AOS PROTOCOL AND PROCEDURE: AQUATIC PLANT AND MACROALGAE SAMPLING IN LAKES AND NON-WADEABLE STREAMS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	02/06/2014	ECO-01131	Initial release
B	08/29/2014	ECO-02210	Minor updates based on feedback from the field
C	01/09/2015	ECO-02621	Migration to new protocol template
D	06/05/2015	ECO-02724	Minor updates to equipment, shipping instructions, labels and subsampling in the lab, and the addition of sampling dates to appendix.

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1 OVERVIEW

1.1 Background

Aquatic plants and macroalgae are primary producers common in lakes and rivers. They, along with microalgae and microbes, form the autochthonous (i.e., originating within the stream) base of the food web. Additionally, aquatic plants add physical structure to lake and river bottoms and, when densely populated, strongly affect ecosystem structure and function (Bowden et al. 2006, Stream Bryophyte Group 1999). Aquatic plants can alter water velocity and current, filter nutrients, transfer nutrients from the sediments to the water column, settle sediments, stabilize the lake/river bottom, provide substrate for algal epiphytes, and provides shelter and food for macroinvertebrates and fish (Figure 1).

Figure 1. Aquatic plants add structure and colonizable area to the lake or river bottom.

Environmental factors such as wave activity, water level, light attenuation, and nutrient availability strongly affect the aquatic plant community (Wetzel 2001). Wave activity, water depth, and light availability are the most important environmental factors affecting aquatic plants and algae in lakes and rivers. Aquatic plants are often limited to shallow, littoral areas in deeper lakes but may grow in deeper areas in lakes with clear water and deeper light penetration.

As a key part of the aquatic ecosystem, it is important to collect data on primary producers such as aquatic plants and algae to determine changes in community structure, abundance, and biodiversity over time, as well as changes in biogeochemical cycles. Aquatic plants and macroalgae can act as indicators of changes in watershed activity by integrating the effects of changing nutrient loads, toxicity, and land-cover. Invasive species are also an increasing threat in many aquatic environments and are often spread among lakes and rivers by boating. Common invasive aquatic flora across the continental U.S. include (but are not limited to) 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 these taxa are spreading into NEON Aquatic sites, and what the impacts of invasive species are on native aquatic flora.

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

This protocol is based on the standard operation procedures of the North Temperate Lakes Long-Term Ecological Research (LTER; 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.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.014051	Field Audit Plan
AD[06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.002195	Datasheets for AOS Protocol and Procedure: Aquatic Plant and Macroalgae Sampling in Lakes and Non-Wadeable Streams
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[09]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[10]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[11]	NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[12]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

2.3 Acronyms

Acronym	Definition
AFDM	ash-free dry mass
C	carbon
^{o}C	degrees Celsius
D(#)	domain (#)
DNR	Department of Natural Resources
EPA	U.S. Environmental Protection Agency
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
N	nitrogen
SCUBA	Self-contained underwater breathing apparatus
SD	secure digital (flash memory card)
TOS	Terrestrial Observation System
USGS	U.S. Geological Survey

2.4 Definitions

Aquatic plant: "Large" vascular aquatic plant with root system. Aquatic plants are classified based on the following life forms:

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

Figure 2. Emergent aquatic plants.

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

Figure 3. Floating-leaved aquatic plants, rooted in the sediments.

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

Figure 4. Submerged vegetation. No part of the aquatic plant breaks the water surface.

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

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

Autochthonous: Originating within the lake/river where found.

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

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

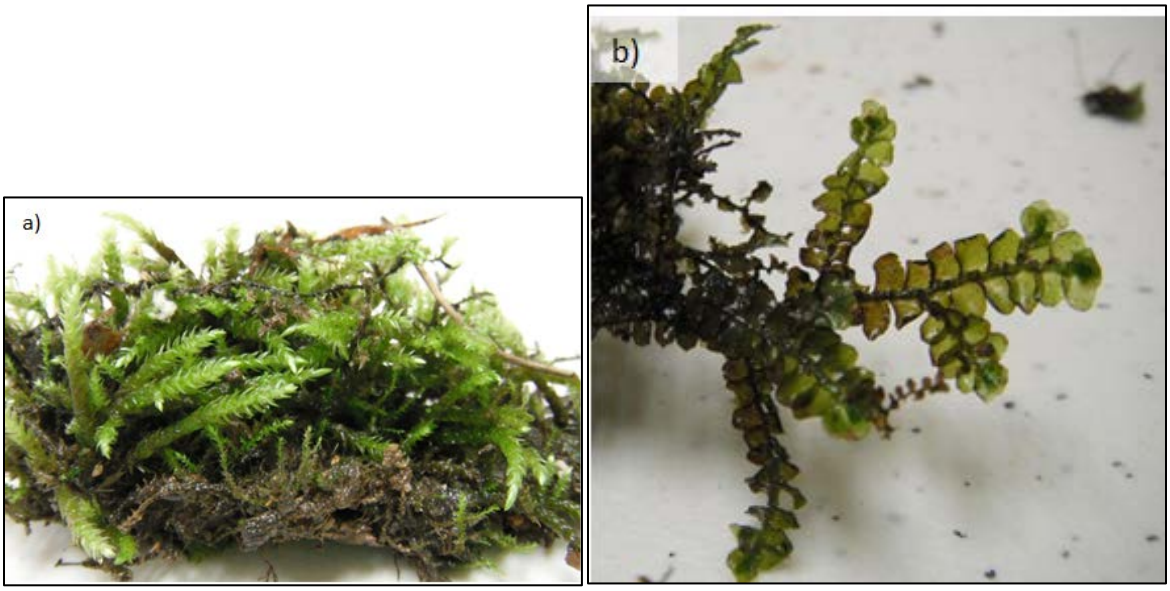


Figure 6. Bryophytes are a group of non-vascular plants including a) mosses and b) liverworts, that may be submerged in the stream or growing at the stream edge.

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

Figure 7. Lichens, a symbiotic relationship between fungi and algae.

Littoral zone: Near-shore area of the lake which extends from the high-water mark to the shallow, submerged area of the lake; typically the area near the shore where sunlight reaches the lake bottom.
Macroalgae: "Large" algae. Multicellular, photosynthetic algae visible to the naked eye. In streams, these algae are typically filamentous (Figure 8).

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

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

3 METHOD

The goals of the Aquatic Plant and Macroalgae Sampling in Lakes and Non-wadeable Streams are: 1) to determine percent cover of aquatic plants on the lake/river bottom; and 2) to collect aquatic plant and macroalgae samples for identification and biomass measurements. Samples are collected using a point-intercept sampling method, which allows for data collection in many areas of the lake or river, rather than along select transect lines (Berg 2009).

