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

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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.
B	02/08/2017	ECO-04376	Update NEON template; Clarify point transect and ashing SOPs; Update datasheets to match Fulcrum; Decrease biomass sampling to once per year; Update sample ID template
C	02/13/2018	ECO-05326	Resolving morphospecies, revise sample timing with regard to bathymetry, clarify overhanging veg in transects, move datasheets to appendix, require Wiley mill grinding for plant CN samples, update randomized point selection procedure, update D14 bout dates, limit macroalgae sample collection along transects
D	12/19/2018	ECO-05967	Simplify bryophyte packet, add contingencies and rules for stream drying
E	04/06/2021	ECO-06520	New template, new flowcharts, add contingent decisions for rivers, morphospecies ID instructions, add algae and plant separation guidelines in domain lab, add external lab label generator and noxious weed instructions, clarify vouchers, revise bryophyte packet instructions
F	03/16/2022	ECO-06781	• Update to reflect change in terminology from relocatable to gradient sites

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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 structural complexity to the lake or stream bottom, and, when abundant, 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, as well as changes in biogeochemical cycles, over time. 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 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.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: Data Management
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.004257	NEON Standard Operating Procedure (SOP): Decontamination of Sensors, Field Equipment and Field Vehicles
RD[09]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[10]	NEON.DOC.003045	AOS Protocol and Procedure: Periphyton 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

RD[18]	NEON.DOC.003564	NEON Standard Operating Procedure (SOP): Plant Pressing and Mounting (Herbarium techniques)
RD[19]	NEON.DOC.003282	NEON Protocol and Procedure: Site Management and Disturbance Data Collection
RD[20]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation
RD[21]	NEON.DOC.005224	NEON Protocol and Procedure: Shipping Ecological Samples and Equipment
RD[22]	NEON.DOC.003600-NEON.DOC.003618	Aquatic Site Sample Design – NEON Domain ##

2.3 Acronyms

Acronym	Definition
AFDM	ash-free dry mass
C	carbon
°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: Vascular plant with root system living in a body of water. Aquatic plants will be categorized by the following growth 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

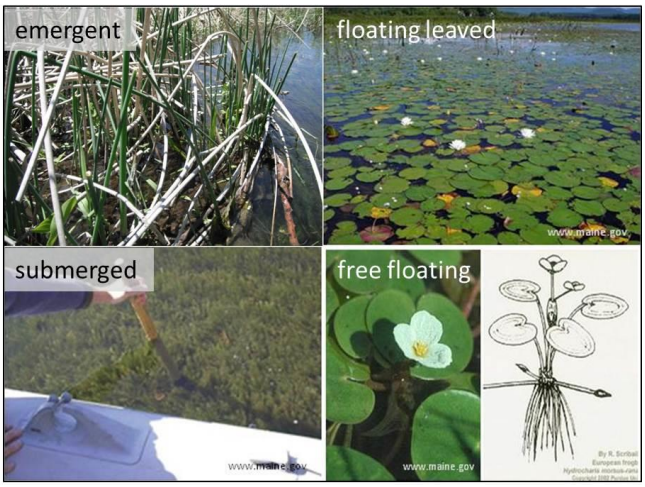

Figure 2. Aquatic plant growth forms: emergent, floating-leaved, submerged, and free-floating.

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).

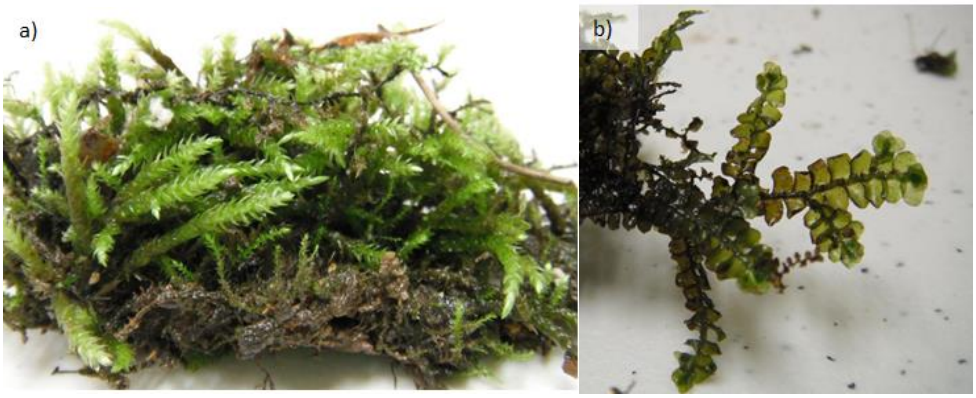

Figure 3. Bryophytes are a group of non-vascular plants including a) mosses and b) liverworts which may be submerged or growing in the "splash zone".

Fulcrum: Software tool used to create NEON electronic data entry applications.

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.

Limnetic zone: Zone of a lake where light does not penetrate to the bottom, typically further from shore than the littoral zone.

Littoral zone: Zone of a lake where light penetrates to the bottom, allowing plant growth (typically light = 1% surface value). This zone is often near shore and relatively shallow.

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

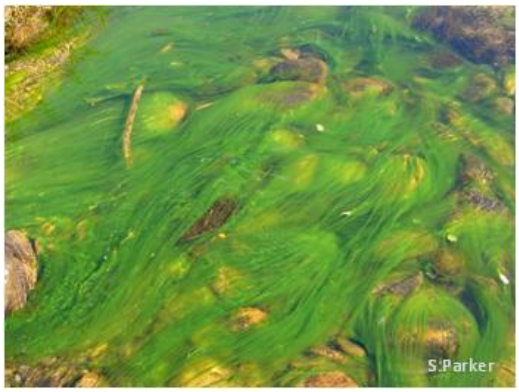

Figure 5. Macroalgae are large, visible algae that often form blooms in streams and lakes

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 for the reach, 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, causing fine sediments to deposit in pools. Pools are generally longer than wide (unless they are plunge pools) and are 1.5 x deeper at their maximum depth than 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.

ServiceNow: Software tool used for problem/incident tracking and resolution.

Step pool: High-gradient streams (steep) where water cascades over a rock or woody snag, dropping into a pool. Due to the gradient and surrounding geology, this pattern continues down the stream: step (drop)—pool—step—pool—etc. (**Figure 6**).

Figure 6. Step pools occur in high-gradient streams where there are channel-spanning pools separated by boulder/cobble steps.

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 rivers will be estimated via the bathymetry protocol, RD[16]); 2) to determine plant presence and absence at randomized points in lakes and rivers; and 3) to collect aquatic plant, bryophyte, and macroalgae samples for identification, biomass measurement, and chemical analysis. Plants encountered on point transects are identified *in situ* where possible using photo keys based on NEON voucher specimens and regional keys. However, specimens should be collected for taxonomic identification and curation in the domain herbarium if the ecologist is unable to make a positive identification in the field. Voucher specimens may also be collected opportunistically during sites visits when this protocol is not performed. Samples collected for taxonomic ID and biomass and plant chemistry processing are collected and returned to the Domain Support Facility for processing and shipping to appropriate taxonomists (RD[21]). Macroalgae specimens will always be collected and sent to expert taxonomists, and not identified in the field, with the exception of *Didymosphenia geminata* at limited sites that have been vetted by NEON Science.

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 can be calculated by data users over time. Percent cover in wadeable streams can be estimated using point transects, a method modified from the standard point-frame method in terrestrial ecosystems (Bowden et al. 2006). In lakes and rivers, samples are collected using a randomized point sampling method, which allows for data collection at 10 points distributed within the lake or river (Figure 7), 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 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.

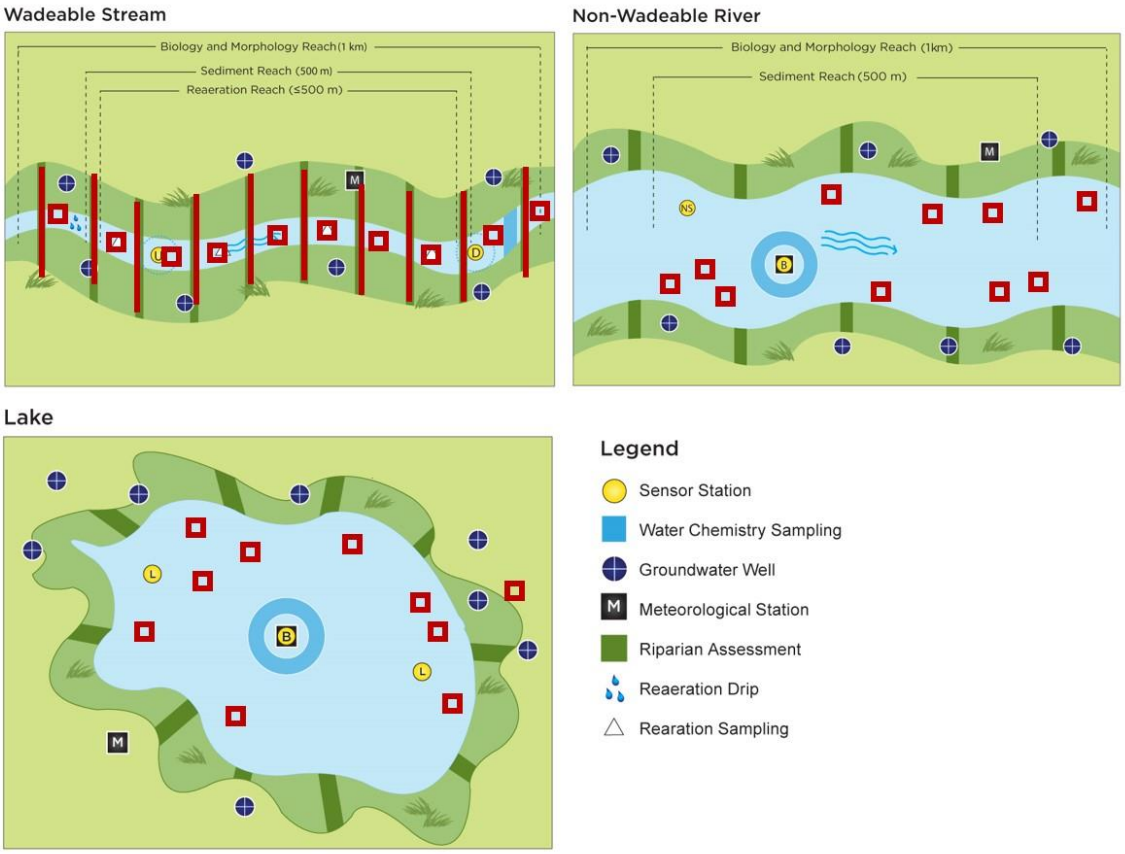

Figure 7. Generic river, lake, and wadeable stream site layouts with aquatic plant, bryophyte, lichen and macroalgae sampling locations. Red lines represent wadeable stream transects, while red boxes are quadrats or rake grab samples.

Percent cover of lakes and rivers is estimated every 5 years (or more frequently if a major weather event occurs) 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 analyst and will show what portions of the lake or stream bottom are colonized by plants and can be used for selecting random points prior to sampling for this protocol. Biomass sampling will allow researchers to determine the contribution of each taxon to the aquatic flora at a site. 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.

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[06]).

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 be scheduled to occur within the first 21 days of the 1 month window specified in Appendix E with a minimum of two weeks between sampling dates. Accommodations for local weather conditions (e.g., late ice-off or high stream flows) 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.

Wadeable streams

- Point transects: Data collection occurs during 3 bouts per year, spring, summer, and fall. Data collection happens entirely in the field. Samples may be collected for expert identification.
- Clip harvest biomass sampling: Quadrat sampling occurs once per year during the mid-summer sampling date (Bout 2) with point transect data collection. Samples are returned to the DSF for processing.
- Voucher sample collection: opportunistic, occurs at times other than regularly scheduled sampling

Lakes and rivers

- Clip harvest presence/absence: Data collection occurs during 2 bouts per year, spring and fall. Data collection happens entirely in the field.
- Clip harvest biomass: Rake sampling occurs once per year during the mid-summer sampling date (Bout 2). Samples are returned to the DSF for processing.
- Voucher sample collection: opportunistic, occurs at times other than regularly scheduled sampling

Sonar (bathymetry) surveys **of lake and river** bottoms will be performed once every five years. In years when bathymetry data are collected, plant sampling will be scheduled within two weeks of bathymetry during sampling Bout 2 survey so that plant data can be used for habitat mapping ([RD16]). Accommodations for local weather conditions may be made that cause plant sampling and the bathymetry survey to date to occur more than two weeks apart.

Table 1. Sampling frequency for Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling procedures on a per SOP per site type basis.

SOP	AOS Site Type	Location	Bout Duration	Bouts Per Year	Remarks
SOP C.1	Lake/river	10 random points	1 day	3	Point sampling in lakes and rivers (Clip Harvest). Bio Bouts 1 & 3 for presence absence, Bout 2 for collection
SOP C.2	Stream	10 quadrats*	1 day	1	Biomass sampling in wadeable streams (clip harvest). Bio Bout 2 only
SOP C.3	Stream	10 transects*	1 day	3	Point sampling in wadeable streams. Bio Bouts 1, 2, & 3

*Note that MCDI has 5 quadrats and transects.

Scheduling Considerations

1. ***Field Work and Laboratory Processing*:** During Bout 2 (mid-summer), , the following points are critical with respect to timing after biomass samples are collected from a given sampling point/transect:
    a. Keep plant samples cold until they are processed in the laboratory. Change cooler cold packs every 12 h.
    b. Process collected samples in the laboratory as soon as possible, within 24-48 hours of collection.

