AOS PROTOCOL AND PROCEDURE: AQUATIC PLANT, BRYOPHYTE, LICHEN, AND MACROALGAE SAMPLING IN WADEABLE STREAMS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	10/31/2012	ECO-00680	Draft release
B	02/07/2014	ECO-01091	Initial production release
C	08/29/2014	ECO-02210	Minor updates based on feedback from the field
D	01/09/2015	ECO-02621	Migration to new protocol template
E	06/05/2015	ECO-02724	Minor updates to equipment, shipping, labeling and sample processing, addition of sampling contingencies, STREON reach scale sampling, and sampling dates to appendix.
F-OBSOLETE	01/22/2016	ECO-03470	OBSOLETE and superseded by NEON.DOC.003039 AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling

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1 OVERVIEW

1.1 Background

Aquatic plants, bryophytes, lichens, and macroalgae are primary producers common in streams. They, along with algae and microbes, form the autochthonous (i.e., originating within the stream) base of the food web. Additionally, aquatic plants and bryophytes add complexity to the stream or pond bottom, and, when abundant, strongly affect ecosystem structure and function (Bowden et al. 2006, Stream Bryophyte Group 1999). Aquatic plants can alter water velocity and current, take up nutrients, settle sediments, stabilize the stream bed, provide substratum for algal epiphytes, and provide shelter and food for macroinvertebrates and fish (Figure 1). High densities of aquatic plants and bryophytes can substantially increase the abundance and influence the community structure of local fauna (e.g., aquatic invertebrates).

Figure 1. Aquatic plants add structure and colonizable area to the stream bottom.

Environmental factors such as flooding and scouring, water level, light, and nutrient availability have strong effects on the aquatic plant community. Light quantity and quality, current velocity, and flow regime are the most important environmental factors affecting aquatic plants and bryophytes. Aquatic plants are typically unable to survive in habitat patches with high current velocity, as fast water may erode the substratum and break plant stems. Studies have shown that the threshold velocity that prohibits aquatic plant and bryophyte colonization in flowing water is 0.9-1.0 m s^{-1} (Bowden et al. 2006).

As a key part of the aquatic ecosystem, it is important to sample primary producers such as aquatic plants, bryophytes, lichens, and macroalgae to determine changes in community structure, abundance, and biodiversity over time. Invasive species are an increasing threat in many aquatic environments and are typically spread among watersheds by people. Common invasive aquatic flora across the continental U.S. include blooms of the diatom *Didymosphenia geminata* and aquatic plants such as purple loosestrife (*Lythrum salicaria*), Eurasian water milfoil (*Myriophyllum spicatum*), water chestnut (*Trapa natans*), and hydrilla (*Hydrilla verticillata*) (www.invasivespeciesinfo.gov/aquatics, USDA 2011). By sampling primary producers, NEON data products can be used to determine whether or not invasive taxa are spreading into NEON Aquatic sites and to investigate potential effects that invasive species have on native aquatic flora.

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, which are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

Thanks to Dave Barnett of the NEON FSU team for assistance with plant pressing lab methods. Protocols for point transect and quadrat sampling are based on those set forth in Bowden et al. 2006. Methods for aquatic plant preservation and pressing are based on the sampling protocols suggested by the State of Washington Department of Ecology (www.ecy.wa.gov).

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.002196	Datasheets for AOS Protocol and Procedure: Aquatic Plant, Bryophyte,

		Lichen, and Macroalgae 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.001153	AOS Protocol and Procedure: Wadeable Stream Morphology Mapping
RD[10]	NEON.DOC.000691	AOS Protocol and Procedure: Periphyton and Seston Sampling in Wadeable Streams
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.000691	AOS Protocol and Procedure: Periphyton and Seston 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
AOS	Aquatic Observation System
^{o}C	degrees Celsius
cm	Centimeter
CPOM	coarse particulate organic matter
CWD	coarse woody debris
DI	deionized water
FPOM	fine particulate organic matter
GPS	Global positioning system
HDPE	High-density polyethylene
m	Meter
mL	Milliliter
mm	Millimeter
$m\ s^{-1}$	meters per second
oz	Ounce

2.4 Definitions

Aquatic plant: "Large" vascular plants with root system living in freshwater bodies of water. Aquatic plants are classified based on the following life forms:

1. **Emergent**: Growing above the water's surface (Figure 2)

Figure 2. Emergent aquatic plants: Rooted in the sediments, stems and leaves protrude above water surface

2. **Floating-leaved**: Permanently submerged plants, rooted at the bottom but producing leaves that float on the water's surface (Figure 3).

Figure 3. Floating-leaved aquatic plants: Rooted in the sediments, leaves float on water surface

3. **Submerged**: Plants entirely underwater (Figure 4).

Figure 4. Submerged vegetation: Neither stems nor leaves break the water surface, however some flowering structures (if present) may break the water surface

4. **Free-floating**: Not attached to substratum (Figure 5).

Figure 5. Free-floating aquatic plants: leaves float on water surface, but plant is not rooted in the sediments

Autochthonous: Originating within the lake/river where found.

Bryophyte: Nonvascular plant, including mosses, liverworts, and hornworts (Figure 6). May often be submerged or in the "splash zone" (annual floodplain).

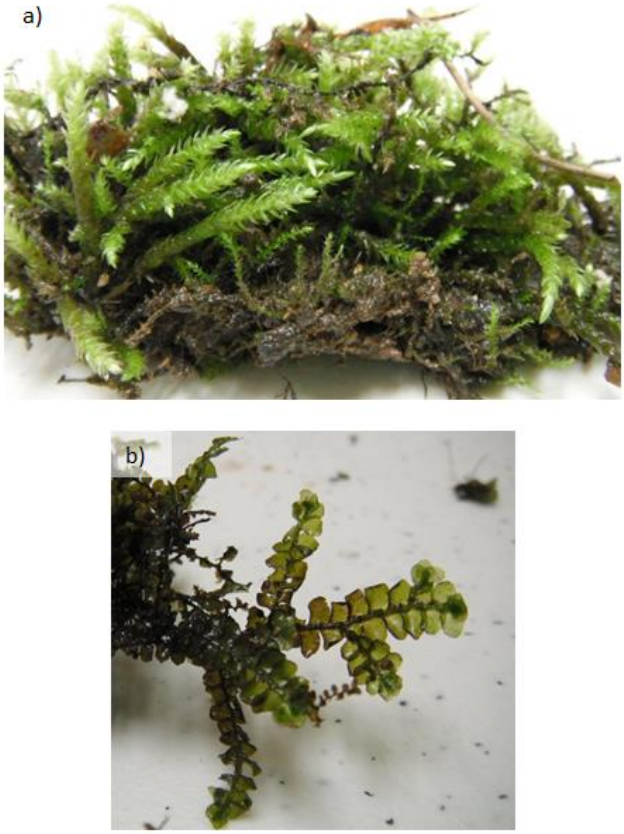

Figure 6. Bryophytes are a group of non-vascular plants including a) mosses and b) liverworts, that may be submerged in the stream or growing at the stream edge

Lichen: Symbiosis of an alga and a fungus, typically not submerged, but may be underwater for short periods of time during high water (Figure 7).

Figure 7. A crustose lichen growing on a rock near a stream channel

Macroalgae: "Large" algae. Multicellular, photosynthetic algae visible to the naked eye. In streams, these algae are typically filamentous (Figure 8).

Figure 8. Macroalgae are large algae that often form blooms in streams and lakes

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

3 METHOD

The goals of this protocol are: 1) to determine percent (%) cover of aquatic plants on the stream bottom; 2) to collect aquatic plant, bryophyte, and macroalgae samples for identification and biomass measurement; and 3) to identify lichens *in situ*. Taxa are identified *in situ* where possible using photo keys based on NEON Construction Voucher specimens. However, additional voucher specimens may be collected during this Operations Protocol if the field technician is unable to make a positive identification in the field. Small samples are collected and returned to the Domain Support Facility for processing and shipping to appropriate taxonomists (see SOP G). Voucher specimens are sampled using grab samples near the transect surveys (percent cover) and/or quadrat (biomass) sampling.

To track changes in the flora of the stream bottom (e.g., arrival of invasive species or the loss of or decline of native taxa), percent cover and biomass of macroalgae, aquatic plants, and bryophytes will be calculated over time. Percent cover is estimated using point transects, a method modified from the standard point-frame method in terrestrial ecosystems (Bowden et al. 2006). If the percent cover of a particular taxon decreases dramatically (to ~5-10% of the stream bottom), biomass sampling methods may be reassessed so as not to extirpate species from the stream.

We expect that field personnel will collect only new or unique taxa for voucher specimens at each site to prevent additional destructive sampling. It may be difficult for an untrained field technician (non-taxonomist) to differentiate among species of aquatic plants, bryophytes, and lichens in the field. Field technicians will receive appropriate training and be supplied with a photo key of previously collected specimens.

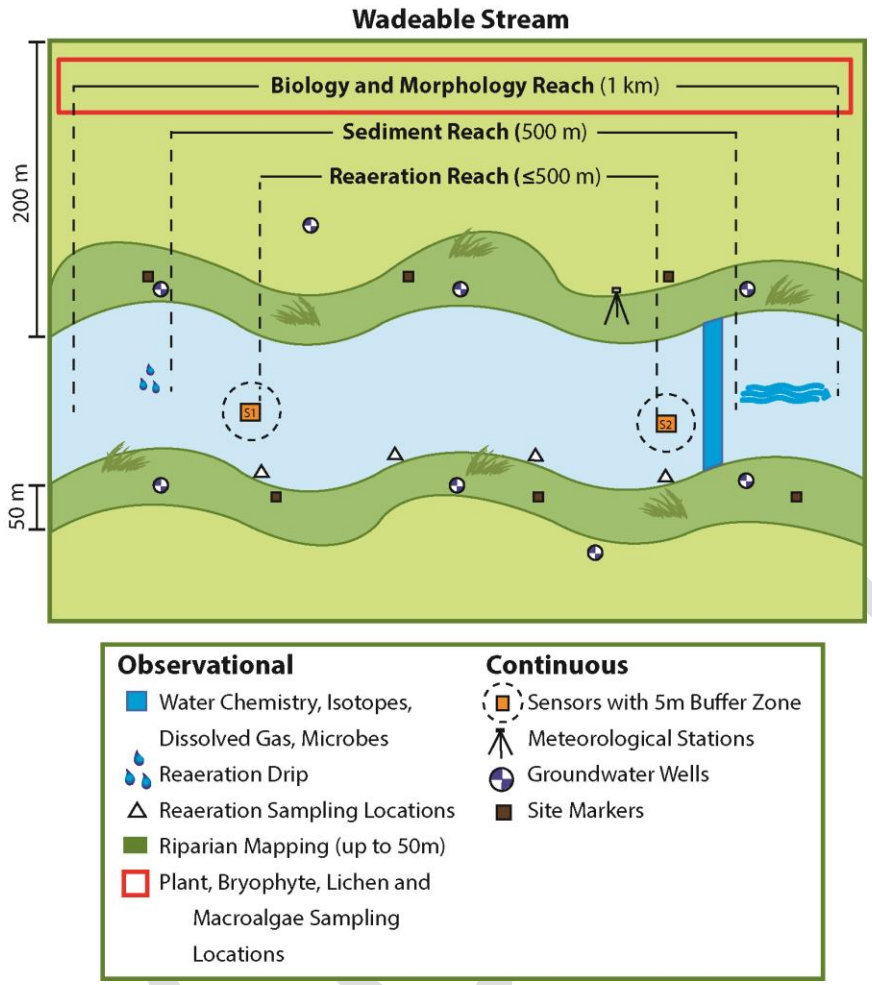

Figure 9. A generic wadeable stream site layout with aquatic plant, bryophyte, lichen and macroalgae sampling locations.