Samples are collected following the EPA National Lakes Assessment (Neuman 2008, Wisconsin DNR 2008) and USGS Long Term Resource Monitoring Program (Yin et al. 2000) where aquatic plants and macroalgae are collected from a boat using a double-sided rake. In many other monitoring programs, quantitative plant surveys and biomass removal are completed using SCUBA diver surveys (Downing and Anderson 1985). However, SCUBA surveys will not be used for NEON data collection due to logistical and financial constraints.

Taxa are identified *in situ* where possible using photo keys based on NEON Construction Voucher specimens. However, additional voucher specimens may be collected during this Operations Protocol if the field technician is unable to make a positive identification in the field. Small samples should be collected and returned to the Domain Support Facility for processing and shipping to appropriate taxonomists (see SOP F). These collections and field identification keys will allow domain staff to identify taxa in the field or in the Domain Support Facility without sending all subsequent samples to external taxonomists.

To track changes in the flora of the lake/river bottom (i.e., arrival of invasive species or the loss or decline of a native taxa), total percent cover of the lake or river bottom is estimated over time as part of the Bathymetry and Morphology for Lakes and Non-Wadeable Streams Protocol (RD[09]). Data collected during echo sounder surveys are processed by a trained technician and will show what portions of the lake or river bottom are colonized by plants when put into the modeling program. Similarly, biomass sampling will allow researchers to determine the contribution of these taxa to the stream-bottom flora. If the biomass of a particular taxon decreases dramatically (to ~5-10% of the lake or river bottom), the sampling methods may be reassessed so as not the extirpate species from the lake/river.

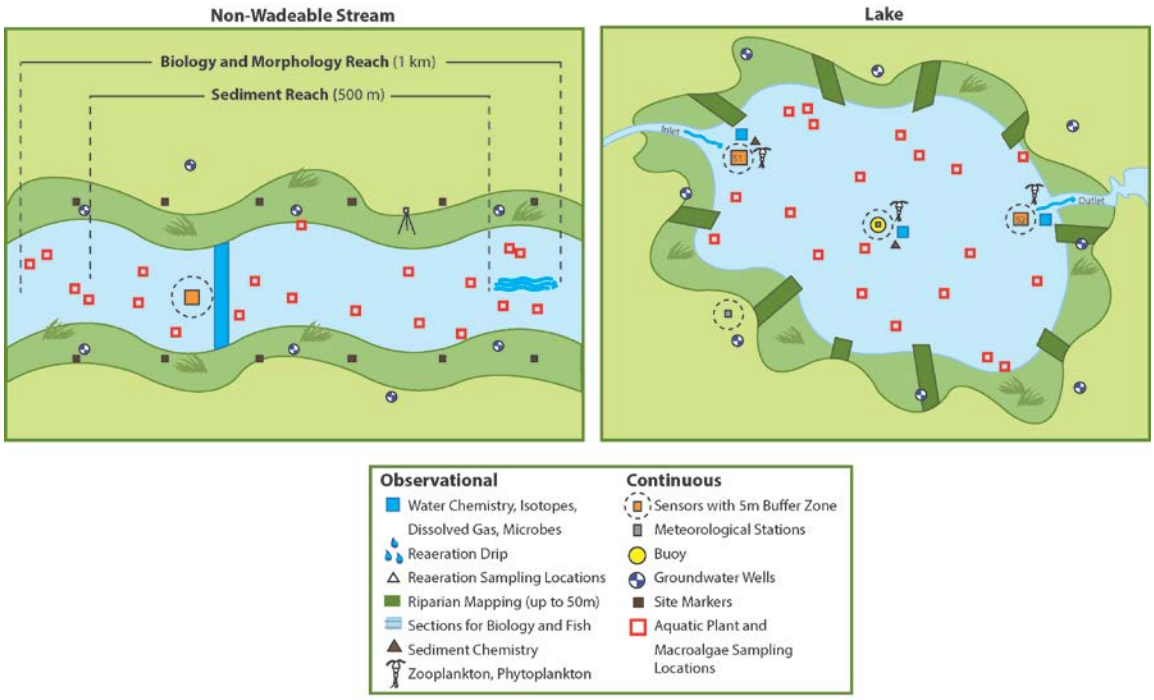


Figure 9. Generic site layouts for lakes and non-wadeable streams with aquatic plant and macroalgae sampling locations.

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

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

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

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

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

4.2 Criteria for Determining Onset and Cessation of Sampling

A range of dates for each site were determined *a priori*, based on historical data including ice on/ice off (for lake sites), river flow (for non-wadeable stream sites), the accumulation of degree days, weather, and riparian phenology (Appendix G).

4.3 Timing for Laboratory Processing and Analysis

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

4.4 Sampling Timing Contingencies

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

Table 1. Contingent decisions

Delay/Situation	Action	Outcome for Data Products
Hours	If circumstances occur that impede sampling (e.g., wildlife, weather), discard samples and start over the next day that conditions permit.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the weather requirements below, continue to collect samples.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If weather conditions deteriorate and the lake/non-wadeable stream becomes too windy (>9 km hr^{-1}) to hold the boat stationary over a sampling point, return to shore and wait in a safe location for 30 minutes. If wind subsides, resume sampling, if not, return to the Domain Support Facility and sample at another time.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If you are able to return to the lake/non-wadeable stream to sample within 24 hours, you may keep samples from the previous day. If you are not able to return within 24 hours, discard any previously collected samples in the lake/non-wadeable stream or at the Domain Support Facility and start over.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
5 or More Days	Samples shall be taken a minimum of 5 days following a major flow event (>25% change in flow within 15 minutes and/or turbidity levels are double the monthly average), allowing for recolonization before sampling occurs.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.

4.5 Sampling Specific Concerns

1. Large amounts of plant biomass may be encountered at some sites (e.g., "wetland" habitat), necessitating larger sampling containers (e.g., trash bags, large coolers).

Table 2. Sampling specific contingent decisions

Delay/Situation	Action	Outcome for Data Products
If an endangered or threatened plant species is discovered (based on the Endangered Species List, Appendix E)	**Do not collect**. Take photos and note location in stream using handheld GPS. Submit a problem ticket to report findings to EHS.	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.

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.

In addition the following safety requirements are sought:

1. Due to site-specific hazards that may be encountered, technicians may conduct sampling from the boat without dismounting from the vessel. In addition, technicians are required to use extra caution in waters where alligators are present and to make sure a safe distance from hazards is maintained.
2. All personnel must be wearing a personal flotation device prior to entering the boat.
3. All employees shall have access to a form of communication with other team members such as a two-way radio.
4. Technicians should be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.)

