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

PREPARED BY	ORGANIZATION	DATE
Stephanie Parker	AQU	11/18/2015

APPROVALS	ORGANIZATION	APPROVAL DATE
Andrea Thorpe	SCI	01/20/2016
Mike Stewart	PSE	01/14/2016

RELEASED BY	ORGANIZATION	RELEASE DATE
Jennifer DeNicholas	CM	01/22/2016

See configuration management system for approval history.

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	01/22/2016	ECO-03470	Initial release, supersedes protocols NEON.DOC.000692 and NEON.DOC.001203. Updates to lake point selection methods and remove cover class.

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1 OVERVIEW

1.1 Background

Aquatic plants, bryophytes, lichens, and macroalgae are primary producers common in aquatic ecosystems. They, along with algae and microbes, form the autochthonous (i.e., originating within the ecosystem) base of the food web. Additionally, aquatic plants and bryophytes add complexity to the lake or stream 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 lake or stream bottom, 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 lake or stream bottom.

Environmental factors such as flooding and scouring, wave activity, water level, light attenuation, and nutrient availability have strong effects on the aquatic plant community (Wetzel 2001). Light quantity and quality, water depth, wave activity, 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). In lakes and rivers, aquatic plants are often limited to shallow, littoral areas in deeper lakes but may grow in deeper areas in lakes with clear water and deeper light penetration.

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, invasive species extent, abundance, and biodiversity over time, as well as changes in biogeochemical cycles. Aquatic plants and macroalgae can act as indicators of changes in watershed activity by integrating the

effects of changing nutrient loads, toxicity, and land-cover. 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 (but are not limited to) the 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, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

Protocols for point transect and quadrat sampling are based on those set forth in Bowden et al. 2006. Sampling procedures in lakes are based on the standard operation procedures of the North Temperate Lakes Long-Term Ecological Research (North Temperate Lakes LTER 2011), the Wisconsin Department of Natural Resources (DNR; Deppe and Lathrop 1992), the US Geological Survey (USGS) long term monitoring program (Yin et al. 2000), and the US Environmental Protection Agency (USEPA) National Lake Assessment (Neuman 2008). Thanks to Dave Barnett of the NEON Terrestrial Observation System (TOS) team for assistance with plant pressing lab methods. 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

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1-4 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.003040	Datasheets for AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling
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.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[10]	NEON.DOC.003045	AOS Protocol and Procedure: Periphyton, Seston and Phytoplankton Sampling
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.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD[15]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbial Sampling
RD[16]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[17]	NEON.DOC.001702	NEON Herbarium Specimen Label & Annotation Generation

2.3 Acronyms

Acronym	Definition
AFDM	ash-free dry mass
C	Carbon
^{o}C	degrees Celsius
cm	Centimeter
CPOM	coarse particulate organic matter
CWD	coarse woody debris
D(#)	domain (#)
DI	deionized water
DNR	Department of Natural Resources
FPOM	fine particulate organic matter
g	grams
GIS	Geographic Information System
GPS	Global positioning system
HDPE	High-density polyethylene
hp	horsepower
HVAC	heating, ventilation and air conditioning
m	Meter
mL	Milliliter
mm	Millimeter
MOB	Man overboard
m s^{-1}	meters per second
N	nitrogen
oz	Ounce
PFD	personal flotation device
SCUBA	Self-contained underwater breathing apparatus
SD	secure digital (flash memory card)
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey

2.4 Definitions

Aquatic plant: "Large" vascular plants with root system living in freshwater bodies of water. Aquatic plants will be classified based on the following life forms (Figure 2):

1. **Emergent**: Growing above the water's surface but rooted in the sediments
2. **Floating-leaved**: Permanently submerged plants, rooted at the bottom but producing leaves that float on the water's surface
3. **Submerged**: Plants entirely underwater
4. **Free-floating**: Not attached to substratum, leaves float on water surface

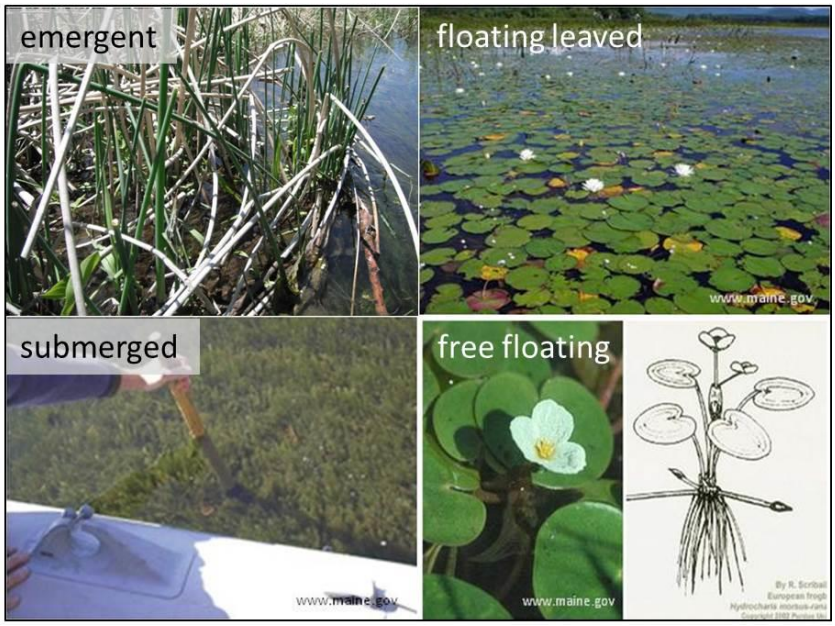

Figure 2. Aquatic plant growth forms: emergent, floating-leaved, submerged, and free-floating.

Autochthonous: Originating within the body of water where found.

Benthic zone: Area of the sediment-water interface, or bottom, of the body of water where aquatic plants are rooted.

Bryophyte: Nonvascular plant, including mosses, liverworts, and hornworts (Figure 3). May often be submerged or in the "splash zone" (annual floodplain).

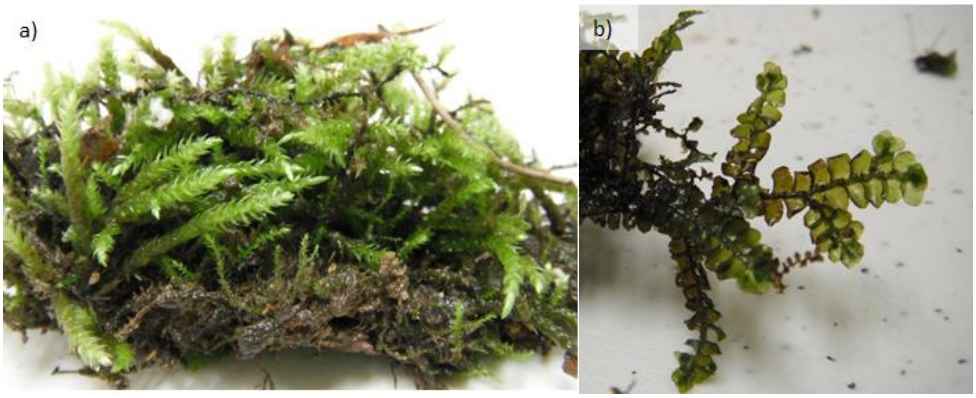

Figure 3. Bryophytes are a group of non-vascular plants including a) mosses and b) liverworts which may be submerged or growing in the "splash zone".

Lichen: Symbiosis of an alga and a fungus, typically not submerged, but may be underwater for short periods of time during high water (Figure 4).

Figure 4. Lichens, a symbiotic relationship between fungi and algae.

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

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

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

Pelagic: Area not near the shore; middle or deepest parts of the lake.

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

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

Run: An aquatic habitat with swiftly flowing water but no surface water agitation, with relatively uniform flow.

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

3 METHOD

The goals of this protocol are: 1) to determine percent cover of aquatic plants on the **wadeable stream** bottom (percent cover in **lakes and non-wadeable streams** will be estimated via the bathymetry protocol, RD[16]); 2) to collect aquatic plant, bryophyte, and macroalgae samples for identification and biomass measurement; and 3) to collect lichen specimens for identification. Plants encountered on point transects are identified *in situ* where possible using photo keys based on NEON Construction Voucher specimens and applicable regional keys. However, additional voucher specimens should be collected if the field technician is unable to make a positive identification in the field. Voucher specimens are collected and returned to the Domain Support Facility for processing and shipping to appropriate taxonomists (see SOP F). Macroalgae specimens will always be collected and not identified

in the field. Voucher specimens are sampled using grab samples near the point transects in **wadeable streams**.

To track changes in the flora of the site (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 in **wadeable streams** is estimated using point transects, a method modified from the standard point-frame method in terrestrial ecosystems (Bowden et al. 2006). In **lakes and non-wadeable streams**, samples are collected using a randomized point sampling method, which allows for data collection in many areas of the lake or river (Figure 6), rather than along select transect lines (Berg 2009). Samples are collected following the EPA National Lakes Assessment (Neuman 2008, Wisconsin DNR 2008) and USGS Long Term Resource Monitoring Program (Yin et al. 2000) where aquatic plants and macroalgae are collected from a boat using a double-sided rake. In many other monitoring programs, quantitative plant surveys and biomass removal are completed using SCUBA diver surveys (Downing and Anderson 1985). However, SCUBA surveys will not be used for NEON data collection due to logistical constraints.