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 aquatic plant, bryophyte, lichen, and macroalgae sampling in wadeable streams occurs three times during the growing season at each site, roughly spring, summer, and autumn. Sampling must occur within the 1 month window specified in Appendix G 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 based on historical data including streamflow, the accumulation of degree days, weather, and riparian phenology (Appendix G).

4.3 Timing for Laboratory Processing and Analysis

All plant processing shall begin within 24-48 hours of field sampling. Samples should be shipped to the external lab or taxonomist within 30 days of sampling. For additional storage and shipping timelines see SOP G.

4.4 Sampling Timing Contingencies

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

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If circumstances occur that impede sampling (e.g., wildlife, weather), discard samples and start over the next day that conditions permit.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the 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 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.	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.
3 – 7 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 3-7 days after water level drops to near-baseflow conditions (within 25% of baseflow as determined by sensor data) to allow the invertebrate community to recolonize.	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. Small, headwater streams may be negatively affected by biomass harvest due to their small size and the relatively slow speed with which bryophytes, which tend to dominate communities at those sites, propagate. Reduced sampling may be conducted at these sites as determined on a site-by-site basis by the site host and the NEON Aquatic Ecologist.
2. Large amounts of plant biomass may be encountered at some sites (e.g., “wetland” habitat), necessitating larger sampling containers (e.g., trash bags, large coolers).

Table 2. Sampling specific contingent decisions

Delay/Situation	Action	Outcome for Data Products
If an endangered or threatened plant species is	**Do not collect**. Take photos and note location in stream using	Plants are more difficult to identify via photos, so accurate identification

discovered (based on the Endangered Species List, Appendix E)	handheld GPS. Submit a problem ticket to report findings to EHS.	may be less certain. It will not be possible to obtain biomass data for the specimen.

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 x 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 02]).

Safety Data Sheets (SDS) shall be made available for chemicals which may be used in this work: glutaraldehyde. Whenever chemicals are used, follow requirements of the site-specific Chemical Hygiene and Biosafety Plan (AD[03]) and general Hazard Communication Plan (AD[01])

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 3. Equipment list – General equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	S	Clipboard	Recording data	1	N
	R	Cooler, 9-28 quart	Storing samples	1	N
	R	Ice packs	Keeping samples cool	2	N
	R	Waders (hip or chest)	Wading	1 pair per person	N
Consumable items					
	R	General Field Metadata Sheet, RD[06] (all-weather paper)	Recording metadata	1	N
	R	Field data sheets (all-weather copier paper, write in pencil; RD[05])	Recording data	2	N
	R	Pre-printed all-weather paper labels	Labeling aquatic plant, bryophyte, and lichen samples	1 sheet	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Pre-printed adhesive labels (all-weather, 2”x4”)	Labeling macroalgae samples	20	N
	R	Pencils	Recording data	2	N
	R	Permanent markers	Labeling samples	2	N
	S	Extra batteries	Backup for GPS, Camera	4	N
	S	Flagging tape (roll)	Flagging plant transect locations	1	N

R/S=Required/Suggested

Table 4. Equipment list – Transect establishment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
RD[09]	R	Site-specific Stream Morphology Map	Determining sampling locations	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Handheld GPS (with batteries, ± 1 m accuracy)	Recording transect locations	1	N
	R	Plot markers	Marking transect locations	10	N
Consumable items					
		(none)			N

R/S=Required/Suggested

Table 5. Equipment list – Sampling equipment

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
	R	Plastic stake (large) or chaining pin	Anchoring the transect tape	All	2	N
	R	Spring clamp	Anchoring the transect tape	All	2	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Meter tape (50 m)	Transect tape	All	1	N
MX100315	S	View bucket (Plexiglas bottom)	Underwater viewing for point-transect measurements	All	1	N
	S	Forceps – featherweight	Separating macroalgae from plants	All	1	N
	S	Forceps – fine point	Separating macroalgae from plants	All	1	N
	R	Scissors or hand clippers	Removing aquatic plants from the biomass quadrat	All	1	N
	S	Mallet	Collecting lichen specimens	Cobbles and boulders	1	N
	S	Chisel	Collecting lichen specimens	Cobbles and boulders	1	N
	S	Trowel	Collecting aquatic plant specimens	Soft sediments	1	N
	S	Aquarium dip net	Collecting floating plants	All	1	N
MX100304	R	Collapsible quadrat (0.5 x 0.5 m)	Biomass quadrat sampling	All	1	N
	S	Modified quadrat (10 x 10 cm)	Biomass quadrat sampling, small headwater streams	All	1	N
	R	Digital camera, waterproof (with	Photographing specimens	All	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		battery, memory card)				
	R	Field identification key (site-specific)	Identifying specimens in the field	All	1	N
Consumable items						
	S	Single-edged razor blades	Collecting mosses and liverworts	Cobbles and boulders	5	N
	R	Electrical tape	Added to the quadrat every 10 cm to estimate percent cover	All	1 roll	N
	R	Whirl-pak® bags, 24 oz.	Sample container	All	30	N
	R	Resealable bags (gallon)	Organizing samples, collecting large plant specimens	All	10	N
	S	Trash bags	Collecting large biomass samples	Heavy plant cover	5	N

R/S=Required/Suggested

Table 6. Equipment list – Laboratory processing: Ash-free dry mass

Item No.	R/S	Description	Purpose	Quantity	Special Handling

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Plastic larval tray	Measuring dry weight of large specimens	1	N
	S	Weigh boats (plastic, large)	Measuring dry weight of small specimens	20	N
	R	Weigh boats (aluminum)	AFDM sampling processing	20	N
	R	Sieve, 1 mm mesh	Rinsing sample to remove sediment and debris	1	N
	R	Soft brush	Cleaning aluminum weigh boats	1	N
	R	Analytical balance	Measuring weight	1	N
	R	Muffle furnace	Burning organic material for ash-free dry mass calculation	1	N
	R	Crucible tongs	Safe handling of equipment in the muffle furnace	1	N
	R	Thermal gloves	Safe handling of equipment in the muffle furnace	1 pair	N
	S	Aluminum baking pan	Sample organization in muffle furnace	2	N
	R	Desiccator (bench top)	Storing dried samples	1	N
Consumable items					
	R	Desiccant packs	For bench top desiccator	1-2	N
	R	Lab data sheets	Recording data	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Paper lunch bags	Drying samples in the drying oven	10	N
	R	Laboratory tissues (box)	Blotting small specimens	1	N
	R	Paper towels	Blotting large specimens	1	N
	S	Aluminum foil	Separating aluminum weigh boats in muffle furnace	1 roll	N
MX106351	R	Borosilicate glass vials, PTFE-lined cap, 4 mL	Sample container for CN subsamples	20	N

R/S=Required/Suggested

Table 7. Equipment list – Laboratory processing: Aquatic plant pressing and mounting

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Standard plant press	Pressing plants	1	N
	R	Cardboard ventilators	Pressing plants	12	N
	R	Standard driers (sheets)	Pressing plants	24	N
	S	Forceps (blunt point)	Handling specimens	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Handheld digital camera	Photographing specimens	1	N
	R	Camera battery	Photographing specimens	1	N
	R	Camera memory card	Photographing specimens	1	N
Consumable items					
	R	Herbarium mounting paper	Herbarium mounting	1 package	N
	R	Herbarium mounting glue, bottle	Herbarium mounting	1	N
	R	Newspaper	Pressing plants	12 sheets	N
	R	All-weather copier paper, 8 1/2” x 11”	Labelling plants in plant press	15 sheets	N
	R	Seed envelopes (3.23” x 4.50”)	Storing small plant parts	10	N
	R	Herbarium labels, paper	Labeling herbarium mounts	10	N

R/S=Required/Suggested

Table 8. Equipment list – Laboratory processing: Macroalgae preservation

Item No.	R/S	Description	Purpose	Quantity	Special Handling

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
		(none)			
Consumable items					
	R	Preservative (glutaraldehyde)	Preserving macroalgae samples	1 L	Y
	R	60 mL widemouth HDPE polypropylene sample bottle with cap	Shipping macroalgae samples	10	N

R/S=Required/Suggested

Table 9. Equipment list – Shipping Supplies

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
		(none)			
Consumable items					
	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	TBD	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	S	Cardboard box (~9”x7”x7”)	Shipping taxonomy samples	1	N
	R	Bubble wrap	Padding taxonomy and CN samples	TBD	N
	R	Shipping inventory (RD[13])	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]).

Personnel will be trained in the field protocols associated with this document, and trained in safe working practices for lake- and river-based field work. Technicians must also be trained in field identification based on the local stream flora and 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 2 technicians for 3-8 hours per site, plus travel to and from the site. Lab processing requires 1-2 technicians for 2-8 hours within 48 hours of field sampling, 1 technician for 3-8 hours on the second lab day, and 1 technician for 2-3 hours on the third lab day.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

1. Collect and prepare all equipment, including sample bottles, sample bags, and pre-printed labels.
2. Have ice or ice packs frozen and ready for transportation cooler.
3. Charge batteries for digital camera and check batteries (bring extras) for handheld GPS unit.
   a. Ensure that the camera memory card is empty before sampling.
4. See Laboratory Preparation procedures in Section E.1 for additional pre-sampling activities (e.g., weigh boat preparation).
5. 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

1. Is this the first time the site is being sampled for aquatic plants?
   a. If YES, go to Step 2.
   b. If NO, go to Step 4.
2. Determine percent cover of habitat types throughout the sampling reach using the Stream Morphology Map (RD[09]) and go to Step 3.
   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 replicate transects must be taken from the same habitat types 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 2.4)
      1) Riffle
      2) Run
      3) Pool (only sample pools that can be waded safely)
3. Does the habitat type account for >20% of the reach throughout the year?
   a. If yes, go Sample Collection in the Field: Establishing transects (SOP C).
   b. If no, ignore this habitat type.
4. Has the channel morphology changed significantly since the last sampling date (e.g., riffles are now pools)?
   a. If yes, stop work and submit a problem ticket to determine if new transects are required.
   b. If no, proceed with Field Procedure (SOP C).