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

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 3. Equipment list – General equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Site-specific Bathymetry Map	Determining sampling locations	1	N
	R	Handheld GPS unit or Humminbird	Navigating to sample locations	1	N
	S	Clipboard	Recording data	1	N
	R	Cooler, 9-28 quart	Field sample storage; use size appropriate to samples being collected	1	N
	R	Knee boots or waders (pair)	Boating or wading	1 per person	N
Consumable items					
RD[06]	R	General Field Metadata Sheet	Recording metadata	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
RD[05]	R	Field data sheets (print on all-weather paper, write in pencil)	Recording data	2	N
	R	Pre-printed all-weather paper labels	Labeling aquatic plant, bryophyte, and lichen samples	1 sheet	N
	R	Pre-printed adhesive labels (all-weather, 2”x4”)	Labeling macroalgae samples	20	N
	R	Pencils	Recording data	2	N
	R	Permanent markers	Labeling samples	2	N
	R	Ice packs	Keeping samples cool	2	N
	R	Extra batteries (GPS, camera)	Backup for GPS, Camera	4	N

R/S=Required/Suggested

Table 4. Equipment list – Sampling equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX100576	R	Double sided rake with handle	Sample collection	1	N
	R	Braided polyester line marked in 20 cm increments for rake	Sample collection	1	N
	R	Dive weight (10 lbs.) or Secchi disk weight – attach to rake with cable ties if using rope	Weighing down rake	1	N
MX100304	S	Collapsible quadrat (0.5 x 0.5 m)	Sample collection for floating plants	1	N
MX100543	R	Depth finder	Determining depth at the sampling point	1	N
	S	Scissors	Removing aquatic plants, bryophytes and macroalgae	1	N
	R	Digital camera, waterproof (with battery, memory card)	Photographing specimens	1	N
	R	Camera battery	Photographing specimens	1	N
	R	Camera memory card (SD)	Photographing specimens	1	N

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

R/S=Required/Suggested

Table 5. Equipment list – General boating equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Boat		1	Y
	R	Anchor with rope		2	N
	R	Oars		2	N
	R	Trolling Electric Motor		1	Y
	R	Battery (12 volt)		1	Y
	R	Safety kit for boat (e.g., flares, bailer, float with rope)		1	Y
	R	First Aid Kit		1	N
	R	Personal Flotation Devices (PFDs)		1 per person	N
Consumable items					
		(none)			

R/S=Required/Suggested

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

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

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Consumable items					
	R	Desiccant packs	For bench top desiccator	1-2	N
	R	Lab data sheets	Recording data	1	N
	R	Paper lunch bags	Drying samples in the drying oven	10	N
	R	Laboratory tissues (box)	Blotting small specimens	1	N
	R	Paper towels	Blotting large specimens	1	N
	S	Aluminum foil	Separating aluminum weigh boats in muffle furnace	1 roll	N
MX106351	R	Borosilicate glass vials, PTFE-lined cap, 4 mL	Shipment container for subsample ground specimen	20	N

R/S=Required/Suggested

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

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Standard plant press	Pressing plants	1	N
	R	Cardboard ventilators	Pressing plants	12	N
	R	Standard driers (sheets)	Pressing plants	24	N
	S	Forceps (blunt point)	Handling specimens	1	N
	R	Handheld digital camera	Photographing specimens	1	N
	R	Camera battery	Photographing specimens	1	N
	R	Camera memory card	Photographing specimens	1	N
Consumable items					
	R	Herbarium mounting paper	Herbarium mounting	1 package	N
	R	Herbarium mounting glue, bottle	Herbarium mounting	1	N
	R	Newspaper	Pressing plants	12 sheets	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	All-weather copier paper, 8 1/2” x 11”	Labelling plants in plant press	15 sheets	N
	R	Seed envelopes (3.23” x 4.50”)	Storing small plant parts	10	N
	R	Herbarium labels, paper	Labeling herbarium mounts	10	N

R/S=Required/Suggested

Table 8. Equipment list – Laboratory processing: Macroalgae preservation

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

R/S=Required/Suggested

Table 9. Equipment – Shipping Supplies

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
		(none)			
Consumable items					
	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	TBD	N
	S	Cardboard box (~9”x7”x7”)	Shipping taxonomy samples	1	N
	R	Bubble wrap	Padding taxonomy and CN samples	TBD	N
	R	Shipping inventory (RD[12])	Provides sample information to external lab	1	N

R/S=Required/Suggested

6.2 Training Requirements

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

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

Personnel will be trained in the field protocols associated with this document, and trained in safe working practices for lake- and river-based field work. Technicians must also be trained in field identification based on the local lake/river flora and in safe handling of glutaraldehyde (see reference to Safety Data Sheets (AD[01] and AD[03])).

6.3 Specialized Skills

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

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

Field sampling requires 2 technicians for 3-8 hours per site, plus travel to and from the site. Lab processing requires 1-2 technicians for 2-8 hours within 48 hours of field sampling, 1 technician for 3-8 hours on the second lab day, and 1 technician for 2-3 hours on the third lab day.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

1. Collect and prepare all equipment, including sample bottles, sample bags, and pre-printed labels.
   a. Load GPS sampling coordinates on handheld GPS unit.
2. Have ice or ice packs frozen and ready for transportation cooler.
3. Charge batteries for digital camera and check batteries (bring extras) for handheld GPS unit.
   a. Ensure that the camera memory card is empty before sampling.
4. See Laboratory Preparation procedures in Section D.1 for additional pre-sampling activities (e.g., weigh boat preparation).
5. Fill out general aquatic field metadata sheet (RD[06]) upon every field sampling visit.

SOP B Determining Point-Intercepts

Point-intercepts will be determined at headquarters and provided to field technicians unless GIS software is available at the domain support facility.

1. Using the most recent bathymetric map of the lake/river (it may be from the previous summer; RD[09]) determine the colonizable depth based on existing aquatic plant cover, and generate a polygon using GIS software of the portions of the lake/river bottom that are equal to or shallower than this depth (Figure 10).
   a. This represents areas of potential plant growth, not necessarily where plants are known to be rooted.

