AOS PROTOCOL AND PROCEDURE: ASC – SEDIMENT SAMPLING FOR CHEMICAL AND PHYSICAL PROPERTIES

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	01/18/2022	ECO-06751	• Initial release of merged protocols (Supersedes NEON.DOC.001193, NEON.DOC.001191 which are now OBSOLETE)

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1 OVERVIEW

1.1 Background

This document describes the required protocol for conducting field sampling and domain lab processing of sediment sampling for the purpose of quantifying chemical and physical properties at an external laboratory. These data can be used quantify the stock of carbon and nutrients in the benthic environment, reveal benthic redox conditions, and monitor concentrations of trace metals. Sediment is also archived at the NEON biorepository to provide the opportunity for the user community to run analyses beyond those that NEON provides. Because sediment chemical and physical properties are key elements of the benthic environment, sediment sampling is collected in conjunction with other biological sampling activities, including benthic algae, microbes, invertebrate, and fish sampling. The goal is that NEON sediment data will be used to address a variety of questions about biogeochemical cycling and monitoring at the reach scale (**Table 1**).

Sediment is solid material in aquatic ecosystems originating from weathering and erosion of rock and soil. Eroded material is transported to aquatic ecosystems by a number of processes, including but not limited to, wind, water, ice, mass wasting, and tectonic activity (Gellis et al. 2009). Once it enters the aquatic ecosystem, sediment can be suspended in the water column or deposited to the benthic environment. Sediment, as defined in this protocol, is the portion that is deposited to the benthic environment of wadeable streams, rivers, and lakes.

Sediment can be a sink (event of long-term accumulation) for many elemental classes in aquatic systems, such as carbon, nutrients, trace metals, and organic contaminants. Consistent sediment deposition in systems with sustained inputs or flow will bury material, keeping naturally-occurring and pollutant compounds from resuspension. Thus, sediment in freshwater, estuarine, and marine systems is recognized as one of the largest global repositories of pollutants (Larsen et al. 2010). Under certain environmental conditions, sediment can be resuspended to the water column and subsequently redistributed in the aquatic ecosystem, becoming a source of compounds, both naturally-occurring and pollutant, to aquatic food-webs. While resuspended sediment can provide essential nutrients and energy for aquatic organisms, it also poses a potential toxicity threat to aquatic food-webs through bioaccumulation of pollutant material (Phipps et al. 1993, Ingersoll et al. 1995).

Sediment provides essential habitat for a vast number of freshwater organisms from algae, to mollusks and crustaceans, to emergent insects, to benthic-feeding fish, reptiles, and amphibians. As such, sediment can be a hotspot of biogeochemical activity where nutrients and energy are cycled in and subsequently buried, assimilated, transformed, recycled, or released (McClain et al. 2003). The chemical properties of sediment can be traced across food-webs, most notably through the bioaccumulation of pollutants (Phipps et al. 1993, Ingersoll et al. 1995), but also through the movement of carbon and nutrients within an aquatic system and even across systems (aquatic-riparian and aquatic-terrestrial

subsidies). Therefore, understanding the chemical and physical properties of sediment is key to understanding carbon, nutrient, and pollutant dynamics in aquatic ecosystems.

Table 1. Summary of measurements associated with NEON Sediment Sampling for Chemical and Physical Properties.

Measurement	Rationale	Frequency
Carbon, total organic (TOC) and total (TC)	C stock, benthic energy budget, decomposition, pollutant potential	pre-2017: 3x per year 2017-2021: 1x per year (fall) 2022-present: 2x per year
Nitrogen, total, ammonium, nitrate, nitrite	N stock, N availability	Pre-2017: 3x per year 2017-present: 2x per year
Total Metals	Watershed geology, trace metal contamination, sediment toxicity	Pre-2017: 3x per year 2017-present: 2x per year
Conductivity, pH, Alkalinity	Redox conditions, context for chemical measurements	Pre-2017: 3x per year 2017-present: 2x per year
Grain size, soil texture classification	Sedimentation, watershed geology, context for chemical measurements	Pre-2017: 3x per year 2017-present: 2x per year
Sediment archive	Provides community access to conduct measurements not being made by NEON	2022-present: 1x per year (fall)
Organic contaminants (PAHs, PCBs)	Organic contamination	pre-2017: 3x per year 2017-2019: 1x per year Discontinued in 2020

The sample strategy described in this protocol focuses on recently-deposited fine-grained surficial (1-3 cm in depth; Golterman et al. 1983; Keith 1991) sediments from depositional zones during low-flow conditions (USGS 1994). A depositional zone is defined as an area in a water body with a low energy regime. In streams, depositional zones are typically found at the inside of bends, within riffles, at pool lips, and downstream from obstacles (USGS 1994) (**Figure 1A**). In large, deep rivers, depositions zones are typically found at the inside of bends (**Figure 1B**). In lakes, deposition zones are often delimited as the deepest zone of the lake and areas with low gradients adjacent to high erosional and/or inflow regions (**Figure 1C**).