B.2 Contingent decisions

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 10 transects/quadrats without causing harm to the stream. Reduce sampling by collecting samples only in the dominant habitat type (5 transects/quadrats 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]).
The site is a small headwater stream or first/second order stream dominated by bryophytes	Use the small 10 cm x 10 cm quadrat	Lower stream coverage for biomass metrics	The size of the quadrat used must be clearly documented in the field datasheets.

SOP C Establishing Transects

1. Do not sample within a 5 m radius of the aquatic instrumentation.
2. Select the two most common habitat types that accounts for >20% of the area of the reach (using the existing Stream Morphology Map, RD[09]) and place 5 transects in each habitat type. If only one habitat is present that accounts for >20% of the reach, place all 10 transects within that habitat type.
   a. If this is a STREON site, transects must be in the same habitat type in both the AQU and STREON reaches.
   b. If working at a STREON site, all transects must be located between the STREON sensor sets.
      1) If the sensor reach is too short to accommodate this volume of sampling, place a limited number of transects downstream of STREON S2 within 200 m.
   c. Alternate sampling transects between different types of habitat along the reach if possible (Figure 10).
   d. If it is not possible to separate each transect by a different habitat, transects should be located a minimum of 10 m apart.
   e. Take care not to place transects in locations where you or others have been walking in the stream or are obvious crossing areas for wildlife (e.g., beavers, cows). Transects should not be located within 5 m of the aquatic sensor sets or discharge transect location due to heavy foot traffic in those areas.

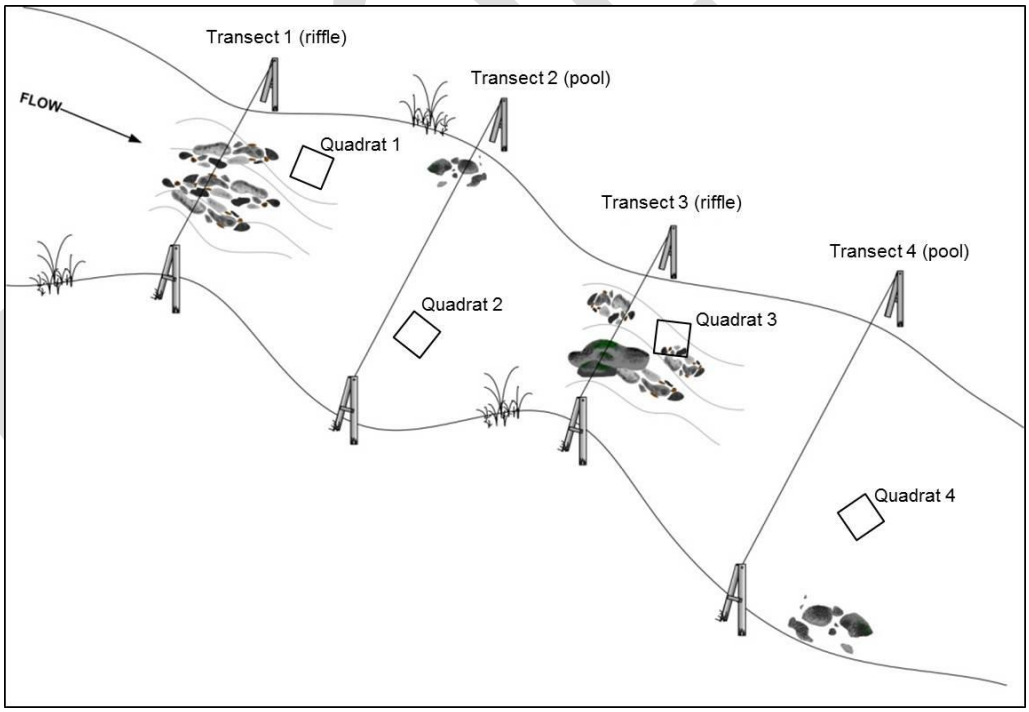

Figure 10. Layout of riffle and pool transects and quadrats within the stream reach

3. Start with the most downstream location and work upstream to avoid suspending sediments that will affect your sampling area.

4. Choose transect locations and place a plot marker on the right bank or left bank (keep the bank consistent throughout all transects for consistency).
5. Record transect end point locations using the GPS.
    a. If GPS coverage is not available at the site, triangulate from nearby geomorphic survey markers (Figure 11) using the following steps:
        1) Find the two closest survey markers from Stream Geomorphology Protocol (RD[09]), one on each bank. Both markers should either be upstream or both should be downstream of the transect.
        2) Record both survey marker numbers on the Point Transect Locations datasheet (Figure 12, RD[05]).
        3) Stretch a meter tape from each survey marker to the edge point of the transect on the right bank.
        4) For consistency, always place zero on right bank.
        5) Measure and record the distance from each survey marker to the edge of the transect.
        6) Repeat Steps 3)-5) for the left bank.
        7) There should be 4 measurements total, 2 for each edge of the transect.

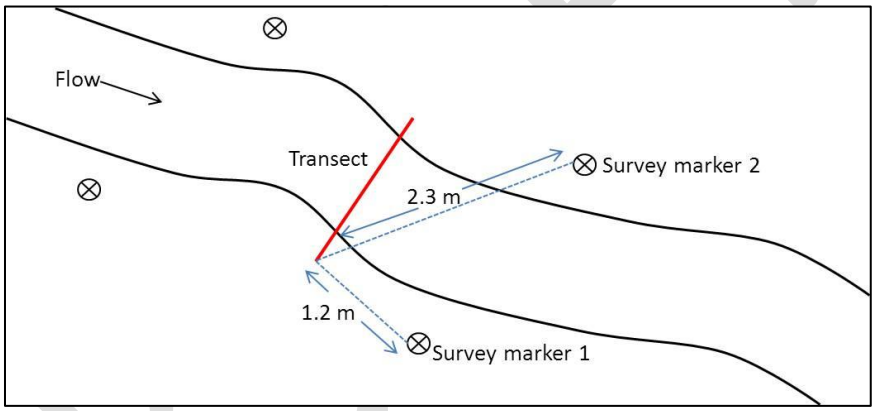

Figure 11. Triangulating from survey markers to determine location of the transect

6. Reassess the current stream morphology map and transect locations each year to ensure that the habitats are still the same. If stream morphology has changed significantly, establish new transect locations according to the steps listed above. Otherwise, continue to use the same locations.

NEON Aquatic Plant/Macroalgae Field Data Sheet - TRANSECT ESTABLISHMENT

Wadeable streams

Site (4-letter code): *ARIK* | **Recorded by:** *sparker*

Date (YYYYMMDD): *20140702* | **Collected by:** *kgoodman*

Local time (HH:MM): *10:00* | **Sampling protocol & Rev.:** *NEON.DOC.000692vB*

Transect Establishment - GPS

Transect ID	Habitat	Plot marker ID	Plot marker bank (R or L)	Latitude	Longitude	Notes
1	*riffle*	*TBD*	*R*	*39.758342°*	*-102.448190°*	
2	*run*	*TBD*	*R*	*39.758308°*	*-102.447502°*	*very slow water*
3	*riffle*	*TBD*	*R*	*39.758334°*	*-102.447098°*	
4	*run*	*TBD*	*R*	*39.758757°*	*-102.446593°*	

NEON Aquatic Plant/Macroalgae Field Data Sheet - TRANSECT ESTABLISHMENT

Transect Establishment (page 2) - triangulation (use only if GPS service is unavailable)

Transect ID	Habitat	Plot marker ID	Plot marker bank (R or L)	Survey marker ID	Survey marker bank (R or L); upstream or downstream	Distance from survey marker (m)
1	*riffle*	*TBD*	*R*	*123*	*R upstream*	*1.2*
1	*riffle*	*TBD*	*R*	*124*	*L upstream*	*2.3*
1	*riffle*	*TBD*	*R*	*123*	*R upstream*	*4.1*
1	*riffle*	*TBD*	*R*	*124*	*L upstream*	*1.4*
2	*run*	*TBD*	*R*	*127*	*R downstream*	*2.6*

Figure 12. Data sheet for recording the location of transects using GPS or triangulation from nearby survey markers (RD[05]).

SOP D Field Sampling

D.1 Biomass Quadrats

Biomass sampling is inherently destructive; therefore, biomass sampling at sites takes place near, but not directly on, the point transects. A location for biomass sampling is haphazardly selected 2-4 m downstream (see below for details) of each set of point transects so as not to disturb the aquatic plants growing along the point transect. Quadrats are 0.5 m per side (0.25 m^2). Field technicians may drill holes in the PVC to allow for the quadrat to sink in the water, and should add electrical tape to the frame of the quadrat every 10 cm to aid in estimating percent cover. In small headwater streams, a modified 10 cm x 10 cm quadrat may be used so as not to destructively sample the bryophyte community. This decision will be made by the site host and NEON HQ.

NOTE: Quadrats are not heavy enough to settle to the stream bottom in areas of fast water (e.g., riffles). A second field technician may need to assist in holding the quadrat when sampling these areas.

1. Start at the most-downstream point transect and work upstream.
2. Haphazardly choose a location for the quadrat.
    a. Stay within the same habitat unit (e.g., within the same riffle) as the point transect.
    b. Toss the quadrat into the channel downstream of the transect, a minimum of 2 m and a maximum of 4 m downstream from the point transect.
        1) If the site has habitat units that are <4 m long, this requirement may be changed to 1-3 m from the transect.
    c. Ensure that the quadrat location is underwater and appears to be underwater at baseflow.
        1) Do not place quadrat on an island populated with terrestrial plants.
        2) If the stream is dominated by boulders that protrude above the water, the transect may not be fully underwater. In this case, sample all obligate aquatic vegetation on the substrata, and separate by “underwater” and “above water”.
        3) Keeping the quadrat underwater may require using the smaller 10 cm x 10 cm quadrat at some sites.
        4) This protocol is designed to capture the underwater community, thus this method may result in 0 plants being in the quadrat even though more plant material may be present on the dry substrata in the channel.
    d. If the stream flow is relatively fast, you may need to have an assistant hold the quadrat in place, or step on the sides of the quadrat to hold it in place.
3. Visually estimate percent cover of each species rooted within the quadrat using the Cover Scale Classes (Table 10**Error! Reference source not found.**). Percent cover estimates are subjective, so it is best for the same technician to estimate all of the quadrats on a given day.
    a. Use the view bucket to assist in seeing the stream bottom if necessary (Figure 13).
    b. Refer to vegetation categories in the transect section (SOP D.2, Step 5).
    c. If you are unable to identify a species in the field, give it a distinct, descriptive name. Take photos *in situ* if possible. These will be identified in the lab.

Table 10. Cover scale classes for percent cover estimates (Bowden et al. 2006).

Cover Scale Classes	Percent Cover
1	≤5%
2	6-25%
3	26-50%
4	51-75%
5	76-100%

Figure 13. A field technician estimates the percent cover of the biomass quadrat using the view bucket: the quadrat is divided into a grid of 25 squares to aid in the percent cover estimate.