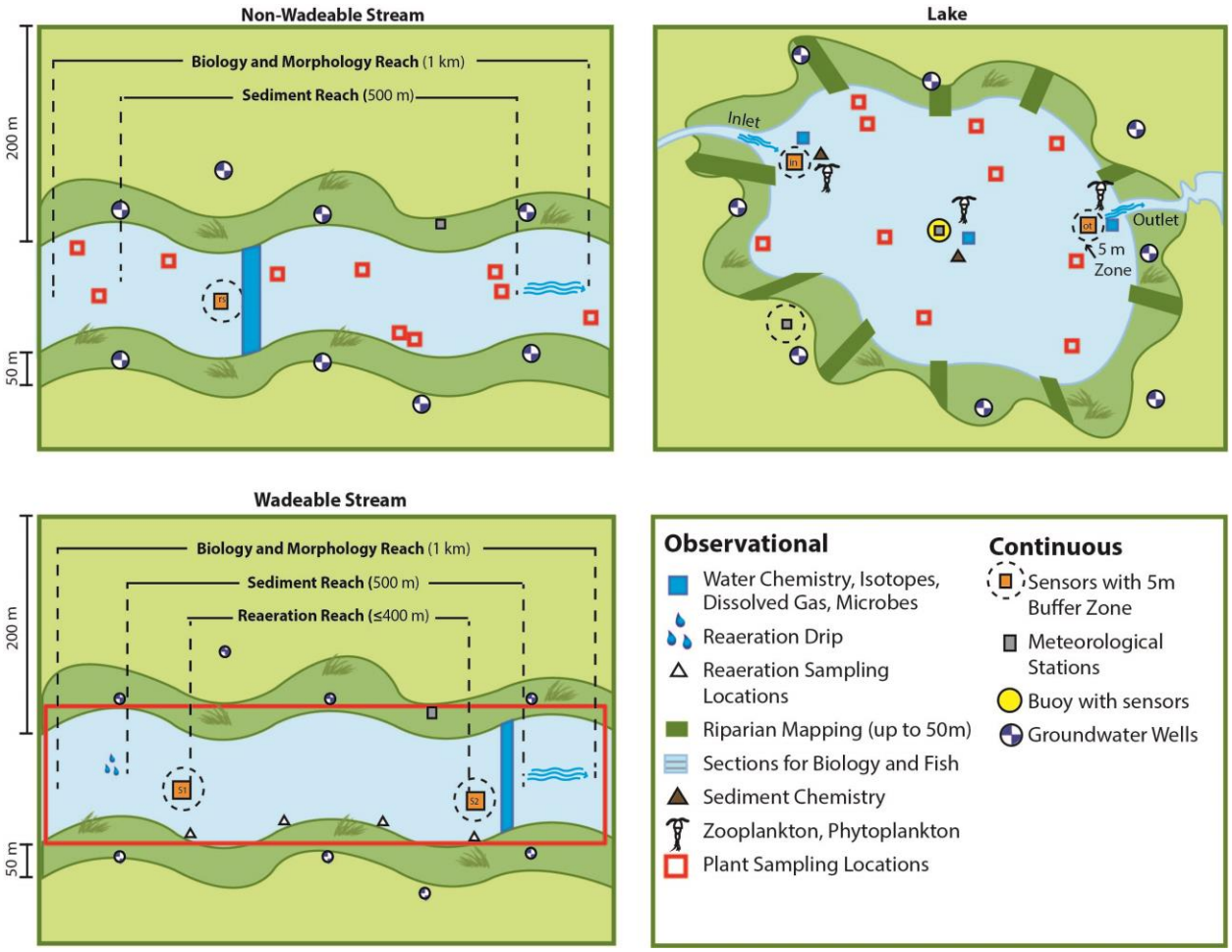

Figure 6. Generic non-wadeable stream, lake, and wadeable stream site layouts with aquatic plant, bryophyte, lichen and macroalgae sampling locations.

Percent cover of **lakes and non-wadeable streams** is estimated over time as part of the Bathymetry and Morphology for Lakes and Non-wadeable Streams Protocol (RD[16]). Data collected during echo sounder surveys are processed by a trained technician and will show what portions of the lake or stream bottom are colonized by plants, and will be used for selecting random points. Biomass sampling will allow researchers to determine the contribution of these taxa to the aquatic habitat flora. If the percent cover of vegetation decreases dramatically (to ~5-10% of the bottom), the sampling methods may be reassessed so as not to extirpate species from the body of water.

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

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Benthic aquatic plant, bryophyte, lichen, and macroalgae sampling occurs three times during the growing season at each site, roughly spring, summer, and autumn. Sampling must occur within the first 21 days of 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. Use NEON's problem reporting system to report sampling efforts that take place outside of the defined sampling window.

Echo sounder surveys **of lake and non-wadeable stream** bottoms will be performed once per year during the time of highest biological activity (sampling Bout 2) and will be performed at approximately the same time each year for continuity. One of the three aquatic plants and macroalgae sampling dates at lakes and rivers should be performed within 1 week (either before or after) the annual sonar survey so data can be compared.

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 ice on/ice off (for lake sites), water flow (for wadeable and non-wadeable stream sites), 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:

1. Day 1: 2-8 hours for sorting and weighing fresh samples
2. Day 2: 2-8 hours for weighing dried samples
3. Day 3: 2-3 hours for weighing ashed samples

Dried samples may be stored between each lab processing day if necessary, days do not need to be consecutive. Samples should be shipped to the external lab or taxonomist within 30 days of sampling. For additional storage and shipping timelines see SOP F.

4.4 Sample 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 weather conditions deteriorate and conditions become unsafe (e.g. approaching thunderstorm, rapid increase of water level in the **wadeable stream**), or the **lake/non-wadeable stream** becomes too windy (>32 km hr^{-1}) and has unsafe wave heights (>1 m) so that the boat cannot be held stationary over a sampling point while at anchor, return to shore and wait in a safe location for 30 minutes. If conditions improve, resume sampling, if not, return to the Domain Support Facility and sample at another time.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the streamflow or weather requirements, continue to collect samples after the delay. If conditions do not improve, discard any previously collected samples at the site or at the Domain Support Facility and start over on the next appropriate sampling day.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
14 Days	If flooding occurs on or prior to the targeted sampling date in a **wadeable stream** (>3x median discharge for the preceding year; Clausen and Biggs 1997) or unsafe wading conditions occur (Lane and Fay 1997), wait a minimum of 5 days after water level drops below 3x median discharge and is safely wadeable and macroalgae can 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.
6 Months	Preserved macroalgae samples may be held for up to 6 months at 4 ^{o}C in the domain lab if circumstances do not allow shipping to the external lab.	Holding samples >30 days affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected and samples do not need to be flagged if held for ≤6 months.
	Dried/ground plant CN samples may be held for up to 6 months, dry, at room temperature	Holding samples >30 days affects external lab schedules, staffing, and budgets and

	in the domain lab if circumstances do not allow shipping to the external lab.	delays data release on the NEON portal. However, sample integrity is not affected and samples do not need to be flagged if held for ≤6 months.
	Dried/pressed plant/moss/liverwort/lichen taxonomy samples may be held for up to 6 months, dry, at room temperature in the domain lab if circumstances do not allow shipping to the taxonomic facility.	Holding samples >30 days affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected and samples do not need to be flagged if held for ≤6 months.

4.5 Sampling Specific Concerns

1. If an endangered or threatened plant species is discovered (based on the Endangered Species List, Appendix E), **do not collect**. Take photos and note location within the site using handheld GPS. Submit a problem ticket to report findings to NEON Permitting.
   a. Plants are more difficult to identify via photos, so accurate identification may be less certain. It will not be possible to obtain biomass data for the specimen.
2. If sampling at a small headwater stream dominated by bryophytes, use the smaller 10cm x 10cm quadrat, determined on a site-by-site basis by the site host and/or NEON Aquatic Ecologist. Quadrat size must be recorded in order to process data.
3. If sampling at a site with large amounts of plant biomass, collect biomass samples using larger containers such as trash bags, use subsampling lab procedures.

5 SAFETY

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

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

Safety Data Sheets (SDS) shall be made available for all chemicals used in this work (glutaraldehyde). Whenever chemicals are used, follow requirements of the site-specific Chemical Hygiene and Biosafety Plan (AD[03]) for laboratory safety and NEON EHS Safety Policy and Program Manual (AD[01]), Section HC-03, Hazard Communication.

See Section 10 in the NEON Operations Field Safety and Security Plan [AD02]). In addition, the following safety requirements are sought:

1. Activities in **wadeable streams** should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 10 ft^2/s (0.93 m^2/s; Lane and Fay 1997).
2. In **lakes and non-wadeable streams**, site-specific hazards may be encountered that cause technicians to conduct sampling from the boat, without dismounting from the vessel. In addition, technicians are required to use extra caution in waters where alligators are present and to make sure a safe distance from hazards is maintained.
3. All personnel must be wearing a personal flotation device (PFD) prior to entering the boat.
4. All employees shall have access to a form of communication with other team members such as a two-way radio.
5. Technicians should be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.)

