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
G	01/17/2023	ECO-06921	• Update sampling impractical instructions • Add contingency for wide stream transects • Add identification recommendations for plants • Clarify lake/river random point generation • Add required plant colonization transect in lake/river • Restructure field collection SOPs • Add island option to point transects • Clarify plant voucher collection, new SOP • Add option for initial subsample in domain lab • Updated flow diagrams

H	01/06/2026	ECO-07166	• Use inventory app for samples in DSF > 2 days • Update definition of macroalgae • Change workflow for lakes and rivers randomized point selection • Shorten -80C freezer time for taxonomy specimens to 48 hrs • Clarified lab plant sorting • Updated muffle furnace pan safety information • Simplified endangered plants list from permitting • Updated site layout design (Figure 7)

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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. Most aquatic plants are 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 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.050005	Field Operations Job Instruction Training Plan
AD[05]	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: DMP – 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: ALG – Periphyton and Phytoplankton Sampling
RD[11]	NEON.DOC.014037	TOS Protocol and Procedure: HBP – Measurement of Herbaceous Biomass
RD[12]	NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[13]	NEON.DOC.003046	AOS Protocol and Procedure: INV – Aquatic Macroinvertebrate Sampling
RD[14]	NEON.DOC.003044	AOS Protocol and Procedure: AMC – Aquatic Microbial Sampling
RD[15]	NEON.DOC.001197	AOS Protocol and Procedure: BAT – Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[16]	NEON.DOC.003564	Standard Operating Procedure: HRB – Plant Pressing, Mounting, and Labeling (Herbarium Techniques)
RD[17]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[18]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation

RD[19]	NEON.DOC.005224	NEON Protocol and Procedure: SCS – Shipping Ecological Samples and Equipment
RD[20]	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
DSF	Domain Support Facility
DNR	Department of Natural Resources
FPOM	fine particulate organic matter
FWD	fine woody debris
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 usually with a 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

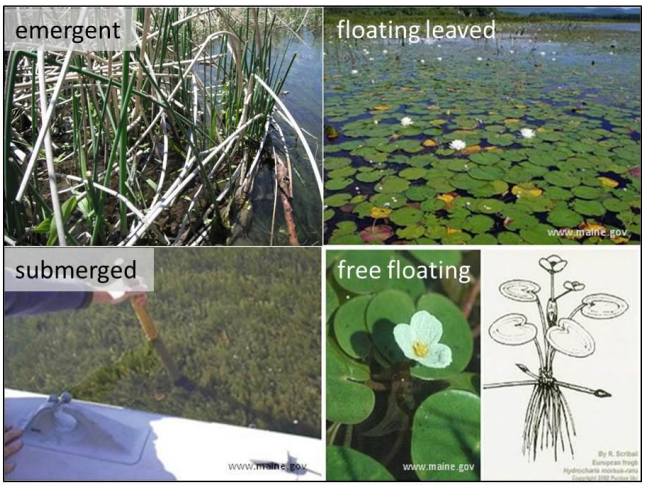

Figure 2. Aquatic plant growth forms: emergent, floating-leaved, submerged, and free-floating.

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).

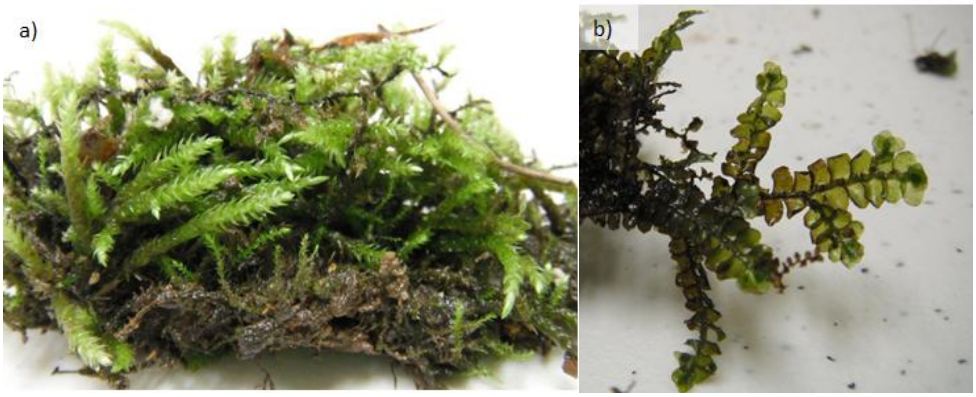

Figure 3. Bryophytes are a group of non-vascular plants including a) mosses and b) liverworts which may be submerged or growing in the "splash zone".

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 that can be seen without microscopy. Multicellular, photosynthetic algae visible to the naked eye. These algae are typically filamentous but may also appear as balls or "blobs" (**Figure 5**).

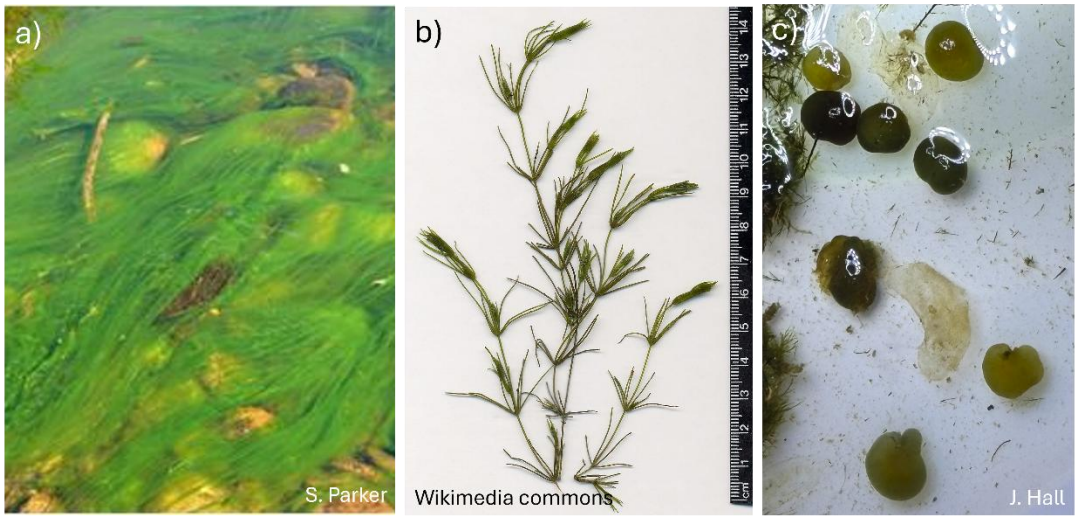

Figure 5. Macroalgae are large, visible algae that often form blooms in streams and lakes: a) filamentous macroalgae; b) *Chara* sp. (resembles a vascular plant); c) *Nostoc* sp. (resembles green balls)

Pool: An aquatic habitat unit in a river or stream created by local scour or impoundment and having a structural control. 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 (upstream boundary), are characterized as areas of the sub-reach that exhibit relatively slow velocity, and are sources of fine sediment deposition.

Riffle: A shallow part of the stream where water flows swiftly over completely or partially submerged obstructions to produce surface agitation.

Run: A relatively shallow part of a stream with moderate velocity and little or no surface turbulence. Runs typically bridge pool and riffle habitat units.

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

Step pool: Areas within high-gradient streams where water cascades over a series of rock ledges or woody snags, dropping into small pools that form between the features. (**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[15]); 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 (DSF) for processing and shipping to appropriate taxonomists (RD[19]). Macroalgae specimens will always be collected and sent to expert taxonomists, and not identified in the field, with the exception of *Didymosphenia geminata* or *Chara* sp. 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.

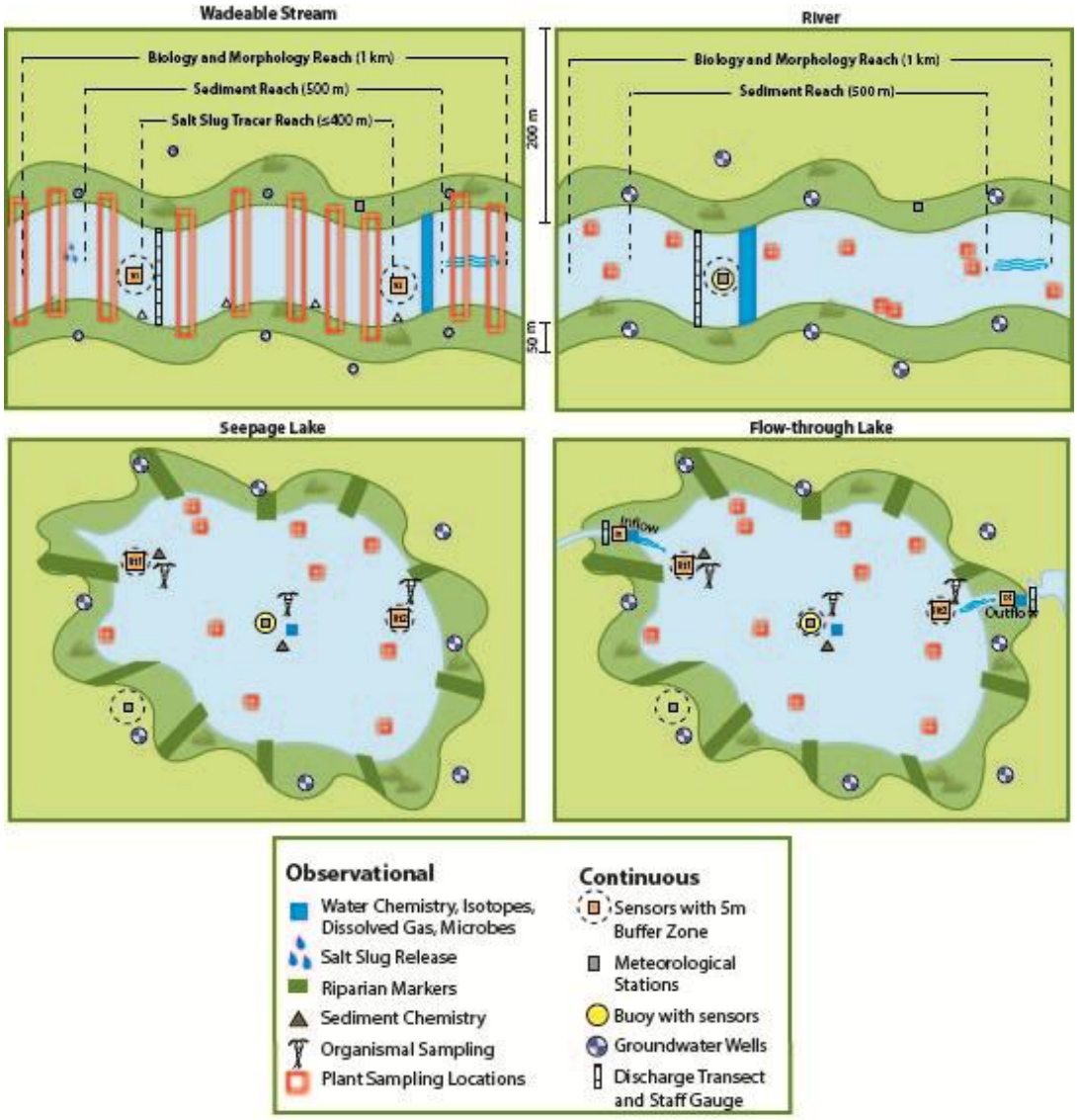

Figure 7. Generic river, lake, and wadeable stream site layouts with aquatic plant, bryophyte, lichen and macroalgae sampling locations. Red lines represent wadeable stream transects, while red boxes are quadrats or rake grab samples.

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[15]). 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[05]).

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 (Parker and Utz 2022, Appendix E). Sampling must be scheduled to occur within the first 21 days of the 1 month window specified in Appendix E, this helps to allow for rescheduling and weather contingencies. Observe a minimum of two weeks between the sampling date and the start of the next scheduled bout window for Bouts 1 and 2. For Bout 3, allow for 30 days to reschedule missed sampling. 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. See the Aquatic Site Sampling Design (RD[20]) for your domain for additional details and scheduling preferences. 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 identification at the Domain Support Facility or to ship for expert identification.
- Clip harvest biomass sampling: Quadrat sampling occurs once per year during the summer sampling date (Bout 2) on the same day as 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 sampling: Rake sampling occurs once per year during the 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
- 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.2	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.3	Stream	10 quadrats*	1 day	1	Biomass sampling in wadeable streams (Clip Harvest). Bio Bout 2 only
SOP C.4	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 (summer), the following details 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

4.3.1 Clip Harvest Processing Timing (Bout 2)

All plant processing shall begin within on or within 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) and press or preserve samples for taxonomy
    a. Preserve macroalgae (**Table 2**) for taxonomic analysis
    b. Press vascular plants for taxonomic analysis and DSF herbarium
    c. Dry non-vascular plants in packets for taxonomic analysis and DSF herbarium
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[19].