4. Record percent cover numbers and associated taxa on the **Quadrat Field Data Sheet** (**Figure 14**, RD[05]).

NEON Aquatic Plant/Macroalgae Field Data Sheet - QUADRAT SAMPLING

Wadeable streams

Site (4-letter code): *ARIK*	**Recorded by:** *sparker*
Date (YYYYMMDD): *20140702*	**Collected by:** *cbohall*
Local time (HH:MM): *14:45*	**Sampling protocol & Rev.:** *NEON.DOC.00692vB*

Cover classes: 1 (≤5%), 2 (6-25%), 3 (26-50%), 4 (51-75%), 5 (76-100%)

Quadrat ID	Plot marker ID	Sample type (quadrat size)	Habitat	Species or temporary ID	Growth form: emergent (E), floating-leaved (FL), submerged (S), free-floating (FF)	Cover class	Sample ID
Q1	*P52*	*0.25 m^2*	*run*	*duckweed1*	*FF*	*2*	*ARIK.20140702.duckweed1.Q1*
"	*"*	*"*	*"*	*Sparganium eurycarpum*	*E*	*1*	*ARIK.20140702.SPEU.Q1*
"	*"*	*"*	*"*	*macroalgae2*	*S*	*1*	*ARIK.20140702.macroalgae2.Q1*
"	*"*	*"*	*"*	*macroalgae4*	*S*	*1*	*ARIK.20140702.macroalgae4.Q1*

Figure 14. Example of field data sheet for biomass quadrats in wadeable streams (RD[05]).

5. After percent cover is estimated and recorded, remove all aquatic plants, bryophytes, and macroalgae from the quadrat by species (do not remove lichens as they grow more slowly and may not replace themselves – ID *in situ* based on field identification key).
   a. Only remove plants that are rooted in the quadrat, and collect the entire plant even if it hangs outside of the quadrat.
   b. Clip plants at the sediment-water interface for above-ground biomass. Floating plants may be scooped using an aquarium dip net or hands. Mosses and liverworts are most easily removed using a single-edged razor blade.
   c. If the entire quadrat cannot fit underwater, collect both the above-water and underwater specimens and keep the separate.
   d. Scoop up plants floating above the quadrat boundary using hands or small aquarium net.
   e. Only remove macroalgae if they form filaments (Figure 8) that can be easily sampled by hand. Some algae form amorphous clouds near the substrate that are difficult to pick up, these will be sampled in the periphyton protocol (RD[10]).
6. Wash sample gently in stream water to remove excess sediments (this saves cleaning time in the lab). Take extra care when rinsing algae to not lose the sample material.
7. Place each species in a separate Whirl-pak® or gallon resealable bag. This makes sorting back in the lab easier.
   a. If there is too much material for one bag, split the sample over two or more bags and make appropriate labels with "#1 of 2" and "#2 of 2" etc. on the labels.
8. Write a label on all-weather paper and add to sample bag (Figure 15**Error! Reference source not found.**, RD[05]). The sample name on the label must match the name on the field data sheet (Figure 14**Error! Reference source not found.**).

NEON Sample ID: *ARIK.20140702.macroalgae2.Q1* Transect/Quadrat/Point ID: *Q1* Species ID: *macroalgae 2* Collected by: *sparker*	**NEON** Sample ID: *ARIK.20140702.macroalgae3.Q1* Transect/Quadrat/Point ID: *Q1* Species ID: *macroalgae 3* Collected by: *sparker*
NEON Sample ID: *ARIK.20140702.SPEU.Q1* Transect/Quadrat/Point ID: *Q1* Species ID: *Spargium eurycarpum* Collected by: *sparker*	**NEON** Sample ID: *ARIK.20140702.duckweed1.Q1* Transect/Quadrat/Point ID: *Q1* Species ID: *duckweed 1* Collected by: *sparker*

Figure 15. Example labels for biomass sample collection (RD[05]).

9. Close and seal sample bag (Figure 20).
10. Place all labeled and sealed sample bags in a cooler with frozen ice packs for transportation to the Domain Support Facility.
    a. Samples should remain refrigerated at 4^{o}C ±3 ^{o}C.
    b. The total time from collection to the start of sample processing in the lab must not exceed 48 hours in order to minimize decomposition of samples.

D.2 Point-Transects

1. Use the pre-recorded plot markers and coordinates (or survey marker numbers and triangulation distances from the Point Transect Locations datasheet) to locate each transect.
    a. If using triangulation:
        1) Find the two survey markers associated with the transect as noted on the datasheet.
        2) Stretch 1 meter tape from each survey marker toward the first transect location (Figure 10) so that both tapes form a "V". Locate the point where the tapes intersect at the distances listed on the Point Transect Location datasheet (Figure 10).
2. Place a plastic tent stake or chaining pin at each end of the transect. String the meter tape from one stake to the other. Fasten the tape to the stakes with spring clamps.
    a. For consistency, always place the zero end of the meter tape on the right bank.
    b. The tape should be perpendicular to thalweg.
3. Sampling points should be evenly spaced in 10 cm (minimum) to 50 cm (maximum) intervals across the transect, depending on the size of the stream. The goal is to have ≥20 sampling points within the wetted channel across each transect.
    a. If the wetted width of the stream is <2 m, sampling points should be spaced 10 cm apart.
    b. For streams >2 m wide, points should be spaced at intervals approximately equaling the stream transect width divided by 20.
    c. Maximum distance between sampling points is 50cm.

4. Use the view bucket to see the stream bottom at each sampling point along the transect. Line up the reference point on the bottom of the view bucket with the sampling point on the meter tape (Figure 16).

    a. Survey points in the wetted channel only.
    b. Set the bottom of the view bucket on the surface of the water, and then push slightly below the water. This will allow you to see underwater through the bottom of the bucket.
    c. If the clear bottom of the view bucket is difficult to see through, rinsing or leaving a thin layer of water in the bottom of the bucket may aid in viewing.
    d. Record all vegetation or substratum type (if no vegetation present) under each point.

        1) If multiple layers of vegetation lie below the point, record all vegetation layers.
        2) If transient leaf litter (e.g., during autumn sampling bouts) has fallen into the stream, record then move out of the way so you can see what rooted plants lie on the stream bottom.

Figure 16. Example of the view bucket (a clear-bottomed bucket) with the reference point marked in the center of the viewing window

5. Record the all vegetation and its growth form (see Definitions Section 2.4) organic matter, or substrate that lies directly below the reference point at each location across the transect on the **Point Transect Field Data Sheet** (Figure 17, RD[05]). If you are not able to determine the plant species from the groups listed below, collect a corresponding voucher specimen off the transect (SOP D.3). Categories include:
    a. Species or type of vegetation
        1) **"Bare substratum"** – Record if no rooted plants are present. Identify substratum type. Substrata are likely to be covered with periphyton, which will be sampled in the periphyton protocol (RD[10]).
        2) **Filamentous algae** or **macroalgae** – Collect samples for identification in the lab, see Step 6 below (Figure 8).
        3) **Algae, other** – Non-filamentous "clouds" of algae that may be found in slow-moving streams, collect for taxonomic analysis.
        4) **Moss** – Use the field key to identify to species if possible, or collect a voucher specimen.

5) **Liverwort** – Use the field key to identify to species if possible, or collect a voucher specimen.
6) **Aquatic plant** – Use the field key to identify to species if possible, or collect a voucher specimen (Figure 2-Figure 5).
7) **Lichen** – Use the field key to identify to species if possible, collect a voucher specimen.
8) **Leaf litter** – Terrestrial leaves from trees that have fallen into the water.
9) **Coarse woody debris** – (CWD) large pieces of wood, sticks.
10) **Fine particulate organic matter** – (FPOM) fine particles of organic matter.
11) **Dry** – area is not underwater at the time of sampling.
12) **Other** – additional categories may be added as necessary (e.g., tumbleweed, terrestrial plant).

b. Growth form (see Definitions Section 2.4)
1) **Emergent (E)**
2) **Floating-leaved (FL)**
3) **Submerged (S)**
4) **Free-floating (FF)**

c. Substratum
1) **Silt** < 0.063 mm
2) **Sand** = 0.063-2.0 mm
3) **Gravel** = 2.0-63 mm
4) **Cobble** = 63-200 mm
5) **Boulder** = 200-630 mm
6) **Bedrock**
7) **Other**

6. Collect all filamentous macroalgae for taxonomic analysis. Do not identify in the field.
a. Only remove macroalgae if they form filaments (Figure 8) that can be easily sampled by hand. Amorphous clouds of algae will be sampled in the periphyton protocol (RD[10]).

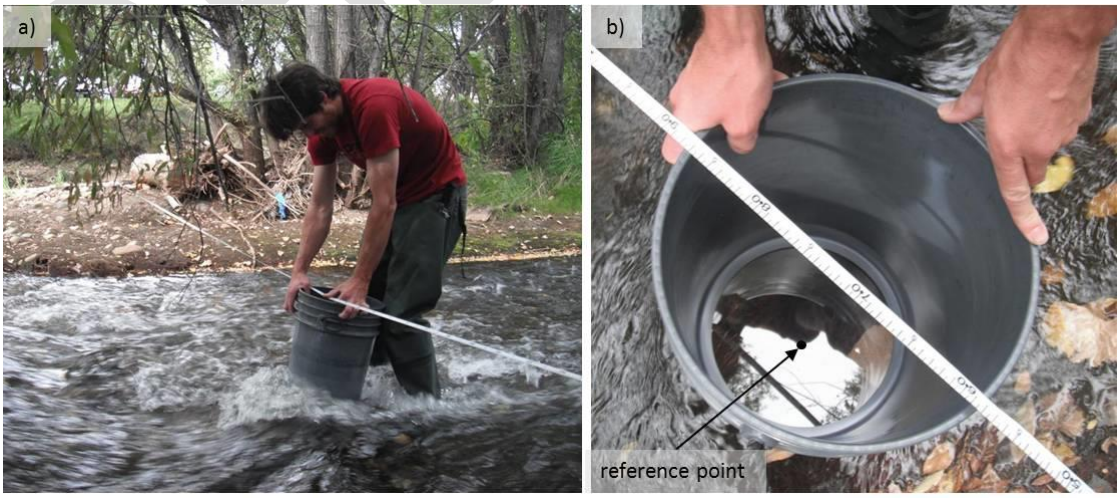

Figure 17. a) A field technician holds the view bucket in the water, under the meter tape transect. b) Hold the view bucket so that the reference point lines up with the point you want to sample on the meter tape.