The sediment that is retained for analysis of chemical and physical properties in this protocol is defined as the fraction < 2 mm in grain size. Variation in the composition of bed surface sediments results from the inherent heterogeneity of the surrounding watershed and airshed. Many nutrients, metals, and other elements are most likely to be concentrated in sediments typified by fine particle size samples and high organic matter content (Lakhan et al. 2003). This association is largely dependent on the sorption capacity of fine sediments and organic matter imparted by their surface charges. Hence, even though element concentrations in the water column may be low, surficial bed sediments can contain large elemental concentrations.

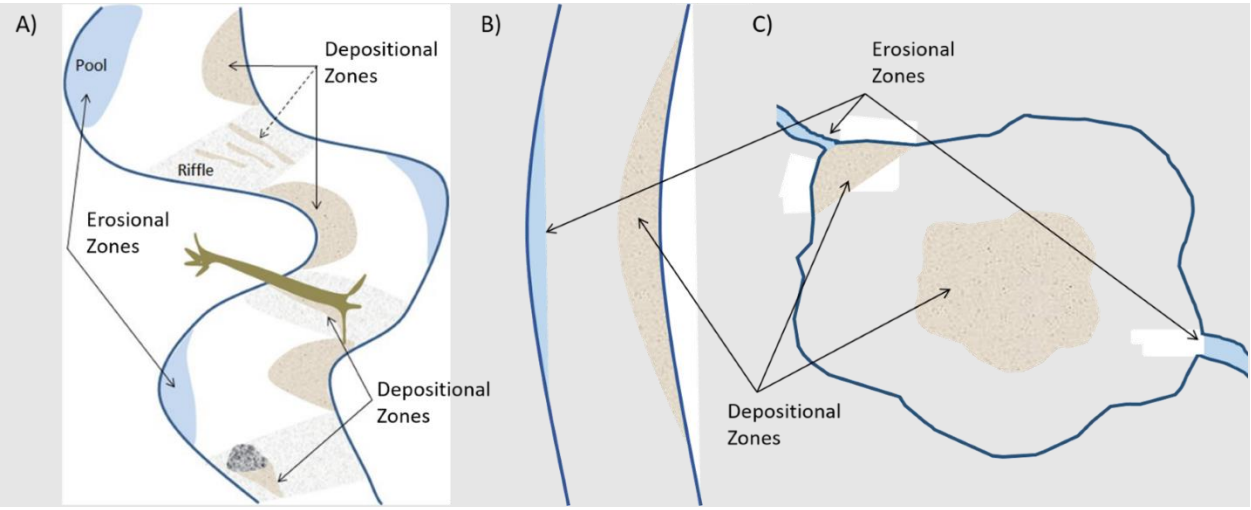

Figure 1. Examples of depositional zones in A) wadeable streams, B) rivers, and C) lakes.

In streams and rivers Sediment deposition patterns are a direct consequence of the flow characteristics of a waterbody. The appropriate season and hydrologic conditions for sampling stream bed sediment are determined by current and antecedent discharge conditions. Access to the sampling site can be limited during seasonal high-flow conditions. Unusually high flows can wash out, redistribute, or bury substantial parts of sediment deposits; therefore, sampling should be delayed following major discharge events to allow fresh sediment to deposit. When sampling for bed sediment during summer or autumn, low-flow conditions are recommended to provide maximum direct access to the stream bed and to minimize seasonal streamflow variability. In lakes, the deepest part of the lake is considered the equivalent to the depositional zones of a stream, since most sediment is transported to deeper zones over time through wind and current induced turbulence, a process known as focusing. However, caution must be exercised, since areas in a lake typified by large inflows and aeration also represent important depositional environments and areas of higher oxygen exchange and should be sampled accordingly.

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 modified versions of the following protocols or SOP documents:

United States Geological Survey (1994), Guidelines for Collecting and Processing Samples of Stream bed Sediment for Analysis of Trace Elements and Organic Contaminants for the National Water-Quality Assessment Program., Larry R. Shelton and Paul D. Capel, U.S. GEOLOGICAL SURVEY Open-File Report 94-458, Sacramento, California

U.S. Environmental Protection Agency (1994), Technical Standard Operating Procedure SOP #EH-02 Sediment Sampling (Adapted from ERT/REAC SOP #2016 Rev 0.0), 1994.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: Data Management
RD[05]	NEON.DOC.005326	Datasheets for AOS Protocol and Procedure: Sediment Sampling for Chemical and Physical Properties
RD[06]	NEON.DOC.003282	NEON Protocol and Procedure: Site Management and Disturbance Data Collection
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.004257	NEON Standard Operating Procedure: Decontamination of Sensors, Field Equipment and Field Vehicles
RD[09]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[10]	NEON.DOC.001197	AOS Protocol and Procedure: BAT – Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[11]	NEON.DOC.003045	AOS Protocol and Procedure: ALG – Periphyton and Phytoplankton Sampling
RD[12]	NEON.DOC.005224	NEON Protocol and Procedure: Shipping Ecological Samples and Equipment