4.3.2 Point Transect Processing Timing (Bout 1, 2, or 3)

1. Processing may begin on the same day as collection, but must occur within 48 hours of collection.
    a. Preserve macroalgae (**Table 2**) for taxonomic analysis
    b. Press vascular plants for taxonomic analysis and DSF herbarium
    c. Dry non-vascular plants in packets for taxonomic analysis and DSF herbarium

Table 2. Sample 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 or transects per site as detailed in this protocol) because of the fluctuating nature of aquatic habitats. Spreading sample collection over multiple days increases the likelihood of variability among samples. Sampling may be rescheduled due to weather or environmental conditions (e.g., high or low water) within the bout window (+ 3 days) provided in the Site Sampling Designs (RD[20], Appendix C). An incident ticket must be issued if sampling is rescheduled >3 days past the end of the bout window. A minimum of 2 weeks between sample collection and the start of the next bout window shall be observed, with the exception of specific domains with a limited growing season (e.g., D18).

Table 3. Contingent decisions.

Delay/ Situation	Action	Outcome for Data Products
Hours	Stream, lake, river: If weather conditions deteriorate and conditions become unsafe during sampling (e.g., approaching thunderstorm, rapid increase of stream/river water, 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.
5 Days	Stream: If flooding occurs on or prior to the targeted sampling date (>3x median discharge; 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, 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.
	River: If flooding occurs on or prior to the targeted sampling date (>3x median discharge; Clausen and Biggs 1997) or unsafe boating conditions occur), wait a minimum of 5 days after water level drops below 3x median discharge, 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.
Delay sampling to occur after fish sampling	At wadeable streams, if both plants and fish sampling are delayed near the end or after the end of the biological sampling window, and fish schedule changes are inflexible due to the labor and time requirements of the protocol, staff can sample plants after fish. To sample plants after fish, wait a minimum of 5 d after fish sampling ends for recolonization.	A Schedule Change Request must be submitted and approved by Science if plants are to be sampled after fish. Flag data with biophysicalCriteria = 'conditions not met: sampled after fish'.
6 Months	Preserved macroalgae samples may be held for up to 1 year at 4 °C in the domain lab if circumstances do not allow shipping to the external lab.	Holding samples past the scheduled shipping date affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected.

Delay/ Situation	Action	Outcome for Data Products
	Dried/ground plant CN samples may be held indefinitely, dry, at room temperature in the domain lab if circumstances do not allow shipping to the external lab.	Holding samples past the scheduled shipping date 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 1 year, dry, at room temperature in the domain lab.	Holding samples past the scheduled shipping date 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[17]).

Missed or Incomplete Sampling Terms

- **Canceled Sampling**: Incidence of *scheduled sampling* that did not, and will not, not occur. Canceled 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 Canceled Sampling event – i.e., why sampling did not occur. This field is also used to indicate any individually missed samples within an otherwise successful sampling bout, e.g., if a stream is drying and contingent decisions are used.
- **Biophysical Criteria**: This field name associated with a controlled list of values that is included in the data product to explain changes to the schedule.

- **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**).

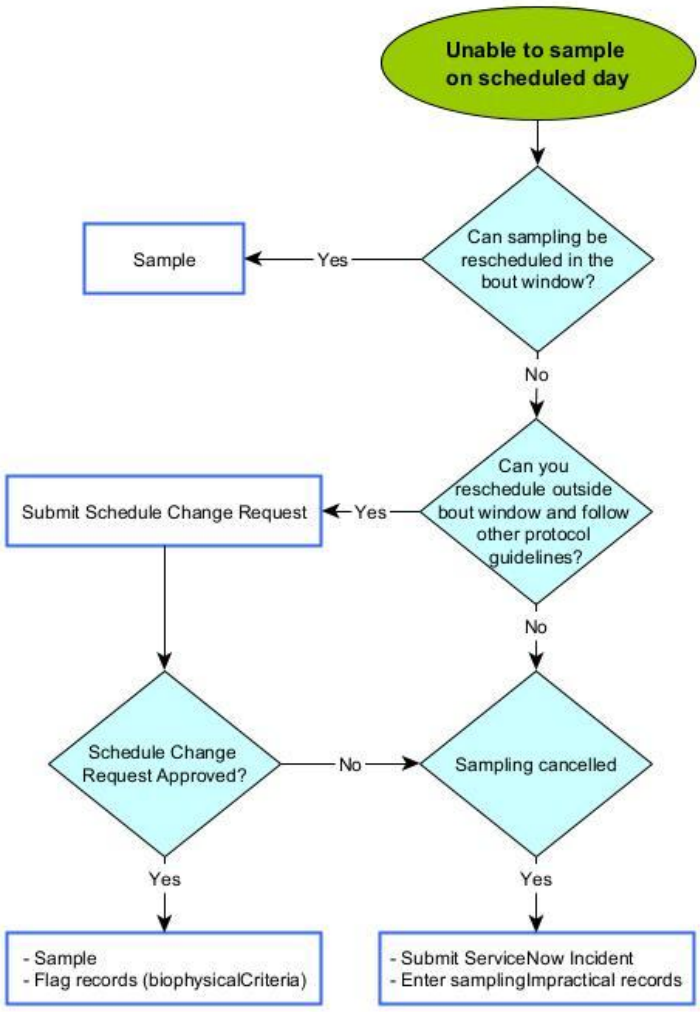

Figure 8. The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) 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. Canceled or rescheduled sampling must be communicated to Science by a ServiceNow Incident or Schedule Change Request.

a. The lead Field Ecologist should consult the Delayed or Cancelled Activities table to best determine when reporting is required (**Figure 8**).

2. **Canceled sampling:** For each missed sample, a **Sampling Impractical** field must be populated in the mobile collection device.
   a. Create one parent record and 10 child records for 10 missed transects, points, or quadrats.
   b. Create a child record for each missed transect, even if the rest of the sampling bout was successful (e.g., one transect was dry, create a sampling impractical record for the transect).
   c. 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.
3. For **Rescheduled** sampling events that occur within the biology bout window, sample and record data as normal. Rescheduling within the bout window does not need to be reported to science unless sampling will occur after fish sampling or another disturbance event.
4. **Rescheduled** sampling events that occur outside of the defined AOS biology sampling bout window must be approved by Science in a Schedule Change Request.
5. Sampling events that are **Rescheduled** ±3 days outside the sampling date require an entry in Biophysical Criteria (**Figure 8**).
   a. biophysicalCriteria – An indicator of whether sampling coincided with the intended biophysical conditions (i.e., within the AOS biology sampling bout window)

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
Lake/River: Randomized Point Sampling Stream: Point Transects or Biomass Quadrats	> 3 days outside bio bout window or rescheduling after fish (streams only)	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.

Field name	Dropdown list options	Description
Sampling Impractical	High water velocity	Water velocity too high to survey stream transect
Sampling Impractical	Location dry	Entire stream transect is dry
Sampling Impractical	Location frozen	Water at stream transect location is frozen
Sampling Impractical	Location snow covered	Stream transect location covered in snow

Field name	Dropdown list options	Description
Sampling Impractical	Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)
Sampling Impractical	Other	Reason not outlined above, make a remark in the record to describe the issue
Biophysical Criteria	OK - no known exceptions	Sampling occurred on schedule, no known issues
Biophysical Criteria	OK - schedule change but conditions met	Sampling occurred not within defined sampling window but reflects the target biophysical conditions
Biophysical Criteria	Conditions not met – outside of sampling window	Sampling was conducted outside of the AOS sampling window
Biophysical Criteria	Unknown - logistical	Sampling not possible due to logistical considerations
Biophysical Criteria	Conditions not met: sampled after fish	Sampling does not reflect the target biophysical conditions, benthic sampling occurred after benthos was disturbed during seasonal fish sampling
Biophysical Criteria	Other	Other potential sample timing inconsistencies described in the remarks

A common occurrence in stream aquatic plant sampling is loss of habitat due to channel drying or permitting restrictions. Contingent decisions in **Table 6** 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 6. 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[13], RD[14]).
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.
Wide **wadeable stream** that cannot be completely sampled in 1 work day	For sites that are wider than average and routinely have 40 points per transect following this protocol, distance between points may be reduced to 1 m if sampling cannot be completed for the entire site in 1 day. Change must be approved by Science. This contingency is currently allowed at D11 BLUE during any bout.	Lower resolution for diversity and density metrics, however, statistical analyses of the plant and macroalgae community were not significantly different as long as 200 point target is maintained.	Change to point distance must be recorded in the Point Count app

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 7. 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 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 8 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 Field Operations Job Instruction Training Plan (AD[04]). Additional protocol-specific required skills and safety training are described here.

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

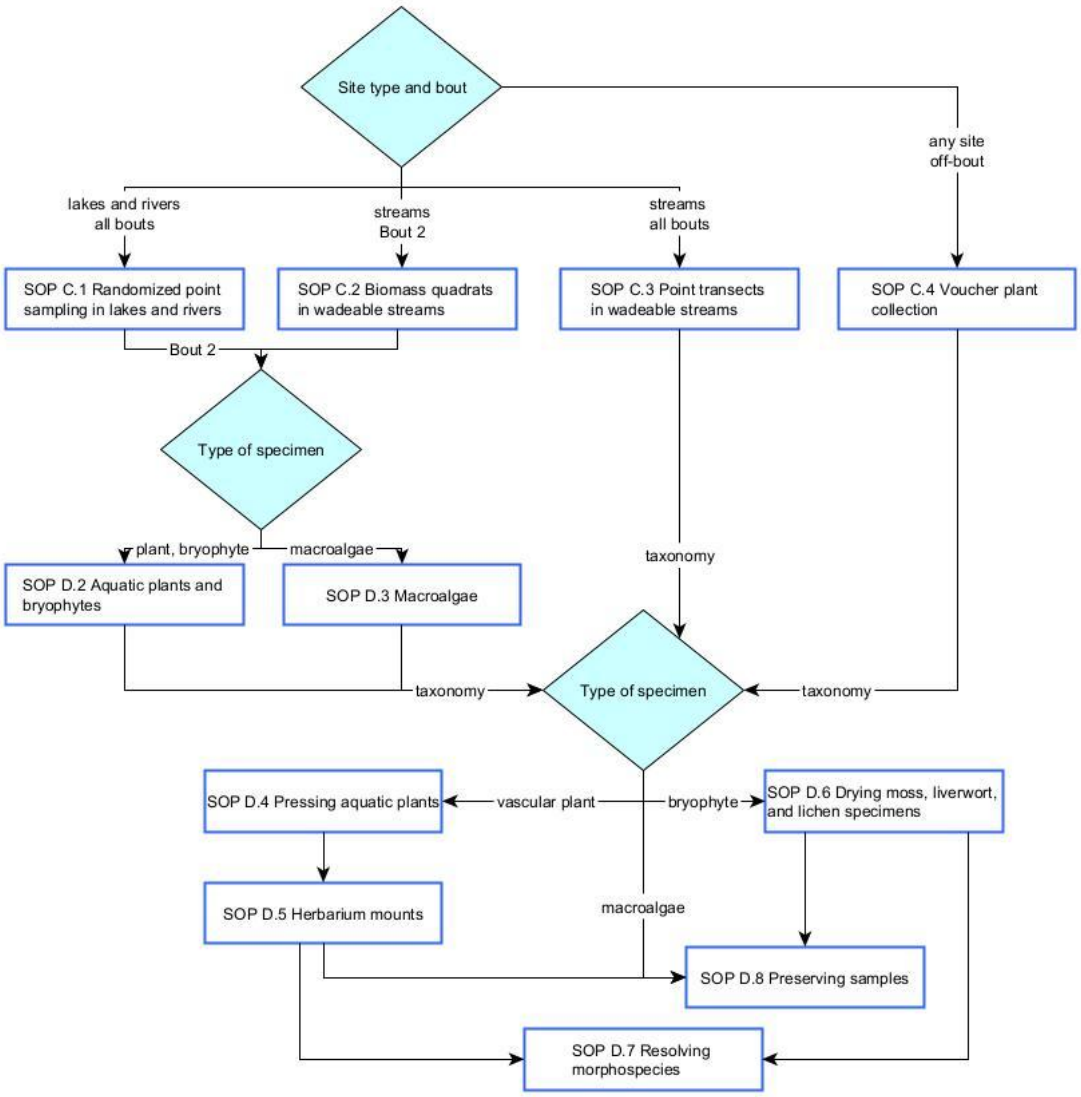

Figure 9. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.