NEON Aquatic Plant/Macroalgae Field Data Sheet - POINT TRANSECTS

Wadeable streams

Site (4-letter code): *ARIK*	**Recorded by:** *sparker*
Date (YYYYMMDD): *20140702*	**Collected by:** *kgoodman*
Local time (HH:MM): *14:30*	**Sampling protocol & Rev.:** *NEON.DOC.000692vB*

Transect Sampling

Transect ID	Plot marker ID	Habitat	Point #	Distance (m)	Species, temporary ID, or substratum (if no plant under point)	Growth form: emergent (E), floating-leaved (FL), submerged (S), free-floating (FF)	Sample ID (macroalgae or associated voucher specimen)
T1	*P52*	*run*	*1*	*0*	*BANK*		
"	*"*	*run*	*2*	*0.1*	*sand*		
"	*"*	*run*	*3*	*0.2*	*sand*		
"	*"*	*run*	*4*	*0.3*	*macroalgae1*	*S*	*ARIK.20140702.macroalgae1.T1*
"	*"*	*run*	*5*	*0.4*	*Sparganium eurycarpum*	*E*	
"	*"*	*run*	*6*	*0.5*	*duckweed1*	*FF*	*ARIK.20140702.duckweed1.T1*

Figure 18. Example of a field data sheet for a point transect in a wadeable stream (RD[05]).

D.3 Voucher Collection

Voucher specimens should be collected when plants cannot be positively identified in the field. Flowers or fruit are often necessary to identify many plant species (especially grasses), so specimens may be collected at any time during the year, not just during aquatic plant sampling. Collect obligate aquatic species only. Field technicians should be prepared on any field visit with zip-top bags and labels. Voucher specimens will be collected in duplicate, with one specimen archived at the domain herbarium and one specimen archived by the external taxonomist.

1. For any non-endangered aquatic plants, mosses, liverworts, or lichens that cannot be positively identified *in situ* using the field identification key, collect a small voucher specimen within the same habitat unit, but not directly on the transect.
    a. Collect specimens only if the species is abundant within the reach. If the species is rare (covering less than ~5% of stream bottom), photograph and note location.
        1) **Aquatic plants:** collect at least 2 full plants (roots, stems, leaves, flowers) so one can be kept at the domain lab and one can be sent to the taxonomist
        2) **Mosses** and **liverworts:** collect a sample that is no larger than the size of your palm
        3) **Lichens**: collect 2 specimens approximately 1”-2” in diameter
    b. Write an all-weather paper label for the specimen in pencil and record on the data sheet (Figure 19, RD[05]).
    c. Place the specimen and the all-weather paper label inside a Whirl-pak® (Figure 20) or zip-top bag and seal.

NEON

Sample ID: *ARIK.20140702.macroalgae1.T1*

Transect/Quadrat/Point ID: *T1*

Species ID: *macroalgae1*

Collected by: *sparker*

Figure 19. Example label for voucher specimen collections. Note that "Sample #" corresponds to the "Notes" section of the Field Data Sheet in Figure 18.

d. Keep samples in a cooler with ice or ice packs (~4 °C) until returning to the lab.

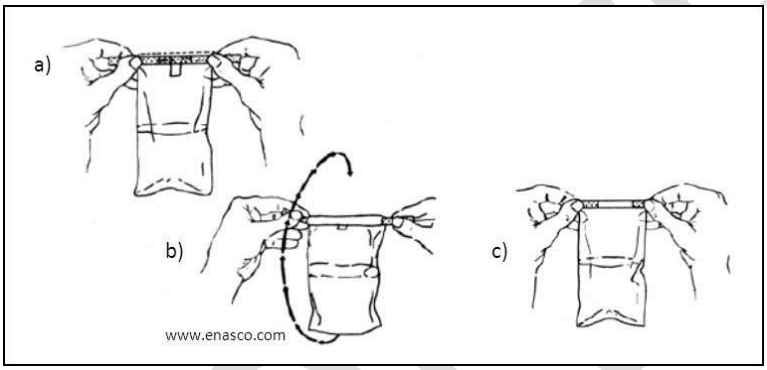

Figure 20. How to close a Whirl-pak® bag: a) hold the wire tabs; b) whirl the bag 3 complete revolutions (or fold the top over), and c) fold the wire ends over to close

D.4 Special Considerations

1. **DO NOT COLLECT ENDANGERED OR THREATENED SPECIES.** Lists of endangered/threatened taxa in the region will be provided for each domain, along with a photo key.
   a. Take good quality photos and record which section of the reach the specimen was found in (e.g., "50 m downstream of S2"). Collect a GPS coordinate of the specimen's location if possible.
      1) If invasive species are identified, submit a problem ticket to report findings to NEON Permitting.

D.5 Sample Preservation

1. Keep samples refrigerated (4 °C ±3 °C) and in the dark until processing at the Domain Support Facility.
2. Samples must be returned to the Domain Support Facility and processed within 48 hours.

D.6 Ending the Sampling Day

1. Refreshing the sampling kit
   a. Replace Whirl-pak® and resealable bags.

b. Print new field labels and field data sheets on all-weather copier paper.
c. Check and charge all batteries, replace if necessary.
d. Place ice packs in the freezer.

2. Equipment maintenance, cleaning and storage
   a. Decontaminate all equipment that has come in contact with stream water according to the NEON Aquatic Decontamination Protocol (RD[08]).
   b. Dry all equipment thoroughly between sites and before storage.

SOP E Laboratory Sampling and Analysis

At the Domain Support Facility, aquatic plant specimens are identified using voucher specimens and keys, weighed and dried for measurements of fresh weight and ash-free dry mass (Figure 22). Macroalgae are preserved and sent to an external lab for identification (Figure 24).

E.1 Preparation

1. If aluminum boats are new and unlabeled:
2. Label ~20 boats by inscribing a unique number on the bottom of each boat with a pencil (e.g., A1, A2, A3, etc.; Figure 21).
   a. NOTE: It doesn't matter what the labels are as long as they are unique and easy to read.

Figure 21. Unique identifiers inscribed on the bottom of the aluminum weigh boats

   b. Place new empty labeled boats in the muffle furnace (500 ^{o}C) for 6 hours to burn off any residue.
3. After 6 hours, carefully remove boats from the furnace and allow to cool to room temperature.
4. If aluminum weigh boats have been previously labeled and used, clean with a soft brush to remove any residual ash.
5. If samples are small and enough material is not available for all analyses, process according to the following hierarchy:
   a. Voucher specimen for taxonomy
   b. AFDM
   c. CN

E.2 Aquatic Plants

The following steps use samples collected from quadrats.

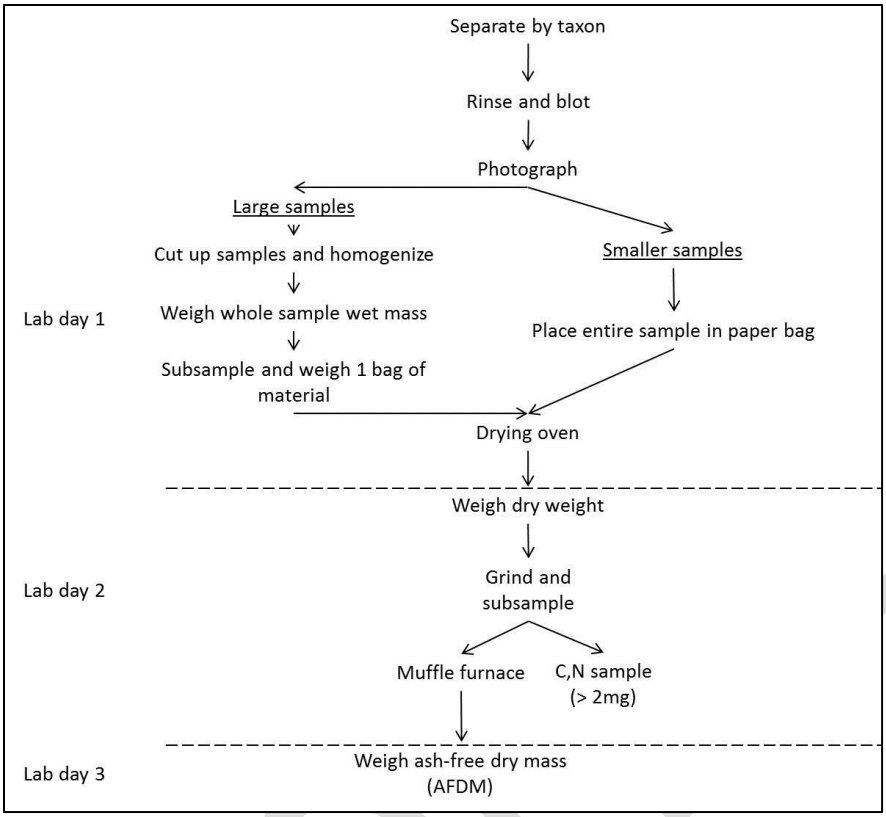

Figure 22. After separating each species from the original sample, follow the steps above for Domain Support Facility aquatic plant processing.

1. **Day 1**: Remove plant/bryophyte sample and field label from sample bag from quadrat sampling. Set label aside.
2. Gently rinse the sample with tap water over a 1 mm sieve to remove sediment, large epiphytes, and debris.
    a. Some aquatic plants are very fragile and may break easily during rinsing. The sieve will collect any plant fragments but allow sediments to be washed away.
3. Separate sample into individual taxa.
    a. Each species will be a unique specimen.
    b. Floating the sample in a tray of water may help facilitate separation of taxa.
    c. At some sites, it may be difficult to separate macroalgae from plant material. After spreading the entire sample out on a tray, spend no more than 5 minutes sorting the sample.
    d. If there is not enough specimen to complete all analyses, prioritize samples as follows:
        1) Taxonomy
        2) AFDM
        3) CN
4. Clean plant fragments out of the sieve and add to the respective specimen.

5. Spread cleaned specimen on a standard drier (blotting paper), gently blot with laboratory tissues (smaller specimens) or paper towels (larger specimens) to remove some water content before placing in the drying oven. **DO NOT CRUSH** specimen or attempt to wring the water out.
    a. For large-volume specimens, you may need multiple standard driers.
6. Photograph specimen, be sure to include roots, leaves, and flowers if present. Ensure that the subject is in focus. Include sample label in photo for identification.
7. Identify the specimen using field key and/or voucher specimens.
    a. If specimen cannot be identified at the Domain Support Facility with at least 85% confidence, use temporary species ID and send a voucher specimen to external taxonomist.
8. For large samples: Cut samples to a size that will fit into paper bags and homogenize.
    a. Weigh entire sample on mass balance and record as **Wet mass** of lab data sheet.
    b. Select a well-homogenized subsample that will fit in one paper bag and weigh. Record as **Wet mass subsample** on lab data sheet.
9. Using a permanent marker, label a clean, paper lunch bag with sample ID and species ID from the field sample label.
10. Place wet sample (or wet mass subsample) in the labeled clean paper lunch bag.
    a. If the specimen is fragile, small pieces may cling to the standard drier. If it is a small amount in relation to the entire sample, it is ok if you are unable to get every fragment off of the standard drier.