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as 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[09] or RD[16]	R	Site-specific Stream Morphology or rapid habitat assessment, or Bathymetry Map	Determining sampling locations	1	N
	R	Handheld GPS unit (with batteries, ±4 m accuracy) or Humminbird	Navigating to sampling locations	1	N
	S	Clipboard	Recording data	1	N
	R	Cooler, 9-28 quart	Storing samples	1	N
	R	Ice packs or water ice	Keeping samples cool	2	N
	R	Waders (hip or chest) or knee boots	Boating or wading	1 pair per person	N
Consumable items					

Item No.	R/S	Description	Purpose	Quantity	Special Handling
RD[06]	R	Aquatic Field Metadata Sheet (all-weather paper)	Recording metadata	1	N
RD[05]	R	Field datasheets (all-weather copier paper, write in pencil)	Recording data	2	N
	R	Pre-printed all-weather paper labels	Labeling aquatic plant, bryophyte, and lichen samples	10-20	N
	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) or pin flags	Flagging plant transect locations	1	N

R/S=Required/Suggested

Table 3. Equipment list – Transect establishment in **wadeable streams**

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
RD[09]	R	Site-specific Stream Morphology Map or rapid habitat assessment	Determining sampling locations	1	N
	R	Handheld GPS (with batteries, ± 4 m accuracy)	Recording transect locations	1	N
	R	AOS plot markers	Marking transect locations	10	N
Consumable items					
		(none)			

R/S=Required/Suggested

Table 4. Equipment list – Sampling equipment for **wadeable streams**

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
	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, use with chisel	Cobbles and boulders	1	N
	S	Chisel	Collecting lichen specimens, use with mallet	Cobbles and boulders	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	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	Headwater streams	1	N
	R	Digital camera, waterproof (with battery, memory card)	Photographing specimens	All	1	N
	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	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	5	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
				cover		
	R	Cable ties (6 inch), package	Attaching weight to rake	Lakes/Non-wadeable Streams	1	N

R/S=Required/Suggested

Table 5. Equipment list – Sampling equipment for **lakes and non-wadeable streams**

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX103032	R	Double sided thatching rake with handle	Sample collection	1	N
	R	Braided polyester line marked in 20 cm increments for rake	Sample collection	1	N
	R	Dive weight (10 lb) or Secchi disk weight – attach to rake with cable ties if using rope	Weighing down rake	1	N
MX100304	S	Collapsible quadrat (0.5 x 0.5 m)	Sample collection for floating plants	1	N
MX109276	R	Depth finder	Determining depth at the sampling point	1	N
	S	Scissors	Removing aquatic plants, bryophytes and macroalgae	1	N
	R	Digital camera, waterproof (with battery, memory card)	Photographing specimens	1	N
	S	Field identification key (site specific), created over time at the domain	Identifying specimens	1	N
	S	Tray, tub, or 5 gallon bucket	Sorting samples in the field	1	N
Consumable items					

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Cable ties (6 inch), package	Attaching weight to rake	1	N
	R	Whirl-pak® bags	Sample container	30	N
	R	Resealable zip-top bags (gallon)	Whirl-pak® container	10	N

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

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Plastic larval tray	Measuring dry weight of large specimens	1	N
	S	Large tray or Tupperware container	Floating plant material to separate taxa and macroalgae	1-3	N
	S	Weigh boats (plastic, large)	Measuring dry weight of small specimens	20	N
MX108050 or MX106267	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

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Desiccator (bench top)	Storing dried samples	1	N
Consumable items					
	R	Desiccant packs	For bench top desiccator	1-2	N
	R	Lab datasheets	Recording data	1	N
	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	24	N
	R	Standard driers (sheets)	Pressing plants	24	N
	S	Forceps (blunt point)	Handling specimens	1	N
	R	Handheld digital camera, battery, and 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
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
	S	Cardboard box (~9”x7”x7”)	Shipping taxonomy samples	1	N
	R	Bubble wrap	Padding taxonomy and CN samples	TBD	N
RD[13]	R	Shipping inventory	Provides sample information to external lab	1	N

R/S=Required/Suggested

6.2 Training Requirements

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

All personnel required to operate a boat shall be trained through an approved program. All others shall be aware of boating safety procedures.

Personnel will be trained in the field protocols associated with this document, and trained in safe working practices for aquatic-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

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

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as a 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 2-8 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. Load GPS sampling coordinates on handheld GPS unit (±4 m accuracy).
3. Print labels on all-weather paper (Figure 7).

NEON Sample ID: *ARIK.20140702.unkfern.T1* Transect/Quadrat/Point ID: *T1* Species ID: *unkfern* Collected by: *jstewart@neoninc.org*	**NEON** Sample ID: *ARIK.20140702.macroalgae1.T1* Transect/Quadrat/Point ID: *T1* Species ID: *macroalgae1* Collected by: *jstewart@neoninc.org*
NEON Sample ID: *ARIK.20140702.macroalgae2.Q1* Transect/Quadrat/Point ID: *Q1* Species ID: *macroalgae2* Collected by: *jstewart@neoninc.org*	**NEON** Sample ID: *ARIK.20140702.unkpoaceae1.Q1* Transect/Quadrat/Point ID: *Q1* Species ID: *unkpoaceae1* Collected by: *jstewart@neoninc.org*

Figure 7. Example of all-weather field labels for aquatic plant and macroalgae collection.

4. Have ice or ice packs frozen and ready for transportation cooler.
5. Charge batteries for digital camera and check batteries (bring extras) for handheld GPS unit.
6. See Laboratory Preparation procedures in Section D.1 for additional pre-sampling activities (e.g., weigh boat preparation).
7. Fill out general aquatic field metadata sheet (RD[06]) upon every field sampling visit.

SOP B Determining Sampling Locations

B.1 Lakes and Non-wadeable Streams – Randomized Point Selection

1. Using the most recent bathymetric map of the lake/river received from NEON HQ (it may be from the previous summer; RD[09]) determine the colonizable depth based on existing aquatic plant cover, and generate a polygon using GIS software of the portions of the lake/river bottom that are equal to or shallower than this depth (Figure 8).
   a. This represents areas of potential plant growth, not necessarily where plants are known to be rooted.
   b. If the plant cover map is not available, estimate maximum depth of plant colonization using the transect method (USEPA 2011) in Step 3.
2. Create 20 random coordinates within the defined polygon of potential plant colonization using the "Create Random Points" function in ArcMap. If the plant cover bathymetry maps are not available, create 40 random points within the wetted perimeter of the lake, and throw out points below the maximum plant colonization depth (Step 3 below).
   a. Ensure that you have received the polygon (shapefile) for your site. This will be created from the perimeter mapping effort in the bathymetry protocol and/or the plant cover bathymetry map.
   b. Open new blank map in ArcMap.
   c. Click the 'Add data' button and navigate to the folder where your polygon is (click 'Connect to Folder' button on top right or screen if you haven't done so already).
   d. Select the shape file for your site.
   e. Go to ArcToolbox.
   f. Select 'Data Management Tools' > 'Feature Class' > 'Create random points'
   g. Specify output location in pop up window and title of feature class
      i. 'Constraining feature class': choose the polygon you are working with
      ii. Number of points = 20 (or ≥40)
      iii. Select 'decimal degrees'
      iv. Click 'ok'
   h. Go to 'Data Management Tools' > 'Features' > 'Add XY Coordinates'
      i. 'Input feature': choose the shapefile of points you just created
      ii. Click 'ok'
   i. Label the random points, 1-20 (or ≥40)
      i. Right click the random points layer and select "Properties". Select the "Labels" tab.
      ii. Select the box "Label Features in this Layer"
      iii. Select the label field "OID" in the drop down menu and apply.
   j. To export attribute table: go to 'ArcToolbox' > 'Conversion Tools' > 'Excel' > 'Table to Excel'
      i. Specify attribute table and where you want the output file to be saved
   k. Number coordinates "1" through "20" and print a copy to take in the field or load into GPS unit.

3. **Perform this step prior to sampling only if the plant cover bathymetric map is not available.** In the field, choose a transect location in an area where you are reasonably sure that plants are growing. Avoid placing the transect at the boat ramp.
   a. Run the transect perpendicular from a point chosen on the shoreline toward the buoy location.
   b. Ideally, at least 6 points along the transect should be sampled.
   c. If the lake is ≤4.0 m deep, collect rake grab samples at the following points:
      i. 0.5 m depth (±0.2 m), 1.0 m, 1.5 m, 2.0 m, 2.5 m, 3.0 m or until you reach one of the stop criteria below:
   d. If the lake is >4.0 m deep, collect rake grab samples at the following points:
      i. 0.5 m depth (±0.2 m), 1.0, 2.0, continue sampling at each meter depth until you reach one of the stop criteria:
   e. STOP sampling criteria:
      i. STOP if you have samples two points in a row with 0 plants/macroalgae on the rake
      ii. STOP if you reach the buoy location.
   f. The last point with plant/macroalgal material on the rake is the estimated maximum sampling depth of plant colonization. Be sure that this depth makes sense given the lake/river you are sampling. If you reach the buoy location with plant material on each rake grab, consider maximum plant depth to be the same as the maximum lake depth.
   g. If it seems that the transect is not representative of plant cover and plant colonization depth at the lake, perform an additional transect to determine plant colonization depth.
4. Only 10 of the random coordinates will be sampled, starting at the beginning of the list. However if conditions (bottom substrate, location is dry, depth, etc.) are not conducive to sampling at a given coordinate, you may move on to the next coordinate on the list. Record both the coordinate samples and the coordinate uncertainty (i.e., error on GPS unit or error associated with navigation and anchoring at the point) on the field data sheet.
   a. If substrata and depth are appropriate for sampling and plant cover is 0, the point is still a valid sampling location.
   b. Do not sample at depths greater than the maximum depth of plant colonization as determined above or on the plant cover bathymetry map.
   c. Do not sample within a 5 m radius of the aquatic instrumentation.