2.3 External References

ER[01]	YSI Inc. 1998. Handheld Oxygen, Conductivity, Salinity and Temperature System, Operations Manual. ITEM # 038503, Revision E https://www.ysi.com/File%20Library/Documents/Manuals/605056-YSI-Pro2030-User-Manual-RevC.pdf

ER[02]	Wildco. 2010. Product Manual: 2424-A and 2424-B Series Hand Corer Instructions, Version 6/10, https://o0zmv2dxqw2iyiagw41cud1b-wpengine.netdna-ssl.com//wp-content/uploads/2017/04/2424-B-Hand-Corer.pdf
ER[03]	Wildco. 2013. Product Manual: 1728-G30/1728-G40 Petite Ponar Grab, Version 8/04, https://o0zmv2dxqw2iyiagw41cud1b-wpengine.netdna-ssl.com//wp-content/uploads/2017/04/1728-G30-1728-G40-petite-ponar.pdf

2.4 Acronyms

Acronym	Definition
C0	Station ID code for the deep lake center station
C	Celsius
$CaCO_3$	Calcium carbonate
cm	Centimeter
d	D
DI	Deionized
DO	Dissolved Oxygen
ft^2	Square foot
h	h
HNO_3	Nitric acid
IN	Station ID code for the shallow littoral station
km	Kilometer
L	Liter
LWD	Large woody debris
m^2	Square m
µm	Micrometer
µS	Microsiemen
MCB	Mid-channel bar
mg	Milligram
min	Min
mL	Milliliter
mm	Millimeter
N	Nitrogen
oz	Ounce
PFD	Personal flotation device
PROD	Production-level mobile application
S1	Sensor Set 1
S2	Sensor Set 2
s	Second
TOC	Total organic carbon
TC	Total carbon
TN	Total nitrogen

2.5 Definitions

Bout: Sampling event.

Benthic: Ecological region at the lowest level of a body of water such as an ocean, lake, or stream, including the sediment surface and some sub-surface layers

Decant: To draw off (a liquid) without disturbing the sediment or the lower liquid layers.

Depositional Zone: Area where sediments settle and accumulate on the stream bottom.

Flow Regime: A stream's naturally occurring changes in water flow through the course of a year.

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

Leaf-off: Time period where there is not foliage on flora species in the area of the site.

Leaf-on: Time period where there is foliage on flora species in the area of the site.

Point Sample: A single sediment grab sample that is composited within a station.

Pool: A stretch of a stream in which water velocity is low and the water depth is above average.

Reach: A stretch of a stream which includes riffles, pools and runs. NEON defines this area as the permitted reach for sampling.

Riffle: A stretch of a stream denoted as shallow and coarse bedded where water flows at a higher velocity and turbulence.

Run: A stretch of a stream where water flows smoothly.

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

Stage: The water level above some arbitrary point in the stream.

Station: Sediment sampling area in a site from which the composite sample is collected, representing the mean chemical and physical properties for a given area.

Thalweg: Deepest part of the stream or channel, usually the line of fastest water flow.

3 METHOD

In wadeable streams, the spatial distribution of sediment depositional zones depends on processes such as flow, turbulence, channel morphology, chemical precipitation, and turbidity, on physical factors and on catchment characteristics such as underlying geology, the number and size of inflows and land use (Ashley 1995; Kumke et al. 2005). In rivers, the channel is less sinuous, and the sediment sampling reaches are located within large bends in the channel. Sediment depositional zones in rivers are therefore distributed along the inside bank of bend, whereas the outside bank is an area of erosion (**Figure 1**). At stream and river sites the sediment sampling reach is defined as spanning 250 m upstream and downstream of the mid-way point of the 1 km biology and morphology reach (500 m sediment reach; (**Figure 2a,b**). Samples are collected from an upstream station (Station 1) located above the mid-way point of the reach and a downstream station (Station 2) located below the mid-way location of the reach (**Figure 2a,b**). At each station, 5 to 10 depositional zones are sampled.

At lake sites, sediment samples are collected from a littoral zone (Station IN) and at the deep center near the buoy infrastructure (Station C0; **Figure 2c,d**). The IN and C0 stations represent zones of recent deposition and long-term accumulation of sediment, respectively. At each station, 2 to 5 depositional zones are sampled.

Within a single depositional zone, 1 to 10+ point samples are collected depending on the sampling strategy (see **SOP B** for sampling strategies by site type) and are composited with samples from other depositional zones within the same station. Collecting a composite sample smooths spatial heterogeneity in sediment chemical and physical properties and allows for more accurate long-term monitoring of reach-scale sediment biogeochemistry. The number of samples from an individual zone will be based on the zone's surface area of (i.e., the larger the area of the zone, the greater the number of point samples collected). Generally, the sampling distribution should follow approximately 5 point samples per m^2. The priority is to collect a sample representative of the entire sampling reach; therefore, staff should prioritize collecting sediment from the maximum number of depositional zones rather than collecting the maximum number of point samples from each zone. Partially wetted depositional zones (< 5 cm water) should only be sampled when no other zones are available, and sampling conditions should be documented in the field notes and data records as a potential outlier.