NEON Aquatic Plant and Macroalgae Lab Data Sheet - AFDM

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.000692vB*

PERIODICALLY CHECK THAT BALANCE IS ZEROED!

Sample ID	Species ID	Total wet mass (g)	Subsample wet mass (g)	Total or subsample dry mass (g)	Boat ID	Boat mass (g)	Boat + dry mass (g)	Boat + ash mass (g)
ARIK.20140702.SPEU.Q1	*Sparganium eurycarpum*	*2.5631*	*1.5240*	*1.4589*	*A1*	*2.1340*	*3.5929*	*3.0752*
ARIK.20140702.dusckweed1.Q	*duckweed1*	*1.4859*	*1.4859*	*0.6189*	*A2*	*2.0561*	*2.6750*	*2.4261*
ARIK.20140702.algae1.Q1	*macroalgae1*	*3.4524*	*1.7262*	*1.4387*	*A3*	*2.2247*	*3.6634*	*3.0148*

Figure 23. Example of lab data sheet for aquatic plant AFDM (RD[05]).

11. Place paper bags containing samples in the drying oven for a minimum of 12 hours at 60°C or until constant weight is achieved (i.e., mass varies by <2% over a one-hour period; RD[11]).
    a. Use TOS "Lab Drying QC Datasheet" in Measurement of Herbaceous Biomass datasheets (RD[12]).
    b. Specimens may be split across multiple labeled paper bags to facilitate drying.
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 large, clean, plastic weigh boat (small specimen) or tray (large specimen) on analytical balance. Tare (zero) balance. Place dry specimen in the plastic weigh boat/tray and record as **Dry Weight** on Lab Data Sheet.
14. Samples may be crushed to fit into weigh boat. If samples are large, grind sample in Wiley mill using 20 mesh (0.85 mm) screen to homogenize.
    a. Subsamples for CN and AFDM
        1) Place a subsample of ground or crushed material in aluminum weigh boat. Record **Boat ID** on lab data sheet.
        2) Remove ≥ 2 mg for C and N analysis. Place in 4 mL PTFE-capped glass vial, apply adhesive label, and set aside for shipping.
            a) Samples sent to external lab for CN analysis do NOT need to be ground on the Wiley Mill. Use the Wiley Mill to homogenize large samples before subsampling for CN. Small samples may be crushed by hand.
    b. Clean grinding mill thoroughly with compressed air between samples and with ethanol after finishing the sampling bout.
15. Place remaining specimen subsample in a clean, pre-labeled aluminum weigh boat. Record **Boat ID** on lab data sheet.
    a. If the ground specimen is too large for the aluminum weigh boat, put only a portion of the subsample in the boat (~1-2 g).
16. Weigh boat on analytical 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.
20. **Day 3**: After 6+ hours, remove covered pans/aluminum boats carefully from the muffle furnace using oven gloves and crucible tongs.
21. 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.
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.

E.3 Macroalgae

1. Weigh several aluminum weigh boats and record as **Boat weight** along with **Boat ID** on lab data sheet.
2. **Day 1**: Remove macroalgae specimens from sample bag. Set label aside.
   a. If macroalgae are combined in a sample bag with aquatic plants, carefully separate using forceps and/or fingers.
   b. Floating the sample in a tray of water may help facilitate separation of macroalgae from plant material.
   c. At some sites, it may be difficult to separate macroalgae from plant material. After spreading the entire sample out on a tray, spend no more than 5 minutes sorting the sample.
3. Gently rinse specimen to remove sediment or other non-algal material (e.g., leaves, twigs) over a 1 mm sieve.

   a. Take care not to lose any sample material.
   b. Biomass estimates for macroalgae can be elevated in error due to non-algal material trapped in the filaments. Take care to clean specimen well.
4. Clean algal strands out of the sieve and add to the specimen.
5. Spread cleaned specimen on standard drier, gently blot dry with laboratory tissues or paper towels. **DO NOT CRUSH** sample or attempt to wring the water out.

   a. How dry is dry? Blot specimens dry until they no longer drip water when you pick them up.
6. Weigh entire sample on mass balance and record as **Wet mass** on lab data sheet.
   a. **Taxonomy subsample**: Remove >20 mL of sample to preserve for taxonomic identification and place in 60 mL HDPE bottle and add enough DI water to cover sample. Proceed to Sample Preservation, SOP E.7. Label according to Figure 25.
   b. **AFDM subsample**: Remove 10-50% of original sample and place in an aluminum weigh boat. Weigh subsample and record as **Wet mass subsample** on Lab Data Sheet along with appropriate **Boat ID** and **Boat weight**.

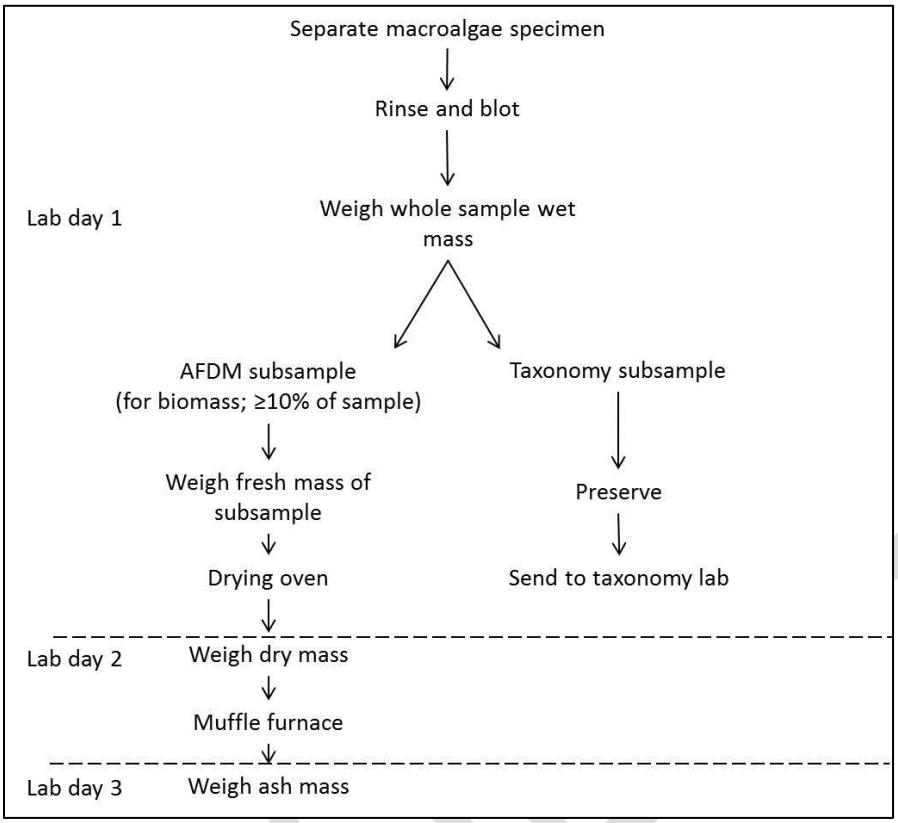

Figure 24. Schematic for macroalgae processing at the Domain Support Facility.

7. Repeat above steps until all samples have been processed.
    a. Place all aluminum boats + subsamples in drying oven for a minimum of 12 hours at 60°C or until constant weight is achieved (i.e., mass varies by <2% over a one-hour period; RD[11]). Cover boats with a cardboard lid or piece of paper to prevent samples from blowing out of the boats once in the oven. **NOTE:** It is helpful to place a number of boats + subsamples in shallow cardboard trays in the drying oven. These can be moved in and out of the oven more efficiently than moving one sample at a time. Aluminum boats + samples can stay in the drying oven longer than 12 hours if needed.
8. **Day 2**: Carefully remove boats from drying oven and let cool to room temperature before weighing. Place boats in a desiccator until weighing. Take care not to let the dried sample blow out of the boats.
    a. Samples may be left in desiccator or plastic bags for up to 30 days before proceeding to the next step.
9. Weigh the boat + dry sample on tared analytical balance, and record as **Dry Weight + Boat** on the Lab Data Sheet.
10. Repeat above steps until all samples have been processed.
11. Place aluminum boats + samples 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. Aluminum foil may be used to separate stacked boats.
b. Place boats on an approved muffle furnace pan (loaf pan) if available before placing in the furnace. This makes it easier and safer to handle samples.

12. Leave samples in the muffle furnace at 500°C for at least 6 hours.
    a. Sample may be left in muffle furnace for longer than 6 hours (e.g., overnight) if necessary.
13. **Day 3**: After 6+ hours, remove pans/aluminum boats carefully from the muffle furnace using oven gloves and crucible tongs.
14. Cover pans/aluminum boats with aluminum foil to prevent ash from blowing out of boats and set aside to cool on a heat-resistant surface without drafts from windows, doors, or HVAC ventilation. Clearly label hot surface for safety.
15. 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.
16. After cooling to room temperature, weigh boats again on analytical balance, record as **Ash Weight** on Lab Data Sheet.
17. After weighing, discard ash in trash can and clean the boat with a soft brush or paper towel.
18. Set clean boats aside to be used again.

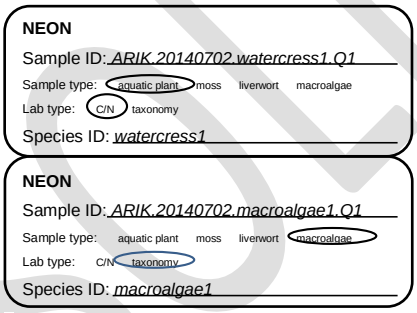

Figure 25. Example of adhesive labels for macroalgae taxonomy and plant CN subsamples.

E.4 Pressing Aquatic Plants (Taxonomy)

1. Open plant press.
2. Place 1 cardboard ventilator on plant press.
3. Place 1 standard drier on top of cardboard ventilator.
4. Place pre-weighed newspaper (2 sheets, opened) on top of the standard drier.
    a. Ensure that newspaper weight has been recorded on Lab Data Sheet (Figure 23, RD[05]).
5. Copy information from the specimen label onto the newspaper using a permanent marker and fill out taxonomy label file (Appendix F, RD[05]) for plant taxonomist.
6. Arrange specimen on newspaper, spread leaves and stems apart. Try to lay all parts flat, open any flowers so they lay flat on the paper.
    a. Do not allow leaves or specimens to overlap.
7. Place specimen label on newspaper (for additional identification).
8. Fold opened newspaper sheet over the sheet that the specimen is on.

9. Place another standard drier on top of newspaper.
10. Place a cardboard ventilator on top of the standard drier.
11. Repeat Steps 2-10 with Specimen #2. Continue until all specimens have been pressed.
12. Place wooden top of plant press on top of last cardboard ventilator.
13. Fasten compression straps, pull tightly to apply even pressure to the press.
14. Set press in a warm, ventilated, dry location. Check periodically to ensure that no mildew forms on the press.
15. Leave plants in press until they are shipped to taxonomist.
16. Proceed to Herbarium Mounts (SOP E.6).

