07	Walker Branch	*	*
D08	Mayfield Creek	Runs (epixylon)	Pools (epipsammon)
D10	Arikaree River	Runs (epiphyton)	Runs (epipsammon)
D11	Pringle Creek	*	*
D12	Bozeman Creek	Riffles (epilithon)	Riffles/Runs (epixylon)
D12	Blacktail Deer Creek	Riffles (epilithon)	Riffles/Runs (epixylon)
D13	Como Creek	Riffles (epilithon)	Pools (epipsammon)
D13	*Site to be determined*	*	*
D14	Sycamore Creek	*	*
D15	Red Butte Creek	Riffles (epilithon)	*
D16	McRae Creek	Riffles (epilithon)	*
D16	Planting Creek	Riffles (epilithon)	*
D17	*Site to be determined*	*	*
D17	*Site to be determined*	*	*
D18	Oksrukuyik Creek	Riffles (epilithon)	Pools (epipelon)
D19	Caribou Creek	Riffles (epilithon)	*