Each point sample should consist of the surficial 1 to 3 cm of bed sediment. In wadeable streams the optimal sampler type depends on depth, flow, the presence of obstructions/debris, and sediment size (**Figure 3**; USGS 1994). A hand corer (**SOP B.3**) or hand scoop (**SOP B.4**) can be used to extract sediment in wadeable streams with minimal impact on the surface sediments depending on in-stream conditions. The hand corer is the optimal sampler type when collecting sediment in deeper (> 30 cm) and faster flowing (noticeable turbulence on water surface) zones because there is high potential for sample loss if an open-mouth hand scoop is used in these conditions (**Figure 3**; USGS 1994). It is only appropriate to use the hand scoop under the aforementioned conditions if 1) the sediment is obstructed to where the corer cannot properly extract, or 2) the corer was attempted but failed to retain the sample due to the sediment being too large or unconsolidated (**Figure 3**). The hand scoop is the optimal sampler type

when collecting sediment in shallower (< 30 cm) zones with little to no flow (no noticeable turbulence on water surface) (**Figure 3**; USGS 1994). Both the hand corer and scoop can be used interchangeably within a single station. For example, if sampling a shallow pool lip, the scoop is the optimal sampler type, then, in a stream run, the hand corer can be used to sample deeper sediment material. In rivers and lakes, a petite Ponar® sampler (**SOP B.7**) is used to collect deep sediments (**Figure 3**; USEPA 1994). When collecting sediment in the littoral zone of a lake site, the hand scoop may be used to collect sediment if it is safe/practical to sample outside the boat (**Figure 3**).

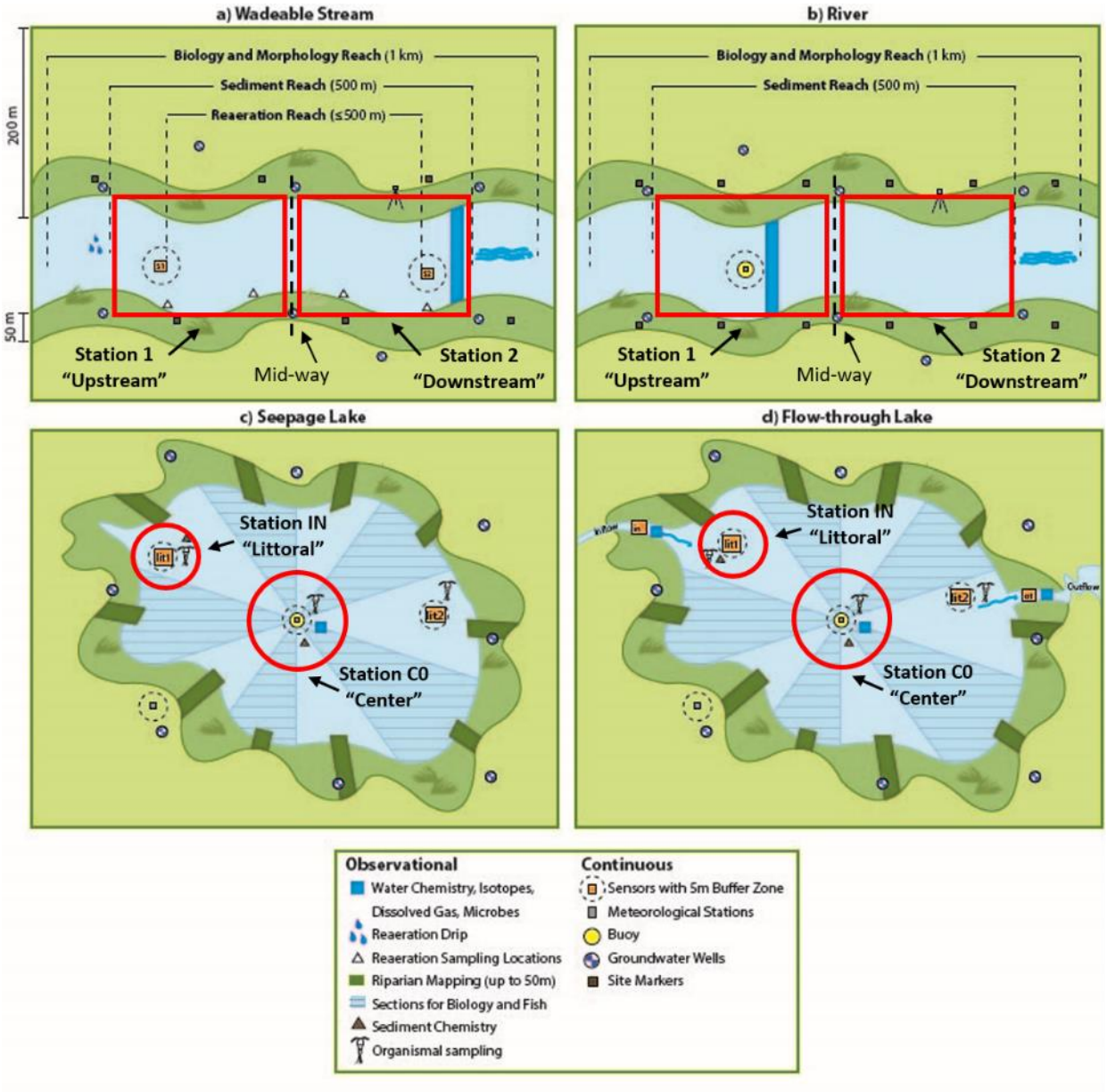

Figure 2. Generic site layouts for a) wadeable streams, b) rivers, c) seepage lakes, and d) flow-through lakes. Sediment sampling stations are outlined in red.