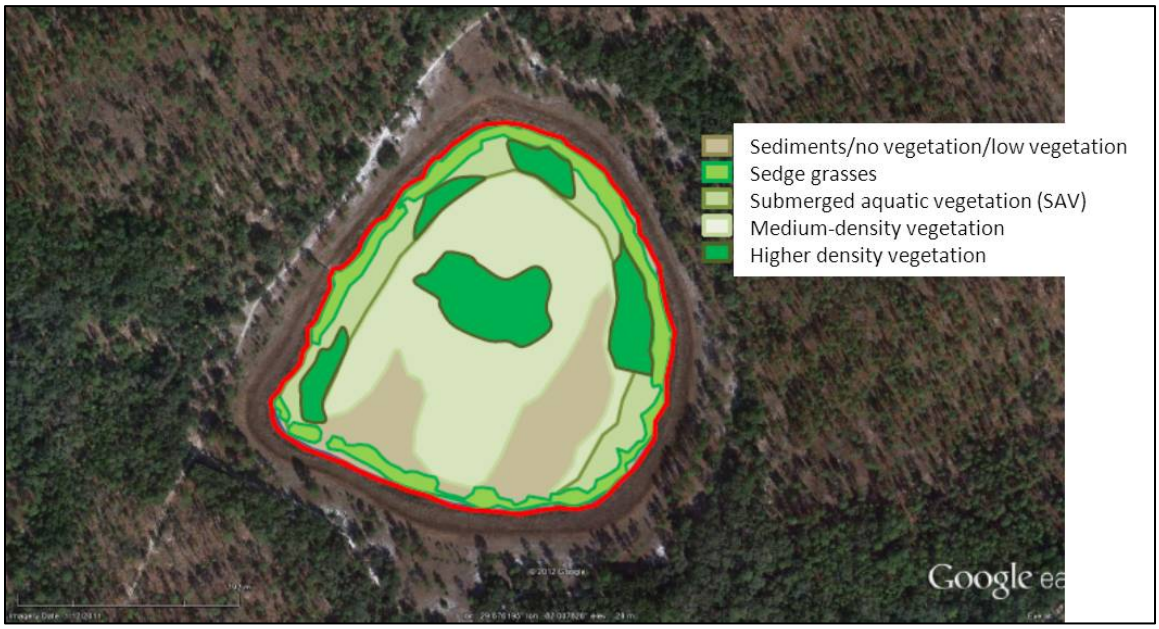


Figure 10. Bathymetric map of Barco Lake (D04) showing location and percent coverage of aquatic plants.

2. Create 20 random coordinates within the defined polygon using the "Create Random Points" function.
   a. GIS work may be done at NEON Headquarters by experienced GIS personnel.
3. Number coordinates "1" through "20" and print a copy to take in the field.
   a. 10 of the 20 coordinates will be sampled, starting at the beginning of the list. However if conditions (bottom substrate, depth, etc.) are not conducive to sampling at a given coordinate, you may move on to the next coordinate on the list.
      1) If substrata are appropriate for sampling and plant cover is 0, the point is still a valid sampling location.
   b. Do not sample within a 5 m radius of the aquatic instrumentation.

SOP C Field Sampling

C.1 Point-Intercept Sampling (Rake)

1. Collect samples at 10 points.
   a. Three rake tows equal 1 composite sample at each point.
2. Navigate to the pre-determined sampling point on the lake/river using the handheld GPS unit (Figure 14, RD[05]).
   a. If you are unable to sample at this point due to natural bed conditions (e.g., bedrock, large boulders), anchors, etc., or it is too close (within 5 m) or aquatic instrumentation, continue to the next point on the list. Note on field data sheet (0).
3. Anchor the boat at the bow and stern to keep the boat in the desired location.
4. Determine depth at the sampling point using the depth-finder and record on the Field Data Sheet (Figure 14).
   a. If the depth is <1 m, use the rake with a handle (Figure 11)
   b. If the depth is >1 m, use the rake with a rope and dive weight attached (Figure 11).

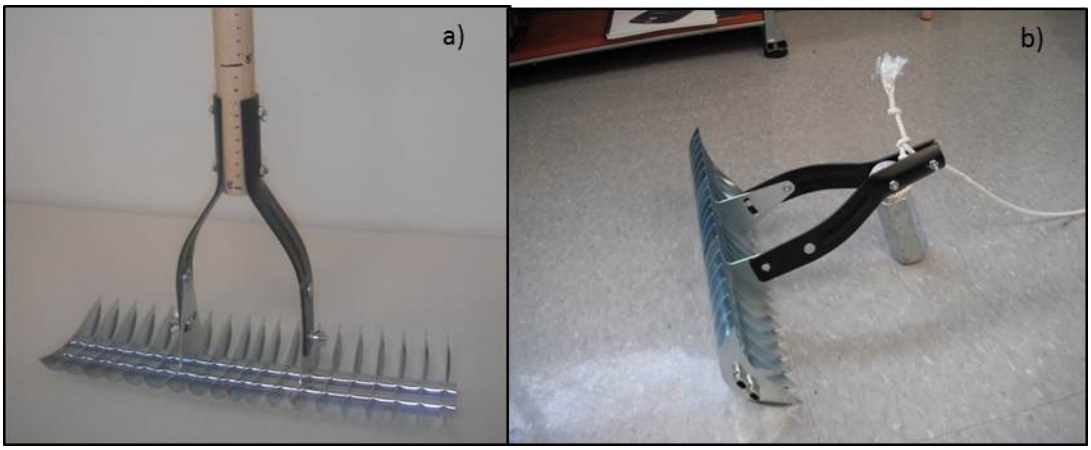


Figure 11. Double-sided sampling rake: a) Rake with handle b) Rake with rope and weight

5. If plants are rooted, proceed to Step 6 and use the rake. If plants are floating, put out the 0.5 x 0.5 m quadrat and collect all floating plants material, then proceed to Step 11.
6. Lower the rake head to the lake/river bottom by casting away from the boat.
7. Pull the rake along the lake/river bottom, toward the boat, for approximately 1.5 m.
   a. This can be measured by leaving 1.5 m of extra rope above the water surface, and pulling that in toward the boat or marking 1.5 m on the gunwale of the boat and towing that distance.
8. Twist rake 180 degrees as you begin to lift it toward the boat. Twisting minimizes the loss of plants from the rake, but twisting more than 180^{o} may cause plants to fall off.
9. Pull the rake and plants into the boat (Figure 12).
   a. NOTE: Do not rinse plants or algae in the lake or river to prevent sample loss.

b. **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.

1) If sampling a rare, threatened, or endangered species, take high resolution photos and record what section of the lake/river the specimen was found in (e.g., "50 m northwest of sensor set") and return the specimen to the water. Collect and record a GPS coordinate of the specimen's location on the field data sheet.
2) Lists and photo keys of local endangered taxa will be provided before sampling and are attached in Appendix E.

Figure 12. Double-sided rake head with plant sample being brought into the boat.

10. Visually estimate the amount of the rake that is covered and assign a "cover rating". Cover rating is assigned on a scale of 0 to 5 (Figure 13).
    a. Record cover rating for plants and macroalgae separately (if it's possible to separate) on field data sheet (Figure 14, RD[05]).
    b. If you are unable to separate plants and algae, make a note on the data sheet.

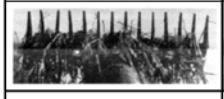
	Cover Rating	Cover %
	5	81-100
	4	61-80
	3	41-60
	2	21-40
	1	1-20
Empty rake	0	0

Figure 13. Aquatic plant cover on a rake head sample (from Deppe and Lathrop 1992) and associated cover ratings (Yin et al. 2000).