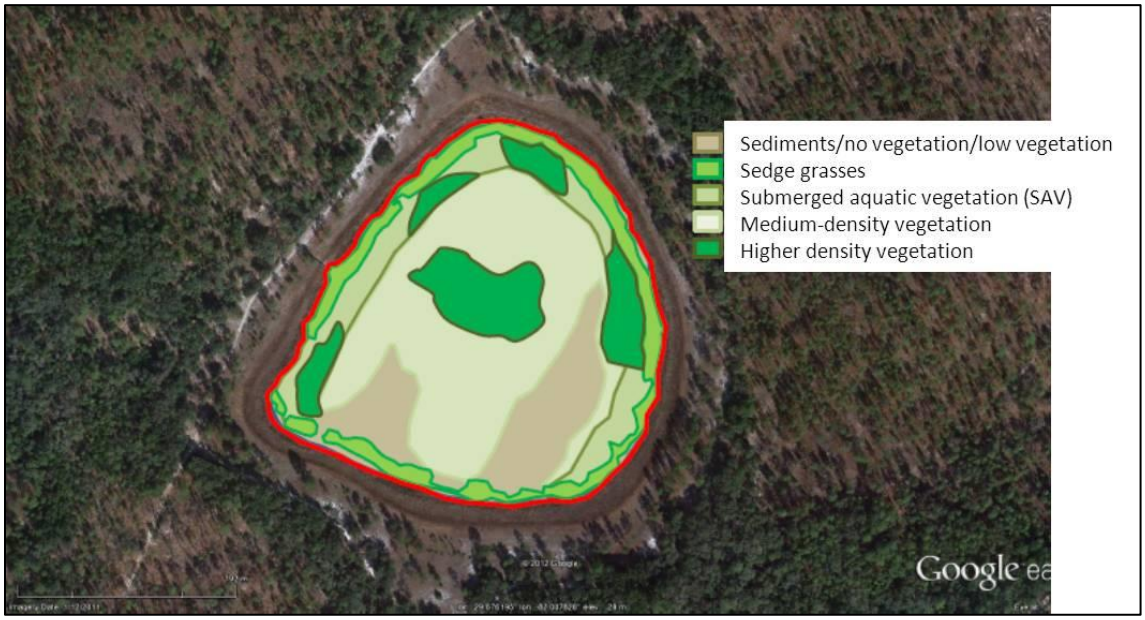

Figure 8. Bathymetric map of Barco Lake (D04) showing location and percent coverage of aquatic plants.

B.2 Wadeable Streams – Transect Establishment

1. Establish transects on the initial sampling bout at the site, or if stream geomorphology has changed necessitating moving a previously established transect(s).
2. Determine percent cover of habitat types throughout the sampling reach using the Stream Morphology Map (RD[09]) or rapid habitat assessment.
   a. The habitat type(s) chosen should be present during all sampling bouts.
   b. The habitat type(s) chosen should account for >20% of the area of the reach (RD[09])
   c. All replicate transects must remain in the same habitat types on each sampling bout, unless a major event (i.e., a flood) causes significant changes to the stream channel.
      i. Reasons for relocating a transect include a change in the habitat type at that location (e.g., riffles are now pools).
      ii. If the stream is dewatered such that a transect is dry, record as 'Y' for "Location dry" on the field datasheet.
   d. Habitats chosen should be the types as those chosen for RD[10], RD[14], and RD[15].
   e. Targeted habitat types (see Definitions, Section 2.4)
      i. Riffle
      ii. Run
      iii. Pool (only sample pools that can be waded safely)
3. 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. Alternate sampling transects between different types of habitat along the reach if possible (Figure 9).

i. If it is not possible to separate each transect by a different habitat, transects should be located a minimum of 10 m apart.

b. 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.

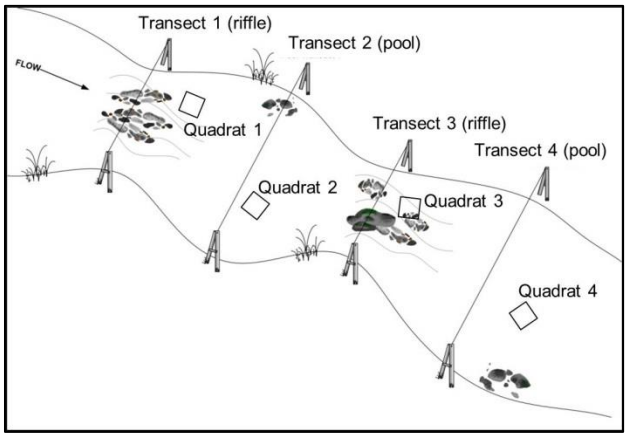

Figure 9. Layout of riffle and pool transects and quadrats within the stream reach

4. Start with the most downstream location and work upstream to avoid suspending sediments that will affect your sampling area.
5. Choose transect locations approximately in the center of the habitat unit, leaving space downstream for quadrat sampling. Place a plot marker on the right bank or left bank (keep the bank consistent throughout all transects for consistency).
   a. Avoid placing transects across islands or braids in the stream channel.
6. Record the coordinate at the plot marker location or transect end point as well as the coordinate uncertainty on the Transect Establishment datasheet (Figure 10, RD[05]) location using the GPS (accuracy ±4 m).
   a. Plot marker coordinates will be surveyed the next time data are collected for the stream geomorphology protocol (RD[09]).
7. Reassess the current stream morphology map or rapid habitat assessment 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 and submit a problem ticket. Otherwise, continue to use the same locations.

NEON Aquatic Plant/Macroalgae Field Data Sheet - TRANSECT ESTABLISHMENT

Wadeable streams

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

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

Local time (HH:MM): *10:30* **Sampling protocol:** *NEON.DOC.003039* **Rev:** *A*

Transect ID	Plot marker ID	Plot marker bank (R or L)	Latitude	Longitude	Coordinate uncertainty (m)	Notes
1	*P1*	*R*	*1806.822*	*6659.198*	*0.34*	
2	*P2*	*R*	*1806.865*	*6659.177*	*1.02*	
3	*P3*	*R*	*1806.889*	*6659.181*	*0.25*	
4	*P4*	*R*	*1806.935*	*6659.202*	*0.21*	

Figure 10. Datasheet for recording the location of transects using GPS (RD[05]).

B.3 Contingent decisions

Situation	Action	Outcome for Data Products	Considerations
Wadeable stream site with <200 m aboveground stream length due to stream size	If establishing transects, habitat available may be insufficient to accommodate all 10 transects/quadrats without causing harm to the stream. Reduce sampling by setting up transects/quadrats 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[10], RD[14], RD[15]).
Wadeable stream site with seasonal drying	If the stream experiences seasonal drying such that established transects are completely dry during a bout, visit each transect and note that it is dry on the datasheet.	None.	Transects should be established in locations that are typically wetted, although seasonal or atypical drying may occur.
The site is a small headwater **wadeable 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 Field Sampling

C.1 Biomass Quadrats in Wadeable Streams

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 1-4 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. In small headwater streams, a modified 10 cm x 10 cm quadrat may be used so as to minimize destruction to the bryophyte community. This decision will be made by the site host and aquatic ecologists at NEON HQ.

NOTE: Quadrats are not heavy enough to settle to the stream bottom in areas of fast water (e.g., riffles). Step on the sides of the quadrat to hold it in place or use a second field technician to assist in holding the quadrat when sampling these areas.

1. Start at the most-downstream point transect and work upstream to minimize suspended sediments in the stream.
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.
      i. 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 completely underwater and appears to be underwater at baseflow. If the initial placement of the quadrat is not underwater, shift it to the nearest location that meets this requirement.
      i. If the stream is small, keeping the quadrat underwater may require using the smaller 10 cm x 10 cm quadrat at some sites. This quadrat size may also be required by permitting.
      ii. Do not place quadrat on an island populated with terrestrial plants.
      iii. If the stream is dominated by boulders that protrude above the water and the quadrat cannot be place fully underwater, sample all aquatic vegetation within the quadrat and separate by “underwater” and “above water”. Note on field datasheet.
      iv. 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 or moss material may be present on the dry substrata in the channel.

NEON Aquatic Plant/Macroalgae Field Data Sheet - QUADRAT SAMPLING

Wadeable streams

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

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

Local time (HH:MM): *14:30* **Sampling protocol:** *NEON.DOC.003039* **Rev:** *A*

Quadrat ID	Plot marker ID	Location dry (Y/N)	Target taxa present (Y/N)	Sample type (quadrat size)	Habitat	Taxon ID	Morphospecies ID	Dominant growth form: emergent (E), floating-leaved (FL), submerged (S), free-floating (FF)	Sample ID
Q1	*P1*	*Y*	*N*						*NA*
Q2	*P2*	*N*	*Y*	*0.5 x 0.5*	*run*	*SCAC3*		*E*	*ARIK.20150324.SCAC3.Q1*
Q2	*P2*	*N*	*Y*	*0.5 x 0.5*	*run*		*unkfern*	*E*	*ARIK.20150324.unkfern.Q1*
Q2	*P2*	*N*	*Y*	*0.5 x 0.5*	*run*		*unkpoaceae1*	*E*	*ARIK.20150324.unkpoaceae1.Q1*
Q2	*P2*	*N*	*Y*	*0.5 x 0.5*	*run*		*macroalgae1*	*S*	*ARIK.20150324.macroalgae1.Q1*
Q3	*P3*	*N*	*N*	*0.5 x 0.5*	*riffle*				

Figure 11. Example of field datasheet for biomass quadrats in wadeable streams (RD[05]).

3. 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. If the habitat unit is dry, enter "Y" in **Location dry** on field datasheet and move on to the next quadrat.
   b. If no plants are present in the quadrat, enter "N" in **Target taxa present** on field data sheet and move on to the next quadrat.
   c. For rooted plants, collect only those that are rooted in the quadrat and collect the entire plant even if it hangs outside the quadrat. Do not collect plants rooted outside the quadrat, even if they overhang the quadrat.
   d. For free floating plants, collect any that are directly above the quadrat
   e. 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.
   f. Only collect material that represents this year's growth (i.e., not senesced vegetation that is clearly from the previous year).
   g. If the entire quadrat cannot fit underwater, collect both the above-water and underwater specimens and keep them separate.
   h. Only remove macroalgae if they form filaments (Figure 5) 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]).