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 stream and river sites), the accumulation of degree days, weather, and riparian phenology (Appendix C).

4.3 Timing for Laboratory Processing and Analysis

All plant processing shall begin within 24-48 hours of field sampling (clip harvest only):

1. Day 1: 2-8 hours for sorting and weighing fresh samples (occurs within 24-48 hours of collection)
2. Day 2: 2-8 hours for weighing dried samples (may occur anytime between Day 1 and Day 3)
3. Day 3: 2-3 hours for weighing ashed samples (may occur anytime between Day 2 and the ship date)

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 on the schedule set by Collections and Laboratory Analysis. For additional storage and shipping timelines see RD[21].

Table 2. Samples types and their appropriate storage criteria.

Sample Type	Field Storage	Post-processing Lab Storage	Domain Hold Time
Quadrat biomass/Rake samples	Cooler with ice packs	Refrigerate until further subsampling and processing 4° C	Process within 24-48 hours of collection
AFDM	NA	Desiccator, drying oven, or muffle furnace	Once subsampled and dry, samples can be held
Macroalgae Taxonomy	NA	Preserved subsamples may be stored at 4 °C (±3 ° C)	Up to 30 days until shipping
Plant CN	NA	Labeled vials stored dry at room temperature	Dried samples may be held up to 6 months or longer if the lab is unable to receive samples
Voucher	Cooler with ice packs	Refrigerator until further subsampling and processing 4° C	Process within 24-48 hours. Voucher to be dried and pressed. Stored in dry ventilated area until shipped or stored in herbarium

4.4 Sampling Timing Contingencies

All samples from one sampling bout must be collected within one day (i.e., all samples per site as detailed in this protocol). A minimum of 2 weeks between sample periods shall be observed. If > 10 days or > 3 days past bio bout window a schedule change request or incident ticket or incident ticket for cancellation should be made on ServiceNow.

Table 3. Contingency decisions.

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and conditions become unsafe during sampling (e.g., approaching thunderstorm, rapid increase of water level in the **wadeable stream**), or the **lake/river** 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, discard samples and data, 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 may be flagged.

Delay/ Situation	Action	Outcome for Data Products
5 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 may be flagged.
5 days	If flooding occurs on or prior to the targeted sampling date in a **river** (defined by USGS and NOAA "flood stage" at the nearest USGS gage), wait until the water level drops below flood stage and allow at least 5 days before sampling.	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data may be flagged.
6 Months	Preserved macroalgae samples may be held for up to 6 months at 4 °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.
	Dried/ground plant CN 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 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.
	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.

4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more plots or sampling locations in a given bout. For example:

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., deep snow, flooding, inclement weather, or ice cover
- Management activities – e.g., controlled burns, pesticide application

Instances such as those listed above must be documented for scheduling, tracking long-term plot suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[06]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates (Appendix C).
- **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled**: Missed Sampling is rescheduled for another time according to one of the scenarios documented in Figure 8, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 8**).

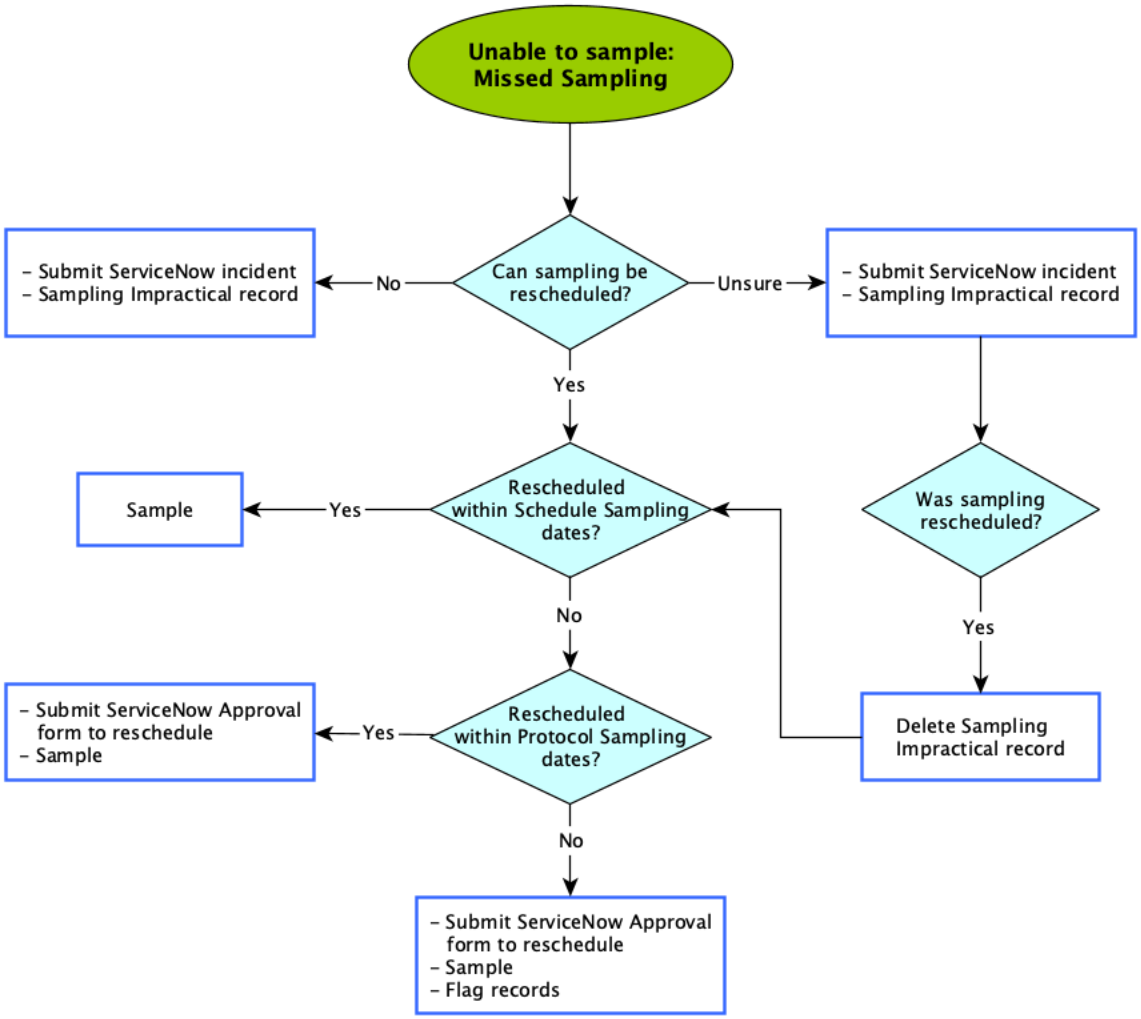

Figure 8. The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management record, or e) some combination of (a) – (d).

To Report Missed or Incomplete Sampling:

1. Missed or Incomplete Sampling that cannot be rescheduled within the Scheduled Sampling Dates must be communicated to Science by a ServiceNow Incident.
   a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 8**).
   b. Consult **Table 4** below to determine required actions if scheduled activities are delayed or canceled. Guidance for this and other NEON protocols is summarized for ease of use in a table posted to a Field Science Sharepoint library. However, this protocol is the ultimate source of information should any discrepancy exist.

2. Create a Fulcrum record for each Missed Sampling event in the field that cannot be rescheduled. That is, if data are recorded in the field at the plot level, a record must be made for each plot missed.
    a. For example, create one parent record and 10 child records for 10 missed transects, points, or quadrats.
    b. Missing data in downstream applications (e.g., Lab apps) are not recorded. For example, plant domain lab data records are not created if biomass field sampling cannot be completed.

For each Missed Sampling record, the **Sampling Impractical** field must be populated in the field apps on the mobile collection device (

3. **Table 5**). One record per plant transect, random point, or quadrat.
4. For Rescheduled sampling events that occur outside of the defined Protocol Sampling Dates, a protocol-specific Flag must also be recorded (**Figure 8**).

    For AOS plant sampling, this would be indicated in in the biophysicalCriteria field.

Table 4. Guidance for responding to delays and cancellations encountered during implementation of the APL-Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling protocol.

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
AOS aquatic plants	> 10 days or > 3 days past bio bout window	IS/OS Schedule Change Request	Submit incident ticket

Table 5. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that more than one is applicable, choose the dominant reason sampling was missed. In lakes and rivers, new randomized points can be selected during sampling to avoid most of the issues below.

Sampling Impractical reason	Description
High water velocity	Water velocity too high to survey stream transect
Location dry	Entire stream transect is dry
Location frozen	Water at stream transect location is frozen
Location snow covered	Stream transect location covered in snow
Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)
Other	Reason not outlined above, make a remark in the record to describe the issue

4.6 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the

time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Table 6. Estimated staff and labor hours required for implementation of the APL-Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling protocol.

SOP	Estimated time	Suggested staff	Total person hours
SOP A: Preparing for sampling	0.5-1 h	1	1 h per bout
SOP B.1: Determining Sampling Locations – Lakes and Rivers Randomized Point Selection	0.5-1 h	1	1 h per bout
SOP B.2: Determining Sampling Locations – Wadeable Streams Transect Establishment	3-8 hours (one time only)	2	6-16 h the first time the stream is sampled
SOP C: Field Sampling	3-8	2	6-16 h per bout
SOP D: Laboratory Sampling and Analysis	2-8 h per bout (Only bout 2)	1-2	2-16 h per bout
SOP E: Data Entry and Verification	1-2 h per bout	1	1-2 h per bout
SOP F: Sample shipment	1-2 h per bout	1	1-2 h per bout

Field sampling requires 2 ecologists for 3-8 hours per site, plus travel to and from the site. Bout 2 may fall on the longer end of the time estimate as it includes clip harvest sample collection. Lab processing only occurs during Bout 2 (clip harvest) and requires 1-2 ecologists for 2-8 hours within 48 hours of field sampling, 1 ecologist for 2-8 hours on the second lab day, and 1 ecologist for 2-3 hours on the third lab day.

4.7 Criteria for Permanent Reallocation of Sampling within a Site

Aquatic plant, bryophyte, lichen, and macroalgae sampling will occur on the schedule described above at 10 transect locations per site in two different habitat types in wadeable streams, and 10 randomly-chosen locations in lakes and rivers. Ideally, plant sampling will occur at designated transects or at random locations within a lake/river site for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (gradient sites). However, circumstances may arise requiring that sampling within a site be moved from one location to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given location becomes compromised, use NEON's problem reporting system to report to Science.

There are two main pathways by which sampling can be compromised. Sampling locations can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot becomes permanently flooded or a stream channel moves after a flood). Alternatively, sampling locations may be located in areas that are logistically impossible to sample on a schedule that is biologically meaningful.

A common occurrence in stream aquatic plant sampling is loss of habitat due to channel drying or permitting restrictions. Contingent decisions in Table 7 should be followed if the wetted area of sampling is decreased, and reported using NEON's problem reporting system. If water returns to the reach within the sampling window, full sampling should resume. Lateral movement of the stream channel is expected and does not necessarily compromise sampling.

Table 7. Contingent decisions for sampling in a wadeable stream.

Situation	Action	Outcome for Data Products	Considerations
Wadeable stream site with <500 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 in the mobile app.	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 mobile app.

4.8 Sampling Specific Concerns

1. If an endangered or threatened plant species is discovered (based on the Endangered Species List, Appendix D), **do not collect**. Take photos and note location within the site using handheld GPS. Use the problem reporting system to notify 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 field ecologists, site host and/or NEON Science. 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.
4. If stream water levels change over time, keep transects at their current locations and record the type of habitat at the transect on the day of sampling, even if this is different from the habitat type that was present during transect establishment.

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 EHSS Policy, Program and Management Plan (AD[01]).

See Section 9 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 rivers**, site-specific hazards may be encountered that necessitate sampling from the boat, without dismounting from the vessel. In addition, use extra caution in waters where alligators are present and maintain a safe distance from hazards.
3. All personnel must be wearing a personal flotation device (PFD) prior to entering the boat.
4. All personnel shall have access to a form of communication with other team members such as a two-way radio.
5. Be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.)

6 PERSONNEL

6.1 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

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. Personnel must also be trained in field identification based on the local stream flora and in safe handling of glutaraldehyde (AD[03]).

6.2 Specialized Skills

Where applicable, personnel will be trained to operate a boat and able to safely handle a motor and drive a boat safely. Personnel should also have the ability to identify regionally specific plants and bryophytes by site and with a dichotomous key.