NEON Plant/Macroalgae Field Data Sheet

Lakes and Non-wadeable Streams

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

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

Local time (HH:MM): *10:15* | **Sampling protocol & Rev.:** *NEON.DOC.001202vA*

Point ID	Waypoint	Sample ID	Depth (m)	Rake rep	Cover rating	Dominant growth form (emergent, floating-leaved, submerged, free-floating)
1	29.690443, -82.016842	BARC.20140702.rake.P1	5.2	1	3	submerged
				2	4	submerged
				3	4	submerged
2	29.680187, -82.014522	BARC.20140702.rake.P2	6.1	1	5	submerged
				2	5	submerged
				3	5	submerged

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

11. Open a Whirl-pak® bag (smaller samples) or gallon zip-top bag (larger samples). Fill out a field label (all-weather paper) and place inside sample bag (Figure 15, RD[05]).

NEON Sample ID: *BARC.20140702.hyacinth.P1* Transect/Quadrat/Point ID: *P1* Species ID: *water hyacinth* Collected by: *sparker*	**NEON** Sample ID: *BARC.20140702.SAV1.P1* Transect/Quadrat/Point ID: *P1* Species ID: *SAV1* Collected by: *sparker*
NEON Sample ID: *BARC.20140702.macroalgae1.P1* Transect/Quadrat/Point ID: *P1* Species ID: *macroalgae1* Collected by: *sparker*	**NEON** Sample ID: *BARC.20140702.macroalgae2.P1* Transect/Quadrat/Point ID: *P1* Species ID: *macroalgae2* Collected by: *sparker*

Figure 15. Example field label for aquatic plant samples.

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

    a. Hold the rake over a 3 or 5 gallon bucket to prevent samples loss.
    b. Take care when removing plants as the rake tines are very sharp.
    c. Separate by taxa (this is easier to do now than in the lab), place each taxon in a separate sample bag with an individual label. Record on data sheet, along with growth form (i.e., emergent, floating-leaved, submerged, or free-floating).
    d. If the samples do not fit in one bag, use multiple bags and label, for example, as "1 of 2" and "2 of 2". You may fold plants if necessary to fit them in the sample bag.
    e. Place contents of all 3 tows in the same sample bag to form a composite sample.
13. Repeat Steps 5 through 12 until three tows have been completed at each point.
14. Close the Whirl-pak® by holding the wire tabs at either side of the bag (Figure 16), gently press out excess air, then whirl the bag at least 3 complete revolutions to form a leakproof seal. Rather than whirling, you may also fold the top over as tightly as possible at least 3 times. Bend the wire ends over onto the bag to complete. If using a resealable zip-top bag, seal top firmly.

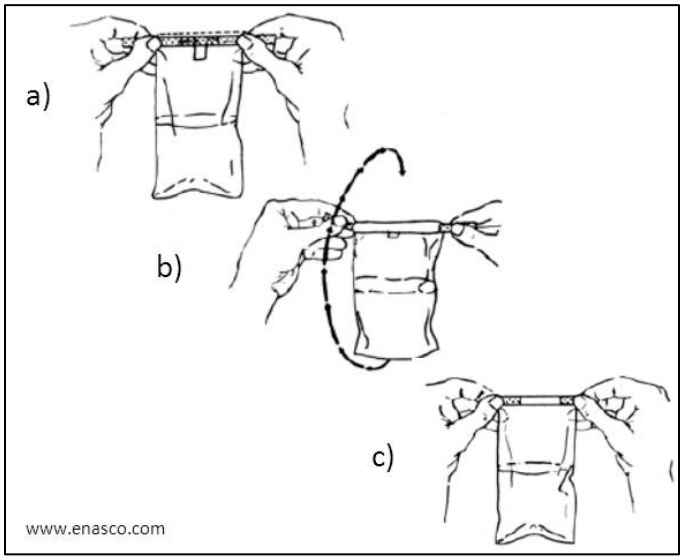


Figure 16. How to close a Whirl-pak® bag: a) hold the wire tabs; b) whirl the bag 3 complete revolutions (or fold the top over), and c) fold the wire ends over to close.

15. Place all labeled and sealed sample bags in a dark cooler with frozen ice packs for transportation to the Domain Support Facility. Store samples at the Domain Support Facility as per SOP D.
    a. Samples should remain refrigerated at 4°C (±3°C).
    b. The total time from collection to the start of sample processing in the lab must not exceed 48 hours in order to minimize decomposition of samples.
16. Repeat Steps 1 through 15 until 10 points (3 tows at each point) have been completed in the lake/river.

C.2 Special Considerations: Threatened, endangered, and invasive species

If threatened or endangered species are accidentally collected, make a note of the location on the field data sheet so that location will not be visited again. These records should be added to the lake bathymetric map.

Retain the collected material, press and send to external taxonomists for identification (SOP D.4) and submit a problem ticket to report findings to NEON Permitting.

If known invasive species are discovered at the site (e.g., *Hydrilla*), submit a problem ticket to report findings to EHS to inform equipment decontamination procedures.

C.3 Sample preservation

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

C.4 Ending the Sampling Day

1. Refreshing the sampling kit
   a. Replace Whirl-pak® and resealable bags.
   b. Print new field labels and field data sheets on all-weather copier paper.
   c. Check and charge all batteries, replace if necessary.
   d. Place ice packs in the freezer.
2. Equipment maintenance, cleaning and storage
   a. Check depth markings on rake handle/rope, refresh markings if necessary.
   b. Decontaminate all equipment that has come in contact with lake/non-wadeable stream water according to the NEON Aquatic Decontamination Protocol (RD[08]).
   c. Clean boat and motor; remove aquatic plants by hand to prevent spread of invasive taxa. Allow boat and motor to dry completely.
   d. Dry all equipment thoroughly between sites and before storage.

SOP D Laboratory Sampling and Analysis

Aquatic plant specimens will be identified using voucher specimens and keys, weighed and dried for measurements of fresh weight and ash-free dry mass (Figure 18). Macroalgae will be preserved and sent to an external lab for identification (Figure 18).

D.1 Preparation

1. If aluminum boats are new and unlabeled:
2. Label ~20 boats by inscribing a unique number on the bottom of each boat with a pencil (e.g., A1, A2, A3, etc.; Figure 17).
   a. NOTE: It doesn't matter what the labels are as long as they are unique and easy to read.