- SOP A: Preparing for Sampling
- SOP B: Determining Sampling Locations
- SOP C: Field Sampling
- SOP D: Laboratory Sampling and Analysis
- SOP E: Post-Field Sampling Tasks
- SOP F: Data Entry and Verification
- SOP G: 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 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.

NEON Sample ID: PRLA.20200723.P1 Collected by: S. Parker	**NEON** Sample ID: PRLA.20200723.P2 Collected by: S. Parker
NEON Sample ID: HOPB.20200723.Q1=9 Collected by: D. Monahan	**NEON** Sample ID: HOPB.20200723.Q10 Collected by: D. Monahan

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

2. Adhesive barcode labels are added to sample containers that are sent to an external facility and scanned by the mobile app. Barcode labels are not used on AOS plant and macroalgae field samples, only on samples and specimens that are sent to an external facility.

   a. In the field, keep a physical human-readable label (**Figure 10**) on each bottle or in each bag with a minimum of the sample ID printed to assist with organization and shipping.

   b. For pressed and dried non-vascular plants, use a Type II barcode because samples spend some time at -80 °C. Do not adhere the barcode to the newspaper or packet, rather keep it on the label backing paper and paper clip it to the herbarium label (**Figure 11**).

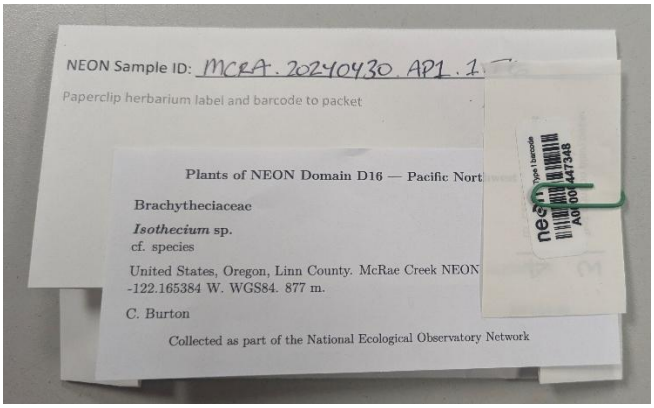

Figure 11. Bryophyte packet with paperclipped labels.

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.

Figure 12. An example of a Type II barcode. These large-size, freeze-tolerant barcodes have a prefix of 'B' followed by 11 numbers.

Table 8. Sample ID and 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
Clip harvest	Field sample in lake or river	CRAM.20161027.rake.1	(AOS) Plant Biomass - Field	Bag or Whirl pak	None	No	0
Clip harvest	Field sample in lake or river	MAYF.20161027.quadrat.1	(AOS) Plant Biomass - Field	Bag or Whirl pak	None	No	0
Clip harvest	Field sample in lake or river	WLOU.20161027.mini.1	(AOS) Plant Biomass - Field	Bag or Whirl pak	None	No	0
Plant voucher	Voucher grab sample	TOOK.20220801.AP5	(AOS) Plant Voucher Collection	Bag or Whirl pak (field) | Newspaper or envelope (lab)	Type II	Required if shipping externally	1 per specimen on pressed or dried label
Point transect sample for external ID	Vascular or non-vascular plant	BLUE.20220719.AP3.1.T4	(AOS) Plant Point Counts	Bag or Whirl pak (field) | Newspaper or envelope (lab)	Type II	Required if shipping externally	1 per specimen on pressed or dried label
Point transect sample for domain herbarium	Pressed specimen or Dried specimen in envelope	BLUE.20220719.AP3.1.T4.H	(AOS) Plant Point Counts	Bag or Whirl pak (field) | Herbarium mount	Type II	Always Required	1 per specimen

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required?	Barcode Qty
Point transect sample	Macroalgae	WLOU.20220816.macroalgae6.T3	(AOS) Plant Point Counts	60 mL Nalgene	Type I	Always Required	1
Vascular or non-vascular plant taxonomy for external ID	Pressed specimen or Dried specimen in envelope	*Stream:* ARIK.20221027.AP1.Q2 *Lake/river:* LIRO.20220707.AP2.P1	(AOS) Plants - Lab	Pressed in newspaper	Type II	Always Required	1 per pressed specimen, paper clipped to herbarium label
Vascular or non-vascular plant taxonomy for domain herbarium	Pressed specimen or Dried specimen in envelope	*Stream:* ARIK.20221027.AP1.Q2.H *Lake/river:* LIRO.20220707.AP2.P1.H	(AOS) Plants – Lab	Herbarium mount	Type II	Always Required	1 per specimen
Macroalgae taxonomy	Preserved macroalgae	ARIK.20161027.MACROALGAE1.Q2	(AOS) Plants - Lab	60 mL bottle	Type I	Always Required	1 per preserved specimen
CN sample	Scintillation vials with plant CN material for lab analysis	*Stream:* ARIK.20161027.AP1.Q2.CN *Lake/river:* LIRO.20220707.AP2.P1.CN	(AOS) Plants - Lab	Scintillation vial	Type IV	Always Required	1 per vial

A.4 Plant Identification

Use the following steps to help identify vascular and non-vascular plants during stream transect sampling or DSF lab processing. This section only applies to macroalgae if your site has been approved to identify select macroalgae species.

1. *TAXON LIST:* Assemble a taxon list for your site using previously collected and identified NEON data. This will help narrow down the choices for identification at your site. Science recommends creating a photo guide using this taxon list to keep on hand in your shared DSF documents. Enter these taxa in the 'plantTaxa' mobile collection app for your domain, so the limited list can be accessed during plant identification in stream point transect sampling and DSF lab processing.
2. *DOMAIN HERBARIUM:* Adding specimens to and maintaining the DSF herbarium is important for plant identification. For this protocol, every specimen shipped to an expert taxonomist has a paired sample archived in the DSF herbarium. Access NEON plant data from the data portal and

match sample IDs with the paired specimens in the herbarium to positively identify what is in the herbarium. Refer to the existing identified herbarium specimens when doing plant identification for this protocol.

3. *MORPHOSPECIES:* Morphospecies is a temporary designation for an individual or group of plants that cannot be positively identified and look the same.
    a. Split groups that look similar but not identical into different morphospecies. It's easier to lump them together than to split them out later.
    b. Allow the Fulcrum apps to generate the morphospecies IDs, but describe each morphospecies in the morphospeciesID remarks with a professional description that helps you tell one morphospecies from another.
    c. Do not use scientificNames in the morphospecies remarks.
    d. Morphospecies IDs for aquatic plants are only good at a site for 1 year, with the assumption that the taxon will be identified by the expert taxonomist before the next sampling year. The Fulcrum app will prompt the user for a new morphospecies ID in the new year.
    e. It is expected the field ecologists will eventually resolve plant and bryophyte morphospecies using the (AOS) Plant and Macroalgae Morphospecies List app and taxonomic identification received from the expert taxonomists.
4. *IDENTIFICATION QUALIFIERS:* Identification qualifiers contain information about the certainty of identification at a specific taxonomic rank. If you are not certain of the species, identify the specimen to a taxon rank that you think is reasonable (can still be species with uncertainty) and use an identification qualifier to indicate your uncertainty.
    a. "cf." + taxon rank – roughly equal to but not sure about the identification at the selected taxon rank
    b. "aff." + taxon rank – similar to another taxon but you know it's definitely not that taxon (e.g., "it looks like a daisy but I know it's an aquatic plant")
5. *FIELD AND DOMAIN LAB IDENTIFICATION:*
    a. You can identify the plant to species with >75% certainty: Select the taxonID in the field or domain lab app. You do not need to send this to the external taxonomist.
    b. You can identify the plant with <75% certainty or to a taxon rank higher than species: Collect a specimen and assign a taxonID with identification qualifier and/or morphospecies using the data collection apps. If you encounter the same plant again, you can refer to the original specimen and morphospecies identifier. Send a specimen to the expert taxonomist and keep a paired specimen in the DSF herbarium.
    c. You can identify to a higher taxon rank but diagnostic parts are not available: Often when flowers or fruit are not present, plants can be difficult to identify to a low taxon rank (e.g.,

Poaceae spp.- grasses). Consider the specimen at hand and the diagnostic parts available prior to sending to an expert taxonomist. If you are able to identify the specimen to family or genus and there are no diagnostic parts, it is unlikely that an expert taxonomist can do any better than that.

d. You know you have collected this before, but you don't know what taxon it is: Find a previously created morphospecies ID that describes this taxon to apply to this specimen.

   i. Also look in the domain herbarium for a similar specimen that you can use to identify.

   ii. If you are able to identify the specimen later, edit the mobile record or request a data edit from Science ("Magpie" request).

   iii. If you are still not sure of a taxonomic identification after checking your resources, send a specimen to the expert taxonomist and keep a paired specimen in the DSF herbarium.

e. There are two or three consistently indistinguishable species: Create "slash" taxa (e.g., TRSA5/TRAE2 terrestrial species) to indicate that you can't tell them apart. Cryptic species issues arise when two species are morphologically indistinguishable from each other and co-occur in the field. They do not need to be in the same genus. If not available in the taxon list, create a request to Science to add slash taxa to the taxon table. Send a specimen to the expert taxonomist and keep a paired specimen in the DSF herbarium.

f. The specimen looks sort of like something you've seen before, but you're not sure: Collect a specimen and assign a new morphospecies ID. You can identify to a higher taxonomic level, such as family, and use an identification qualifier to indicate your level of certainty. Send a specimen to the expert taxonomist and keep a paired specimen in the DSF herbarium.

g. You have no idea what taxon this is and you have never seen it before: Collect a specimen and create a new morphospecies ID, send a specimen to the expert taxonomist and keep a paired specimen in the DSF herbarium.

6. *GENERAL TIPS:*

   a. Start with family and narrow down from there.

   b. Use the identification qualifiers, especially affinity (aff.) to indicate that it looks like something else.

   c. Use identification apps such as iNaturalist to narrow down species in your area.

   d. Ask terrestrial botanists at the DSF or local experts for help.

   e. Check online plant image collections, such as the NEON Biorepository Portal or the SERENEC herbarium consortium.

SOP B Determining Sampling Locations

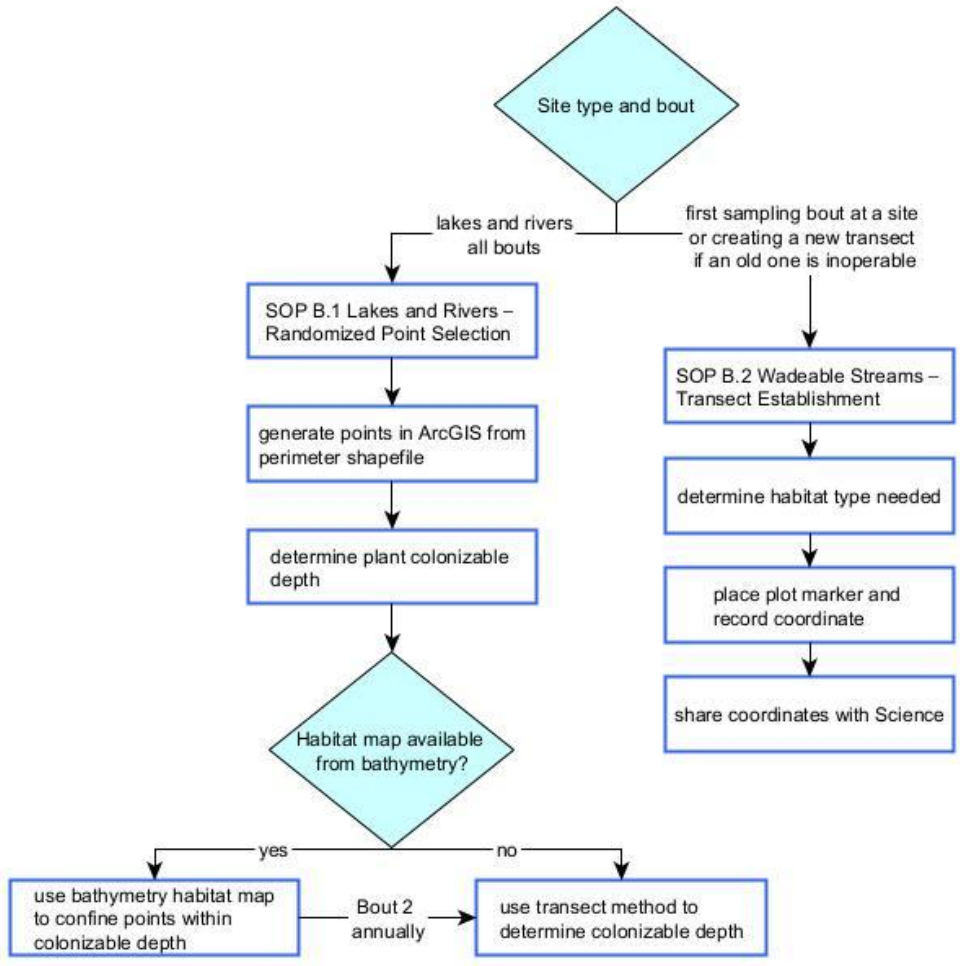

Figure 13. Expanded diagram for the workflow of SOP B.