7 STANDARD OPERATING PROCEDURES

SOP Overview

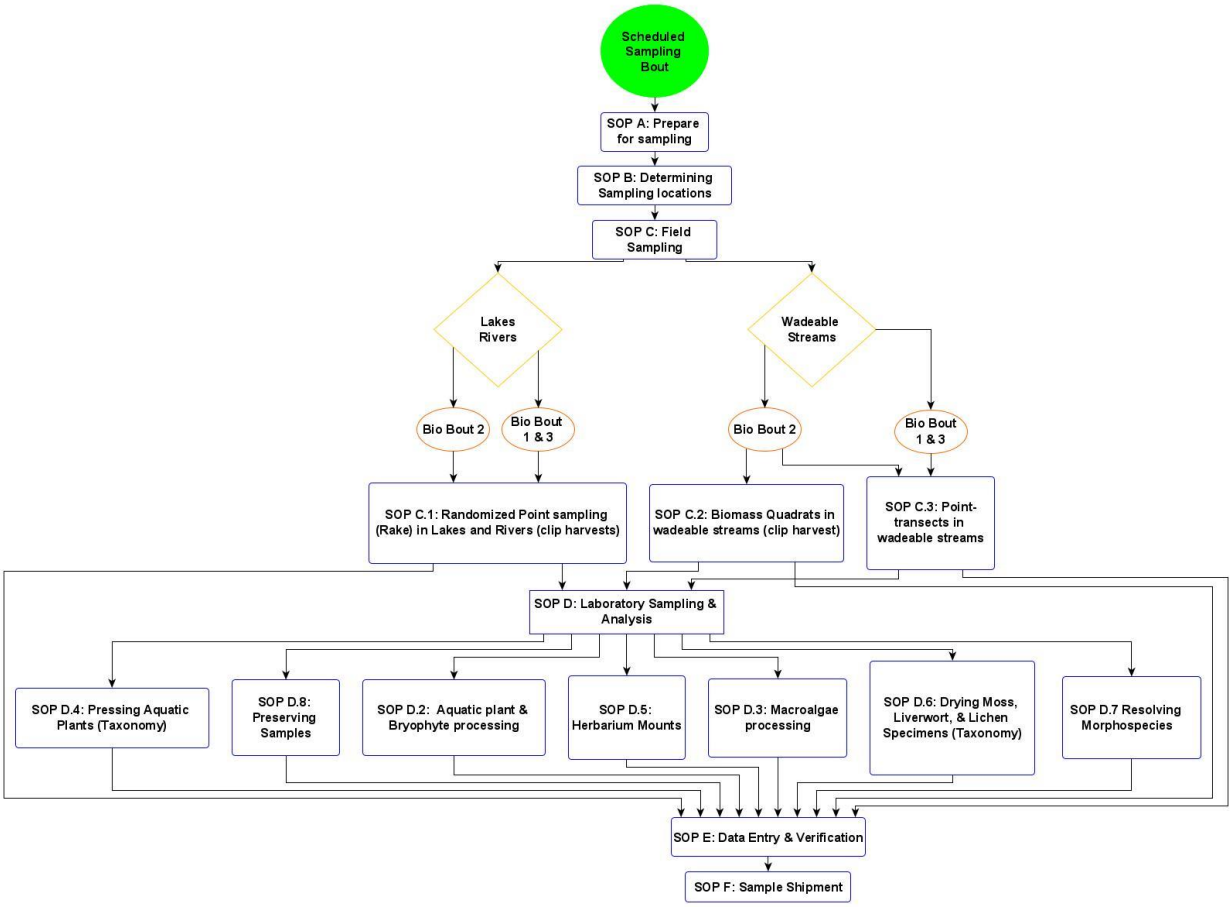

Figure 9. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.

- SOP A: Preparing for Sampling
- SOP B: Determining Sampling Locations
- SOP C: Field Sampling
- SOP D: Laboratory Sampling and Analysis
- SOP E: Data Entry and Verification
- SOP F: Sample Shipment

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged at the beginning of each field day, whenever possible.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

A.2 Preparing for Field Sampling

1. Plan and save sampling routes for field teams using standard site navigation procedures (RD[07]). Route planning enhances sampling efficiency and helps avoid accidental foot traffic at NEON sites. Load GPS sampling coordinates on handheld GPS unit (±4 m accuracy) for lakes/rivers.
2. Collect and prepare all equipment, including sample bottles, sample bags, and pre-printed labels.
3. Have ice or ice packs frozen and ready for transportation cooler.
4. Charge batteries for digital camera and check batteries (bring extras) for handheld GPS unit.
5. See Laboratory Preparation procedures in Section D.1 for additional pre-sampling activities (e.g., weigh boat preparation).
6. Fill out general aquatic field metadata app on the mobile app. General field metadata only need to be filled out once per site per day, even if multiple protocols are implemented.

A.3 Labels and Identifiers

1. Print field labels on all-weather paper (**Figure 10**). You may also use adhesive labels for macroalgae bottles.
   a. Note that plant biomass collection only occurs during Bout 2, however macroalgae may be collected during any bout for taxonomic identification.

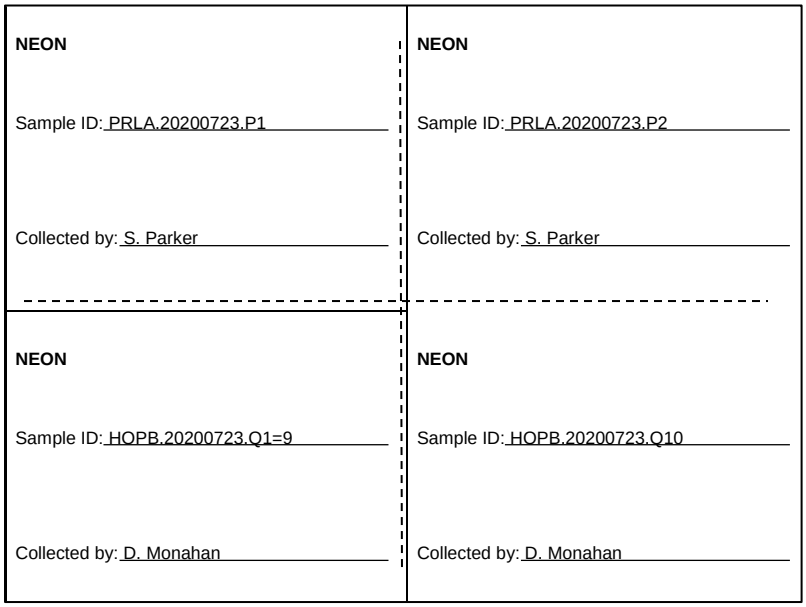

Figure 10. Example of all-weather field labels for bulk aquatic plant and macroalgae sample collection.

2. Adhesive barcode labels will be added to sample containers that are sent to an external facility and scanned by the mobile app. Barcode labels will not be used on field samples, only on samples and specimens that are sent to an external facility.
   a. Keep a physical human-readable label (**Figure 10**) on each bottle/bag with a minimum of the sample ID printed to assist with organization and shipping.
3. Sample IDs will be generated by the mobile app as follows (**Table 8**). Sample IDs written on the physical human-readable label must match the sample ID generated by the mobile app.

Table 8. Examples of field sample IDs generated by the mobile app.

Sampler type	Site type	Field app populates as	Example field sample ID
rake	lake, river	rake	CRAM.20161027.rake.1
benthic quadrat	stream, lake, river	quadrat	MAYF.20161027.quadrat.1
floating quadrat	lake	quadrat	SUGG.20161027.quadrat.1
mini quadrat	stream	mini	MAYF.20161027.mini.1

Figure 11. An example of a Type I barcode. These large-size, field-tolerant barcodes have a prefix of 'A' followed by 11 numbers.

About Barcode Uses and Placement

Although it is always acceptable to use barcodes, in some cases barcodes are absolutely required. **Table 9** provides a quick reference to the types of sample this protocol generates that require barcodes. The rule of thumb is that the primary field sample will ALWAYS need a barcode due to its importance in generating future samples. Likewise, the final disposition of all vialed samples must have a barcode affixed to assist in the shipping and receipt of samples destined for the Biorepository or an external laboratory.

Table 9. Barcode requirements for sample types generated by the APL-Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling protocol. Barcodes are only required for subsamples sent to external facilities.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required?	Barcode Qty
CN sample	Scintillation vials with plant CN material for lab analysis	ARIK.20161027.LEMNA.Q2	(AOS) Plants - Lab	Scintillation vial	Type I	Always Required	1 per vial
Vascular plant taxonomy	Pressed specimen	ARIK.20161027.LEMNA.Q2	(AOS) Plants - Lab	Pressed in newspaper	Type I	Always Required	1 per pressed specimen
Bryophyte taxonomy	Dried specimen in envelope	ARIK.20161027.BRYO.Q2	(AOS) Plants - Lab	Dried in envelope	Type I	Always Required	1 per envelope
Macroalgae taxonomy	Preserved macroalgae	ARIK.20161027.MACROALGAE1.Q2	(AOS) Plants - Lab	60 mL bottle	Type I	Always Required	1 per pressed specimen

SOP B Determining Sampling Locations

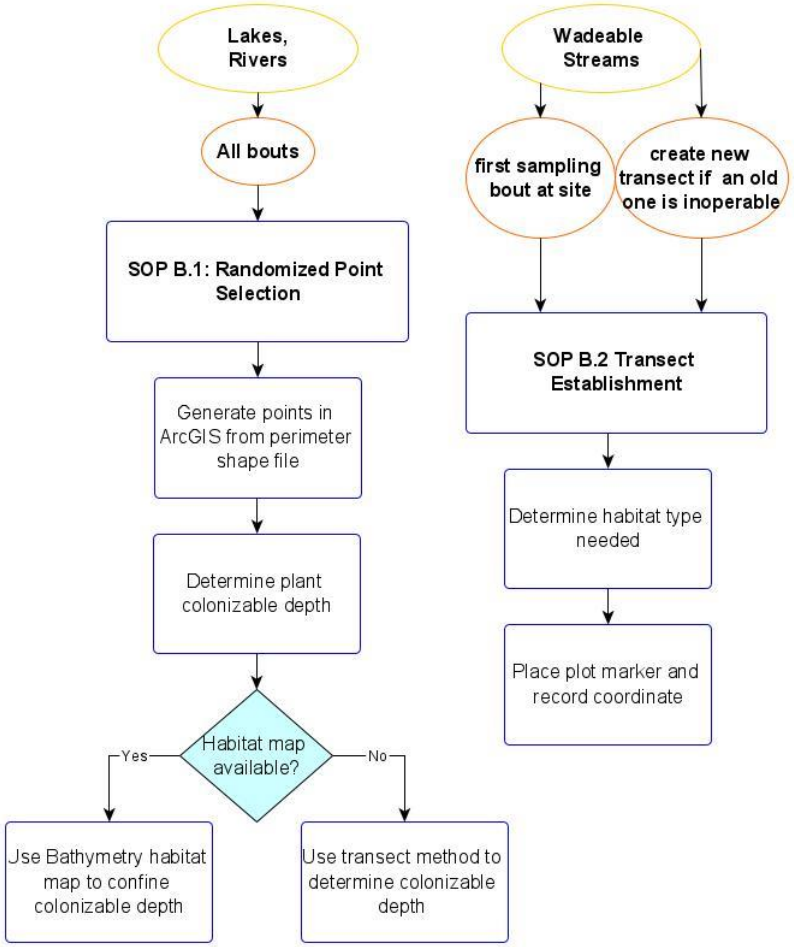

Figure 12. Expanded diagram for the workflow of SOP B.

B.1 Lakes and Rivers – Randomized Point Selection

1. Using the most recent perimeter from the bathymetric map of the lake/river received from NEON HQ (it may be from a prior summer; RD[16]) determine the colonizable depth based on existing aquatic plant cover. If available and representative of the site at the current water level, use the habitat map from the NEON data portal generated as part of the bathymetry data product to determine where plants colonize. Generate a polygon using GIS software of the portions of the lake/river bottom that are equal to or shallower than this depth.
   a. The perimeter shape file can be provided with different depth options to accommodate changing water levels. Contact Science/GIS to get the suite of layers for your site.
   b. If conditions (e.g., water level, hurricane impacts) have changed significantly since the last bathymetry survey, see the bathymetry protocol (RD[16]) for guidance on scheduling an additional survey. If habitats at your site do not match the current map so that the habitat layers

are not useful for predicting plant sampling locations, use the plant colonization criteria in Step 2 below.

c. This represents areas of potential plant growth, not necessarily where plants are known to be rooted.

d. At sites where plants are floating, include all areas of lake where plants are floating regardless of depth.

e. If the plant cover map is not available, estimate maximum depth of plant colonization using the transect method (USEPA 2011) in Step 2.

f. Lakes where the perimeter cannot be determined from the bathymetry protocol (i.e., D03 Suggs) may use a perimeter determined from AOP data or aerial photos (e.g. Google Earth).

g. The ArcGIS ArcMap software is available through the Citrix Receiver using the XD GIS-NoGPU virtual desktop. Contact IT helpdesk if this is not available for your Citrix Receiver profile

2. **Plant colonization depth transects: perform this step prior to sampling if the plant cover bathymetric map is not available or is not representative of the site at the current water level.** 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. Re-check the plant colonizable depth once per year during Bout 2 sampling. NOTE: Keep in mind that water levels/depth may vary throughout the year.

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 sampling criteria:

a) STOP if you have samples two points in a row with 0 plants/macroalgae on the rake

b) STOP if you reach the buoy location.

e. 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.

f. If it seems that the transect is not representative of plant cover and plant colonization depth at the lake, perform an additional transect targeting an area where plants are known to be present to determine plant colonization depth.