4. 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.

5. 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.
6. Proceed to sample labeling and storage in SOP C.4.

C.2 Point-Transects in Wadeable Streams

1. Use the pre-recorded plot markers and coordinates (Figure 10) to locate each transect.
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. If the entire transect is dry (i.e., the habitat unit is dry), enter "Y" in **Location dry** on field datasheet and move on to the next transect.
4. Sampling points should be evenly spaced in 10 cm (minimum) to 50 cm (maximum) intervals across the wetted portion of 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 50 cm.
5. 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 12).
   a. Survey points in the wetted channel only.
   b. Set the bottom of the view bucket on the surface of the water, 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.
      i. If no plants are present, enter "N" in **Target taxa present** on field data sheet and move on to the next point.
      ii. If plants are present, enter "Y" in **Target taxa present** on field data sheet fill in the remaining information in Step 6.
      iii. If multiple layers of vegetation lie below the point, record all vegetation layers.
      iv. 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.

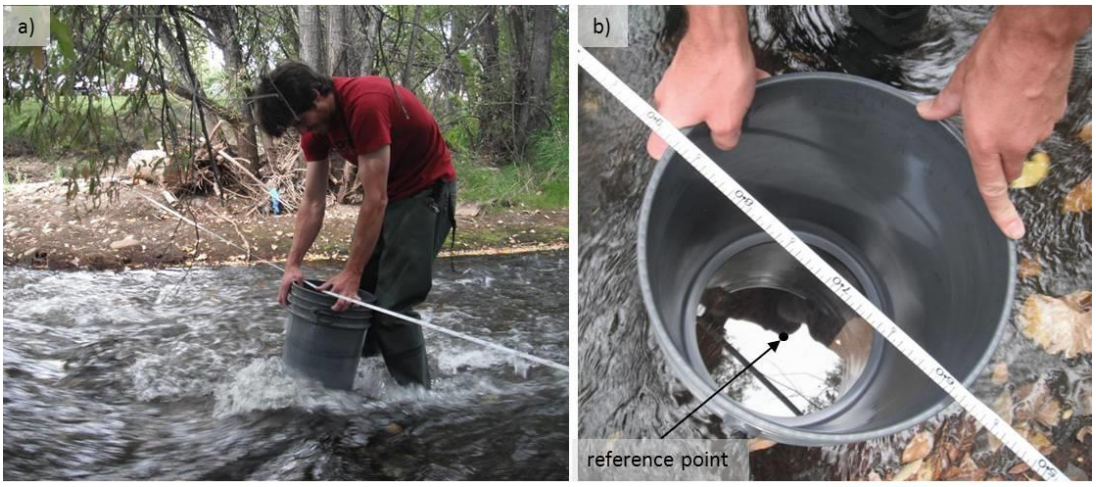

Figure 12. a) A field technician holds the view bucket in the water, under the meter tape transect. b) Hold the view bucket so that the reference point lines up with the point you want to sample on the meter tape.

6. 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 Datasheet (Figure 13, RD[05]). If the specimen can be identified in the field, record the taxon ID (species or 4 letter USDA code). If the specimen cannot be identified in the field, enter a descriptive morphospecies ID. Categories include:
   a. Target taxa:
      i. **Aquatic plant** – Use the field key to identify to species if possible, or collect 2 voucher specimens off-transect (Figure 2) and record morphospecies ID.
      ii. **Moss** – Use the field key to identify to species if possible, or collect a voucher specimen and record morphospecies ID.
      iii. **Liverwort** – Use the field key to identify to species if possible, or collect a voucher specimen and record morphospecies ID.
      iv. **Lichen** – Use the field key to identify to species if possible, collect a voucher specimen and record morphospecies ID.
      v. **Macroalgae** – Collect samples or filamentous algae for identification at the external lab, see Step 7 below (Figure 5).
   b. Substrate (record only if target taxa are not present):
      i. **Coarse woody debris** – (CWD) large pieces of wood, sticks.
      ii. **Dry** – point is not underwater at the time of sampling.
      iii. **Fine particulate organic matter** – (FPOM) fine particles of organic matter.
      iv. **Leaf litter** – Terrestrial leaves from trees that have fallen into the water (do not collect).
      v. **Senesced vegetation** – Dead vegetation rooted in the stream from the previous season.
      vi. **Substratum size class** – Record only if there is no plant or other organic material above is present
         a. **Silt** < 0.063 mm

b. **Sand** = 0.063-2.0 mm
c. **Gravel** = 2.0-63 mm
d. **Cobble** = 63-200 mm
e. **Boulder** = 200-630 mm
f. **Bedrock**
g. **Other**

vii. **Other –** additional categories may be added as necessary (e.g., tumbleweed, terrestrial plant), record descriptive ID.

c. Growth form (see Definitions Section 2.4)
   i. **Emergent (E)**
   ii. **Floating-leaved (FL)**
   iii. **Submerged (S)**
   iv. **Free-floating (FF)**

7. Collect all filamentous macroalgae for taxonomic analysis. Do not identify in the field.
   a. Only remove macroalgae if they form filaments (Figure 5) that can be easily sampled by hand. Amorphous clouds of algae will be sampled in the periphyton protocol (RD[10]).
8. If you are not able to determine the plant species from the groups listed above, collect a corresponding voucher specimen off the transect and give the specimen and descriptive morphospecies ID.
   a. 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.
   b. 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.
      i. 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.
         a. **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
         b. **Mosses** and **liverworts:** collect a sample that is no larger than the size of your palm
         c. **Lichens**: collect 2 specimens approximately 1”-2” in diameter
   c. Record voucher information on the datasheet (RD[05]) and proceed to sample labeling and storage in SOP C.4.

NEON Aquatic Plant/Macroalgae Field Data Sheet - POINT TRANSECTS

Wadeable streams

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

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

Local time (HH:MM): *14:30* **Sampling protocol:** *NEON.DOC.003039* **Rev:** *A*

Transect ID	Plot marker ID	Location dry (Y/N)	Habitat	Point #	Distance (m)	Target taxa present (Y/N)	Substrate (if no plant under point)	Taxon ID	Morphospecies ID	Growth form	Sample ID (macroalgae or associated voucher specimen)
T1	P1	N	run	1	0.1	N	CWD				
T1	P1	N	run	2	0.2	N	sand				
T1	P1	N	run	3	0.3	N					
T1	P1	N	run	4	0.4	Y			macroalgae1	S	ARIK.20140702.macroalgae1.T1
T1	P1	N	run	5	0.5	Y		SPEU		E	
T1	P1	N	run	6	0.6	Y			duckweed1	FF	ARIK.20140702.duckweed1.T1
T2	P2	Y									

Figure 13. Example of a field datasheet for a point transect in a wadeable stream (RD[05]).

C.3 Randomized Point Sampling (Rake) in Lakes and Non-Wadeable Streams

1. Collect samples at 10 points.
   a. Three rake tows equal 1 composite sample at each point.
2. Navigate to the pre-determined sampling point on the lake/river using the handheld GPS unit.
   a. Due to error associated with the GPS unit and error associated with holding the boat over an exact waypoint, sample within 2 m of the pre-determined sampling.
   b. If you are unable to sample at this point due to natural bed conditions (e.g., bedrock, large boulders), anchors, the location is dry, or is too close (within 5 m) or aquatic instrumentation, continue to the next point on the list.
   c. Record the coordinates of the sampling location and the coordinate uncertainty on the field datasheet (Figure 14, RD[05]).

NEON Plant/Macroalgae Field Data Sheet

Lakes and Non-wadeable Streams

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

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

Local time (HH:MM): 10:15 | **Sampling protocol:** *NEON.DOC.003039* **Rev:** *A*

Point ID	Waypoint (Latitude, Longitude)	Coordinate uncertainty (m)	Depth (m)	Rake rep	Dominant growth form (emergent, floating-leaved, submerged, free-floating)	Temporary Sample ID *SITE.DATE.rake.pointID* (replace 'rake' with Taxon ID in the lab)
1	29.690443, -82.016842	4.2	5.2	1	submerged	BARC.20140702.rake.P1
				2	submerged	
				3	submerged	
2	29.680187, -82.014522	2.1	2.3	1	submerged	BARC.20140702.rake.P2
				2	submerged	
				3	submerged	

Figure 14. Example field sheet for aquatic plant rake samples.

3. Anchor the boat at the bow and stern to keep the boat in the desired location. Get as close to the desired sampling coordinate as possible, however there will be error associated with anchoring and allowing the boat to drift. Include an estimate of offset in the coordinate uncertainty.
4. Determine depth at the sampling point using the depth-finder and record on the Field Datasheet (Figure 14).
   a. If the depth is <1 m, use the rake with a handle (Figure 15a)
   b. If the depth is >1 m, use the rake with a rope and dive weight attached (Figure 15b).