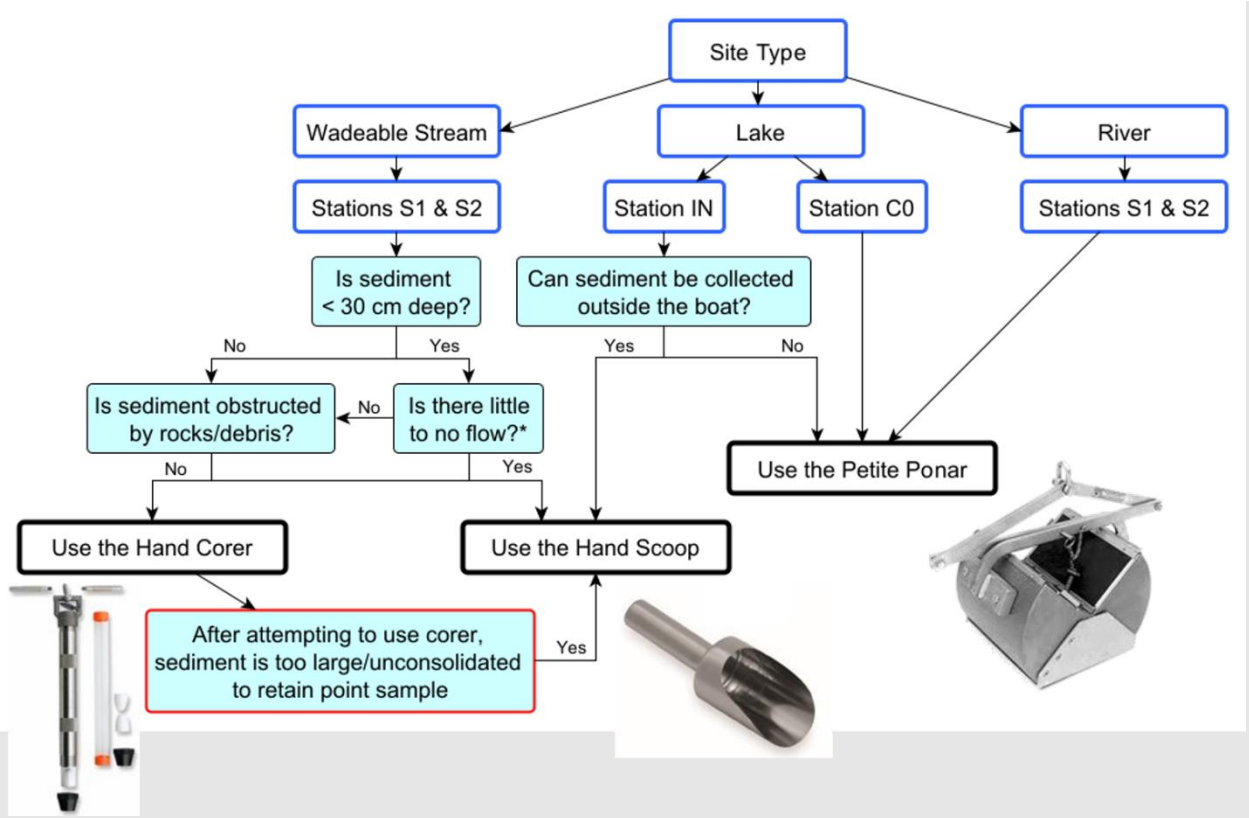

Figure 3. Decision flow chart for determining appropriate sampler type to use in the field by site type. The wadeable stream branch of the decision tree is based on the USGS 1994 sediment sampling SOP.

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 scientists **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 field scientists 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 Science Performance QA/QC Plan (AD[06]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Two stations per site are sampled twice per year; mostly during biological sampling bouts bout 1 and bout 3 (spring and fall). Sediment samples are collected after most other biological sampling activities, except fish sampling, in order to avoid disturbance of benthic habitats. Other benthic sampling activities conducted prior to sediment sampling (benthic algae and microbes) are not conducted on depositional zones, so there should be no disturbance to recently-deposited sediment prior to sampling.