3. 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 (as determined in Step 2).

a. Ensure that you have received the polygon (shp file) for your site from GIS at headquarters. This will be created from perimeter mapping in the bathymetry protocol.
b. Open ArcMap
c. Open ArcCatalog to Connect to Folder where your polygon is located
d. Click "Connect to Folder" icon and point to folder where your lake/river outline shapefile is located. Use the most recent polygon available. After selecting, click "OK".
e. In ArcMap, expand the folder you just connected to using the "+" icon. Drag your polygon into the blank map. You should now see the water layer in your map.
f. Go to ArcToolbox
g. Select 'Data Management Tools' > 'Feature Class' > 'Create random points' OR select the "AOS_randomPointModel" tool if available
h. Specify output location in pop up window and name Output Point Feature Class
    i. 'Constraining feature class': choose the polygon you are working with (e.g., "Lake Polygon")
    ii. Number of points = 20 (or ≥40)
    iii. Select 'decimal degrees' if option is available
    iv. Name the output feature
    v. Choose the location where the output file will be exported. Add the ".xls" extension.
    vi. Click 'ok'
    vii. Random points now appear on your polygon
        a) If points do not appear:
            1) Check your feature name, change to something more simple with no characters
            2) Click the error message (if present, at bottom right of screen) to see where the error has occurred
            3) Use NEON's problem reporting system to notify a NEON GIS specialist for help
i. Go to 'Data Management Tools' > 'Features' > 'Add XY Coordinates'
    i. 'Input feature': choose the shapefile of random points layer you just created
    ii. Click 'ok' (nothing happens on the map)
j. 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 click "Apply".
k. 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
    ii. Print a copy of random points coordinates to take in the field or load into GPS unit.
l. Export your map and print
    i. Center your map in the ArcMap window
    ii. Use File-> Export to JPG, save map and print – Do not print directly from ArcMap as it will freeze
m. If you have many points….
    i. Right click your random points layer in ArcGIS

ii. Select "Open Attribute Table"

iii. Select the row on the table to highlight the point on the map as you go through

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 sampled and the coordinate uncertainty (i.e., the larger of 1) the uncertainty reported on the GPS unit or 2) how far you are away from your intended 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. If the lake contains thick floating vegetation maps such that you are unable to maneuver a boat to the sampling point (i.e. D03 Suggs Lake), get the boat as close as possible and record the target coordinate as well as the coordinate uncertainty (distance you are from that point when sampling).
   c. Do not sample at depths greater than the maximum depth of plant colonization as determined above or on the plant cover bathymetry map.
   d. Do not sample within a 5 m radius of the aquatic instrumentation.

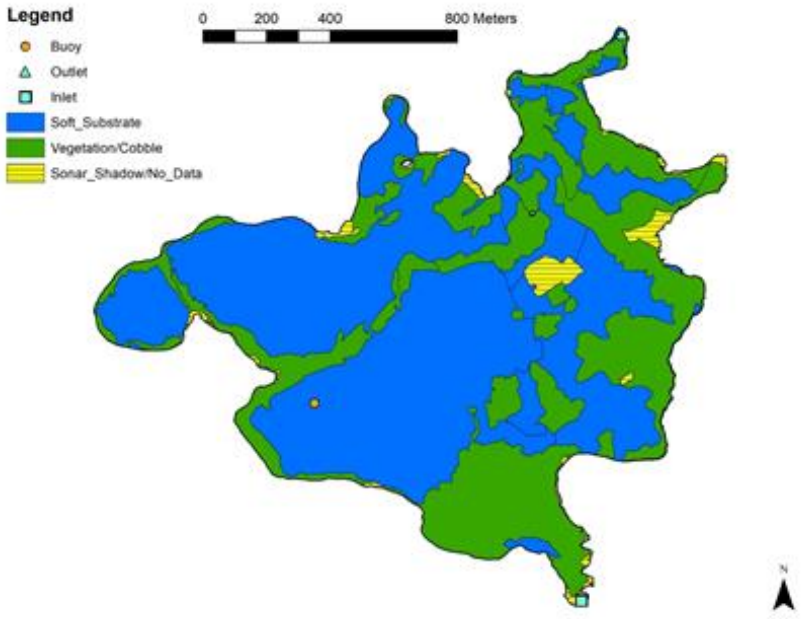

Figure 13. Bathymetric map of Toolik Lake (D18) showing habitat patches of vegetation, cobble, and soft substrates. (Figure courtesy of Spencer Phillips).

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. Transects must remain in the same location on each sampling bout, even if flow conditions cause the habitat types to change. Transects should not be moved unless a transect is no longer in the stream channel.
    i. Reasons for relocating a transect include a change in the stream morphology such that the channel no longer flows through the transect.
    ii. If the stream is dewatered such that a transect is dry at the time of collection, record as 'Location dry' for "Sampling Impractical" in the mobile app.
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)
    iv. Step pool

2. 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 14**).
    b. If it is not possible to separate each transect by a different habitat, transects should be located a minimum of 10 m apart
    c. 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.

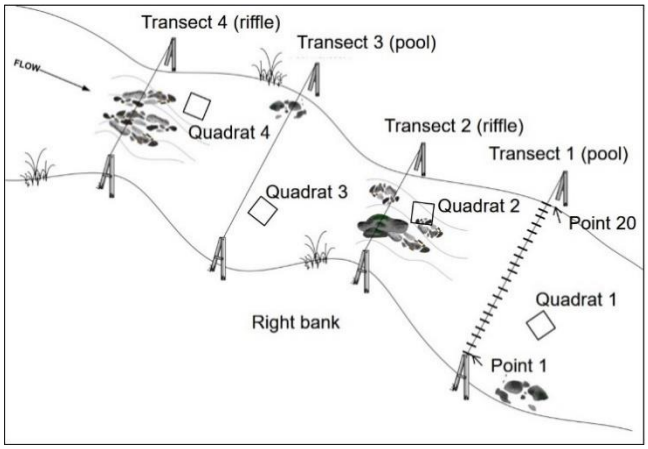

Figure 14. Layout of riffle and pool transects and quadrats within the stream reach.

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

4. Transects should be spread throughout the 1000m sampling reach as evenly as possible.
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). Plant transects used for stream morphology surveys may have plot markers on both banks (RD[09]).
   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 using the Trimble data dictionary for AOS locations. If the Trimble is not available at the time of transect selection, record on the Transect Establishment datasheet (RD[05]) and return at a later date to record locations using the Trimble.
   a. Plot marker coordinates will be surveyed the next time data are collected for the stream geomorphology protocol (RD[09]).

SOP C Field Sampling

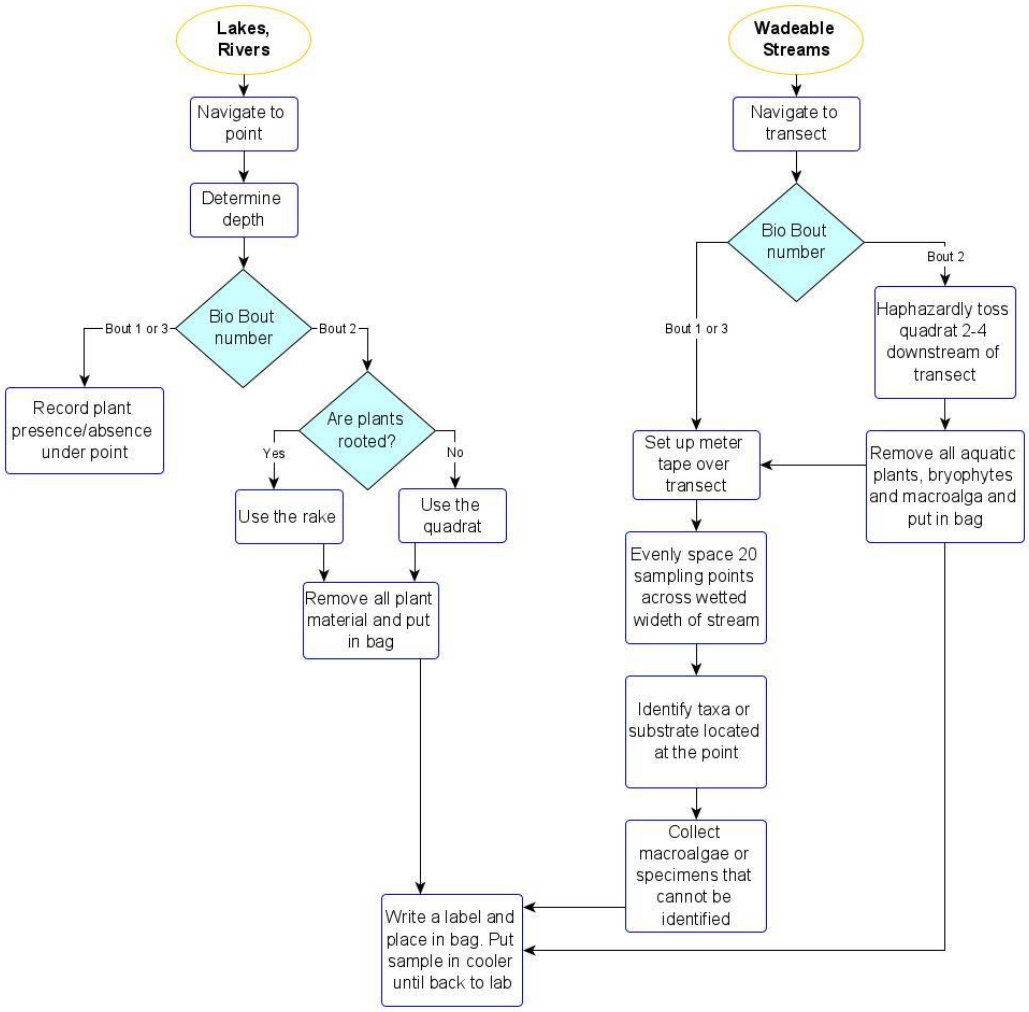

Figure 15. Expanded diagram of the workflow for field sampling SOP C.

Do not collect known rare, threatened, or endangered species. If specimens are accidentally collected, follow permitting regulations for the specific site.

C.1 Randomized Point Sampling (Rake) in Lakes and Rivers (clip harvest)

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) to aquatic instrumentation, continue to the next point on the list.
c. Record the coordinates of the sampling location and the coordinate uncertainty in the mobile app.

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.
   a. Coordinate uncertainty is the larger of 1) the uncertainty reported on the GPS unit or 2) how far you are away from your intended point.
4. Determine depth at the sampling point using the depth-finder and record in the mobile app (Figure 15).
   a. If the depth is <1 m, use the rake with a handle (**Figure 16a**).
   b. If the depth is >1 m, use the rake with a rope and weight attached (**Figure 16b**).

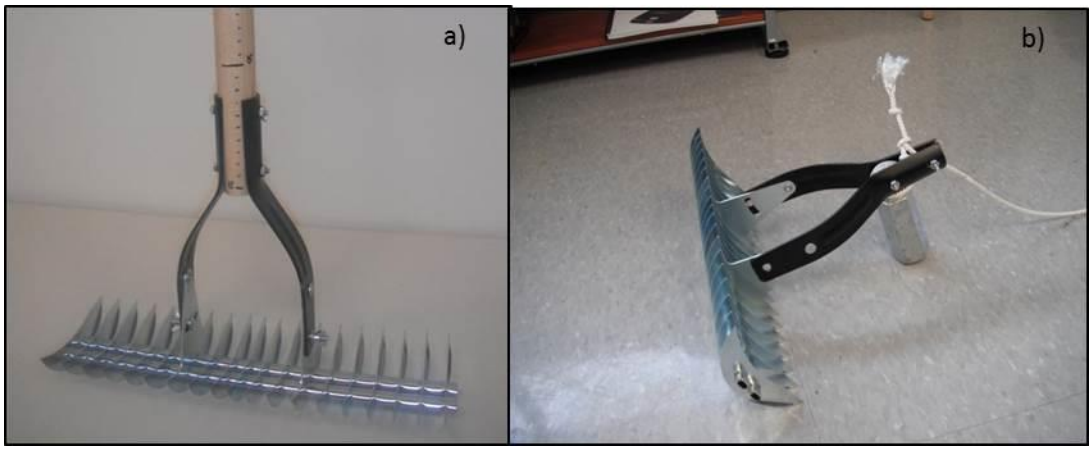

Figure 16. Double-sided sampling rake: a) Rake with handle b) Rake with rope and weight

5. If plants are rooted, use the rake. If plants are floating, put out the 0.5 x 0.5 m quadrat and collect all floating plant material within the quadrat, then proceed to Step 10c.
   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).
   b. For floating plants, include roots as aboveground biomass.
   c. 3 rake tows will be composited per sample. 1 quadrat will be used per sample.
6. Lower the rake head to the lake/river bottom by casting away from or dragging alongside the boat.
7. Pull the rake slowly along the lake/river bottom, toward the boat, for approximately 1.5 m. Be careful not to pull too fast causing the rake to skip off the substrate.
   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. If using the handle, 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 17**).
   a. NOTE: Do not rinse plants or algae in the lake or river to prevent sample loss.

Figure 17. 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 within the parent sample bag. Do not spend more than 10 minutes per tow separating species in the field. Lump all individual species bags from one point in one parent bag with the assigned parent field sample ID (*SITE.DATE.samplerType.pointID*) from the Fulcrum app.
       ii. If lumping the sample in the field, assign a field sample ID (*SITE.DATE.samplerType.pointID*) and replace "rake" in the ID with the taxon/species ID in the lab. Sampler type may be "rake" or "quadrat".
    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 data in the mobile app.
       i. Scan the barcode label with the tablet.
       ii. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app. See SOP C.4 for additional labelling and storage instructions.
    f. Any senesced material collected can be separated from this year's growth in the lab and binned for biomass analysis. Correct identification of senesced material is difficult and unnecessary for this analysis.
11. Repeat steps above until three tows have been completed at each point. Each of the three tows should sample different strips of the lake bottom. For example, use the left side, right side, and front of boat.