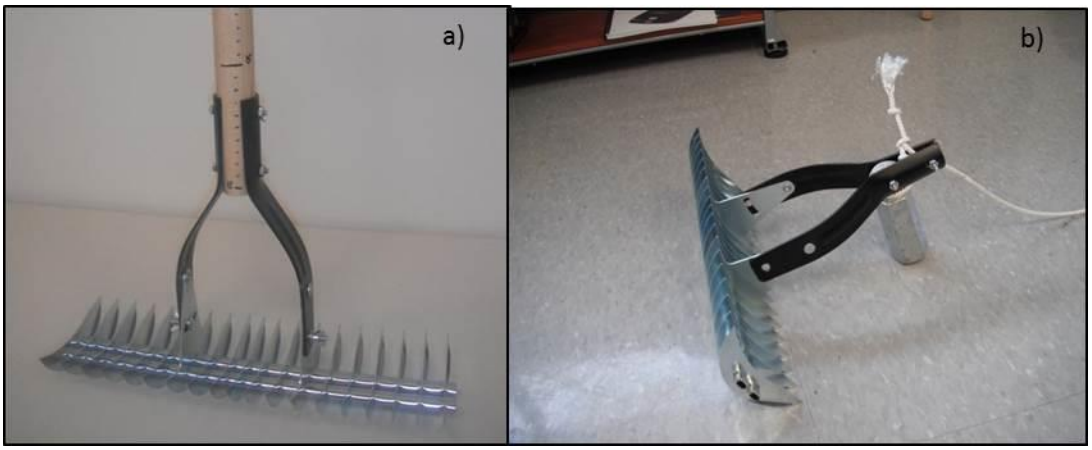

Figure 15. Double-sided sampling rake: a) Rake with handle b) Rake with rope and weight

5. If plants are rooted, proceed to Step 6 and use the rake. If plants are floating, put out the 0.5 x 0.5 m quadrat and collect all floating plants material, then proceed to Step 10c. If both are present, collect the floating vegetation first, then the rooted vegetation.

a. NOTE: If the vegetation is too firmly rooted to be sampled with the rake (e.g., cattails or rushes), follow procedure for biomass quadrat sampling as done in wadeable streams (SOP C.1).

6. Lower the rake head to the lake/river bottom by casting away from or dragging alongside the boat.
7. Pull the rake along the lake/river bottom, toward the boat, for approximately 1.5 m.
   a. This can be measured by leaving 1.5 m of extra rope above the water surface, and pulling that in toward the boat or marking 1.5 m on the gunwale of the boat and towing that distance.
8. Twist rake 180 degrees as you begin to lift it toward the boat. Twisting minimizes the loss of plants from the rake, but twisting more than 180° may cause plants to fall off.
9. Pull the rake and plants into the boat (Figure 16).

   a. NOTE: Do not rinse plants or algae in the lake or river to prevent sample loss.

Figure 16. Double-sided rake head with plant sample being brought into the boat.

10. Remove all plants or algae from rake and place in labeled Whirl-pak® or gallon resealable bags.

   a. Hold the rake over a 3 or 5 gallon bucket to prevent sample loss.
   b. Take care when removing plants as the rake tines are very sharp.
   c. Samples may be separated by taxa in the field (this may be easier to do now than in the lab) or lumped into a composite sample and separated in the lab.
      i. If separating by species in the field, place each species in a separate sample bag with an individual label that includes that taxon ID or morphospecies ID. Record on datasheet, along with dominant growth form of the rake (i.e., emergent, floating-leaved, submerged, or free-floating). Do not spend more than 10 minutes per tow separating species in the field.
      ii. If lumping the sample in the field, assign a temporary sample ID (SITE.DATE.'rake'.pointID) and replace "rake" in the ID with the taxon/species ID in the lab.
   d. Place contents of all 3 tows (either by taxa or lumped sample) in the same sample bags to form a composite sample.
   e. Record information on datasheet (Figure 14) and proceed to sample labeling and storage instructions in SOP D.4.
11. Repeat steps above until three tows have been completed at each point.

C.4 Sample Labeling and Storage

1. Write a label on all-weather paper for quadrat biomass, voucher, and rake (Figure 7) samples, then add to sample bag. The sample ID on the label must match the ID on the respective field datasheet.
   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. You may fold plants if necessary to fit them in the sample bag.
2. Close and seal sample bag.
   a. Sample bag for transportation from the field to the domain lab may be a Whirl-pak®, zip-tip bag, or trash bag depending on the size of the sample.
3. Place all labeled and sealed sample bags in a dark cooler with frozen ice packs for transportation to the Domain Support Facility.
   a. Keep samples in a cooler with ice or ice packs (~4 ^{o}C) until returning to the lab.
   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.
   c. Store samples at the Domain Support Facility as per SOP C.6.

C.5 Special Considerations

1. **DO NOT COLLECT ENDANGERED OR THREATENED SPECIES.** At sites where endangered taxa are known to occur, special precautions should be taken so threatened/endangered plants are not collected. This may be challenging as submerged species may be difficult to see. If species of concern are known to occur at the site, you will be notified by NEON Permitting ahead of sampling.
2. A list of endangered/threatened taxa in the region will also be provided for each domain.
3. If endangered species are accidentally collected:
   a. Make a note of the location on the field datasheet (e.g., "50 m northwest of sensor set") and record GPS coordinates so that location will not be visited again. These records should be added to the lake bathymetric map RD[16],stream morphology map RD[09], or rapid habitat assessment.
   b. Take high resolution photos
   c. Retain the collected material, press and send to external taxonomists for identification (SOP D.4) and submit a problem ticket to report findings to NEON Permitting .
   d. If invasive species (e.g., *Hydrilla*) are identified (as defined by USDA aquatic nuisance species or local or state lists), submit a problem ticket to report findings to NEON Permitting to inform equipment decontamination procedures.

C.6 Sample Preservation

1. Keep samples refrigerated (4 ^{o}C ±3 ^{o}C) and in the dark until processing at the Domain Support Facility.

2. Samples must be returned to the Domain Support Facility or remote lab facility and processed within 48 hours.

C.7 Ending the Sampling Day

1. Refresh the sampling kit
   a. Replace Whirl-pak® and resealable bags.
   b. Print new field labels and field datasheets 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 lake/stream water according to the NEON Aquatic Decontamination Protocol (RD[08]).
   b. Check depth markings on rake handle/rope, refresh markings if necessary.
   c. Clean boat and motor; remove aquatic plants by hand to prevent spread of invasive taxa. Allow boat and motor to dry completely.
   d. Dry all equipment thoroughly between sites and before storage.

SOP D 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. Macroalgae are preserved and sent to an external lab for identification.

D.1 Preparation

1. If aluminum boats are new and unlabeled:
   a. Label ~20 boats by inscribing a unique number on the bottom of each boat with a pencil (e.g., A1, A2, A3, etc.; Figure 17).
      i. NOTE: It doesn't matter what the labels are as long as they are unique and easy to read.
   b. Place new empty labeled boats in the muffle furnace (500 ^{o}C) for 6 hours to burn off any residue.
   c. After 6 hours, carefully remove boats from the furnace and allow to cool to room temperature.
      i. Use thermal gloves and tongs
      ii. Set boats aside in a safe, heatproof location
      iii. After cooled, weigh boats on analytical balance (0.0001 g) and record boat number and weight (g) on lab data sheet (RD[05]).
   d. This may be done in advance, before field sampling.
   e. Boats may be reused from previous sampling bouts.

Figure 17. Examples of newly labeled aluminum weigh boats.

2. If aluminum weigh boats have been previously labeled and used, clean with a soft brush to remove any residual ash.
3. 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

D.2 Aquatic Plants

The following steps are used to process aquatic plant samples from quadrats (**wadeable streams**) and rakes (**lakes and non-wadeable streams**; Figure 18).

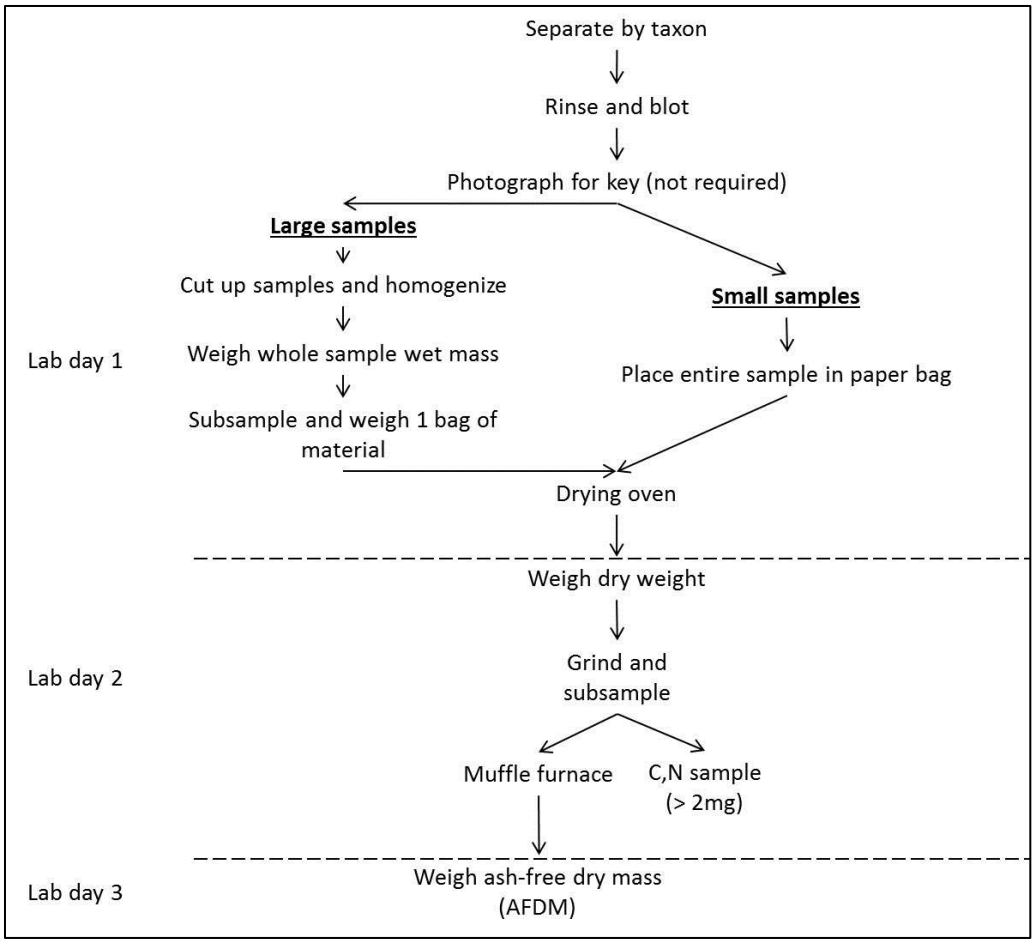

Figure 18. After separating each species from the original sample, follow the steps above for Domain Support Facility aquatic plant processing.