E.5 Drying Moss, Liverwort, and Lichen Specimens (Taxonomy)

1. Print Bryophyte/Lichen packets on all-weather copier paper (Appendix D).
   a. Fold in thirds (like a letter) with the label on the outside.
   b. Open the flap with the label, fold in margins along the dotted lines.
   c. This will create a packet when the label flap is closed.
2. Copy all label information for each specimen to the outside of two paper packets (Appendix D).
3. Gently rinse specimen in tap water to remove sediments. Take care to avoid breaking the specimen.
4. Lay the specimen out on the lab bench, photograph using the macro setting on the camera. Be sure to take photos of any fruiting bodies.
5. Split the specimen into two portions, one to keep at the Domain Herbarium, and one to send to the taxonomist.
6. Place each specimen inside a labeled packet.
   a. **NOTE:** If specimen is a lichen, leave the lichen attached to rock substratum if present.
7. Set packets in a warm, ventilated, dry location. Check periodically to ensure that the specimen is drying without mildew formation.
8. Retain one set of packets at Domain Herbarium; proceed to SOP G for shipping instructions to taxonomists.

E.6 Herbarium Mounts (Aquatic Plants Only)

1. Open plant press carefully.
2. Carefully weigh newspaper plus specimens, record on Lab Data Sheet (RD[05]).
3. Open a newspaper sheet with one specimen on it.
   a. If there are small pieces or reproductive structures that have fallen off of the main samples, you may place these in a bryophyte/lichen packet or seed envelope and adhere the packet to the top left corner of the herbarium paper with the flap facing out so the plant parts can be easily accessed.
4. Separate into two or more specimens.
   a. One specimen will be sent to the aquatic plant taxonomist (keep in newspaper; proceed to SOP G for shipping).
   b. One specimen will be mounted and retained at the Domain Herbarium.

c. Include flowering structures in each specimen if present. If only one specimen has flowers, send this specimen to the taxonomist for complete identification.

5. Fill out paper herbarium label. *These labels will be standardized across TOS and AOS protocols and are to be determined pending NEON Scientific Collections*.
   a. If you have species information from the taxonomist, include this on the label. If not, write the unique sample number on the label and fill in the species information later.
   b. Label should include the following information: Species name, Collector, Date collected, Location collected, and Habitat.
6. Adhere label to the bottom right corner of the herbarium paper using archival glue.
7. Arrange specimen on herbarium paper. If the plant is too long, you may cut it into several sections and place them lengthwise on the paper.
   a. Arranging specimen diagonally allows for more room.
8. Glue specimen to herbarium paper.
   a. Dot archival glue in several locations along the length of the plant roots, stems, and leaves.
9. Allow glue to dry before placing mounted specimen in the Domain Herbarium.

E.7 Preserving Samples

1. Aquatic plant specimens sent out for further taxonomic identification should be dried and pressed in a plant press. Pressed plants should be stored in a dry, well-ventilated area for a maximum of 60 days until shipping.
2. Mosses, liverworts, and lichens sent out for further taxonomic identification should be dried and placed in paper packets. Specimens should be stored in a dry, well-ventilated area for a maximum of 60 days until shipping.
3. Using a disposable pipet, preserve each algae sample with glutaraldehyde to a reach a final concentration of 2% glutaraldehyde in the sample. Preserved samples may be stored at 4^{o}C (±3^{o}C) for up to 30 days until shipping.

E.8 Ending the Processing Day

1. Refreshing the laboratory supplies
   a. Ensure that there is enough preservative for the next sampling date.
   b. Ensure that there is adequate room in the plant presses for new specimens.
2. Equipment maintenance, cleaning and storage
   a. Clean and dry all aluminum weigh dishes.
   b. Store plant presses in a dry, well-ventilated area until removing specimens for shipping.

SOP F 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.

Rename voucher photos similar to sample ID naming convention:
“SITE.DATE.species.transectID/quadratID” (e.g., ARIK.20140702.duckweed.T3).

SOP G 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.

Ship dried and pressed plant specimens in the newspaper they were pressed in. Retain a duplicate of each specimen (excluding macroalgae) in the domain herbarium 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.

G.1 Algal Taxonomy Sample Shipping

1. Taxonomy samples must not freeze, take care to avoid shipping at times when the samples may be subject to sitting for long periods in a frozen warehouse (e.g., take note of lab weekend and holiday receiving schedule).
2. Place bottles into one or several gallon-size resealable zip-top bags, grouped by site.
3. 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 periphyton/seston taxonomy samples if possible.
5. Include shipping inventory/manifest in additional zip-top bag.
6. Ship ground at ambient temperature.

G.2 Plant CN Sample Shipping

1. Organize samples by site/bout in resealable bags.
2. Wrap bagged samples in bubble wrap to cushion during shipping.
3. Include shipping inventory/manifest in additional zip-top bag.
4. Ship ground at ambient temperature.

G.3 Plant Taxonomy Sample Shipping

1. Ship pressed plants in the original newspaper.
2. Stack the newspapers containing specimens, sandwich the bundle of specimens securely between two pieces of cardboard.
3. Place bundle in a cardboard box.
4. Include shipping inventory/manifest in additional zip-top bag.
5. Ship ground at ambient temperature.

G.4 Moss, Liverwort, Lichen Taxonomy Sample Shipping

1. Ship moss, liverwort, and lichen specimens in original envelopes.
2. Organize specimens by site/bout in resealable bags.
3. Place bags in a cardboard box.
4. Wrap bagged packets in bubble wrap to cushion during shipping.
5. Include shipping inventory/manifest in additional zip-top bag.
6. Ship ground at ambient temperature.

G.5 Handling Hazardous Material

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

G.6 Supplies/Containers and Conditions

See Sections G.1-G.4 and **Table 9** for specific shipping materials.

G.7 Timelines

1. Algal 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°C (±3°C) for up to 30 days if necessary.
2. Pressed or dried taxonomy specimens
   a. May be stored in plant press or bryophyte/lichen packet for up to 60 days at room temperature.

G.8 Grouping/Splitting Samples

Group samples by site per bout.

G.9 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).

G.10 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. Email plant taxonomy labels (**Error! Reference source not found.** to aquatic plant taxonomist.

G.11 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

8 REFERENCES

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Stream Bryophyte Group. 1999. Roles of bryophytes in stream ecosystems. Journal of the North American Benthological Society. 18:151-184.

USDA [United States Department of Agriculture]. 2011. National Agricultural Library: National Invasive Species Information Center- Aquatic species. Available: (http://www.invasivespeciesinfo.gov/aquatics/main.shtml)

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 11. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002196	Datasheets for AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling in Wadeable Streams
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass

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

APPENDIX B QUICK REFERENCES

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

Step 2 – Prepare labels (2" * 4").

NEON
Sample ID: *ARIK.20140702.macroalgae2.Q1*
Transect/Quadrat/Point ID: *Q1*
Species ID: *macroalgae 2*
Collected by: *sparker*

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[09]).

Step 5 – Establish transects:

1. If this is the first site visit record transect end point locations using the GPS or establish transects through triangulation if GPS coverage is not available
2. If this is not the first visit return to previously established transects

Step 6 – Biomass quadrats: Toss the quadrat into the channel 2 m to 4 m downstream of the transect, visually estimate percent cover of each species within the quadrat, and remove all aquatic plants, bryophytes, and macroalgae from the quadrat.

Step 7 – Point transects: Space sampling points at even intervals 10 cm to 50 cm apart to have approximately 20 sampling points across each transect. Visually estimate percent cover using the view bucket and collect voucher specimens if plants cannot be positively identified in the field.

Step 8 – Keep samples chilled (~4 ^{o}C) and in the dark until processing at the Domain Support Facility.

Step 9 – Lab processing:

1. Aquatic Plants/Mosses: photograph, measure dry weight, grind and subsample for ash-free dry mass, subsample for C, N sample, press and mount plants for Domain Herbarium and taxonomist.
2. Macroalgae: measure ash-free dry mass, preserve in glutaraldehyde and send to taxonomy lab
3. Moss, Liverwort and Lichen Taxonomy: photograph, subsample for Domain Herbarium, subsample for taxonomist

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare all equipment including labels.
- ☑ Pre-print labels on waterproof paper.
- ☑ Upload GPS locations for the transects.

Sample collection: Be sure to…

- ☑ Determine the dominant habitat and second-most dominant habitat based on the Stream Morphology Map (RD[09])
- ☑ Take care not to place transects in locations where you or others have been walking in the stream or are obvious crossing areas for wildlife (e.g., beavers, cows).
- ☑ Transects should not be located within 5 m of the aquatic sensor sets or discharge transect location due to heavy foot traffic in those areas.
- ☑ Start sampling at the bottom of the reach, working upstream so as not to decrease visibility and disrupt aquatic plant, bryophyte, lichen and macroalgae communities.
- ☑ Take extra care when rinsing samples to not lose the sample material.
- ☑ Remove aquatic plants, bryophytes and macroalgae from the quadrat, but do not remove lichen.
- ☑ **DO NOT COLLECT ENDANGERED OR THREATENED SPECIES.**

Sample processing: Be sure to…

- ☑ **DO NOT CRUSH** samples or attempt to wring the water out.
- ☑ Take care not to let the dried sample blow out of weigh boats.

APPENDIX D BRYOPHYTE AND LICHEN PACKET TEMPLATE

Domain:	**Site:**
Collector:	**Date collected:**
State:	**County:**
Lat:	**Long:**

Site and Substrate Characteristics (circle all that apply)

Light: sunny, open, filtered, partial shade, full shade

Water: dry, mesic, moist, seep, wet, submerged to _____m

Topography: ridge, slope, valley, trail, roadside

Habitat: dense/open/cut forest, woodland, savannah, grassland, heath, chaparral, desert, riparian, spring/seep, meadow, bog/fen, swamp, pond/lake, river/stream, intermittent

Substrate: granitic, metamorphic, sedimentary, volcanic, other _____

Soil: sand, gravel, clay, rocky, litter, humus, peat, moss

Rock: outcrop, cliff, crevice, top/wall of boulder/slab, under-hang

Tree: base, trunk, stump, snag, log, fallen/dead/rotten, branch, bark, leaf, shrub, climber _____m above ground

Type: conifer, hardwood, other _____

Elevation: m	**Aspect:** N, S, E, W exposure
Dominant plants:	
Coll. #:	**Taxon:**