C.2 Biomass Quadrats in Wadeable Streams (clip harvest)

Biomass sampling (clip harvest) 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). Small holes may be drilled 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 person 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. 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.
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 "Location Dry" in **Sampling Impractical** in the mobile app and move on to the next quadrat.
   b. If no plants are present in the quadrat, enter "N" in **Target taxa present** 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 live vegetation (i.e., not senesced vegetation that is clearly from the previous year).
g. 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.
   b. Record data in the mobile app.
      1) Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
6. Proceed to sample labeling and storage in SOP C.4.

C.3 Point-Transects in Wadeable Streams

1. Use the pre-recorded plot markers and coordinates to locate each transect.
2. Place a plastic tent stake or chaining pin at each end of the transect. String the meter tape from the right bank to the left bank. 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 "Location dry" in **Sampling Impractical** in the mobile app 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 21, so that there are 20 points in the water.
   c. Maximum distance between sampling points is 50 cm.
   d. Use the mobile app to enter right and left bank distance and calculate sampling intervals.
5. Use the view bucket to see the stream bottom at each sampling point along the transect. Create a reference point using a permanent marker on the center bottom of the view bucket. Line up the reference point on the bottom of the view bucket with the sampling point on the meter tape (**Figure 18**).
   a. Survey points in the wetted channel only.

b. Set the bottom of the view bucket (if using) 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 aquatic plants are present, enter "N" in **Target taxa present** in the mobile application and note the substrate under the point, then move on to the next point.
    ii. If aquatic plants are present, enter "Y" in **Target taxa present** in the mobile app fill in the remaining information in Step 6.
    iii. If multiple layers of vegetation lie underwater below the point, record all vegetation layers.
    iv. If overhanging riparian vegetation is below the sampling point, record as "Target Taxa Present" = No because it is not an aquatic plant, then select substrate = other and describe the vegetation in the remarks field. If there are target taxa beneath the overhanging vegetation, record that as Target Taxa Present = "Yes".
    v. If an object, such as large woody debris, has entered the transect temporarily such that there is water below, record the conditions in the water below the object.
    vi. If transient leaf litter (e.g., during autumn sampling bouts) has fallen into the stream, move out of the way so you can see what rooted plants lie on the stream bottom. If no plants are present, record as "leaf litter" in the mobile application.

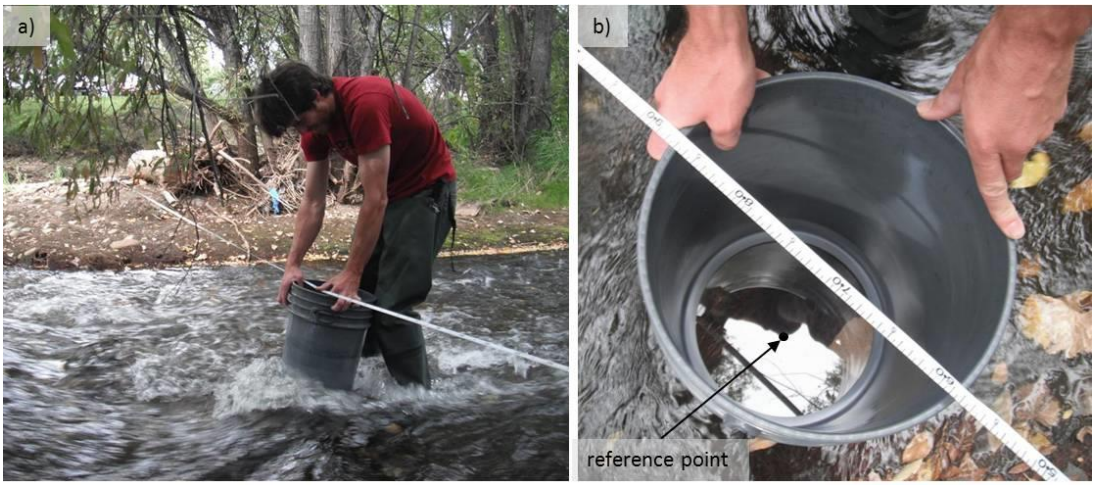

Figure 18. a) Hold the view bucket in the water, under the meter tape transect. b) Hold the view bucket so that the reference point lines up with the point you want to sample on the meter tape.

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 in the mobile app RD[05]). If the specimen can be identified in the field, record the taxon ID (species or 4 letter USDA code). Identify to the lowest taxonomic level that you can, and use the identification qualifier field to indicate if you are unsure about the identification at that level. If you are unable to

identify a taxon or tell it apart from another similar taxon, create a morphospecies ID. Categories include:

a. Target taxa:
   i. **Aquatic plant** – Use the field key to identify to species if possible, or collect a specimen for taxonomic identification off-transect (**Figure 2**) and record morphospecies ID.
   ii. **Moss** – Use the field key to identify to species if possible, or collect a specimen for taxonomic identification and record morphospecies ID. A small specimen may be collected from the transect if you are concerned about identifying a moss off the transect. Limit collection to 1 bout if possible so you do not deplete the colony, and check the data from the eternal taxonomist after identification.
      a) At sites with heavy moss cover where it is difficult to tell species apart, you may collect small specimens from the transect for identification at the domain or an external lab. This decision must be discussed with Science for each site.
   iii. **Liverwort** – Use the field key to identify to species if possible, or collect a specimen for taxonomic identification and record morphospecies ID. A small specimen may be collected from the transect if you are concerned about identifying a specimen elsewhere. Limit collection to 1 bout if possible so you do not deplete the colony.
   iv. **Lichen** – Use the field key to identify to species if possible, collect a specimen for taxonomic identification and record morphospecies ID. A small specimen may be collected from the transect if you are concerned about identifying a specimen elsewhere. Limit collection to 1 bout if possible so you do not deplete the colony.
   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, even if plants or bryophytes are present.
   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) **Pebble** = 2.0-63 mm
      d) **Cobble** = 63-200 mm
      e) **Boulder** = 200-630 mm
      f) **Bedrock**
      g) **Other**

ii. **Other** – additional categories may be added as necessary (e.g., tumbleweed, terrestrial plant), record a descriptive identification of the substrate. Examples include points that include dry and wet substrate beneath (e.g., exposed coarse woody debris with wetted cobble substrate below), or anything that does not fit within the categories above, describe in remarks.

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

7. Collect filamentous macroalgae for taxonomic analysis. Do not attempt to identify in the field unless there is known and previously identified *Didymosphenia geminata* at your site.
   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]).
   b. If *Didymosphenia geminata* or other algae that are both easily identified by eye, and have already been identified by an expert taxonomist, you may enter an algal taxon ID. Contact Science to add new algal taxon IDs to the mobile app.
   c. If macroalgae is abundant at the site and it appears to be similar at several points along the transect, do not collect a sample at each point. Create a macroalgae morphospecies ID in the mobile application and use the sample ID generated by the mobile app. and collect a sample at one point along the transect. Select that morphospecies ID for later points along the transect if the algae appear to be of similar composition, without collecting an additional sample at each point.
      i. Macroalgae morphospecies IDs are generated automatically by the Fulcrum app, use the identifier that is generated.
      ii. Macroalgae morphospecies IDs cannot be shared between transects, create a new morphospecies and sample ID at Transect 2 and collect a new sample for that morphospecies.
      iii. Limit macroalgae collection to <20 samples per site/bout. Contact Science if you have more than 20 samples.
8. If you are not able to determine the plant species from the groups listed above, collect a corresponding specimen off the transect and give the specimen and descriptive morphospecies ID.
   a. Keep morphospecies IDs professional, they will be seen by data users.
   b. Do not use scientific names in the morphospecies, keep them simple.
   c. 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 (use the AOS Plant Voucher App for off-bout voucher collection). Collect obligate aquatic species only. Be prepared on any field visit with zip-top bags and labels. Specimens collected for taxonomic

identification or vouchers will be collected in duplicate, with one specimen archived at the domain herbarium and one specimen archived by the external taxonomist.

d. 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 specimen within the same habitat unit, but not directly on the transect to prevent removal of too much biomass over time from the transect. If you are unable to determine whether off-transect vegetation is the same as on-transect, collect a small specimen from 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.4 Sample Labeling and Storage

1. Write a label on all-weather paper for quadrat biomass, specimen for taxonomic identification, voucher (collected outside of the normal bout sampling), and rake samples, then add inside the sample bag. The sample ID on the label must match the ID generated in the mobile app.
   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-top 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 °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 in the mobile app (e.g., "50 m northwest of sensor set") and record GPS coordinates so the location can be avoided for biomass collection.

b. Take high resolution photos
c. Retain the collected material, press and send to external taxonomists for identification (SOP D.4) and use NEON's problem reporting system to notify NEON Permitting.
d. If invasive species (e.g., *Hydrilla*) are identified (as defined by USDA aquatic nuisance species or local or state lists), use the problem reporting system to notify NEON Permitting, and inform equipment decontamination procedures.
    i. Field Science should become familiar with common invasives in their area by contacting state agencies and/or NEON permitting.

C.6 Sample Preservation

1. Keep samples refrigerated (4 °C ±3 °C) and in the dark until processing at the Domain Support Facility.
2. Samples must be returned to the Domain Support Facility 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. Sync mobile app.
    d. Check and charge all batteries, replace if necessary.
    e. 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.
3. Data QA/QC
    a. Required checks
        i. Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample bottles/bags.
    b. Nice to check
        i. site ID, collect date, sampling protocol version

SOP D Laboratory Sampling and Analysis

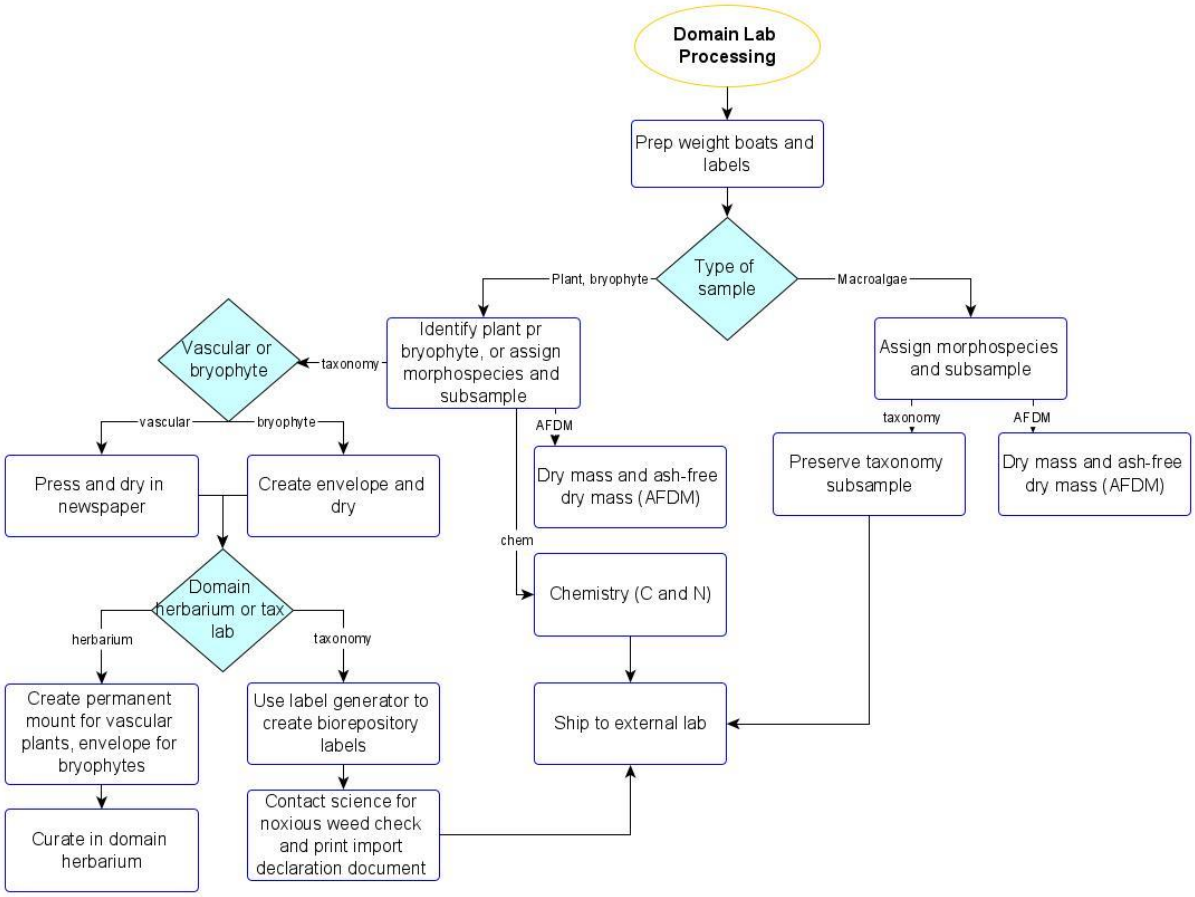

Figure 19. Domain lab workflow.

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 20**).
    b. NOTE: It doesn't matter what the labels are as long as they are unique and easy to read.
    c. Consider that you will be filling the inside of the boat with material, making the ID hard to see from the top. It's ok to put IDs on the boat tab if you have one, but they tend break and fall off. Make sure there is a backup ID on the boat bottom.
    c. Place new empty labeled boats in the muffle furnace (500 °C) for 6 hours to burn off any residue.
    d. 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) in mobile app (RD[05]).

e. This may be done in advance, before field sampling.

f. Boats may be reused from previous sampling bouts.