B.1 Lakes and Rivers – Randomized Points

1. Use the (AOS) Plants Random Point List app to access the current year's randomized point list. target point list. This list is generated annually using site bathymetry data and only includes points that fall within the most recently recorded Plant Colonizable Depth.
2. Confirm the following:
   a. Coordinate Generation Date is within the past year.
   b. Bathymetry Survey Year is the year the most recent survey was conducted.
      i. If conditions (e.g., water level, hurricane impacts) have changed significantly since the last bathymetry survey and the site boundary is not representative, see the bathymetry protocol (RD[15]) for guidance on scheduling an additional survey. Contact Science/GIS support if unsure.

c. Plant Colonizable Depth (m) is accurate for the most recent Bout 2 measurement.

d. Contact SCI if any of these do not line up with expected inputs.

3. A reference map file is generated with labeled target points each year and can be located at the top of the app. To create your own field reference map, download the Fulcrum data and Screen Capture the Map view with the randomized points. These Fulcrum data can also be exported into a handheld GPS if desired.

   a. Fulcrum does not have the same spatial capabilities as a standard GIS so points cannot be labeled in the Map View. This can be done manually though by hovering over specific points in the Web version of Fulcrum and recording them on the field reference map once printed.

   b. A reference map can be useful to chart a sampling path, especially on larger lakes such as D18 TOOK. The first 10 points of the list should be used across the bout but do not need to be sampled in 1-10 order.

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.

5. Record both the coordinate sampled and the coordinate uncertainty (i.e., the larger of 1. the uncertainty reported on the GPS/tablet or 2. how far you are away from your intended point) in the field application.

   a. The tablet functionality to record coordinates and uncertainty is preferred to the handheld GPS.

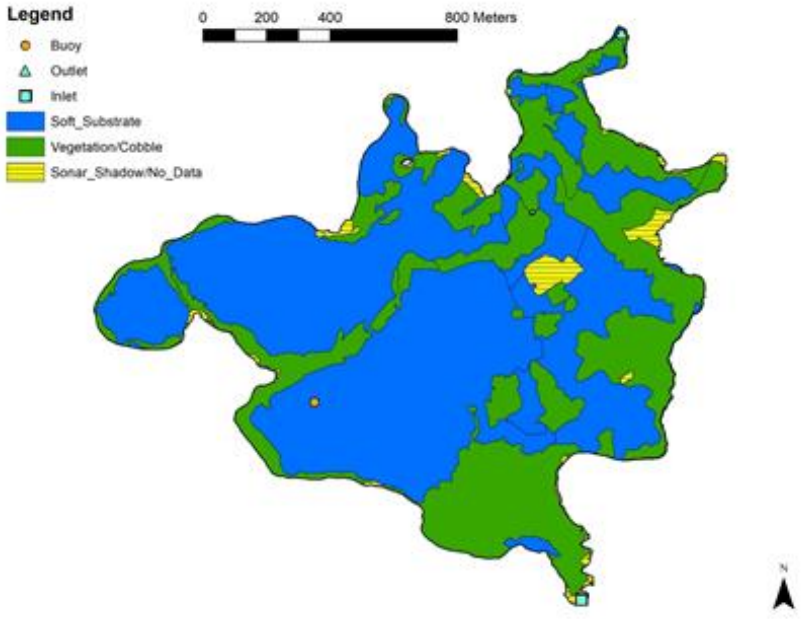

Figure 14. Bathymetric map of Toolik Lake (D18) showing habitat patches of vegetation, cobble, and soft substrates. (Figure courtesy of Spencer Phillips).

B.2 Wadeable Streams – Establishing Transects

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[13], and RD[14].
    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

3. Place 5 transects in each habitat type. If only one habitat is present that accounts for >20% of the reach, place all 10 transects within that habitat type.

   a. Alternate sampling transects between different types of habitat along the reach if possible (**Figure 15**).

   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.

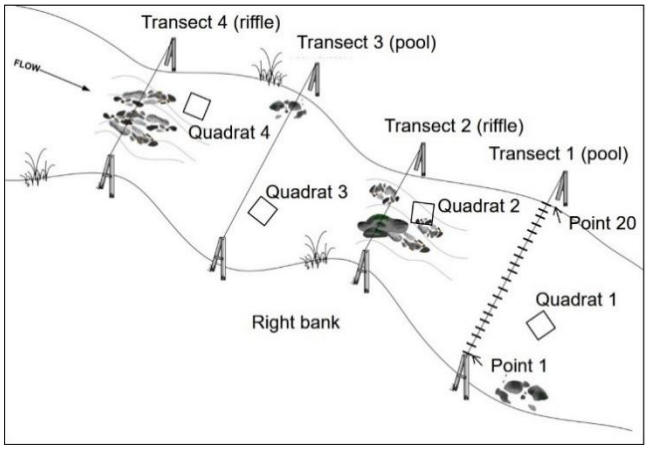

Figure 15. Layout of riffle and pool transects and quadrats within the stream reach.

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

5. Transects should be spread throughout the 1000m sampling reach as evenly as possible.

6. 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 (> 1 m of terrestrial vegetation) or braids in the stream channel.

7. 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

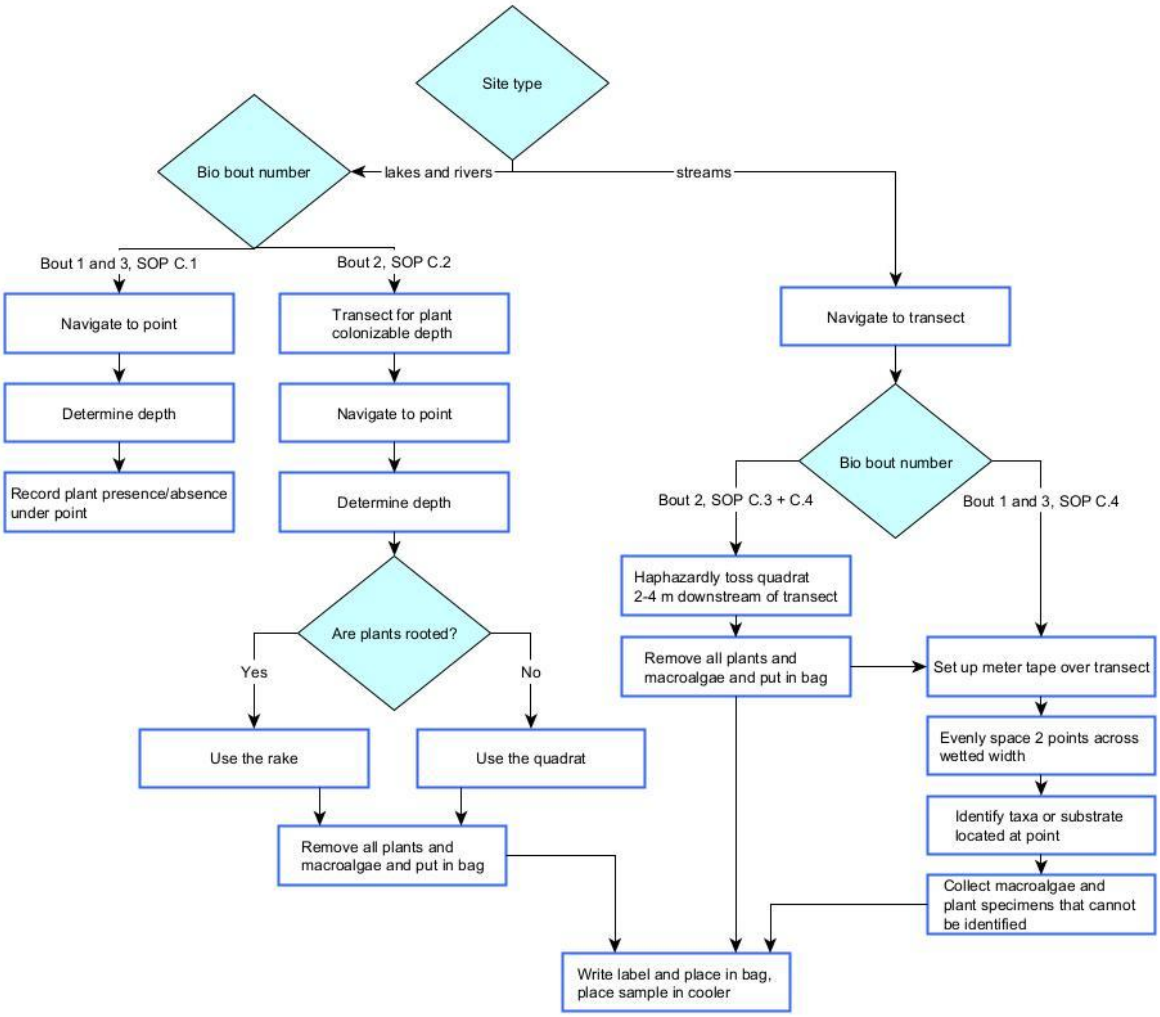

Figure 16. Expanded diagram of the workflow for field sampling SOP C.

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

C.1 <u>Lakes and Rivers</u> – Bout 1 and 3 Randomized Point Presence Absence

1. Randomized points are used during every sampling bout in lakes and rivers.
2. Navigate to the first 10 points on the randomized point list generated in SOP B.1. Skip points that are out of water. In lakes, skip points that are too deep for plant colonization. See below for river guidance.

a. Rivers with frequently fluctuating water levels may make randomized points difficult to predict based on a previous bout's water depth. If the water is too deep, navigate to the intended point and head toward shore until you can see bottom.

3. At the first point, 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. Record the coordinates of the sampling location and the coordinate uncertainty in the mobile app. Maximum coordinate uncertainty is 500 m.

5. Determine depth at the sampling point using the depth-finder and record in the mobile app.

6. Determine whether there are plants growing below the sampling point and record in the mobile app. If conditions are calm and the water is clear to the bottom, you may do a visual assessment. If you cannot see to the bottom, use a view bucket or rake to check for plant colonization.

7. Continue to the remaining points to collect presence/absence data. There is no sample collection during Bouts 1 and 3.

C.2 Lakes and Rivers – Bout 2 Randomized Point Clip Harvest

1. Randomized points are used during every sampling bout in lakes and rivers.

2. Prior to Bout 2 sampling each year, perform plant/macroalgae colonization depth transects. These data are used both for this protocol and for bathymetric map processing. Colonization transects do not need to be in the same place from year to year.

   a. In the field, choose a transect location in an area where you are reasonably sure that plants or macroalgae are growing. Avoid placing the transect at the boat ramp.

   b. Run the transect perpendicular from a point chosen on the shoreline toward the buoy or deepest location.

   c. Check for plants and macroalgae presence on at least 6 points along the transect. If the water is calm and clear to the bottom, you may do a visual assessment. If not, use a view bucket or rake to determine plant and macroalgae presence.

      i. If the lake is ≤4.0 m deep, assess colonization at following points: 0.5 m depth (±0.2 m), 1.0 m, 1.5 m, 2.0 m, 2.5 m, and 3.0 m until you reach one of the STOP SAMPLING CRITERIA below.

ii. If the lake is >4.0 m deep, assess colonization at the following points: 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 below.

d. STOP SAMPLING CRITERIA:

i. STOP if you encounter two points in a row with 0 plants or macroalgae

ii. STOP if you reach the buoy or deepest location

e. The last point with plant or macroalgal material on the rake is the estimated maximum sampling depth of plant or macroalgae colonization, record in the mobile app. Be sure that this depth makes sense given your knowledge of 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 (i.e., plants grow at all depths in the lake).

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

3. Collect clip harvest samples at 10 randomized points. Navigate to the next 10 randomized sampling points (from SOP B.1) 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 intended sampling point.

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.

4. 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. Record the coordinates of the sampling location and the coordinate uncertainty in the mobile app.

b. 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.

5. Determine depth at the sampling point using the depth-finder and record in the mobile app.

a. If the depth is <1 m, use the rake with a handle (**Figure 17a**).

b. If the depth is >1 m, use the rake with a rope and weight attached (**Figure 17b**).