Figure 17. Unique identifiers inscribed on the bottom of the aluminum weigh boats

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

D.2 Aquatic Plants

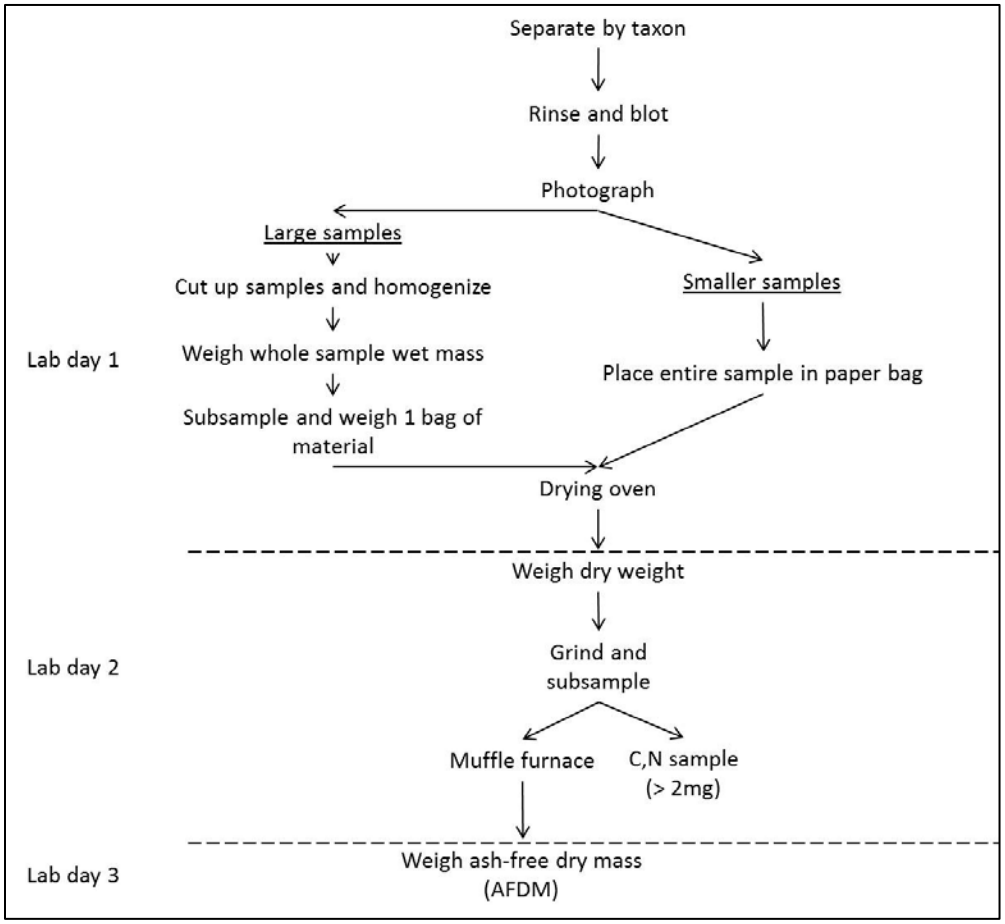


Figure 18. After separating each species from the original samples, follow the steps above for Domain Support Facility aquatic plant processing.

1. **Day 1**: Remove plant/bryophyte sample and field label from sample bag from quadrat sampling. Set label aside.
2. Gently rinse the sample with tap water over a 1 mm sieve to remove sediment, large epiphytes, and debris.
   a. Some aquatic plants are very fragile and may break easily during rinsing. The sieve will collect any plant fragments but allow sediments to be washed away.
3. Separate sample into individual taxa, separate microalgae and set aside if they were included in the sample bag.
   a. Each species will be a unique specimen.
   b. Floating the sample in a tray of water may help facilitate separation of taxa.
   c. At some sites, it may be difficult to separate macroalgae from plant material. After spreading the entire sample out on a tray, spend no more than 5 minutes sorting the sample.

d. If there is not enough specimen to complete all analyses, prioritize samples as follows:
   1) Taxonomy
   2) AFDM
   3) CN

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

NEON Aquatic Plant and Macroalgae Lab Data Sheet - AFDM

Site (4-letter code): *BARC*	**Date analysis finished (YYYYMMDD):** *20140705*
Date collected (YYYYMMDD): *20140702*	**Recorded by:** *sparker*
Date analysis started (YYYYMMDD): *20140703*	**Sampling protocol & Rev.:** *NEON.DOC.001202vA*

PERIODICALLY CHECK THAT BALANCE IS ZEROED!

Sample ID	Species ID	Total wet mass (g)	Subsample wet mass (g)	Total or subsample dry mass (g)	Boat ID	Boat mass (g)	Boat + dry mass (g)	Boat + ash mass (g)
BARC.20140702.hyacinth.P1	*water hyacinth*	*2.5631*	*1.5240*	*1.4589*	*A1*	*2.1340*	*3.5929*	*3.0752*
BARC.20140702.SAV1.P1	*SAV1*	*1.4859*	*1.4859*	*0.6189*	*A2*	*2.0561*	*2.6750*	*2.4261*
BARC.20140702.algae1.P1	*macroalgae1*	*3.4524*	*1.7262*	*1.4387*	*A3*	*2.2247*	*3.6634*	*3.0148*

Figure 19. Example of lab data sheet for ash-free dry mass determination (RD[05]).

11. Place paper bags containing samples in the drying oven for a minimum of 12 hours at 60 ^{o}C or until constant weight is achieved (i.e., mass varies by <2% over a one-hour period; RD[10]).
    a. Use TOS "Lab Drying QC Datasheet" in Measurement of Herbaceous Biomass datasheets (RD[11]).

b. Specimens may be split across multiple bags to facilitate drying.

12. **Day 2**: When dry, remove all bags+samples from drying oven and let cool to room temperature in a plastic bag or desiccator.
    a. Placing samples in a bag or desiccator is important because samples absorb water quickly from the air as they cool. Samples may be left in desiccator or plastic bags for up to 30 days before proceeding to the next step.
13. Place a large, clean, plastic weigh boat (small specimen) or white plastic larval tray (large specimen) on analytical balance. Tare (zero) balance. Place dry specimen in the plastic weigh boat/tray and record as **Dry Weight** on Lab Data Sheet.
14.
15. Samples may be crushed to fit into weigh boat. If samples are large, grind sample in Wiley mill using 20 mesh (0.85 mm) screen to homogenize.
    a. Subsamples for CN and AFDM
        1) Place a subsample of ground or crushed material in aluminum weigh boat. Record **Boat ID** on lab data sheet.
        2) Remove ≥ 2 mg for C and N analysis. Place in 4 mL PTFE-capped glass vial, apply adhesive label, and set aside for shipping
            a) Samples sent to external lab for CN analysis do NOT need to be ground on the Wiley Mill. Use the Wiley Mill to homogenize large samples before subsampling for CN. Small samples may be crushed by hand.
16. Clean grinding mill thoroughly with compressed air between samples and with ethanol after finishing the sampling bout.
17. Place remaining specimen subsample in a clean, pre-labeled aluminum weigh boat. **Record Boat ID** on lab data sheet.
    a. If the ground specimen is too large for the aluminum weigh boat, put only a portion (or "subsample") of the specimen in the boat (~1-2 g).
18. Weight boat on analytical balance, and record **as Dry weight + boat Weight** on Lab Data Sheet.
19. Repeat above steps until all specimens have been processed.
20. 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. Aluminum foil may be used to separate stacked boats.
    b. Place boats on an approved muffle furnace tray (if available) before placing in the furnace. This makes it easier and safer to handle samples.
21. Leave samples in the muffle furnace at 500°C for at least 6 hours.
22. **Day 3**: After 6+ hours, remove covered pans/aluminum boats carefully from the muffle furnace using oven gloves and crucible tongs.