1. **Bout 1 & 3:** Samples are collected for **chemical and physical properties**
    a. Sample containers (per station):
        i. 1x 8 oz (250 mL) glass jar for chemical properties
        ii. 2x 1 qt (1 L) plastic sealable bags for physical properties
            1. Fill 1 bag with 8-16 oz (250-500 mL) sediment depending on how wet the sediment is
            2. Use the 2nd bag to double bag the sample
2. **Bout 3 only:** Samples are collected for **archiving** at the NEON Biorepository
    a. Sample containers (per station):
        i. 4x 2 oz (60 mL) glass jars for sediment archive

4.2 Criteria for Determining Onset and Cessation of Sampling

Sample timing should reflect the influence of chemical and biological inputs and varying chemical conditions. Hence the temporal strategy for NEON samples will include sampling dates reflective of key times of leaf – on, leaf- off and peak greenness. The timing of such samples should reflect the hydrologic nature of the stream and the temporal variability of the system. A range of dates for each site were predetermined (**Appendix C**), based on historical data including streamflow, ice on/off days, the accumulation of degree days, weather, and riparian phenology (Didan 2015). Sediment in wadeable streams and rivers will be collected during periods of stable stream flow, following the same criteria as NEON benthic periphyton sampling (RD[11]).

4.3 Timing for Laboratory Processing and Analysis

Composite samples are to be collected and transported to the DSF on ice in sealed > 2 L glass jars. Upon arrival to the DSF, place composite samples in a refrigerator between 0-6°C (DO NOT FREEZE) and allow to sit undisturbed for a minimum of 2-3 d to allow suspended sediment to settle (USGS 1994). At some sites, particularly those with very fine silty sediment, composite samples may take longer than 2-3 d to settle. In this case, sediment can be left undisturbed to settle for a maximum of 9 d before processing. Once all fine sediment has settled in the composite sample (i.e., the overlying water is clear), staff should immediately decant, subsample, and process subsamples for shipping.

It is recommended that samples are shipped to the external laboratory and the NEON biorepository (Bout 3 only) within 24-72 h following processing and no later than 12 d following original sample collection (to ensure the samples arrive at the external lab within 14 d of collection). However, if it is expected to take longer to ship the samples, be sure to store them in a refrigerator between 0-6°C (DO NOT FREEZE) until the samples can be shipped. Samples must be shipped to the external lab on ice between 0-6°C but not frozen and arrive within 14 d of sample collection. Ship samples "Priority Overnight." DO NOT send them "FedEx First Overnight." If samples are shipped on Friday, send "Priority Overnight with Saturday Delivery." Be sure to mark the Saturday delivery box on the FedEx form. It is recommended that samples collected before a major holiday should be stored (refrigerated) at the DSF and shipped after the holiday to avoid shipping delays. Some Domains will ship via UPS and on a recommended range of d (e.g. Monday – Thursday). Refer to the Domain-specific shipping instructions provided by CLA. The external laboratory is required to return data within 90 d of receiving samples. Analyte-specific hold times are reported in **Table 2**.

Table 2. Analytical hold time for sediment chemical and physical properties by analyte class.

Sample Type	Required Analyses	Hold time
Chemical Properties (samples with ".SC" suffix)	Total organic carbon (TOC)	28 d from collection
	Total carbon (TC)	
	Total Metals: Aluminum, Antimony, Barium, Beryllium, Cadmium, Calcium, Chromium, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Phosphorus, Potassium, Sodium, Tin, Titanium, Zinc	Digested within 6 months of collection Analyzed within 6 months of digestion
	Mercury by Direct Combustion AA	28 d from collection
	Conductivity, Saturated Paste	28 d from collection
	pH, Saturated Paste	28 d from collection
	Total Alkalinity Bicarbonate as CaCO3 Carbonate as CaCO3 Hydroxide as CaCO3	Analysis within 12 d of extraction
	Total Nitrogen (TN)	Analysis within 28 d of extraction
	Nitrate as N, soluble (water)	Analysis within 28 d of extraction
	Nitrate/Nitrite as N, soluble (water)	Analysis within 28 d of extraction
	Nitrite as N, soluble (water)	Analysis within 28 d of extraction
	Nitrogen, ammonia (water)	Analysis within 28 d of extraction
	Grain size	Indefinitely
Physical Properties (samples with ".SS" suffix)	Soil texture analysis	Indefinitely

4.4 Sampling Timing Contingencies

All samples from both stations in one sampling site per bout must be collected within one day because sedimentation is a constant process in streams, rivers, and lakes. Spreading sample collection over multiple days increases data variability among samples. The timing of the sampling is flow dependent in wadeable streams and rivers, and precipitation dependent in lakes. Sediment samples can only be collected when velocity in a **wadeable stream** is below 0.93 m^2/s (Lane and Fay 1997) and when conditions are calm (< 9 km/h) in **river** and **lake** sites in order to ensure no sediment re-suspension. Should a major event occur that disrupts sediments at any aquatic site type, samples should not be collected for at least 5 d following a major event to allow time for safe conditions to return. Waiting at least 5 d following a major event also allows sufficient time for suspended sediments to settle in depositional zones. Sampling may be rescheduled due to weather or environmental conditions within the 10 d of the scheduled date provided that date is within the bout window (+ 3 d) provided in **Appendix C**. An incident ticket must be issued if sampling is rescheduled >10 d past the original scheduled date, or >3 d past the end of the bout window in **Appendix C**.