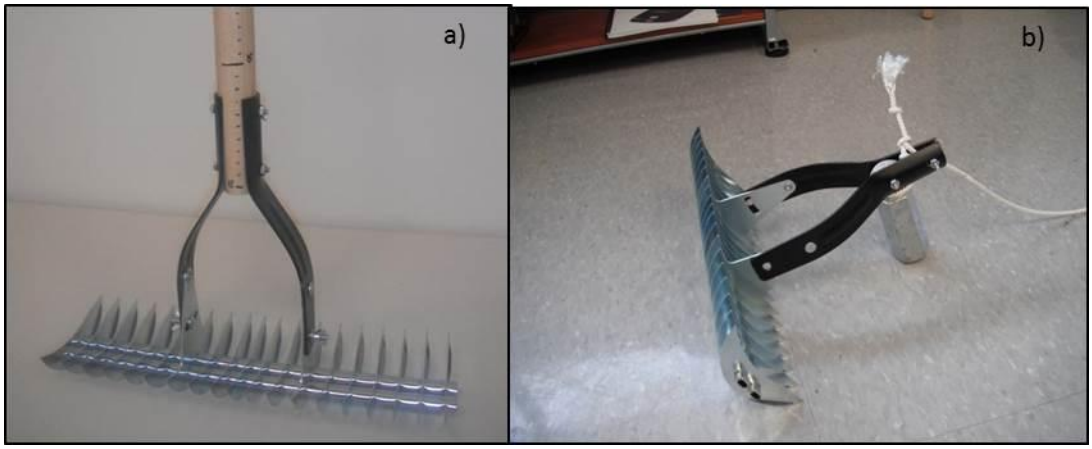

Figure 17. Double-sided sampling rake: a) Rake with handle b) Rake with rope and weight

6. If plants are rooted, use the rake. If plants are floating (i.e., D03 SUGG), put out the 0.5 x 0.5 m quadrat and collect all floating plant material within the quadrat, then proceed to Step 12c.

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.3). Sink the quadrat to the substrate, and clip plants at the sediment-water interface for above-ground biomass.

   a. For floating plants/quadrats, include roots as aboveground biomass. 1 quadrat equals 1 sample.

   b. For rooted plants/rake samples, 3 rake tows will be composited into 1 sample.

7. Lower the rake head to the lake/river bottom by casting away from or dragging alongside the boat.

8. 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.

9. 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^{o} may cause plants to fall off.

10. Estimate the rake fullness rating based on **Table 9**.

   a. Record the average fullness rating for all three rakes in the sample. This is intended to be a qualitative measure of how full the rakes are in each sample. See Table 9 for descriptions.

   b. Record whether plants fall off the rake while pulling the sample up to the boat.

Table 9. Rake fullness rating, based on Hauxwell et al. 2010.

Rake fullness rating	Rake coverage	Description
1		No plants to few plants, plants do not cover the length of the rake
2		Plants cover the length of the rake head, but do not fully cover the tines
3		The rake is completely covered and the tines are not visible

11. Pull the rake and plants into the boat (**Figure 18**).

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

Figure 18. Double-sided rake head with plant sample being brought into the boat.

12. Remove all plants and algae from rake and place in labeled Whirl-paks® 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*) generated by the Fulcrum app.

d. Place contents of all 3 tows (either by taxa or lumped sample) in the same parent sample bag to form the composite sample. 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.

e. Record data in the mobile app. If no plants or macroalgae were present, record as target taxa present = "N".

i. Ensure that the human-readable parent sample ID matches the sample ID generated by the mobile app. See SOP C.5 for additional labelling and storage instructions. Barcodes are not required at this point.

f. Any senesced material collected can be separated from this year's growth in the lab and binned for biomass analysis, this includes both senesced plants and macroalgae. Correct identification of senesced material is difficult and unnecessary for this analysis.

13. Repeat steps above until all 10 points have been sampled.

14. Place all labeled and sealed sample bags and bottles in a dark cooler with frozen ice packs for transportation to the Domain Support Facility.

a. Keep samples in a cooler with ice or ice packs (~4 ^{o}C) until returning to the lab.

15. 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.3 Wadeable Streams Bout 2 – 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. During Bout 2, collect clip harvest samples prior to performing point transect data collection at each transect to minimize suspended sediments that may affect the clip harvest sample collection.
2. Start at the most-downstream transect and work upstream to minimize suspended sediments in the stream.
3. 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 location, 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 (> 1 m across) 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.
4. 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 lichens *in situ* based on field identification key if possible).
    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 location.
    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, balls, or a structure (**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]).

5. 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.

6. Place each species in a separate Whirl-pak® or gallon resealable bag. This makes sorting back in the lab easier. Place any separate taxa in a parent bag with a label containing the parent sampleID generated by 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.

   b. Record data in the mobile app.

      i. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.

7. Place all labeled and sealed sample bags and bottles 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.

8. Proceed to point transect data collection in SOP C.4.

C.4 Wadeable Streams All Bouts – Point-Transect Data Collection

1. Use the pre-recorded plot markers and coordinates to locate each transect.

2. If you are unable to find the plot marker, follow these troubleshooting steps. If you can find the plot marker, skip to Step 3.

   a. Navigate to the plot marker coordinates. Search for coordinates in the named location viewer (navigate in the named location viewer as follows: domain > site> stream > plant parent > transects) or ask Science to help provide coordinates.

   b. Check the GPS coordinate uncertainty. Is the uncertainty less than or equal to 1 m from the original plot marker location?

      i. Yes: Continue sampling, we would consider this to be within the margin of error.

ii. No: If coordinate uncertainty is >1 m, record coordinates and uncertainty, continue sampling, and submit an incident to the problem tracking system for Science to add a data quality flag.

c. Make a plan to locate the missing plot marker(s) (e.g., metal detector) or replace them. Submit problem tracking incidents as necessary.

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. If the transect is otherwise compromised so that you are temporarily unable to access the location (e.g., downed trees), select "other" in **Sampling Impractical** and enter a remark to description. If sampling is impractical for the whole transect, there is no other data collection required. Proceed to the next transect location.

4. 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.

5. Transects may change in appearance over time. If the transect now has an island larger than 1 m across in it, span the meter tape across the island from river right (right fork) to river left (left fork) with the island in the middle. Data points will only be collected in the wetted channels and not on the island itself. Delineate the start and end points of the island in the mobile collection app.

6. 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 (with the exception of D11 BLUE, which is 1 m, **Table 6**).

   d. Use the mobile app to enter right and left bank distance and calculate sampling intervals.

7. 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 19**). You may also hang a small weight from the meter tape (similar to a plumb bob) to help line up the point and reference point in the view bucket.

   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 8. This includes floating plants.

   iii. If multiple layers of vegetation lie underwater below the point, record all vegetation layers.

   iv. If overhanging riparian vegetation, rooted elsewhere, 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 rooted 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.

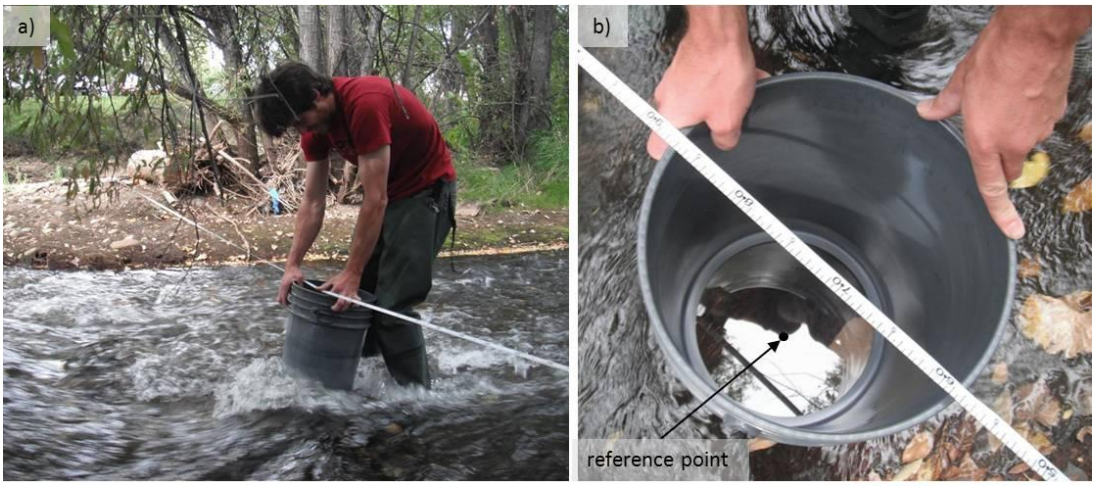

Figure 19. a) Hold the view bucket in the water, under the meter tape transect. b) Hold the view bucket so that the reference point lines up with the point you want to sample on the meter tape.

8. Record 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). See Section A.4 for mor information on identification.

    a. Target taxa:

        i. **Aquatic plant** – Use a field key to identify to species if possible, or collect a representative specimen for taxonomic identification slightly off the transect (**Figure 2**) and record morphospecies ID. This is not a "voucher" collection, this sample ID is be recorded in the Plant Point Count mobile app.

        ii. **Moss** – Use a 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 expert taxonomist after identification.

            1) 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, 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.

        v. **Macroalgae** – Collect samples of macroalgae for identification at the external lab, see Step 9 below (**Figure 5**).

    b. Substrate (record if target taxa are not present):

        i. **Coarse woody debris** – (CWD) large pieces of wood, sticks> 10 cm diameter

        ii. **Fine woody debris** – (FWD) smaller pieces of wood, branches, twigs, sticks, < 10 cm diameter

        iii. **Dry** – point is not underwater at the time of sampling, even if plants or bryophytes are present

        iv. **Fine particulate organic matter** – (FPOM) fine particles of organic matter

        v. **Leaf litter** – Terrestrial leaves from trees that have fallen into the water (do not collect)

vi. **Senesced vegetation** – Dead vegetation rooted in the stream from the previous season

vii. **Substratum size class** – Record only if there is no plant or other organic material above is present

1) **Clay** <0.002 mm

2) **Silt** < 0.063 mm

3) **Sand** = 0.063-2.0 mm

4) **Pebble** = 2.0-63 mm

5) **Cobble** = 63-200 mm

6) **Boulder** = 200-630 mm

7) **Bedrock**

8) **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)**

9. 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, balls, or a structure (**Figure 5**) that can be easily sampled by hand. Amorphous clouds of algae will be sampled in the periphyton protocol (RD[10]).

i. Minimum size is dictated by what you can grab, approximately a 1” by 1” colony.

b. If *Didymosphenia geminata*, *Chara* sp., or other algae that are 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. Field taxonomists must be verified by science or the lead field ecologist and tracked in the domain authorized macroalgae identification spreadsheet available in the sampling support library.

c. If macroalgae is abundant at the site and it appears to be similar at several points along one transect, do not collect a sample at each point. Collect a sample at one point along the transect and use the sample ID generated by the mobile app to link to later points. If algae appear to be of similar composition at later points in the same transect, use the mobile app to select the representative sampleID/morphospecies ID that you already collected, without collecting an additional sample for analysis at each point.

   i. Macroalgae morphospecies IDs and sample IDs are generated automatically by the Fulcrum app, use the identifier that is generated.

   ii. Macroalgae sample IDs can be linked to points within one transect but cannot be linked between transects. Collect a new representative macroalgae sample in the next transect.

   iii. Limit macroalgae collection to <20 samples per site/bout. Contact Science if you expect to have more than 20 samples.

d. Sample collection: Place macroalgae sample in 60 mL Nalgene bottle for transport back to the DSF with an adhesive label on the outside of the container. The sample ID on the label must match the ID generated in the mobile app. See SOP D.8 for preservation instructions.

10. If you are not able to determine the plant or bryophyte species from the groups listed above, collect a corresponding specimen off the transect and give the specimen and descriptive morphospecies ID (see SOP A.4 for identification instructions).

    a. Morphospecies IDs will be generated by the mobile app.

    b. Keep morphospecies remarks professional, they will be seen by data users.

    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, SOP C.5).

    d. Collect obligate aquatic species only. Specimens collected for taxonomic identification are collected in duplicate, with one specimen archived at the domain herbarium and one specimen archived by the external taxonomist.

    e. 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 enough within the reach that you are not depleting the population. If the species is rare (covering less than ~5% of stream bottom), photograph and note location.