23. 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 in an area without drafts from windows, doors, or HVAC ventilation. Clearly label hot surface for safety.
24. When boats have cooled enough to handle, place in desiccator.
    a. Samples may be left in desiccator for up to 30 days before proceeding to the next step.
25. Weigh boats again on analytical balance, record as **Ash Weight** on **Lab Data Sheet**.
26. After weighing, discard ash into the trash and clean the boat with a soft brush or paper towel.
27. Set clean boats aside to be reused.

D.3 Macroalgae

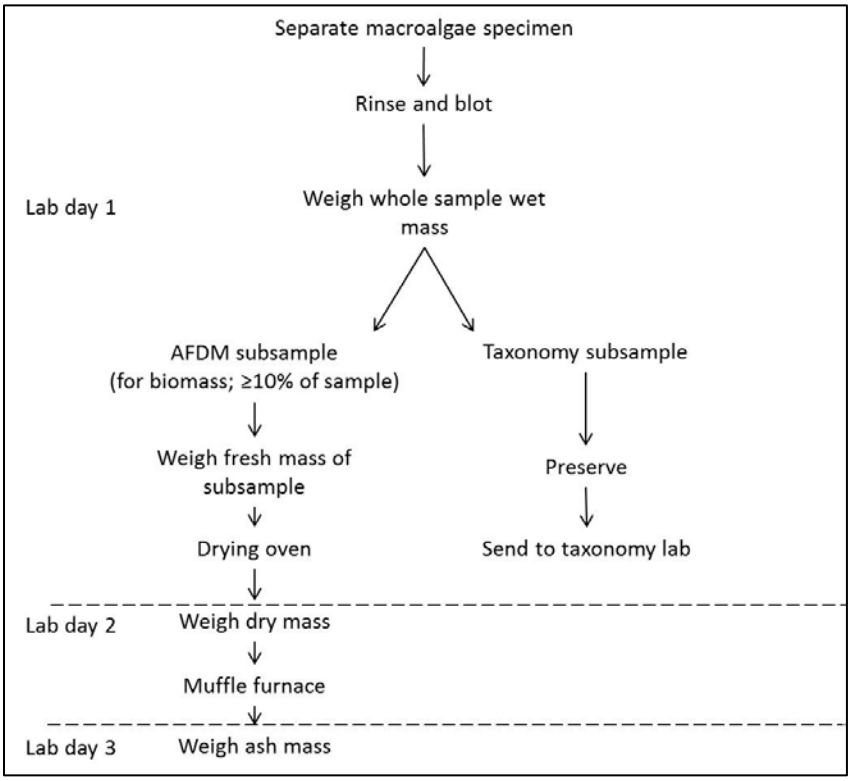


Figure 20. Schematic for macroalgae processing at the Domain Support Facility.

1. Weigh several aluminum weigh boats and record as **Boat weight** along with **Boat ID** on lab data sheet.
2. **Day 1**: Remove macroalgae specimen from sample. Set label aside.
    a. If macroalgae are combined in a sample bag with aquatic plants, carefully separate using forceps and/or fingers.
    b. Floating the sample in a tray of water may help facilitate separation of macroalgae from plant material.

c. At some sites, it may be difficult to separate macroalgae from plant material. After spreading the entire sample out on a tray, spend no more than 5 minutes sorting the sample.

3. Gently rinse specimen to remove sediment or other non-algal material (e.g., leaves, twigs) over a 1 mm sieve.

a. Take care not to lose any sample material.

b. Biomass estimates for macroalgae can be elevated in error due to non-algal material trapped in the filaments. Take care to clean specimen well.

4. Clean algal strands out of the sieve and add to the specimen.

5. Spread cleaned specimen on standard drier, gently blot dry with laboratory tissues or paper towels. **DO NOT CRUSH** sample or attempt to wring the water out.

a. How dry is dry? Blot specimens dry until they no longer drip water when you pick them up.

6. Weigh entire sample on mass balance and record as **Wet mass** on lab data sheet.

a. **Taxonomy subsample**: Remove >20 mL of sample to preserve for taxonomic identification and place in 60 mL HDPE bottle and add enough DI water to cover sample. Proceed to Sample Preservation, SOP D.7. Label according to Figure 21.

b. **AFDM subsample**: Remove 10-50% of original sample and place in an aluminum weigh boat. Weigh subsample and record as **Wet mass subsample** on Lab Data Sheet along with appropriate **Boat ID** and **Boat weight**.

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

8. Place all aluminum boats + subsamples in drying oven for a minimum of 12 hours at 60°C or until constant weight is achieved (i.e., mass varies by <2% over a one-hour period; RD[10]). Cover boats with a cardboard lid or piece of paper to prevent samples from blowing out of the boats once in the oven.

a. NOTE: It is helpful to place a number of boats + subsamples in shallow cardboard trays in the drying oven. These can be moved in and out of the oven more efficiently than moving one sample at a time.

b. Aluminum boats + samples can stay in the drying oven longer than 12 hours if needed.

9. **Day 2**: Carefully remove boats from drying oven and let cool to room temperature before weighing. Place boats in a desiccator until weighing. Take care not to let the dried sample blow out of the boats.

a. Samples may be left in desiccator or plastic bags for up to 30 days before proceeding to the next step.

10. Weigh the boat + dry sample on tared analytical balance, and record as **Dry weight + Boat** on the Lab Data Sheet.

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

12. 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.
b. Place boats on an approved muffle furnace pan if available before placing in the furnace. This makes it easier and safer to handle samples.

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

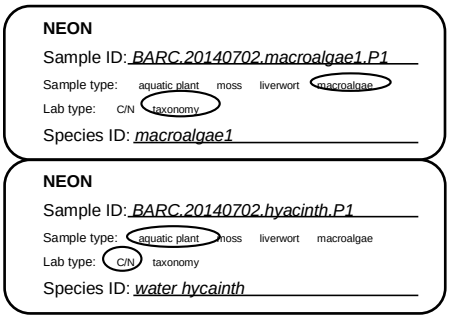


Figure 21. Example of adhesive labels for macroalgae taxonomy and plant CN subsamples.