Figure 20. 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.
   a. Boats should be re-ashed to minimize residue between sampling events.
   b. Boats must be re-weighed prior to every use. Minimize touching the boat with un-gloved hands after weighing. Oils from your hands can skew results.
   c. Best practice is to re-ash boats prior to use, rather than storing ashed and weighed boats for a long period of time (> 2 months) between bouts. Boats may be stored in a closed container (e.g., bag, box, or plastic container) to minimize dust contamination.
3. Sample IDs will be generated by the mobile app as follows in Table 10. Also adhere barcode labels to any containers sent to an external facility (**Figure 21**).

Table 10. Example if taxon-specific sample IDs generated by the mobile app, based on species or morphospecies data entered.

Species or Morphospecies example	Example Final Sample ID
Lemnaceae	ARIK.20161027.LEMNA.Q2
macroalgae1	MAYF.20161027.macroalgae1.Q1
Potomogeton nodosus	SUGG.20161027.PONO2.P7
fuzzy fern	MAYF.20161027.fuzzyfern.T4

Figure 21. Example of adhesive barcode labels.

4. If samples are small and enough material is not available for all analyses, process according to the following hierarchy:
   a. Specimen for taxonomic identification (if needed)
   b. AFDM (enough material to measure on the balance)
   c. CN (CN is never collected for macroalgae; minimum plant CN is 0.2 mg dry mass)

D.2 Aquatic Plants and Bryophytes

The following steps are used to process aquatic plant and bryophyte samples from quadrats (**wadeable streams**) and rakes (**lakes and rivers**).

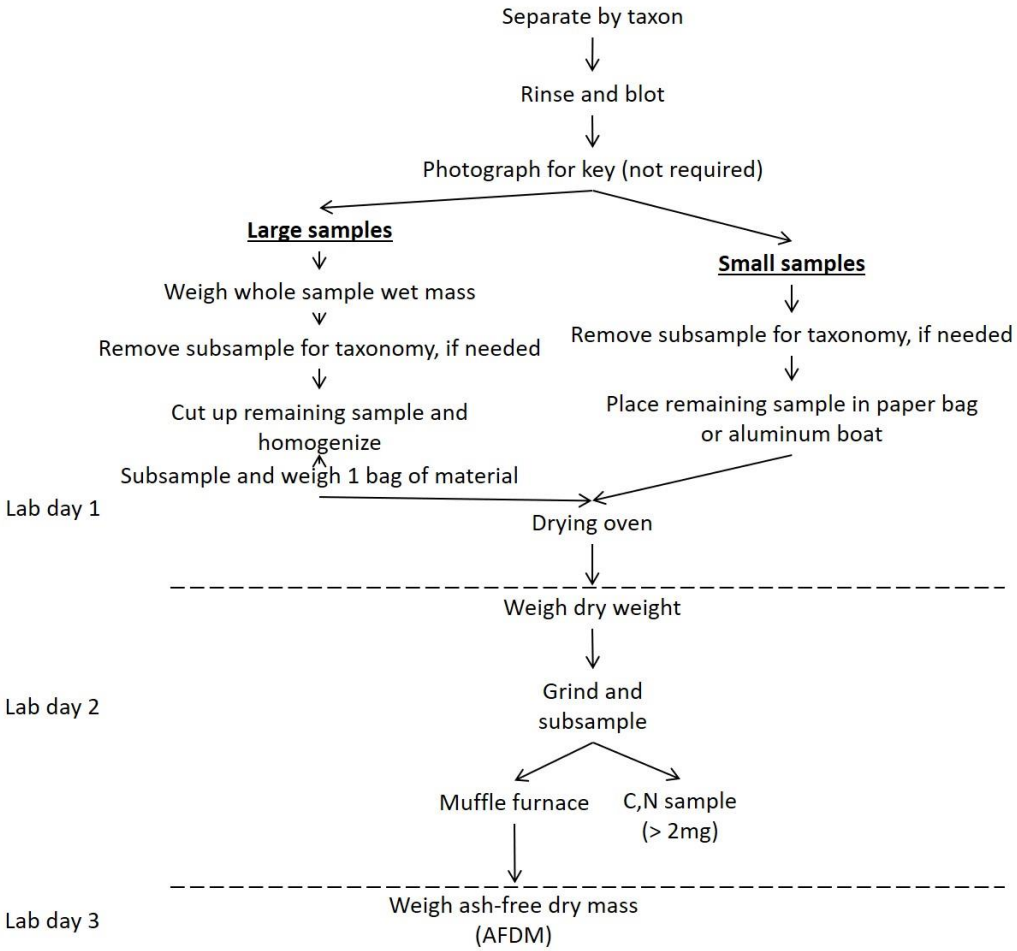

Figure 22. After separating each species from the original sample, follow the steps above for Domain Support Facility aquatic plant processing.

1. **Day 1**: Remove plant/bryophyte sample and field label from sample bag from quadrat/rake 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 plant or bryophyte species will be a unique specimen.
   b. Float 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 or moss taxa from each other. After spreading the entire sample out on a tray, spend no more than 5-10 minutes sorting the sample.

i. For macroalgae entwined with plants, a series of floating the sample and dumping the macroalgae into another container may be helpful for some sites. Do not spend more than 5-10 minutes for any one sample.

d. If there is not enough material in the specimen to complete all analyses, prioritize samples as follows:
   i. Taxonomy: Only subsample for taxonomic identification if specimen cannot be identified in domain lab, or is not needed to contribute to the herbarium. Identify to the lowest taxonomic level that you can, and use the identification qualifier field to indicate if you are unsure about the identification at that level. If you are unable to identify a taxon or tell it apart from another similar taxon, create a morphospecies ID.
   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. Weigh entire sample, prior to subsampling, on mass balance and record as **Total wet mass** in the lab app (. This may occur on the 2-place balance if the sample is large (0.01 g accuracy).
7. Identify the specimen using field key and/or voucher specimens from the domain herbarium.
   a. If specimen cannot be identified at the Domain Support Facility with at least 85% confidence, use temporary morphospecies ID, subsample, and send a specimen to external taxonomist.
   b. Retain a portion of the specimen for curation in the domain herbarium.
   c. For rake samples, replace the temporary rake ID with sample identifier generated by the mobile app.
8. Cut samples, if necessary, to a size that will fit into paper bags and homogenize prior to subsampling further.
   a. For large samples: Select a well-homogenized subsample that will fit in one paper bag and weigh. Record as **Subsample wet mass** in the lab app. The remaining material not used in the subsample may be discarded.
   b. If subsamples are not necessary, **Subsample wet mass** = **Total wet mass**.
   c. **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.
9. Using a permanent marker, label a clean, paper lunch bag with sample ID and species ID from the field sample label.
10. Place wet sample (or wet mass subsample) in the labeled clean paper lunch bag.

a. If the specimen is fragile, small pieces may cling to the standard drier. If it is a small amount in relation to the entire sample, it is ok if you are unable to get every fragment off of the standard drier.

11. Place paper bags containing samples in the drying oven for a minimum of 12 hours at 65 °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.
12. **Day 2**: When dry, remove all bags+samples from drying oven and let cool to room temperature in a closed plastic bag or the desiccator prior to weighing.
    a. Placing samples in a bag/desiccator is important because samples absorb water quickly from the air as they cool.
    b. Samples may be left in the bags/desiccator for up to 30 days before proceeding to the next step.
13. Place a large, clean, plastic weigh boat (small specimen) or tray (large specimen) on analytical balance. Tare (zero) balance. Place dry specimen in the plastic weigh boat/tray and record as **Total or Subsample Dry Mass** in the lab app.
14. Samples may be crushed (with a gloved hand) 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.
15. Subsample for CN and AFDM.
    a. If there is enough material, remove ≥ 2 mg for C and N analysis. Place in 4 mL PTFE-capped glass vial, apply adhesive label, and set aside for shipping. Use a Type 1 barcode label and scan using the mobile app.
        i. Samples sent to external lab for CN must be ground on the Wiley Mill using 40 mesh (**Figure 23**).
        ii. If a sample is too small to process in the Wiley Mill, crush with a gloved hand and place in the sample vial. The external lab will grind sample further.
    b. Place remaining specimen subsample in a clean, pre-labeled, pre-weighed aluminum weigh boat. Record **Boat ID** and **Boat mass** in the lab app prior to placing the subsample in the boat.
        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).
        ii. Handle the weigh boat using tongs or forceps to avoid transferring oils from your hands or powder from your gloves to the sample.
        c. **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.

Figure 23. Photo of open CN vial and crushed contents.

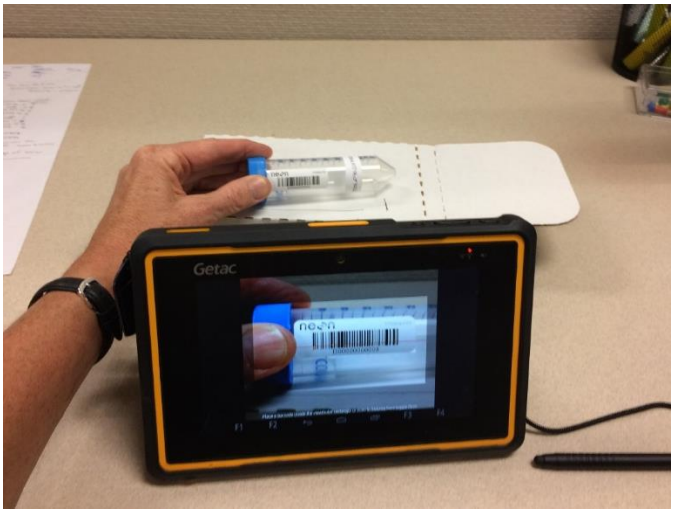

Figure 24. Barcode label scanning.

16. Weigh boat on analytical balance, and record as **Boat + dry mass** in the lab app.
17. Repeat above steps until all specimens have been processed.
18. Place aluminum boats + specimens in the muffle furnace using oven gloves and tongs. **TAKE CARE NOT TO BURN YOURSELF!**
    a. Boats may be stacked on top of each other as long as there is space for air flow between them.

b. 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.
c. Covering the pan with foil will help protect the samples from the rush of air after opening the muffle furnace and avoid sample loss.

19. Leave samples in the muffle furnace at 500 °C for at least 6 hours.
    a. Sample may be left in muffle furnace for longer than 6 hours (e.g., overnight) if necessary.
20. **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.
21. Cover pans/aluminum boats to prevent ash from blowing out of boats and set aside to cool on a heat-resistant surface in an area without drafts from windows, doors, or mechanical building ventilation.
22. When boats have cooled enough to handle, place in the desiccator. Allow samples to cool to room temperature in the desiccator for at least 20 minutes prior to weighing.
    a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
23. After cooling to room temperature, weigh boats again on analytical balance, record as **Boat + ash mass** in the lab app.
    a. **Boat + ash mass** should be a smaller number than **Boat + dry mass**, as the muffle furnace burns off organic material.
24. After weighing, dump ash into the trash and clean the boat with a soft brush or paper towel.
    a. If there have been problems with the balance, consider storing the ashed samples in the desiccator until you have quality checked the data.
25. Set clean boats aside to be used again.

D.3 Macroalgae

The following steps are used to process macroalgae samples from quadrats and rakes. Macroalgae are not subsampled for chemistry (CN).

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.

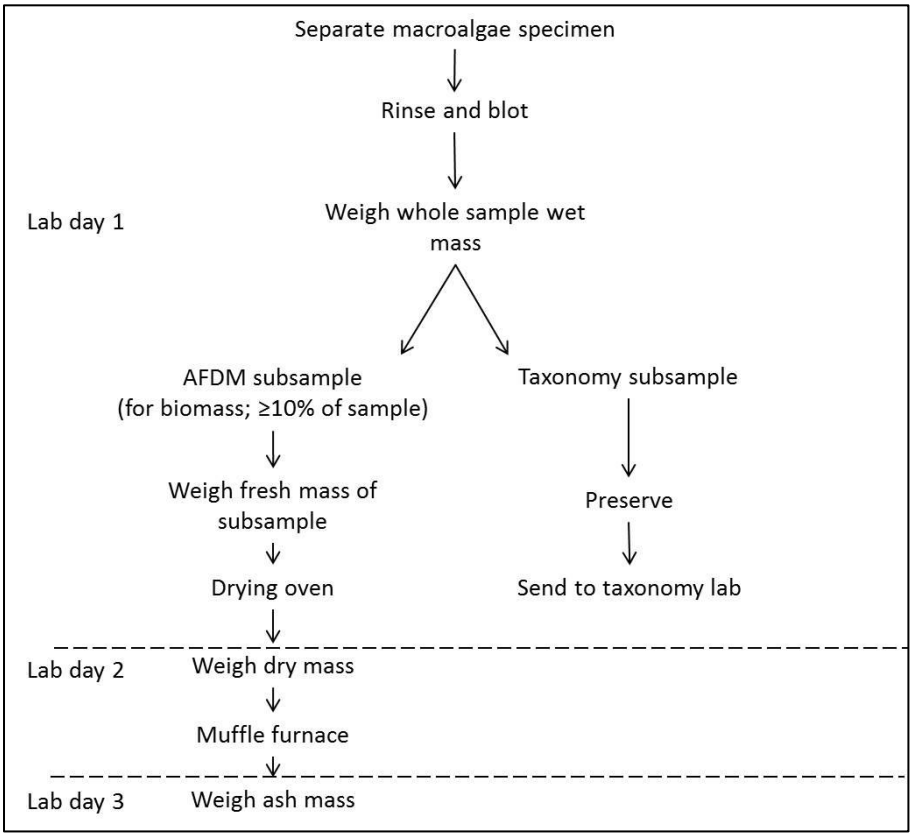

Figure 25. Schematic for macroalgae processing at the Domain Support Facility.