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. Separate macroalgae if they were included in the sample bag and set aside.
   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:
   i. Taxonomy
   ii. AFDM
   iii. 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. How dry is dry? Blot specimens dry until they no longer drip water when you pick them up.
   b. For large-volume specimens, you may need multiple standard driers.
6. 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 morphospecies ID and send a voucher specimen to external taxonomist.
   b. For rake samples, replace the temporary rake ID with a taxon- or morphospecies-specific sample ID.
7. Cut samples, if necessary, to a size that will fit into paper bags and homogenize.
   a. Weigh entire sample on mass balance and record as **Total wet mass** on lab datasheet (Figure 19, RD[05]).
   b. For large samples: Select a well-homogenized subsample that will fit in one paper bag and weigh. Record as **Subsample wet mass** on lab datasheet.
   c. If subsamples are not necessary, **Subsample wet mass** = **Total wet mass**.
8. Using a permanent marker, label a clean, paper lunch bag with sample ID and species ID from the field sample label.
9. 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, Bryophyte, and Macroalgae Lab Data Sheet - AFDM

Site (4-letter code): *ARIK* | **Date analysis finished (YYYY-MM-DD):** *2014-07-06*

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

Date analysis started (YYYY-MM-DD): *2014-07-03* | **Sampling protocol:** *NEON.DOC.003039* **Rev:** *A*

PERIODICALLY CHECK THAT BALANCE IS ZEROED!

Taxon ID	Morphospecies ID	Point, Quadrat, or Transect ID	Sample ID (taxon-specific)	Total wet mass (g)	Subsample wet mass (g)	Total or subsample dry mass (g)	Boat ID	Boat mass (g)	Dry mass + boat (g)	Ash mass + boat (g)	CN collected?
SPEU		*Q1*	*ARIK.20140702.SPEU.Q1*	*2.5631*	*1.5240*	*1.4559*	*A1*	*2.1340*	*3.5899*	*3.0752*	*Y*
	duckweed1	*Q1*	*ARIK.20140702.duckweed1.Q1*	*1.4659*	*1.4659*	*0.6189*	*A2*	*2.0561*	*2.6750*	*2.4261*	*Y*
	algae1	*Q1*	*ARIK.20140702.algae1.Q1*	*3.4524*	*1.7262*	*1.4367*	*A3*	*2.0561*	*3.6614*	*3.0148*	
	poaceae1	*Q1*	*ARIK.20140702.poaceae1.Q1*	*4.2562*	*4.2562*	*3.9752*	*A4*	*2.2247*	*6.1999*	*4.8547*	*Y*

Figure 19. Example of lab datasheet for aquatic plant AFDM (RD[05]).

10. Place paper bags containing samples in the drying oven for a minimum of 12 hours at 60 °C or until constant mass is achieved (i.e., mass varies by <2% over a one-hour period; RD[11]).
    a. Check a subset of at least 3 sample bags to determine whether constant mass has been achieved. Use TOS "Lab Drying QC Datasheet" in Measurement of Herbaceous Biomass datasheets (RD[12]). These data are not entered into the data ingest.
    b. Specimens may be split across multiple labeled paper bags to facilitate drying.
11. **Day 2**: When dry, remove all bags+samples from drying oven and let cool to room temperature prior to weighing. If you are allowing samples to cool for > 1 hour prior to weighing, place 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.
    b. Samples may be left in desiccator or plastic bags for up to 30 days before proceeding to the next step.
12. 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 **Total or Subsample Dry Mass** on Lab Datasheet.
13. 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. Clean grinding mill thoroughly with compressed air between samples and with ethanol after finishing the sampling bout.
14. Subsample for CN and AFDM.
    a. Remove ≥ 2 mg for C and N analysis. Place in 4 mL PTFE-capped glass vial, apply adhesive label, and set aside for shipping.
        i. 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. Place remaining specimen subsample in a clean, pre-labeled, pre-weighed aluminum weigh boat. Record **Boat ID** and **Boat mass** on lab datasheet.
   i. 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).

15. Weigh boat on analytical balance, and record as **Boat + dry mass** on Lab Datasheet.
16. Repeat above steps until all specimens have been processed.
17. Place aluminum boats + specimens in the muffle furnace using oven gloves and tongs. **TAKE CARE NOT TO BURN YOURSELF!**
    a. Boats may be stacked on top of each other as long as there is space for air flow between them.
    b. Boats may be placed in an approved muffle furnace pan (if available) before placing in the furnace. This may make it easier and safer to handle samples.
18. Leave samples in the muffle furnace at 500 ^{o}C for at least 6 hours.
    a. Sample may be left in muffle furnace for longer than 6 hours (e.g., overnight) if necessary.
    b. If necessary, the muffle furnace may cool down prior to removing the samples.
19. **Day 3**: After 6+ hours, remove covered pans/aluminum boats carefully from the muffle furnace using oven gloves and crucible tongs.
    a. The muffle furnace may be turned off and allowed to cool prior to removing specimens. Depending on the model of muffle furnace, a draft may be created if the furnace is not cool before opening the door.
20. Cover pans/aluminum boats to prevent ash from blowing out of boats and set aside to cool on a heat-resistant surface in an area without drafts from windows, doors, or mechanical building ventilation.
21. When boats have cooled enough to handle, weigh immediately or place in desiccator.
    a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
22. After cooling to room temperature, weigh boats again on analytical balance, record as **Boat + ash mass** on Lab Datasheet.
23. After weighing, dump ash into the trash and clean the boat with a soft brush or paper towel.
24. Set clean boats aside to be used again.

D.3 Macroalgae

The following steps are used to process macroalgae samples from quadrats and rakes (Figure 20).

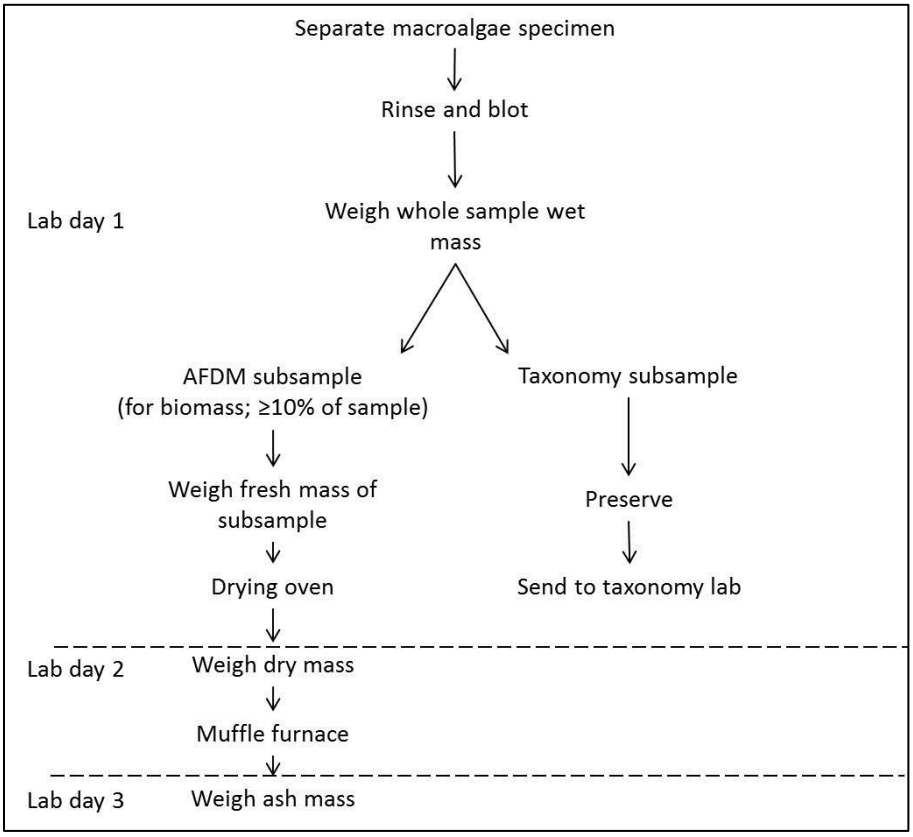

Figure 20. Schematic for macroalgae processing at the Domain Support Facility.

1. **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.
2. 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.
3. Clean algal strands out of the sieve and add to the specimen.

4. 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.
   b. For large-volume specimens, you may need multiple standard driers.
5. For rake samples, replace the temporary rake ID with a morphospecies-specific sample ID.
6. Weigh entire macroalgae sample on mass balance and record as **Total wet mass** on lab datasheet.
   a. Taxonomy subsample: Remove ~10 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 D.7. Label according to Figure 21.
   b. AFDM subsample: Remove 10-50% of original sample and place in a clean, pre-labeled, pre-weighed aluminum weigh boat. Weigh subsample and record as **Subsample wet mass** on Lab Datasheet along with appropriate **Boat ID** and **Boat mass**.
   c. Repeat above steps until all samples have been processed.