1) **Aquatic plants:** collect at least 2 full plants (roots, stems, leaves, flowers) so one can be kept at the domain lab and one can be sent to the taxonomist
2) **Mosses** and **liverworts:** collect a sample that is approximately the size of your palm
3) **Lichens**: collect 2 specimens approximately 1”-2” in diameter

11. Place all labeled and sealed sample bags and bottles 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.5 Voucher Plant and Bryophyte Collection

Voucher collection is opportunistic and occurs during non-bout sampling at any site. For example, if you are at the site and see a flowering plant that that never flowers during sampling bouts, you may collect and record it in the (AOS) Plant Voucher app. Collect obligate aquatic species only. Be prepared on any field visit with zip-top bags and labels. Specimens collected for vouchers will be collected in duplicate, with one specimen archived at the domain herbarium and one specimen archived by the external taxonomist.

Macroalgae is not collected as a voucher. However, if you encounter macroalgae that seems concerning (e.g., algae you have never seen before growing in an unusual location), contact Science. Limited macroalgae voucher collection may occur with Science approval in an attempt to identify and prevent the spread of invasive species.

C.6 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.

4. 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.

    a. Field Science should become familiar with common invasives in their area by contacting state agencies and/or NEON permitting.

C.7 Field Sample Preservation

1. Keep all samples refrigerated (4 ^{o}C ±3 ^{o}C) and in the dark until processing at the Domain Support Facility. This includes plants and macroalgae. No preservative (i.e., glutaraldehyde) is used in the field.
2. Samples must be returned to the Domain Support Facility or remote lab facility and processed within 48 hours.

SOP D Laboratory Sampling and Analysis

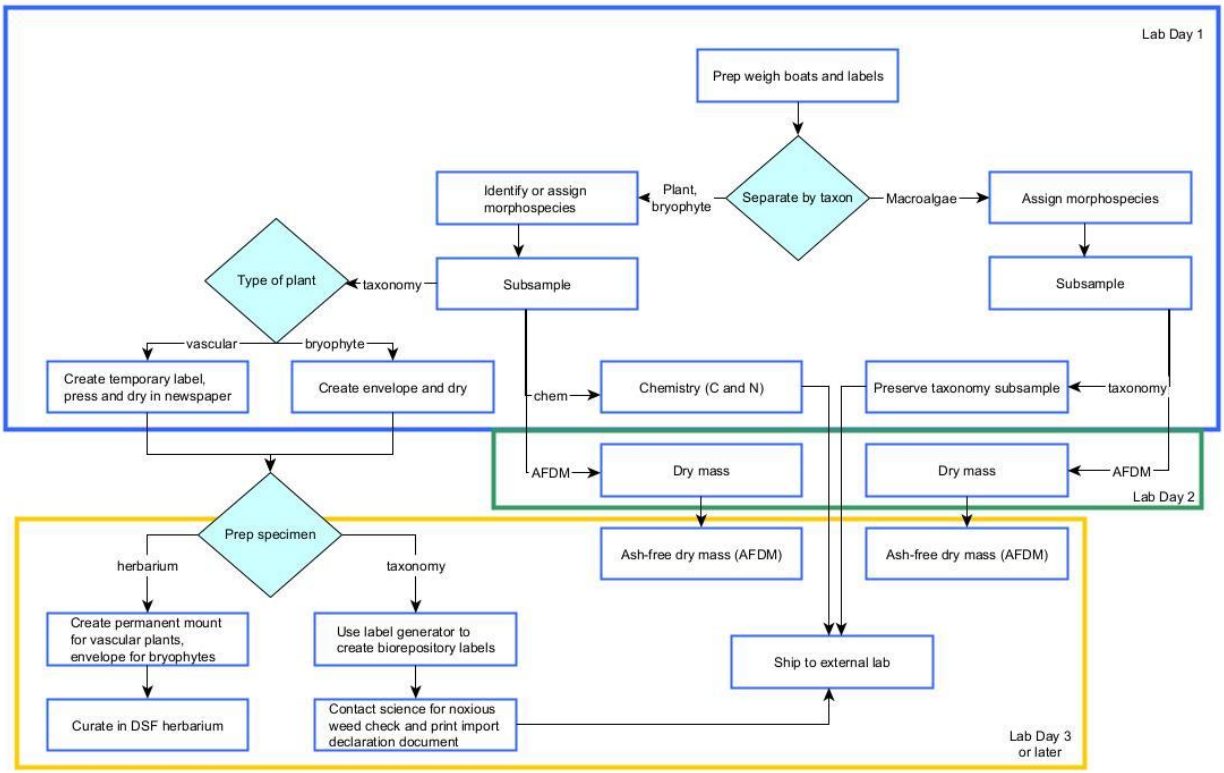

Figure 20. Domain lab workflow. (Blue = day 1, Green = day 2, Yellow = day 3)

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 21**).
   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.
   d. Place new empty labeled boats in the muffle furnace (500 °C) for 6 hours to burn off any residue.
   e. 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. When cool, weigh boats on analytical balance (0.0001 g precision) and record boat number and weight (g) in mobile app (RD[05]).

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

g. Boats may be reused from previous sampling bouts.

Figure 21. 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 8**. Also adhere barcode labels to any containers sent to an external facility (**Figure 12**).

4. If samples are small and enough material is not available for all analyses, process according to the following hierarchy:

   a. Two specimens for taxonomic identification and the DSF herbarium if identification is unknown

      i. Any plant material sent to the expert taxonomist must have a paired specimen stored in the DSF herbarium. If you only have enough for one taxonomy specimen, notify Science.

   b. AFDM (enough material to measure on the balance)

   c. CN (CN is never collected for macroalgae; minimum plant CN is 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**).

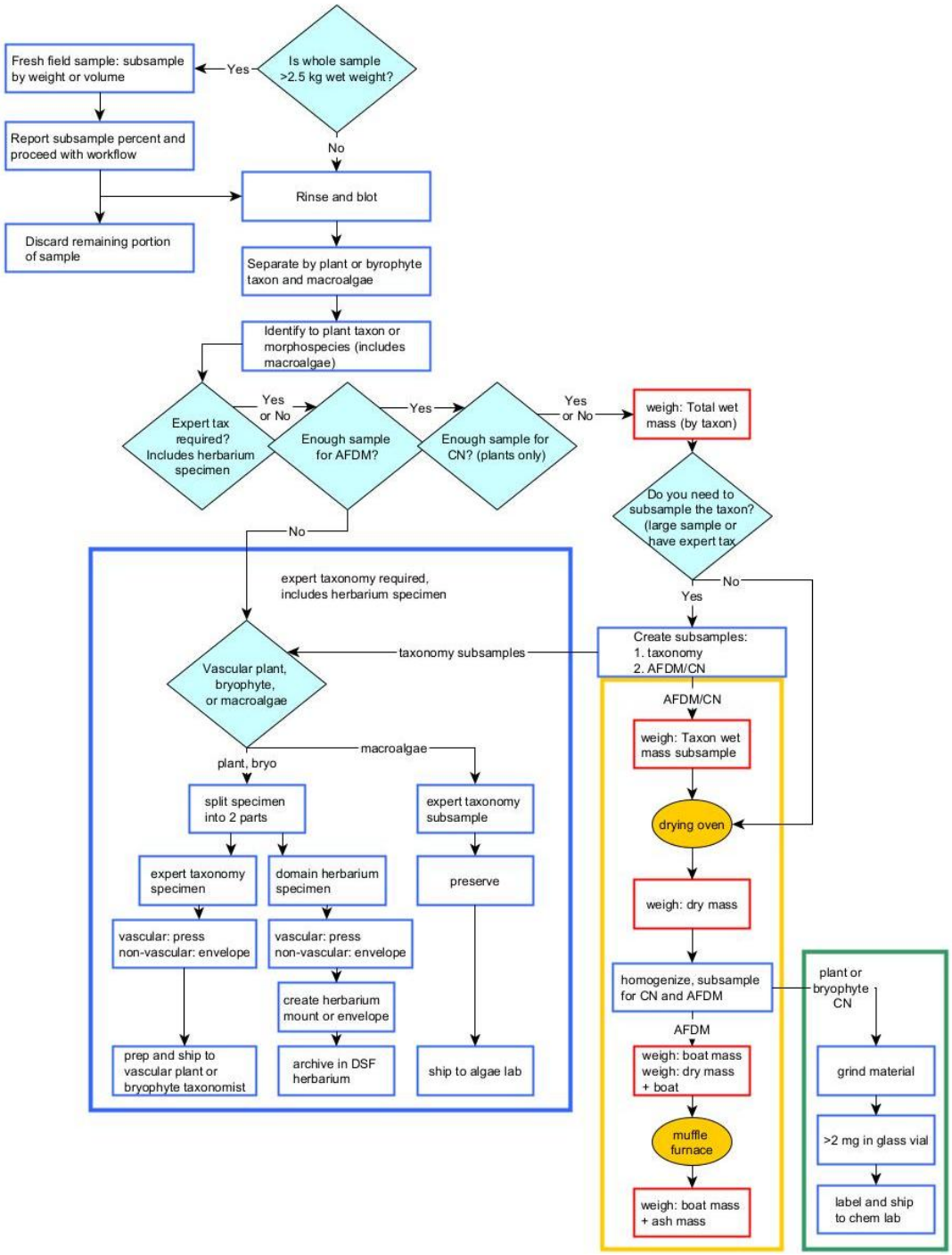

Figure 22. Follow the steps above for Domain Support Facility aquatic plant and macroalgae processing. Red boxes indicate a weighing step with data that need to be recorded in the app. (Blue = expert taxonomy + herbarium, Yellow = AFDM, Green = CN)

1. **Day 1**: Remove plant/bryophyte sample and field label from sample bag from quadrat/rake sampling. Set label aside.
2. Is this sample larger than 2.5 kg fresh mass? You may perform an initial subsample to speed up processing. If no, skip to Step 3.
    a. Homogenize the field sample
    b. Select a subsample that is ≥ ¼ of the original
    c. Determine the subsample percent, i.e., the percentage of sample you are keeping to process. You may do this by mass (weigh the initial fresh mass then the subsample fresh mass) or volumetrically (in a large tray divided into quarters)
    d. Report the subsample percent and proceed
    e. Limited domains have access to this step in the lab processing app. If your domain has samples that are this large, reach out to Science.
3. 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.
4. 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. Floating the sample in a tray of water may help facilitate separation of taxa.
    c. At some sites, it may be difficult to separate macroalgae from plant material or moss taxa from each other. After spreading the entire sample out on a tray, spend no more than 15 minutes sorting the sample.
        i. For macroalgae entwined with plants, or large clumps of moss, a series of floating the sample and dumping the macroalgae into another container may be helpful for some sites. Do not spend more than 15 minutes for any one sample.
        ii. If you are left with a clump of plants/macroalgae/moss that is obviously 2 or more different taxa, do your best to separate large clumps within 15 minutes. If the taxa are intertwined, subsample using the steps below and treat as 1 morphospecies, allowing the taxonomist to make a determination and rating of how dominant each taxon is in the sample (**Figure 23**).

Figure 23. Example of sorting moss fragments at TOOK.

5. Clean plant fragments out of the sieve and add to the respective specimen.

    a. Some taxa may break easily during rinsing (e.g., macroalgae like *Chara*, vascular plants like milfoils, or pieces of moss), leaving a pile of small unidentifiable plant parts in the sieve. If you have a sample like this, do not spend time trying to sort out the broken plant parts. Treat as a separate mixed sample, record as '2PLANT' in the lab collection app, and perform the dry mass and ash-free dry mass steps without taxonomy or CN analysis (**Figure 24**).

Figure 24. Example of leftover fragments from moss, macroalgae, and vascular plant sorting.

6. 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.
7. Weigh entire sample by taxon, prior to subsampling, on top-loading 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). You may use a plastic or aluminum weight boat, tray, or a tared bag for weighing.
8. 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, create a subsample that is approximately the size of your palm, and send a specimen to external taxonomist. Smaller specimens are difficult for the lab to identify.
    b. Retain a portion of the specimen for curation in the domain herbarium and proceed to step 9 for subsampling.
    c. For rake samples, replace the temporary rake ID with sample identifier generated by the mobile app.
9. 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 **Taxon Wet Mass Subsample Created** = “Y” and record the **Subsample wet mass** you will use for further mass measurements in the lab app. The remaining material not used in the subsample may be discarded.
    b. If subsamples are not necessary, record **Taxon Wet Mass Subsample Created** = “N”.
        i. Note that the subsample step is necessary if you are creating an expert taxonomy or herbarium 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.
10. Using a permanent marker, label a clean, paper lunch bag with sample ID and species ID from the field sample label.
11. Place the wet sample (or the 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.

12. Place paper bags containing samples in the (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.

13. **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.