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.
   c. Spend no more than 5-10 minute per sample removing macroinvertebrates from the sample.
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.
   c. If there is not enough specimen to conduct AFDM analysis (less than approximately 10 mL of algae), place macroalgae directly into 60 mL HDPE sample bottle and proceed to D.8.
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** in the lab app.
   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.8. Label according to Figure 26, and also use a barcode label.

1) If there is only ~10 mL of sample, do not process for AFDM, rather contribute all material to the taxonomy sample.
2) Add a Type I barcode label to the sample container and scan into mobile app.

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** in the lab app along with appropriate **Boat ID** and **Boat mass**.
c. Repeat above steps until all samples have been processed.

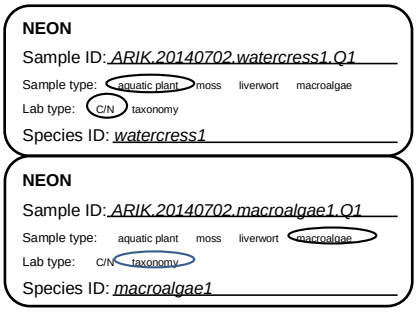

Figure 26. Example of adhesive labels for macroalgae taxonomy and plant CN subsamples.

7. Place all aluminum boats + subsamples in drying oven for a minimum of 12 hours at 65 °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** in the lab app.
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 °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** in the lab app.
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. Plants at some aquatic sites may be covered with epilithic algae. You may remove epilithic algae from leave gently by hand. Algae may be tightly adhered, and it is ok if they remain on the specimen leaves.
2. Open plant press.
3. Place 1 cardboard ventilator on plant press.
4. Place 1 standard drier on top of cardboard ventilator.
5. Place newspaper (1 large sheet with fold down the middle, opened) on top of the standard drier.
    a. Do not use 2 separate sheets of newspaper as these can move during drying and transport, damaging the specimen.
6. Copy information from the specimen label onto the newspaper using a permanent marker and use the NEON label generator Shiny app to generate a label for plant taxonomist.
7. 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.
8. Place specimen label on newspaper (for additional identification). Add a barcode label and scan using the mobile app.
9. Fold opened newspaper sheet over the sheet that the specimen is on.
10. Place another standard drier on top of newspaper.
11. Place a cardboard ventilator on top of the standard drier.
12. Repeat Steps 3-11 with Specimen #2. Continue until all specimens have been pressed.

13. Place wooden top of plant press on top of last cardboard ventilator.
14. Fasten compression straps, pull tightly to apply even pressure to the press.
15. Set press in a warm, ventilated, dry location. Check periodically to ensure that no mildew forms on the press.
16. Leave plants in press until they are shipped to taxonomist. Do not create permanent mounts as the taxonomist needs to be able to move the specimen to identify.
17. Proceed to Herbarium Mounts (SOP D.5) for specimens that are kept in the domain herbarium.

D.5 Herbarium Mounts (Aquatic Plants Only)

Herbarium mounts are created for specimens that are kept in the domain herbarium for reference. Do not mounts specimens that are shipped to the external taxonomist.

1. Refer to the Plant Pressing and Mounting SOP (RD[18]).
2. Open plant press carefully.
3. Open a newspaper sheet with one specimen on it.
    a. If there are small pieces or reproductive structures that have fallen off of the main samples, you may place these in a bryophyte/lichen packet or seed envelope and adhere the packet to the top left corner of the herbarium paper with the flap facing out so the plant parts can be easily accessed.
4. Separate into two or more specimens.
    a. One specimen will be sent to the aquatic plant taxonomist (keep in newspaper, proceed to SOP F and see RD[21] for shipping).
    b. One specimen will be mounted and retained at the Domain Herbarium for reference.
    c. Use sampleIDs generated by the lab app for the taxonomy subsample and the domain herbarium subsample.
    d. Include flowering structures in each specimen if present. If only one specimen has flowers, send this specimen to the taxonomist for complete identification.
5. Follow instructions for pressing and mounting in RD[18].

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

1. Print Bryophyte/Lichen packets on all-weather copier paper.
    a. Fold the bottom third of an 8.5” x 11” sheet of paper up, leaving 3-3.25” of paper revealed (Figure 27 step 2).
    b. Fold 1” to 1.5” margins in from the edges (Figure 27 steps 3-4).
    c. Fold down the top flap, place labels on the outside of flap.
    d. There should not be a gap on the inside hinge of the flap (Figure 27 last step), or the specimen could fall out fo the packet.
2. Adhere a human readable label and barcode to the outside of the packet. If the packet template includes habitat information, fill out as much as you can.
3. Gently rinse specimen in tap water to remove sediments. Take care to avoid breaking the specimen.

4. If using this specimen to create a lab photo key, 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. A photo does not need to be taken for each specimen.
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 (. Add a barcode label and scan using the mobile app.
   a. **NOTE:** If specimen is a lichen, leave the lichen attached to rock substratum if present.

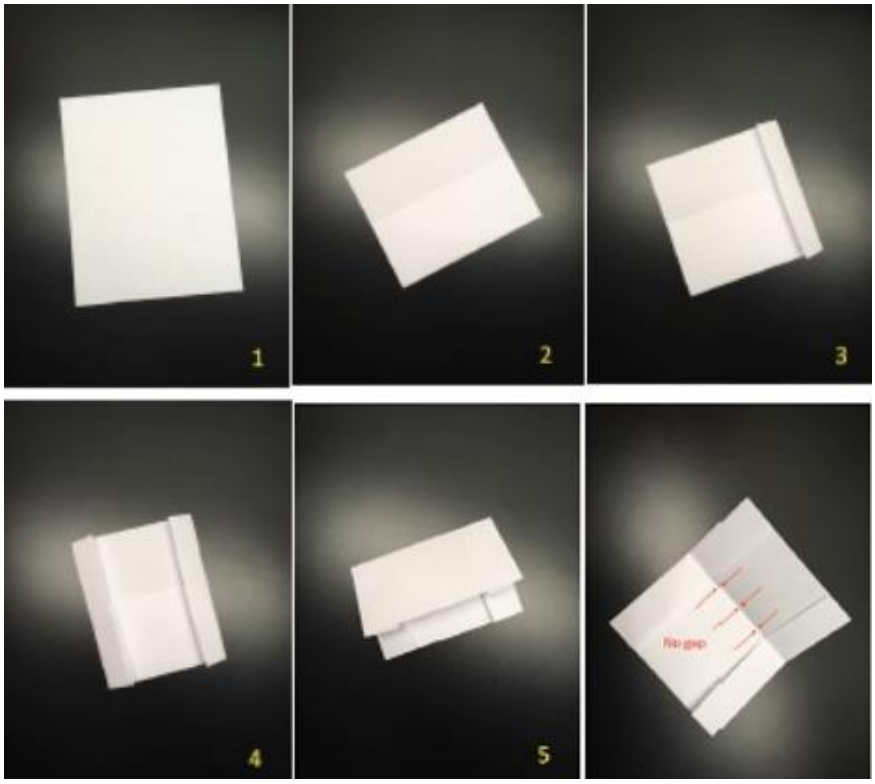

Figure 27. Example of packet folding steps, courtesy of David Kofranek LLC. 1) 8 ½" x 11" paper; 2) fold the bottom 1/3 up; 3) fold 1" margins in on both sides; 5) fold lid over, place labels here.

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 and RD[21] for shipping instructions to taxonomists.

D.7 Resolving Morphospecies

1. Morphospecies names and descriptions are good for 1 calendar year in the mobile app.
2. Morphospecies may be resolved and identified to an accepted taxon ID by field ecologists at any step in the process.

3. If a morphospecies can be resolved (i.e., identified to a name in the plant taxon list), open the morphospecies mobile app, enter the correct taxon ID from the taxon list and mark as "resolved".
4. Ecologists do not need to go back through old data to update these morphospecies, however any data that has not yet been submitted and locked should be updated to reflect the resolved ID.

D.8 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 until shipping. For every specimen sent to a taxonomist, an identical specimen should be mounted and kept in the domain herbarium.
2. **Mosses, liverworts, and lichens** sent out for further taxonomic identification should be dried and placed in paper packets. Specimens should be stored in a dry, well-ventilated area until shipping. For every specimen sent to a taxonomist, an identical specimen should be and kept in the domain herbarium.
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 °C (±3 °C) for up to 30 days until shipping.
    a. For reference, 60 mL of sample (or sample + water) is preserved with 1.2 mL of glutaraldehyde.

D.9 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.
3. Data QA/QC
    a. Required checks
        i. Check that the sample IDs generated by the mobile application(s) match the sample IDs on the sample bottles/packets.
        ii. Check that the barcode labels in in the mobile application(s) match the barcode labels adhered to the samples. At a minimum, check the last few numbers of the barcode.
    b. Nice to check
        i. Site ID, collect date, sampling protocol version
        ii. AFDM measurements

SOP E Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times, however mobile applications should be used when available. 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). These are for use in the domain lab and will not be submitted to the data portal.

Data and sample IDs must be entered digitally and quality checked prior to shipping samples to an external lab

SOP F Sample Shipment

Specimens shipped to external taxonomists for identification

1. All specimens shipped to the external taxonomist will ultimately be housed at the NEON Biorepository. The following steps are required for storage at the biorepository, so will be done at the domain support facility prior to shipped.
2. Create a standardized human-readable label for each specimen with location information using the NEON Label Generator app. See RD[18] for details. Keep the label with the specimen.
3. Freeze all specimens for 2 weeks at -80 °C prior to shipping to kill any pests (RD[18]).
4. Include one import declaration per box prior to shipping. The import declaration documentation is provided by Contracts and Laboratory Analysis (CLA).
5. Submit a request to AOS Science for a noxious weed check prior to shipping. Include the shipping manifest or list of sample identifiers that you plan to ship. You may not ship until this step has been complete.
6. For plant taxonomy, macroalgae taxonomy, and plant chemistry samples, follow instructions in the NEON Protocol and Procedure: Shipping Ecological Samples, Sensors, and Equipment for instructions on shipping samples to external laboratories (RD[08]).

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APPENDIX A 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 rivers**, 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.
    c. At sites where plants are floating, include all areas of lake where plants are floating regardless of depth.

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 specimens if plants cannot be positively identified in the field.
2. In **lakes or rivers**:
    a. Randomized Point Sampling: Collect samples at 10 points, with 3 rake tows from each point.

Step 6 – Keep samples chilled (~4 °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 plants, mount plants for the domain herbarium, or place in packets for Domain Herbarium and taxonomist (if necessary).

Macroalgae: measure ash-free dry mass, preserve in glutaraldehyde and send to taxonomy lab.

APPENDIX B REMINDERS

Before heading into the field:

- Collect and prepare all equipment, including labels on waterproof paper.
- Upload GPS locations to find transects, determine plant points for lakes and rivers.

Sample collection in <u>wadeable streams</u>:

- 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 specimens off transect if necessary.

Sample collection in <u>lakes and rivers</u>:

- Determine the 20 random points based off of the perimeter shapefile from 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:

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

Data QA/QC:

Required checks

- Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample containers.
- Check that the barcode labels in in the mobile application(s) match the barcode labels adhered to the samples. At a minimum, check the last few numbers of the barcode.