APPENDIX E THREATENED AND ENDANGERED PLANTS

Domain	Symbol	Scientific name	Common name	Federal status*	State status*	Habitat type
D1	BOFL3	*Bolboschoenus fluviatilis*	river bullrush		MA (SC)	aquatic (stream)
D1	POCE3	*Podostemum ceratophyllum*	hornleaf riverweed		MA (SC)	aquatic (stream)
D1	CAAL8	*Carex alopecoidea*	foxtail sedge		MA (T)	aquatic (stream)
D1	SPPE3	*Sphenopholis pensylvanica*	swamp oats		MA (T)	riparian (stream, pond)
D1	CALE8	*Carex lenticularis*	lakeshore sedge		MA (T)	riparian (stream, pond)
D1	CAMI15	*Carex michauxiana*	Michaux's sedge		MA (E)	riparian (stream, pond)
D1	CAOL3	*Carex oligosperma*	fewseed sedge		MA (E)	riparian (stream, pond)
D1	CASC9	*Carex schweinitzii*	Schweinitz's sedge		MA (E)	riparian (stream, pond)
D1	CATU2	*Carex tuckermanii*	Tuckerman's sedge		MA (E)	riparian (stream, pond)
D2	ELIN	*Eleocharis intermedia*	matted spikerush		MD (E)	aquatic
D2	PAFL5	*Paspalum fluitans*	horsetail paspalum		MD (E)	aquatic
D2	POAM5	*Potamogeton amplifolius*	largeleaf pondweed		MD (E, X)	aquatic
D2	POFO3	*Potamogeton foliosus*	leafy pondweed		MD (E)	aquatic
D2	PORI2	*Potamogeton richardsonii*	Richardson's pondweed		MD (E, X)	aquatic
D2	PORO2	*Potamogeton robbinsii*	Robbins' pondweed		MD (E, X)	aquatic
D2	POZO	*Potamogeton zosteriformis*	flatstem pondweed		MD (E)	aquatic
D2	SCSU10	*Schoenoplectus subterminalis*	swaying bulrush			aquatic
D2	WOFL	*Wolffiella floridana*	duckweed		MD (E, X)	aquatic
D2	WOGL2	*Wolffiella gladiata*	Florida mudmidget			aquatic
D2	CALA16	*Carex lacustris*	hairy sedge		MD (T)	aquatic (stream, pond)
D2	CATU2	*Carex tuckermanii*	Tuckerman's sedge		MD (E)	aquatic (stream, pond)
D2	CACO14	*Carex conoidea*	openfield sedge		MD (E)	riparian (stream, pond)
D2	CAGL5	*Carex glaucescens*	southern waxy sedge		MD (E)	riparian (stream, pond)
D3	ISBO2	*Isoetes boomii*	Boom's quillwort		FL (E)	aquatic (blackwater stream)
D3	RHCR3	*Rhynchospora crinipes*	mosquito beaksedge		FL (E)	aquatic (blackwater stream)
D3	HYCO5	*Hymenocallis coronaria*	Cahaba lily		GA (E)	aquatic (Piedmont rivers)
D3	HYHE2	*Hymenocallis henryae*	Henry's spiderlily		FL (E)	aquatic (Piedmont rivers)
D3	PTNO	*Ptilimnium nodosum*	piedmont mock bishopweed	E	GA (E)	riparian (stream)
D3	COTU	*Coelorachis tuberculosa*	bumpy jointtail grass		FL (T)	aquatic (pond)
D3	ISHY	*Isoetes hyemalis*	evergreen quillwort		FL (E)	aquatic (pond)
D3	MYLA2	*Myriophyllum laxum*	loose watermilfoil		GA (T)	aquatic (pond)
D3	NYJA	*Nymphaea jamesoniana*	James' waterlily		FL (E)	aquatic (pond)
D3	POFL5	*Potamogeton floridanus*	Florida pondweed		FL (E)	aquatic (pond)
D3	NAFI2	*Najas filifolia*	needleleaf waternymph		FL (T)	aquatic (sandy bottomed lakes)
D5	ARLA22	*Armoracia lacustris*	lake cress		WI (E)	aquatic (pond)
D5	CAHE3	*Callitriche heterophylla*	twoheaded water-starwort		WI (T)	aquatic (pond)
D5	STVA8	*Stuckenia vaginata*	sheathed pondweed			aquatic (pond)
D5	CANA	*Caltha natans*	floating marsh marigold		WI (E)	aquatic (pond)
D5	ELNI	*Eleocharis nitida*	quill spikerush		WI (E)	aquatic (pond)
D5	ELQU	*Eleocharis quadrangulata*	squarestem spikerush		WI (E)	aquatic (pond)
D5	ELRO2	*Eleocharis rostellata*	beaked spikerush		WI (T)	aquatic (pond)
D5	NEAQ2	*Neobeckia aquatica*	lakecress			aquatic (pond)
D5	POPU6	*Potamogeton pulcher*	spotted pondweed		WI (E)	aquatic (pond)
D5	RAGM	*Ranunculus gmelinii*	Gmelin's buttercup			aquatic (pond)
D5	RAGMH	*Ranunculus gmelinii var. hookeri*	Gmelin's buttercup		WI (E)	aquatic (pond)
D5	SPGL	*Sparganium glomeratum*	clustered bur-reed		WI (T)	aquatic (pond)
D5	CASC9	*Carex schweinitzii*	Schweinitz's sedge		WI (E)	aquatic (stream, pond)
D5	CALE8	*Carex lenticularis*	lakeshore sedge		WI (T)	aquatic (stream, pond)
D5	SCHA9	*Schoenoplectus hallii*	Hall's bulrush			aquatic, riparian (pond)
D5	FUPU	*Fuirena pumila*	dwarf umbrella-sedge		WI (E)	riparian (pond)
D5	SCRE	*Scleria reticularis*	netted nutrush		WI (E)	riparian (pond)

*E=endangered, T=threatened, X=extirpated, PX=possibly extirpated SC=special conern, SR=salvage restricted

Domain	Symbol	Scientific name	Common name	Federal status*	State status*	Habitat type
D5	CAMI15	*Carex michauxiana*	Michaux's sedge		WI (T)	riparian (stream)
D5	RHSC5	*Rhynchospora scirpoides*	longbeak beaksedge			riparian (stream)
D5	SESE	*Selaginella selaginoides*	club spikemoss		WI (E)	riparian (stream)
D7	ELNU2	*Elodea nuttallii*	western waterweed		TN (SC)	aquatic (stream, pond)
D7	SCSU10	*Schoenoplectus subterminalis*	water bulrush		TN (SC)	aquatic (stream, pond)
D7	CAAL8	*Carex alopecoidea*	foxtail sedge		TN (PX, E)	riparian (stream)
D7	CAHY2	*Carex hyalina*	tissue sedge		TN (SC)	riparian (stream)
D7	CLGL	*Clematis glaucophylla*	whiteleaf leather flower		TN (E)	riparian (stream)
D7	ELLA	*Eleocharis lanceolata*	daggerleaf spikerush		TN (SC)	riparian (stream)
D7	MATR2	*Marshallia trinervia*	broadleaf Barbara's buttons		TN (T)	riparian (stream)
D7	RHCH2	*Rhynchospora chalarocephala*	loosehead beaksedge		TN (T)	riparian (stream)
D7	VEAM2	*Veronica americana*	American speedwell		TN (SC)	riparian (stream)
D7	VEAN2	*Veronica anagallis-aquatica*	water speedwell			riparian (stream)
D7	CACO8	*Carex comosa*	longhair sedge		TN (T)	riparian (stream, pond)
D7	CAECE	*Carex echinata*	star sedge		TN (SC)	riparian (stream, pond)
D7	CALA16	*Carex lacustris*	hairy sedge		TN (T)	riparian (stream, pond)
D7	CAPE42	*Carex pellita*	woolly sedge			riparian (stream, pond)
D7	ELEQ	*Eleocharis equisetoides*	jointed spikesedge		TN (E)	riparian (stream, pond)
D7	ELIN	*Eleocharis intermedia*	matted spikerush		TN (SC)	riparian (stream, pond)
D7	RHRA2	*Rhynchospora rariflora*	fewflower beaksedge		TN (E)	riparian (stream, pond)
D7	RHWR	*Rhynchospora wrightiana*	Wright's beaksedge		TN (PX, E)	riparian (stream, pond)
D7	CARO6	*Carex rostrata*	beaked sedge		TN (T)	aquatic, riparian (stream, pond)
D8	PTNO	*Ptilimnium nodosum*	piedmont mock bishopweed	E		riparian (stream)
D8	XYTE	*Xyris tennesseensis*	Tennessee yelloweyed grass	E		riparian (stream)
D11	POCL	*Potamogeton clystocarpus*	little aguja pondweed	E	TX (E)	aquatic (pond)
D11	ZITE	*Zizania texana*	Texas wildrice	E	TX (E)	aquatic (San Marcos rivers)
D14	CIPA	*Cirsium parryi*	Parry's thistle		AZ (SR)	riparian (stream)
D14	EPGI	*Epipactis gigantea*	stream orchid		AZ (SR)	riparian (stream)
D14	LIPA2	*Lilium parryi*	lemon lily		AZ (SR)	riparian (stream)

*E=endangered, T=threatened, X=extirpated, PX=possibly extirpated SC=special conern, SR=salvage restricted

APPENDIX F AQUATIC PLANT TAXONOMY LABELS

NATIONAL ECOLOGICAL OBSERVATORY NETWORK (NEON)	
PLANTS OF THE UNITED STATES	
[STATE]	
[SampleID]	
[habitat]	
[breif description of growth form and location]	
[COUNTY]: [Full site name, direction and # of miles from a known point or town, any locational information.	
[Latitude], [Longitude]	
[date collected]	[Collected by]
	[Recorded by]

NATIONAL ECOLOGICAL OBSERVATORY NETWORK (NEON)	
PLANTS OF THE UNITED STATES	
[STATE]	
[SampleID]	
[habitat]	
[breif description of growth form and location]	
[COUNTY]: [Full site name, direction and # of miles from a known point	
[Latitude], [Longitude]	
[date collected]	[Collected by]
	[Recorded by]

APPENDIX G ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

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 H SITE-SPECIFIC 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	Pools
D01	Sawmill Brook	Riffles	Pools
D02	Mill Run	to be determined	to be determined
D02	Posey Creek	Riffles	Pools
D04	Rio Guilarte	Riffles	Pools
D04	Rio Cupeyes	Riffles	Pools
D05	Pickerel Creek	Riffles	Pools
D06	Kings Creek	*	*
D07	Leconte Creek	*	*
D07	Walker Branch	*	*
D08	Mayfield Creek	Runs/riffles	Runs
D10	Arikaree River	Runs	Pools/Runs
D11	Pringle Creek	*	*
D12	Bozeman Creek	Riffles	Riffles/Runs
D12	Blacktail Deer Creek	Riffles	Riffles/Runs
D13	Como Creek	Riffles	*
D13	West St. Louis Creek	Riffles	Pools
D14	Sycamore Creek	Riffles	Runs/Pools
D15	Red Butte Creek	Riffles	*
D16	McRae Creek	Riffles	Step pools
D16	Planting Creek	Riffles	Pools
D17	Convict Creek	Riffles	*
D17	Providence Creek	*	*
D18	Oksrukuyik Creek	Riffles	Pools
D19	Caribou Creek	Riffles	*

**Habitat type to be determined*