14. Place a large, clean, plastic weigh boat (small specimen) or tray (large specimen) on top-loading balance. Tare (zero) balance. Place dry specimen in the plastic weigh boat/tray and record as **Dry Mass** in the lab app. This represents the dry mass of the specimen prior to subsampling, crushing, or grinding.

15. 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.

16. 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 small Type IV barcode label adhered to the vial and scan using the mobile app (**Figure 25**).

        i. Samples sent to external lab for CN must be ground on the Wiley Mill using 40 mesh (**Figure 26**).

        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.

        iii. See the Wiley Mill training in the training center for operation instructions.

    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.

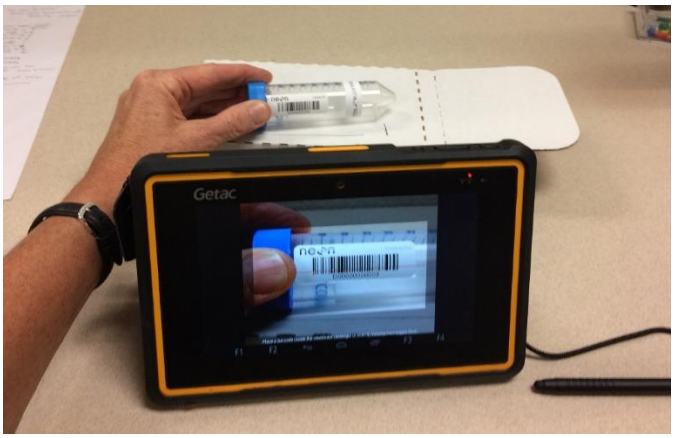

Figure 25. Barcode label scanning.

Figure 26. Photo of CN vial with human readable label and Type IV barcode.

17. Weigh boat on analytical balance, and record as **Boat + dry mass** in the lab app.

18. Repeat above steps until all specimens have been processed.

19. 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) or on top of and under sheets of aluminum foil before placing in the furnace. This may make it easier and safer to handle samples.

   i. Please note that aluminum pans have a relatively short life span (6 months-2 years) when used in a muffle furnace and should not be used after showing signs of warping, discoloration, corrosion, or apparent chemical reaction.

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

20. 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.

21. **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.

22. 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.

23. 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.

24. 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.

25. 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.

26. 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. Macroalgae are algae that can easily be consolidated and picked up by hand.
2. Gently rinse specimen to remove sediment or other non-algal material (e.g., leaves, twigs) over a 1 mm sieve (see **Figure 22**).

    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. If this is a common macroalgae, such as *Chara* sp., that has been previously identified by an expert taxonomist, you may identify in the domain support facility and enter an algal taxon ID in the mobile app. Contact Science to add new algal taxon IDs to the mobile app. Field taxonomists must be verified by science or the lead field ecologist and tracked in the domain authorized macroalgae identification spreadsheet available in the sampling support library.
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, add enough DI to cover specimen, 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 top-loading balance and record as **Total wet mass** in the lab app. After recording the mass, you may start to subsample.
7. Select "Taxon Wet Mass Subsample Created" = Y to indicate that you will have a subsample for taxonomy and a subsample for AFDM.
8. Taxonomy subsample: Remove approximately 10 mL of macroalgae sample to preserve for taxonomic identification. Using forceps, 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 27**, and also use a barcode label.
    a. If there is only ~10 mL of sample, do not process for AFDM, rather contribute all material to the taxonomy sample.
    b. You may also measure the volume of macroalgae and the volume of DI in a graduated cylinder to help with volume calculations for preservation.
    c. This is not a volumetric sample and does not need to add up to 60 mL.
    d. Add a Type I barcode label to the sample container and scan into mobile app.
9. AFDM subsample: Remove 10-50% of original sample and place in a clean, pre-labeled, pre-weighed aluminum weigh boat that you will use for the following analysis.
    a. Record **Boat ID** and **Boat Mass** in the lab app
    b. Weigh the aluminum boat containing the wet subsample. Record the **Subsample wet mass** (subtract the boat mass) in the lab app.
    c. The remaining material not used in the subsample or taxonomy sample may be discarded.
10. Repeat above steps until all samples have been processed.

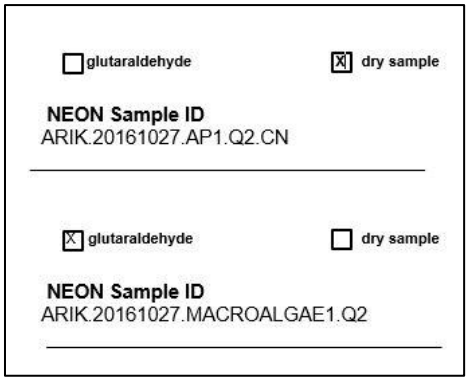

Figure 27. Example of adhesive labels for macroalgae taxonomy and plant CN subsamples.

11. Place aluminum boats containing subsamples in the 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, piece of paper, or loose aluminum foil 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 trays (e.g., cardboard) 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.

12. **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.

13. Weigh the boat + dry sample on tared analytical balance, and record as **Boat + dry mass** in the lab app.

a. The **Dry mass** field does not need to be filled in for macroalgae, it will automatically populate as the 'Boat + dry mass – Boat mass'.

14. Repeat above steps until all samples have been processed.

15. 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) or between sheets of aluminum foil before placing in the furnace. This may make it easier and safer to handle samples.

i. Please note that aluminum pans have a relatively short life span (6 months-2 years) when used in a muffle furnace and should not be used after showing signs of warping, discoloration, corrosion, or apparent chemical reaction.

16. 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.

17. **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.

18. 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.
19. 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.
20. After cooling to room temperature, weigh boats again on analytical balance, record as **Boat + ash mass** in the lab app.
21. After weighing, discard ash in trash can and clean the boat with a soft brush or paper towel.
22. Set clean boats aside to be used again.

D.4 Pressing and Drying Aquatic Vascular Plants

1. This section is used for both vascular plants destined for the expert taxonomist and plants that will stay in the DSF herbarium.
2. Plants at some aquatic sites may be covered with epiphytic algae. You may remove epiphytic algae from leaves gently by hand. Algae may be tightly adhered, and it is ok if they remain on the specimen leaves.
3. Follow instruction in SOP C.1 (pressing) and C.2 (drying) in the Plant, Pressing, and Labeling SOP shared with TOS (RD[16]).
4. Leave plants in press until they are shipped to taxonomist. Do not create permanent mounts for specimens sent to the expert taxonomist as the taxonomist needs to be able to move the specimen around to identify.
5. Proceed to Herbarium Mounts (SOP D.5) for specimens that are going to be kept in the domain herbarium.

D.5 Domain Herbarium Mounts for Vascular Plants

Herbarium mounts are created for specimens that are kept in the domain herbarium for reference. A paired sample should be kept at the DSF herbarium for every taxonomy specimen shipped to the expert taxonomist. Do not create herbarium mounts specimens that are shipped to the external taxonomist.

1. This section is only for aquatic vascular plants that will be archived in the Domain Herbarium.
2. Open plant press carefully.
3. Select a specimen to mount.
4. Follow steps in SOP C.6 and C.7 in the Plant Pressing and Mounting SOP (RD[16]).

D.6 Drying Moss, Liverwort, and Lichen Specimens in Envelopes

1. This section is for all moss, liverwort, and lichen specimens shipped to the expert taxonomist and for archiving in the Domain Herbarium.
2. Create Bryophyte/Lichen packets with all-weather copier paper. Use the packet folding instructions presented here for AOS, not the instructions in the Plant Pressing and Mounting Protocol Appendix C (RD[16]).
    a. Fold the bottom third of an 8.5” x 11” sheet of paper up, leaving 3-3.25” of paper revealed (**Figure 28** step 2).
    b. Fold 1” to 1.5” margins in from the edges (**Figure 28** 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 28** last step), or the specimen could fall out of the packet.
3. 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.
4. Gently rinse specimen in tap water to remove sediments. Take care to avoid breaking the specimen.
5. 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.
6. Split the specimen into two portions, one to keep at the Domain Herbarium, and one to send to the expert taxonomist.
7. Place each specimen inside a labeled packet. Scan the barcode label using the mobile app.
    a. **NOTE:** If specimen is a lichen, leave the lichen attached to rock substratum if present.

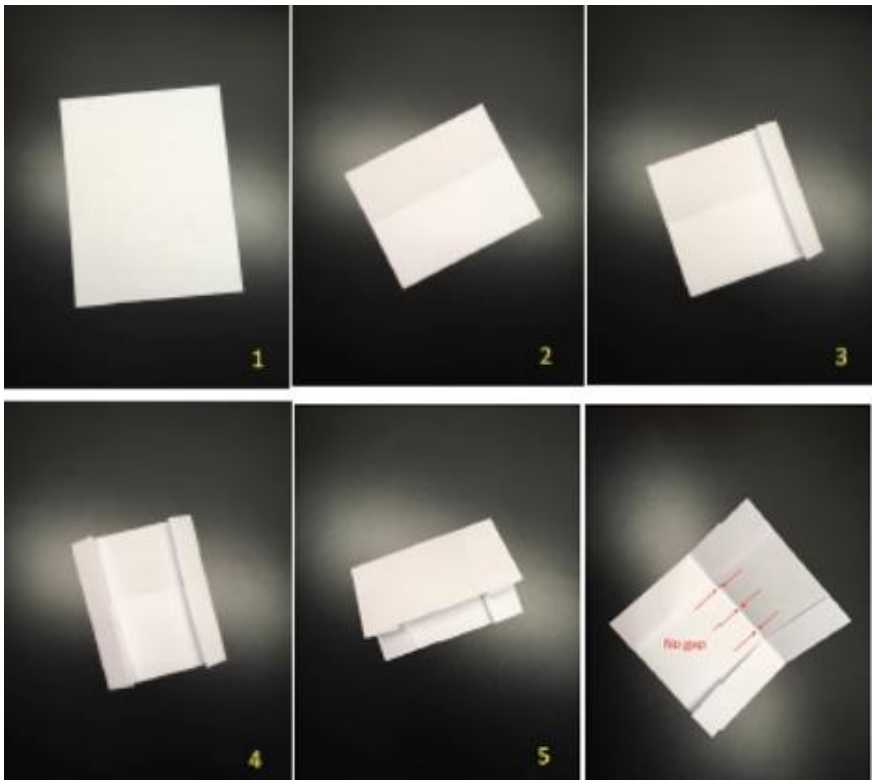

Figure 28. Example of packet folding steps, courtesy of David Kofranek LLC. 1) 8 ½" x 11" paper; 2) fold the bottom 1/3 up; 3) fold 1" margins in on both sides; 5) fold lid over, place labels here.

8. Set packets in a warm, ventilated, dry location with some space between them to allow for air flow. Check periodically to ensure that the specimens are drying without mildew formation.
    a. Ensure specimens are completely dry before shipping to the taxonomist. Do not place packets in plastic bags as they can promote mildew.
9. For each paired specimen, retain one packet at Domain Herbarium and prepare to ship the other packet to the expert taxonomist. Proceed to SOP G and RD[19] for shipping instructions to taxonomists.

D.7 Resolving Morphospecies

1. Morphospecies names and descriptions are good for 1 calendar year in the AOS morphospecies mobile app. When identifying plants, you may select from a list of morphospecies IDs and descriptions already created this year using the link to the morphospecies app provided in the Plants – Lab app. Morphospecies expire each calendar year under the assumption that plants with a morphospecies have been shipped to an external taxonomist for identification if needed.
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 herbarium mounted and kept in the domain herbarium.
2. **Mosses, liverworts, and lichens** sent out for further taxonomic identification should be dried and placed in labeled 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 kept in the domain herbarium.
3. **Macroalgae samples** are preserved using a disposable pipette. Each 60 mL bottle should have macroalgae with enough DI to cover (can be up to 60 mL total). Preserve each sample with glutaraldehyde to reach a final concentration of 2% glutaraldehyde in the sample (note that this concentration is greater than the concentration of glutaraldehyde used in a microalgae sample because there is typically more biological material in the macroalgae sample). Estimate the amount of sample plus water in the bottle, and estimate the amount of glutaraldehyde to add. Preserved samples may be stored at 4 °C (±3 °C) until shipping. It is better to overestimate the preservative needed than to underestimate.
    a. Use C1*V1 = C2*V2 to calculate preservative volume, where:
        i. C1 = concentration of preservative before adding to sample
        ii. V1 = volumes of preservative before adding to sample
        iii. C2 = concentration of preservative in final sample
        iv. V2 = volume of final sample (this may be slightly more than 60 mL after adding the preservative, this is ok)
    b. Example: For a final 60 mL sample preserved to 2% glutaraldehyde, use 4.8 mL of 25% glutaraldehyde. Adjust your final concentration accordingly.
        i. C1 = 0.25 (25% glutaraldehyde)
        ii. v1 = solve for V1
        iii. C2 = 0.02 (2% glutaraldehyde)

iv. V2 = 60 mL

v. Equation for solving V1 is: V1 = (C2*V2)/C1

D.9 Sample Storage

See **Table 2** for sample storage instructions. Any samples stored for longer than 2 days should be entered into the Domain Support Inventory app.