Table 3. Contingent decisions for implementation of the Sediment Sampling for Chemical and Physical Properties protocol.

Delay/ Situation	Action	Outcome for Data Products
Hours	If sampling stirred up sediments or added chemical constituents to the **wadeable stream/lake/river** (i.e., gas additions) within the past hour, allow the water to clear and disturbance to pass, sample upstream/upwind of the disturbance.	No adverse outcome
	If weather conditions deteriorate and conditions become unsafe (e.g., approaching thunderstorm, rapid increase of water level in the **wadeable stream**), or the **lake/river** becomes too windy [> 35 km/h at lake center station or > 9 km/h at littoral station] or 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 min. If conditions improve, resume sampling, if not, discard samples, return to the DSF 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 or More Days	Following a major precipitation event and resulting high flow (> 3x median discharge for the preceding year) at a **wadeable stream** or **river**, wait a minimum of 5 d after flow drops below 3x median discharge to allow time for	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside

	sediment to deposit and safe conditions to return to the stream.	of the sampling window, data may be flagged.

Because sediment is not collected during the biological sampling bout 2, spring sediment sampling (Bout 1) can be conducted < 14 d before the start of biological sampling bout 2 if safe conditions following spring runoff return within that timeframe. If spring sediment sampling can only be collected < 14 d before the start of biological sampling bout 2, an incident ticket must be issued. Bout 1 sediment sampling may still be cancelled if < 14 d before the start of Bout 2 if field staff determine sediment sampling cannot occur without significant disturbance to the benthic habitats in which algae/microbes/invertebrates are sampled.

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
- 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 Disturbance Reporting Protocol for more detail (RD[06]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates (**Appendix C**).
- **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled**: Missed Sampling is rescheduled for another time according to one of the scenarios documented in **Figure 4** 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 4**).