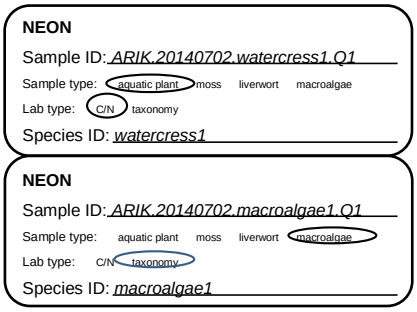

Figure 21. Example of adhesive labels for macroalgae taxonomy and plant CN subsamples

7. Place all aluminum boats + subsamples in 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]). Cover boats with a cardboard lid or piece of paper to prevent samples from blowing out of the boats once in the oven.

   a. **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**: When dry, carefully remove boats from drying oven and let cool to room temperature in a plastic bag or desiccator.

   a. Take care not to let the dried sample blow out of the boats.
   b. Placing samples in a bag or desiccator is important because samples absorb water quickly from the air as they cool.
   c. 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 **Boat + dry mass** on the Lab Datasheet.
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. Boats may be placed in an approved muffle furnace pan (loaf pan) if available before placing in the furnace. This may make it easier and safer to handle samples.
12. Leave samples in the muffle furnace at 500 ^{o}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.
    a. The muffle furnace may be turned off and allowed to cool prior to removing specimens. Depending on the model of muffle furnace, a draft may be created if the furnace is not cool before opening the door.
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 mechanical building ventilation.
15. When boats have cooled enough to handle, weigh immediately or 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 **Boat + ash mass** on Lab Datasheet.
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.

D.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 newspaper (2 sheets, opened) on top of the standard drier.
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 D.5).

D.5 Herbarium Mounts (Aquatic Plants Only)

1. Open plant press carefully.
2. 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.
3. Separate into two or more specimens.
    a. One specimen will be sent to the aquatic plant taxonomist (keep in newspaper, proceed to SOP F 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.
4. Fill out paper herbarium label (RD[17]). These labels will be standardized across TOS and AOS.
    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.
5. Adhere label to the bottom right corner of the herbarium paper using archival glue.
6. 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.
7. Glue specimen to herbarium paper.
    a. Dot archival glue in several locations along the length of the plant roots, stems, and leaves.
8. Allow glue to dry before placing mounted specimen in the Domain Herbarium.
9. Photograph specimen to include in the domain field key.

D.6 Drying Moss, Liverwort, and Lichen Specimens (Taxonomy, if collected)

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 F for shipping instructions to taxonomists.

D.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 (Appendix D). Specimens should be stored in a dry, well-ventilated area for a maximum of 60 days until shipping.
3. **Macroalgae samples** are preserved using a disposable pipet. Preserve each 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 °C) for up to 30 days until shipping.

D.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 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.

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

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

SOP F Sample Shipment

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

Shipments are to include a hardcopy of the "per sample" tab of the shipping inventory (RD[13]) 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 periphyton/seston/phytoplankton taxonomy samples if possible.
5. Include shipping inventory/manifest in additional zip-top bag.
6. Ship ground at ambient temperature. Glutaraldehyde in these concentrations is not considered hazardous.

F.2 Plant CN Sample Shipping

1. Organize sample vials by site/bout in resealable bags.
2. Wrap bagged sample vials in bubble wrap to cushion during shipping.

3. Include shipping inventory/manifest in additional zip-top bag.
4. Ship ground at ambient temperature.

F.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.

F.4 Moss, Liverwort, Lichen Taxonomy Sample Shipping

1. Ship moss, liverwort, and lichen specimens in original envelopes.
2. Organize specimens by site/bout in clean, dry 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.

F.5 Handling Hazardous Material

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

F.6 Supplies/Containers

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

F.7 Timelines and Conditions

1. **Algal taxonomy samples**: Shipping should occur within one week of sampling if possible, but samples may be stored at the domain support facility at 4 ^{o}C (±3 ^{o}C) for up to 30 days if necessary.
   a. Preserved samples may be held for up to 6 months if the lab is not able to receive samples (Table 1).
2. **Pressed or dried taxonomy specimens**: May be stored in plant press or bryophyte/lichen packet for up to 60 days at room temperature.
   a. Dried samples may be held up to 6 months or longer if the lab is not able to receive samples (Table 1).
3. **Plant CN samples**: May be stored in labeled vials for 60 days dry at room temperature.
   a. Dried samples may be held up to 6 months or longer if the lab is not able to receive samples.

F.8 Grouping/Splitting Samples

Group samples of each type by site per bout.

F.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).

F.10 Shipping Inventory

Shipments are to have a hardcopy of the per sample tab of the shipping inventory (RD[13]) sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. Also include the shipment tracking number in the email. Email plant taxonomy labels (Appendix F, RD[05]) to aquatic plant taxonomist.

F.11 Laboratory Contact Information and Shipping/Receipt Days

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

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 10. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.003040	Datasheets for AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass
NEON.DOC.002494	Datasheets for AOS Shipping Inventory

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

APPENDIX B QUICK REFERENCES

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

Step 2 – Prepare labels (2" x 4", all-weather paper).

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

Step 4 – Determine sampling locations:

1. In **wadeable streams**, determine habitat sampling locations from the Stream Morphology Map (RD[09]) or rapid habitat assessment, and establish transects:
    a. If this is the first site visit record transect end point locations using the GPS (accuracy ±4 m)
    b. If this is not the first visit return to previously established transects
2. In **lakes and non-wadeable streams**, determine randomized points:
    a. Determine plant colonization depth using the transect method.
    b. Generate a polygon using the portions of the lake/river bottom that are equal to or shallower than the colonizable depth. Create 20 random coordinates within the defined polygon.

Step 5 –Sample based on aquatic habitat type.

1. In **wadeable streams**:
    a. Biomass Quadrats: Toss the quadrat into the channel 1 m to 4 m downstream of the transect, remove all aquatic plants, bryophytes, and macroalgae from the quadrat.
    b. Point Transects: Space sampling points at even intervals 10 cm to 50 cm apart to have approximately 20 sampling points across each transect. Determine presence-absence using the view bucket and collect voucher specimens if plants cannot be positively identified in the field.
2. In **lakes or non-wadeable streams**:
    a. Randomized Point Sampling: Collect samples at 10 points, with 3 rake tows from each point.

Step 6 – Keep samples chilled (~4 ^{o}C) and in the dark until processing at the Domain Support Facility.

Step 7 – Lab processing:

1. **Aquatic Plants/Mosses**: photograph (if necessary), measure dry weight, grind and subsample for ash-free dry mass, subsample for CN sample, press and mount plants or place in packets for Domain Herbarium and taxonomist (if necessary).
2. **Macroalgae**: measure ash-free dry mass, preserve in glutaraldehyde and send to taxonomy lab.

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 transect or point locations.

Sample collection in wadeable streams: Be sure to…

- Determine the dominant habitat and second-most dominant habitat based on the Stream Morphology Map (RD[09]) or rapid habitat assessment
- 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.**
- Collect point transect data along transects, collect voucher specimens off transect if necessary.

Sample collection in lakes and non-wadeable streams: Be sure to…

- Determine the 20 random points based off of the most recent bathymetric map.
- Determine plant colonizable depth.
- Collect samples at 10 points, with 3 rake tows from each point.
- Take care when removing plants as the rake tines are very sharp.
- Take extra care when rinsing to not lose the samples.
- **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

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

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

*soft sites as of November 2015

APPENDIX H SITE-SPECIFIC INFORMATION: HABITAT AND SAMPLER RECOMMENTATIONS

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

Domain	Site	Habitat 1	Habitat 2	Biomass sampler
D01	Hop Brook	Riffle	Pool	0.5 x 0.5 m quadrat
D02	Mill Run*			
D02	Posey Creek	Riffle	Pool	10 cm x 10 cm quadrat
D03	Ichawaynochaway Creek			
D03	Lake Barco			rake
D03	Lake Suggs			floating vegetation sweep
D04	Rio Guilarte	Riffles	Pool	0.5 x 0.5 m quadrat
D04	Rio Cupeyes	Riffle	Run	0.5 x 0.5 m quadrat
D05	Crampton Lake			rake
D05	Round Lake			rake
D06	Kings Creek	Riffle/run	Pool	0.5 x 0.5 m quadrat
D06	McDowell Creek			
D07	Leconte Creek			10 cm x 10 cm quadrat
D07	Walker Branch			10 cm x 10 cm quadrat
D08	Mayfield Creek	Riffle/run	Run	0.5 x 0.5 m quadrat
D08	Black Warrior River			rake
D08	Tombigbee River			rake
D09	Prairie Lake			rake
D09	Prairie Pothole			0.5 x 0.5 m quadrat for rooted littoral vegetation
D10	Arikaree River	Runs	Pool/Run	0.5 x 0.5 m quadrat
D11	Pringle Creek			0.5 x 0.5 m quadrat
D11	South Pond at Klemme			
D12	Blacktail Deer Creek	Riffle	Riffle/Run	0.5 x 0.5 m quadrat
D13	Como Creek	Riffle	Run/Pool	10 cm x 10 cm quadrat
D13	West St. Louis Creek	Riffle	Pool	10 cm x 10 cm quadrat
D14	Sycamore Creek	Riffle	Run/Pool	0.5 x 0.5 m quadrat
D15	Red Butte Creek	Riffle	Runs	0.5 x 0.5 m quadrat
D16	McRae Creek	Riffle	Step pools	10 cm x 10 cm quadrat
D16	Martha Creek	Riffle	Pools	0.5 x 0.5 m quadrat
D17	Convict Creek*			
D17	Providence Creek*			
D18	Oksrukuyik Creek	Riffle	Pools	0.5 x 0.5 m quadrat
D18	Toolik Lake			rake
D19	Caribou Creek	Riffle		0.5 x 0.5 m quadrat

*soft sites as of November 2015