SOP E Post-Field Sampling Tasks

E.1 Field Sampling

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]) within 48 hours of returning from the field. If sampling occurs on Friday, decontamination can occur the following Monday.
        i. For sites that are downstream of other NEON sites (applies only to D08 TOMB downstream of BLWA), TOMB equipment does not need to be decontaminated if used at BLWA first.
    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 correct number of transects or quadrats are entered, including sampling impractical records.
        ii. 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

E.2 Lab Processing

1. Refresh 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

SOP F 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. Data collected on paper data sheets must be transcribed within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Quality Assurance

Data Quality Assurance (QA) is an important part of data collection and ensures that all data are accurate and complete. Certain QA checks can be conducted in the field (i.e., before a field team leaves a plot or site), while others can be conducted at a later date in the office (typically within a week of collection). Field QA procedures are designed to prevent the occurrence of invalid data values that cannot be corrected at a later time, and to ensure that data and/or sample sets are complete before the sampling window closes. Invalid metadata (e.g. collection dates, sampleIDs) are difficult to correct when field crews are no longer at a sampling location.

Office QA procedures are meant to ensure that sampling activities are ***consistent*** across bouts, that sampling has been carried out to ***completion***, and that activities are occurring in a ***timely*** manner. The Office QA will also assess inadvertently duplicated data and transcription errors to maintain data ***validity*** and ***integrity***. See the Data Management Protocol (RD[04]) for more discussion of QA measures.

Before samples ship to external facilities and/or their digital records load to the NEON database, the data must undergo thorough quality checks. The steps needed to accomplish this are outlined in the APL QC Checklist, which is available on the NEON SSL.

Sample Identifiers & Barcodes

By default, each sample and subsample produced by this protocol receives a sample identifier, which contains information about the location, date, and sample type. Each subsample sent to an external facility will also be associated with a scannable barcode, which will not contain information about sample provenance, but will improve sample tracking and reduce transcription errors introduced by writing sample identifiers by hand.

Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use (at least 30 minutes prior but may be applied at the start of the season). Barcodes are unique, but are not initially associated with a particular sample, thus it is encouraged to apply these in advance. Use the

appropriate barcode label type with each container (i.e., cryogenic Type II barcode labels only used for samples that are stored at -80°C, etc). Note that a barcode label is applied *in addition to* a sample identifier (hand-written or printed).

Barcodes are scanned into the data entry application when a sample is placed into a container; only one barcode may be associated with a particular sample. Do not reuse barcodes. If a barcode is associated with multiple samples, the data ingest system will throw an error and refuse to pull in entered data. For AOS plant clip harvest sampling, barcodes only need to be used during lab processing and entered in the lab app.

SOP G Sample Shipment

1. Follow sample shipping timelines in Section 4 to maintain appropriate sample hold times and storage conditions.
    a. Discrepancies between this protocol document and the Shipping Protocol should be communicated to Science.
2. Follow instructions in the NEON Protocol and Procedure: Shipping Ecological Samples, Sensors, and Equipment in order to ship samples to external laboratories or the biorepository (RD[08]).

G.1 Vascular Plant and Bryophyte Specimens Shipped to the Expert Taxonomist

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 shipping. The following steps do not apply to macroalgae or plant chemistry.
2. Create a standardized human-readable label for each vascular plant or bryophyte specimen with location information using the NEON Label Generator app. See RD[16] for details. Keep the label with the specimen.
3. Freeze all specimens for at least 48 hours at -80 °C prior to shipping to kill any pests (RD[16]).
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 vascular plants or bryophytes (this step is not needed for macroalgae or plant chemistry). 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 and bryophyte 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[19]).

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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. Use random point app to navigate to points.
    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. Bout 2 Clip Harvest: 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. Bouts 1, 2, and 3 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 and rivers**:
    a. Bout 1 and 3: Visit 10 randomized points to determine plant presence. No sample collection.
    b. Bout 2: Collect samples at 10 randomized 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).
2. **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 or 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 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 are based on the NEON temporal sampling strategy (Parker and Utz 2022). Refer to the Aquatic Site Sampling Design for your domain (RD[20]) for bout window start and end dates.

APPENDIX D THREATENED AND ENDANGERED PLANTS

Domain	Site	Scientific Name	Common Name	Specimen Type	Status
D03	FLNT	*Schwalbea americana*	American Chaffseed	Plant	Endangered
D03	FLNT	*Thalictrum cooleyi*	Cooley's Meadowrue	Plant	Endangered
D03	FLNT	*Silene polypetala*	Fringed Campion	Plant	Endangered
D03	FLNT	*Lindera melissifolia*	Pondberry	Plant	Endangered
D03	SUGG/BARC	*Conradina etonia*	Etonia Rosemary	Plant	Endangered
D04	CUPE	*Cordia bellonis*	Cordia Bellonis	Plant	Endangered
D04	CUPE	*Gesneria pauciflora*	Gesneria Pauciflora	Plant	Threatened
D04	CUPE	*Crescentia portoricensis*	Higuero De Sierra	Plant	Endangered
D07	LECO	*Geum radiatum*	Spreading Avens	Plant	Endangered
D07	WALK	*Spiraea virginiana*	Virginia Spiraea	Plant	Threatened
D07	WALK	*Platanthera integrilabia*	White Fringeless Orchid	Plant	Threatened
D08	BLWA	*Arabis georgiana*	Georgia Rockcress	Plant	Threatened
D08	MAYF	*Arabis georgiana*	Georgia Rockcress	Plant	Threatened
D08	TOMB	*Arabis georgiana*	Georgia Rockcress	Plant	Threatened
D13	COMO	*Spiranthes diluvalis*	Ute Ladies'-tresses	Plant	Threatened
D13	COMO	*Platanthera praeclara*	Western Prairie Fringed Orchid	Plant	Threatened
D12	BLDE	*Spiranthes diluvialis*	Ute Ladies'-tresses	Plant	Threatened

APPENDIX E SITE-SPECIFIC INFORMATION

For more information see the Site Specific Sampling Strategy Document.

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 10. Equipment List – General Equipment.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Site-specific Stream Morphology or rapid habitat assessment, or Bathymetry Map	Determining sampling locations	1
	N	Mobile data entry tablet	Field data entry	1
	N	Handheld GPS unit (with batteries, ±4 m accuracy) or Humminbird	Navigating to sampling locations	1
	N	Clipboard	Recording data	1
	N	Cooler, 9-28 quart	Storing samples	1
	N	Ice packs or water ice	Keeping samples cool	2
	N	Waders (hip or chest) or knee boots	Boating or wading	1 pair per person
	N	Aquatic Field Metadata Sheet (all-weather paper)	Recording metadata in case tablet fails	1
	N	Field datasheets (all-weather copier paper, write in pencil)	Recording data in case tablet fails	2
	N	Pre-printed all-weather paper labels	Labeling aquatic plant, bryophyte, and lichen samples	10-20
	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
	N	Permanent markers	Labeling samples	2
	N	Extra batteries	Backup for GPS, Camera	4
	N	Flagging tape (roll) or pin flags	Flagging plant transect locations	1

Table 11. Equipment list – Transect establishment for **wadeable streams**.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Site-specific Stream Morphology Map or rapid habitat assessment	Determining sampling locations	1
	N	Handheld GPS (with batteries, ± 4 m accuracy)	Recording transect locations	1
	N	AOS plot markers	Permanently marking transect locations	10

Table 12. Equipment List – Sampling equipment for **wadeable streams**.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Plastic stake (large) or chaining pin	Anchoring the transect tape	2
	N	Spring clamp	Anchoring the transect tape	2
	N	Meter tape (50 m)	Transect tape	1
	N	View bucket (Plexiglas bottom)	Underwater viewing for point-transect measurements	1
	N	Forceps – featherweight	Separating macroalgae from plants	1
	N	Forceps – fine point	Separating macroalgae from plants	1
	N	Scissors or hand clippers	Removing aquatic plants from the biomass quadrat	1
	N	Mallet	Collecting lichen specimens, use with chisel	1
	N	Chisel	Collecting lichen specimens, use with mallet	1
	N	Trowel	Collecting aquatic plant specimens	1
Amazon Capital Services, Inc./B00H5 1AIYK	N	Aquarium dip net	Collecting floating plants	1
	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
	N	Digital camera, waterproof (with battery, memory card) or phone camera	Photographing specimens	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Field identification key (site-specific)	Identifying specimens in the field	1
	N	Single-edged razor blades	Collecting mosses and liverworts	5
	N	Whirl-pak® bags, 24 oz.	Sample container	30
	N	Resealable bags (gallon)	Organizing samples, collecting large plant specimens	10
	N	Trash bags	Collecting large biomass samples	5
	N	Cable ties (6 inch), package	Attaching weight to rake	1

Table 13. Equipment list – Sampling equipment for **lakes and rivers**

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	Y	ArcMap Software	Generating random points for sampling	1
	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
	N	Collapsible quadrat (0.5 x 0.5 m)	Sample collection for floating plants	1
	N	Depth finder	Determining depth at the sampling point	1
	N	Scissors	Removing aquatic plants, bryophytes and macroalgae	1
	N	Digital camera, waterproof (with battery, memory card) or phone camera	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
	N	Whirl-pak® bags, various sizes	Sample container for smaller samples	30
	N	Resealable zip-top bags (gallon)	Sample container for larger samples	10

Table 14. Equipment list – Laboratory processing: Ash-free dry mass.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Mobile data entry tablet or desktop app	Domain data entry	1
	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
	N	Weigh boats (plastic, large)	Measuring dry weight of small specimens	20
	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
	N	Top-loading balance (0.01 g precision)	Measuring weight	1
	N	Analytical balance (0.0001 g precision)	Measuring weight	1
	N	Muffle furnace	Burning organic material for ash-free dry mass calculation	1
	N	Crucible tongs	Safe handling of equipment in the muffle furnace	1
	N	Thermal gloves	Safe handling of equipment in the muffle furnace	1 pair
	N	Aluminum baking pan	Sample organization in muffle furnace	2
	N	Desiccator (bench top)	Storing dried samples	1
	N	Desiccant packs	For bench top desiccator	2-Jan
	N	Lab datasheets	Recording data if tablet fails	1
	N	Paper lunch bags	Drying samples in the drying oven	10
	N	Laboratory tissues (box)	Blotting small specimens	1
	N	Paper towels	Blotting large specimens	1
	N	Aluminum foil	Separating aluminum weigh boats in muffle furnace	1 roll
	N	Borosilicate glass vials, PTFE-lined cap, 4 mL	Sample container for CN subsamples	20

Table 15. Equipment list – Laboratory processing: Aquatic plant pressing and mounting (RD[16]).

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Standard plant press	Pressing plants	1
	N	Cardboard ventilators	Pressing plants	24
	N	Standard driers (sheets)	Pressing plants	24
	N	Forceps (blunt point)	Handling specimens	1
	N	Handheld digital camera, battery, and memory card	Photographing specimens	1
	N	Herbarium mounting paper	Herbarium mounting	1 package
	N	Herbarium mounting glue, bottle	Herbarium mounting	1
	N	Newspaper	Pressing plants	12 sheets
	N	All-weather copier paper, 8 1/2” x 11”	Labelling plants in plant press	15 sheets
	N	Seed envelopes (3.23” x 4.50”)	Storing small plant parts	10
	N	Herbarium labels, paper	Labeling herbarium mounts	10

Table 16. Equipment list – Laboratory processing: Macroalgae preservation.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Sigma-Aldrich, Inc: G62571L	N	Glutaraldehyde, Grade II, 25% in H2O	Preserving macroalgae samples	1 L
	N	60 mL widemouth HDPE polypropylene sample bottle with cap	Shipping macroalgae samples	10

Table 17. Equipment list – Shipping supplies.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Zip-top bags, gallon	Store samples in bags in vermiculite	TBD
	N	Cardboard box (~9”x7”x7”)	Shipping taxonomy samples	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Bubble wrap	Padding taxonomy and CN samples	TBD
	N	Shipping inventory/manifest	Provides sample information to external lab	1