Nice to check

- Site ID, collect date, sampling protocol version
- AFDM measurements

APPENDIX C 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	Lewis Run	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D02	Posey Creek	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D03	Flint River	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	Little Rock Lake	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D06	Kings Creek	23Mar-20Apr	3Jul-31Jul	3Oct-31Oct
D06	McDiffett Creek	23Mar-20Apr	3Jul-31Jul	3Oct-31Oct
D07	Leconte Creek	15Mar-12Apr	30Jun-28Jul	12Oct-9Nov
D07	Walker Branch	9Mar-6Apr	1Jul-29Jul	19Oct-16Nov
D08	Mayfield Creek	5Mar-2Apr	29Jun-27Jul	31Oct-28Nov
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	Blue River	7Mar-4Apr	1Jul-29Jul	12Oct-9Nov
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	12Jan-11Feb	24Mar-23Apr	3Jun-3Jul
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	Teakettle 2 Creek	10Apr-8May	9Jul-6Aug	25Sep-23Oct
D17	Upper Big Creek	2Apr-30May	9Jul-6Aug	28Sep-26Oct
D18	Oksrukuyik Creek	21May-18Jun	29Jun-27Jul	7Aug-4Sep
D18	Toolik Lake	19Jun-17Jul	27Jul-24Aug	3Sep-1Oct
D19	Caribou Creek	2May-30May	26Jun-24Jul	18Aug-15Sep

APPENDIX D 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)
D17	--	*Peltigera gowardii*	lemon lily		CA**	riparian (stream)

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

**Species of concern as listed by local taxonomic groups

APPENDIX E SITE-SPECIFIC INFORMATION

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	Lewis Run	Run	Riffle	0.5 x 0.5 m quadrat
D02	Posey Creek	Riffle	Pool	10 cm x 10 cm quadrat (mini)
D03	Flint River	Littoral		rake
D03	Lake Barco	Littoral		rake
D03	Lake Suggs	Littoral		floating vegetation sweep
D04	Rio Guilarte	Riffle	Pool	0.5 x 0.5 m quadrat
D04	Rio Cupeyes	Riffle	Run	0.5 x 0.5 m quadrat
D05	Crampton Lake	Littoral		rake
D05	Little Rock Lake	Littoral		rake
D06	Kings Creek	Riffle/run	Pool	0.5 x 0.5 m quadrat
D06	McDiffett Creek	Riffle	Short reach, no habitat 2	0.5 x 0.5 m quadrat
D07	Leconte Creek	Riffle	Pool	10 cm x 10 cm quadrat (mini)
D07	Walker Branch	Riffle	Run	10 cm x 10 cm quadrat (mini)
D08	Mayfield Creek	Riffle	Run	0.5 x 0.5 m quadrat
D08	Black Warrior River	Littoral		rake
D08	Tombigbee River	Littoral		rake
D09	Prairie Lake	Littoral		rake
D09	Prairie Pothole	Littoral		0.5 x 0.5 m quadrat for rooted littoral vegetation
D10	Arikaree River	Run	Pool	0.5 x 0.5 m quadrat
D11	Pringle Creek	Run	Riffle	0.5 x 0.5 m quadrat
D11	Blue River	Run	Riffle	0.5 x 0.5 m quadrat
D12	Blacktail Deer Creek	Riffle	Run	10 cm x 10 cm quadrat (mini)
D13	Como Creek	Riffle	Run	10 cm x 10 cm quadrat (mini)
D13	West St. Louis Creek	Riffle	Pool	10 cm x 10 cm quadrat (mini)
D14	Sycamore Creek	Run	Pool	0.5 x 0.5 m quadrat
D15	Red Butte Creek	Step pool	Run	10 cm x 10 cm quadrat (mini)
D16	McRae Creek	Step pool/Riffle	Step pool/pool	10 cm x 10 cm quadrat (mini)
D16	Martha Creek	Riffle	Pool	0.5 x 0.5 m quadrat
D17	Teakettle 2 Creek	Riffle-cobble	Riffle-bedrock	10 cm x 10 cm quadrat (mini)
D17	Upper Big Creek	Run	Riffle	0.5 x 0.5 m quadrat
D18	Oksrukuyik Creek	Run	Riffle	0.5 x 0.5 m quadrat
D18	Toolik Lake	Littoral		rake
D19	Caribou Creek	Run	Riffle	0.5 x 0.5 m quadrat

APPENDIX F EQUIPMENT

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

Table 11. Equipment List - General Equipment.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Site-specific Stream Morphology or rapid habitat assessment, or Bathymetry Map	Determining sampling locations	1
CDW-G/4452963	N	Mobile data entry tablet	Field data entry	1
Forestry Suppliers, Inc./39481 Cabela's Inc./IK-270217 Recreational Equipment Inc./895022	N	Handheld GPS unit (with batteries, ±4 m accuracy) or Humminbird	Navigating to sampling locations	1
Forestry Suppliers, Inc./52190 Grainger/1GEJ1	N	Clipboard	Recording data	1
	N	Cooler, 9-28 quart	Storing samples	1
	N	Ice packs or water ice	Keeping samples cool	2
Grainger, W.W. Forestry Suppliers, Inc. Cabela's, Simm's	N	Waders (hip or chest) or knee boots	Boating or wading	1 pair per person
Consumable items				
Forestry Suppliers, Inc./49247	N	Aquatic Field Metadata Sheet (all-weather paper)	Recording metadata in case tablet fails	1
Forestry Suppliers, Inc./49247	N	Field datasheets (all-weather copier paper, write in pencil)	Recording data in case tablet fails	2
Forestry Suppliers, Inc./49247	N	Pre-printed all-weather paper labels	Labeling aquatic plant, bryophyte, and lichen samples	10-20
Amazon Capital Services Inc./B00006IBUW	N	Pre-printed adhesive labels (all-weather, 2"x4")	Labeling macroalgae samples	20
	N	Adhesive barcode labels	Labeling sample bottles with barcode-readable	1 sheet
	N	Pencils	Recording data	2
Grainger, W.W./1JU51	N	Permanent markers	Labeling samples	2
	N	Extra batteries	Backup for GPS, Camera	4
Forestry Suppliers, Inc./22312OR	N	Flagging tape (roll) or pin flags	Flagging plant transect locations	1

Table 12. Equipment list- Transect establishment for **wadeable streams**

Supplier/Item no.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Site-specific Stream Morphology Map or rapid habitat assessment	Determining sampling locations	1
Forestry Suppliers, Inc./39481 Cabela's Inc./IK-270217 Recreational Equipment Inc./895022	N	Handheld GPS (with batteries, ± 4 m accuracy)	Recording transect locations	1
	N	AOS plot markers	Permanently marking transect locations	10

Table 13. Equipment List - Sampling equipment for **wadeable streams**

Supplier/Item no.	Exact Brand	Description	Purpose	Quantity
Durable items				
Amazon Capital Services Inc./B001JYXXH8 Forestry Suppliers, Inc./100952	N	Plastic stake (large) or chaining pin	Anchoring the transect tape	2
McMaster-Carr Supply Co./50065A21	N	Spring clamp	Anchoring the transect tape	2
Forestry Suppliers, Inc./213379, 37184	N	Meter tape (50 m)	Transect tape	1
West Marine Products, Inc./2001931	N	View bucket (Plexiglas bottom)	Underwater viewing for point-transect measurements	1
BioQuip Products Inc./4748	N	Forceps – featherweight	Separating macroalgae from plants	1
BioQuip Products Inc./4533	N	Forceps – fine point	Separating macroalgae from plants	1
	N	Scissors or hand clippers	Removing aquatic plants from the biomass quadrat	1
Grainger, W.W./6R335	N	Mallet	Collecting lichen specimens, use with chisel	1
Forestry Suppliers, Inc./162065	N	Chisel	Collecting lichen specimens, use with mallet	1
	N	Trowel	Collecting aquatic plant specimens	1
Amazon Capital Services, Inc./B00H51AIYK	N	Aquarium dip net	Collecting floating plants	1
Grainger, W.W./20JZ18	N	Collapsible quadrat (0.5 x 0.5 m)	Biomass quadrat sampling	1

	N	Modified quadrat (10 x 10 cm)	Biomass quadrat sampling - small headwater streams only	1
B&H Photo Corp/PADMCTS30BL	N	Digital camera, waterproof (with battery, memory card)	Photographing specimens	1
	N	Field identification key (site-specific)	Identifying specimens in the field	1
Consumable items				
Grainger, W.W./4A807	N	Single-edged razor blades	Collecting mosses and liverworts	5
Thomas Scientific, Inc./1303N48	N	Whirl-pak® bags, 24 oz.	Sample container	30
Grainger, W.W./5LH30	N	Resealable bags (gallon)	Organizing samples, collecting large plant specimens	10
Uline/S-5106	N	Trash bags	Collecting large biomass samples	5
	N	Cable ties (6 inch), package	Attaching weight to rake	1

Table 14. Equipment list - Sampling equipment for **lakes and rivers**

Supplier/Item no.	Exact Brand	Description	Purpose	Quantity
Durable items				
	Y	ArcMap Software	Generating random points for sampling	1
Home Depot U.S.A., INC./157369	N	Double sided thatching rake with handle	Sample collection	1
	N	Braided polyester line marked in 20 cm increments for rake	Sample collection	1
	N	Secchi disk weight (3 lb)	Weighing down rake	1
Forestry Suppliers, Inc./279992, 53149	N	Collapsible quadrat (0.5 x 0.5 m)	Sample collection for floating plants	1
Amazon Capital Services Inc./B00X0WT8MQ	N	Depth finder	Determining depth at the sampling point	1
	N	Scissors	Removing aquatic plants, bryophytes and macroalgae	1
B&H Photo Corp/PADMCTS30BL	N	Digital camera, waterproof (with battery, memory card)	Photographing specimens	1
	N	Field identification key (site specific), created over time at the domain	Identifying specimens	1
	N	Tray, tub, or 5 gallon bucket	Sorting samples in the field	1
Consumable items				
Fisher Scientific Company/14955175	N	Whirl-pak® bags, various sizes	Sample container for smaller samples	30

Thomas Scientific, Inc./1303N23, 1303N48				
Grainger, W.W./5LH30	N	Resealable zip-top bags (gallon)	Sample container for larger samples	10

Table 15. Equipment list - Laboratory processing: Ash-free dry mass

Supplier/Item no.	Exact Brand	Description	Purpose	Quantity
Durable items				
CDW-G; 4452963	N	Mobile data entry tablet or desktop app	Domain data entry	1
BioQuip Products Inc. Fisher Scientific Company/NC0095946	N	Plastic larval tray	Measuring dry weight of large specimens	1
	N	Large tray or Tupperware container	Floating plant material to separate taxa and macroalgae	1-3
Fisher Scientific Company/LLC/8732115	N	Weigh boats (plastic, large)	Measuring dry weight of small specimens	20
Fisher Scientific Company, LLC/8732100 Thomas Scientific, Inc./1209Q58	N	Weigh boats (aluminum)	AFDM sample processing	20
	N	Sieve, 1 mm mesh	Rinsing sample to remove sediment and debris	1
	N	Soft brush	Cleaning aluminum weigh boats	1
Fisher Scientific Company, LLC/1910200	N	Analytical balance (0.0001 g precision)	Measuring weight	1
Fisher Scientific Company, LLC/1055423	N	Muffle furnace	Burning organic material for ash-free dry mass calculation	1
Grainger, W.W./5ZPT8 Fisher Scientific Company, LLC/15207 Thomas Scientific, Inc/0241B17	N	Crucible tongs	Safe handling of equipment in the muffle furnace	1
Grainger, W.W./2EWX3	N	Thermal gloves	Safe handling of equipment in the muffle furnace	1 pair
Amazon Capital Services Inc./B000OSNQXQ	N	Aluminum baking pan	Sample organization in muffle furnace	2
Fisher Scientific Company, LLC/0864223B	N	Desiccator (bench top)	Storing dried samples	1
Consumable items				
Fisher Scientific Company, LLC/075783A	N	Desiccant packs	For bench top desiccator	2-Jan
	N	Lab datasheets	Recording data if tablet fails	1
Grainger, W.W./12R027	N	Paper lunch bags	Drying samples in the drying oven	10

Thomas Scientific, Inc./1234Z63	N	Laboratory tissues (box)	Blotting small specimens	1
	N	Paper towels	Blotting large specimens	1
Grainger, W.W./6CHG5	N	Aluminum foil	Separating aluminum weigh boats in muffle furnace	1 roll
Thomas Scientific, Inc./2701B04	N	Borosilicate glass vials, PTFE-lined cap, 4 mL	Sample container for CN subsamples	20

Table 16. Equipment list - Laboratory processing: Aquatic plant pressing and mounting (RD[18])

Supplier/Item no.	Exact Brand	Description	Purpose	Quantity
Durable items				
Forestry Suppliers, Inc./53674 BioQuip Products Inc./3115	N	Standard plant press	Pressing plants	1
Forestry Suppliers, Inc./53741 BioQuip Products Inc./3127	N	Cardboard ventilators	Pressing plants	24
Forestry Suppliers, Inc./53740 Herbarium Supply/223	N	Standard driers (sheets)	Pressing plants	24
Fisher Scientific Company/975350	N	Forceps (blunt point)	Handling specimens	1
B&H Photo Corp/PADMCTS30BL	N	Handheld digital camera, battery, and memory card	Photographing specimens	1
Consumable items				
Herbarium Supply/101		Herbarium mounting paper	Herbarium mounting	1 package
Herbarium Supply/120		Herbarium mounting glue, bottle	Herbarium mounting	1
		Newspaper	Pressing plants	12 sheets
Forestry Suppliers, Inc./010510-1; 49247		All-weather copier paper, 8 1/2” x 11”	Labelling plants in plant press	15 sheets
Herbarium Supply/115		Seed envelopes (3.23” x 4.50”)	Storing small plant parts	10
Herbarium Supply/353		Herbarium labels, paper	Labeling herbarium mounts	10

Table 17. Equipment list - Laboratory processing: Macroalgae preservation

Supplier/Item no.	Exact Brand	Description	Purpose	Quantity
Durable items				
			(none)	
Consumable items				
Sigma-Aldrich, Inc./G62571L	N	Preservative (glutaraldehyde)	Preserving macroalgae samples	1 L
Fisher Scientific Company, LLC/03-313-15B	N	60 mL widemouth HDPE polypropylene sample bottle with cap	Shipping macroalgae samples	10

Table 18. Equipment list - Shipping supplies

Supplier	Supplier ID	Description	Purpose	Quantity
Durable items				
		(none)		
Consumable items				
Grainger, W.W./5LH30	N	Zip-top bags, gallon	Store samples in bags in vermiculite	TBD
Global Equipment Company/B68603 Uline/S-4485	N	Cardboard box (~9”x7”x7”)	Shipping taxonomy samples	1
	N	Bubble wrap	Padding taxonomy and CN samples	TBD
	N	Shipping inventory/manifest	Provides sample information to external lab	1