D.4 Pressing Aquatic Plants (Taxonomy)

1. Open plant press.
2. Place 1 cardboard ventilator on plant press
3. Place 1 piece of standard drier on top of cardboard ventilator.
4. Place pre-weighed newspaper (2 sheets, opened) on top of the standard drier.
    a. Ensure that newspaper weight has been recorded on Lab Data Sheet (Figure 19, RD[05]).
5. Copy information from the specimen label onto the newspaper using a permanent marker and fill out taxonomy label files (RD[05]).
6. Arrange specimen on newspaper, spread leaves and stems apart. Try to lay all parts flat, open any flowers so they lay flat on the paper.
    a. Do not allow leaves or specimens to overlap.
7. Place specimen label on newspaper (for additional identification).

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

D.5 Drying Moss and Liverwort Specimens (Taxonomy)

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

D.6 Herbarium Mounts (Aquatic Plants Only)

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

a. One specimen will be sent to the aquatic plant taxonomist (keep in newspaper, proceed to SOP F for shipping).
b. One specimen will be mounted and retained at the Domain Herbarium.
c. Include flowering structures in each specimen if present.

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

D.7 Preserving Samples

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

D.8 Ending the Processing Day

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

SOP E Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Rename voucher photos similar to sample ID naming convention: SITE.DATE.species.pointID (e.g., BARC.20140702.hyacinth.P7).

SOP F Sample Shipment

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

Ship dried and pressed plant specimens in the newspaper they were pressed in. Retain a duplicate of each specimen (excluding macroalgae) in the domain herbarium. Shipments are to include a hardcopy of the "per sample" tab of the shipping inventory as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. The shipment tracking number (shipment ID) must be included in the electronic version of the shipping inventory as well as the email, but is not necessary on the hard copy.

F.1 Algal Taxonomy Sample Shipping

1. Taxonomy samples must not freeze, take care to avoid shipping at times when the samples may be subject to sitting for long periods in a frozen warehouse (e.g., take note of lab weekend and holiday receiving schedule).
2. Place bottles into one or several gallon-size resealable zip-top bags, grouped by site.
3. Line a cardboard box or 9 qt. cooler with a trash bag to prevent leaks.
4. Place all bottles right-side up inside the liner bag. Add packing material (Vermiculite or other) to take up excess space in container and cushion samples.
    a. If using 9 qt. coolers, include return shipping label for external lab to send cooler back.
    b. Combine shipment with periphyton/seston taxonomy samples if possible.
5. Include shipping inventory/manifest in additional zip-top bag.
6. Ship ground at ambient temperature.

F.2 Plant CN Sample Shipping

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

F.3 Plant Taxonomy Sample Shipping

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

5. Ship ground at ambient temperature.

F.4 Moss, Liverwort, Lichen Taxonomy Sample Shipping

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

F.5 Handling Hazardous Material

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

F.6 Supplies/Containers and Conditions

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

F.7 Timelines

1. Algal taxonomy samples
   a. Shipping should occur within one week of sampling if possible, but samples may be stored at the domain support facility at 4°C (±3°C) for up to 30 days if necessary.
2. Pressed or dried taxonomy specimens
   a. May be stored in plant press or bryophyte/lichen packet for up to 60 days at room temperature.

F.8 Grouping/Splitting Samples

Group samples by site per bout.

F.9 Return of Materials or Containers

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

F.10 Shipping Inventory

Shipments are to have a hardcopy of the shipping inventory (RD[12]) sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. Also include the shipment tracking number in the email. . Email plant taxonomy labels (Appendix F, RD[05]) to aquatic plant taxonomist.

F.11 Laboratory Contact Information and Shipping/Receipt Days

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

8 REFERENCES

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 10. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002195	Datasheets for AOS Protocol and Procedure: Aquatic Plant and Macroalgae Sampling in Lakes and Non-Wadeable Streams
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.001574	Datasheets for TOS Protocol and Procedure: Measurement of Herbaceous Biomass

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

APPENDIX B QUICK REFERENCES

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

Step 2 – Prepare labels (2" * 4").

NEON
Sample ID: *BARC.20140702.hyacinth.P1*
Transect/Quadrat/Point ID: *P1*
Species ID: *water hyacinth*
Collected by: *sparker*

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

Step 4 – Determine point-intercepts:

1. If GIS software is available at the domains, generate a polygon using the portions of the lake/river bottom that are equal to or shallower than the colonizable depth. Create 20 random coordinates within the defined polygon.
2. If GIS software is unavailable, point-intercepts will be determined at headquarters and provided to field technicians.

Step 5 – Point-Intercept Sampling: Collect samples at 10 points, with 3 rake tows from each point.

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

Step 7 – Lab processing:

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

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

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

Sample collection: Be sure to…

- ☑ Determine the 20 random points based off of the most recent bathymetric map.
- ☑ Collect samples at 10 points, with 3 rake tows from each point.
- ☑ Take care when removing plants as the rake tines are very sharp.
- ☑ Take extra care when rinsing to not lose the samples.
- ☑ **DO NOT COLLECT ENDANGERED OR THREATENED SPECIES.**

Sample processing: Be sure to…

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

APPENDIX D BRYOPHYTE PACKET TEMPLATE

Domain: **Site:**

Collector: **Date collected:**

State: **County:**

Lat: **Long:**

Site and Substrate Characteristics (circle all that apply)

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

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

Topography: ridge, slope, valley, trail, roadside

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

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

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

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

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

Type: conifer, hardwood, other _____

Elevation: m **Aspect:** N, S, E, W exposure

Dominant plants:

Coll. #: **Taxon:**

APPENDIX E THREATENED AND ENDANGERED PLANTS

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

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

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

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

APPENDIX F AQUATIC PLANT TAXONOMY LABELS

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

APPENDIX G ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for biological sampling bouts in lakes and non-wadeable streams . Also ee the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

Domain	Site	Bout 1	Bout 2	Bout 3
D03	Ichawaynochaway Creek	21Feb-21Mar	27Jun-25Jul	7Oct-4Nov
D03	Lake Barco	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D03	Lake Suggs	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D05	Crampton Lake	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D06	McDowell Creek	20Mar-17Apr	3Jul-31Jul	27Sep-25Oct
D08	Black Warrior River	19Feb-19Mar	27Jun-25Jul	31Oct-28Nov
D08	Tombigbee River	22Feb-22Mar	26Jun-24Jul	2Nov-30Nov
D09	Prairie Lake	18Apr-16May	5Jul-2Aug	11Sep-9Oct
D09	Prairie Pothole	20Apr-18May	5Jul-2Aug	11Sep-9Oct
D11	South Pond at Klemme	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D18	Toolik Lake	21May-18Jun	29Jun-27Jul	6Aug-3Sep

APPENDIX H SITE-SPECIFIC INFORMATION

See the Site Specific Sampling Strategy Document on AQU's NEON intranet site.