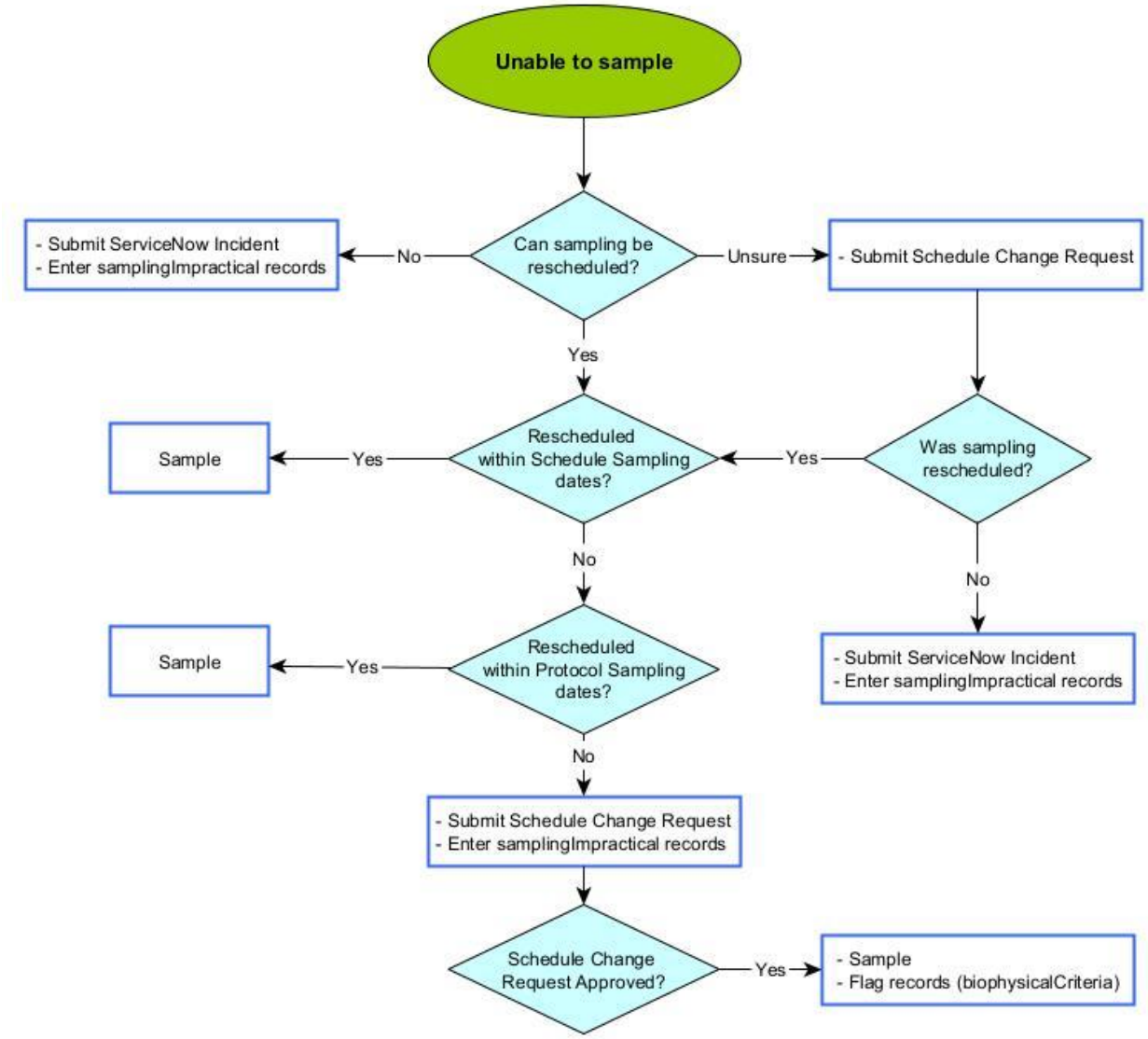

Figure 4. The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a Service Now incident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management record, or e) some combination of (a) – (d).

To Report Missed or Incomplete Sampling:

1. Missed or Incomplete Sampling that cannot be rescheduled within the Scheduled Sampling Dates sampling dates must be communicated to Science by a ServiceNow Incident
    a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 4**).

b. Guidance for this and other NEON protocols is summarized for ease of use in a table posted to a Field Science Sharepoint library. However, this protocol is the ultimate source of information should any discrepancy exist.

2. Create a Fulcrum record for each Missed Sampling event in the field that cannot be rescheduled.
    a. For sediment sampling, create a **Sampling Impractical** record for each station.
3. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device.
4. For Rescheduled sampling events that occur outside of the defined Protocol Sampling Dates, a protocol-specific Flag must also be recorded (**Figure 4**).
    a. Indicate in the **biophysicalCriteria** field.

Table 4. 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.

Sampling Impractical reason	Description
Location dry	Sampling station does not contain sustained flow nor disconnected pools of water.
Location frozen	Sampling station if covered in ice too thick to break through and safely sample sediment
Location snow covered	Sampling station contains snow too thick to remove and safely sample sediment
Logistical	Sampling station access compromised, staffing issues, errors (e.g., equipment not available in the field)
Other	Sampling location inaccessible due to other ecological reason described in the remarks

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.

We estimate wadeable stream sediment chemistry sampling requires 2 field scientists for 2-4 h of field work each sampling day plus travel to and from the site. Sample processing and subsampling at the DSF requires 1-2 field scientist(s) 2 h of laboratory work.

Table 5. Estimated staff and labor hours required for implementation of the Sediment Sampling for Chemical and Physical Properties protocol.

SOP	Estimated time	Suggested staff	Total person hours
SOP A: Preparing for Sampling	1 h	1	1 h
SOP B: Field Sampling	4 h	2	8 h
SOP C: Post-Field Sampling Tasks	1 h	1	1 h
SOP D: Laboratory Processing and Subsampling	2 h	1-2	2-4 h
SOP E: Data Entry and Verification	1 h	1	1 h

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. Activities in **wadeable streams** should only be performed when flows are safe. Do not attempt to wade a stream where flow is ≥ 10 ft^2/s (0.93 m^2/s; Lane and Fay 1997).
2. If the sampling trip involves the use of a boat (**lakes and rivers**), the weather forecast or marine conditions should be obtained prior to departure. The sampling trip should be rescheduled to a later date when conditions are suitable for working on or near water.
3. Due to accessibility constraints at some **lake/river** sites, most sampling will have to take place from the boat, without dismounting from the vessel.
4. When handling hazardous products (such as nitric acid) follow laboratory safety standards and have Safety Data Sheet (SDS) readily available to review prior to handling any chemicals. Wear gloves, a laboratory coat and protective eyewear.
5. Personal flotation devices (PFDs) shall be worn when wading in deep streams. Depth requirements may vary in different regions.
6. In areas with alligators or other submerged wildlife dangers, extra precaution must be taken. The crew will be required to not put hands and feet in the water and to make sure a safe distance is kept from alligators.
7. At extreme cold water sites, additional safety training may be required (example Oksrukuyik Creek) and special equipment (e.g. floatation jacket or PFD) may be needed for added safety.
8. When operating the petite Ponar®, take care to handle it when the boat is properly balanced to avoid dropping the sampler on people (feet), the boat, or equipment. Also, follow the safety guidelines below when opening, closing, deploying, and lifting the Ponar® to prevent injuries to fingers and hands.
    a. When not deploying the Ponar®, place the safety pin through both locking holes.
    b. During Ponar® deployments make sure that the boat is steady and that the sampler is deployed in a controlled manner but with enough velocity to penetrate the sediments and that the jaws close.
    c. Take care when retrieving the sampler when it is filled with sediments. Pull the line up in a steady, even motion. Avoid twisting or jerky motions to prevent back and shoulder injuries. It may be helpful for two or more people to pull up the sampler.

d. Wear protective gloves (e.g., leather gloves) to prevent rope burn when deploying and/or retrieving the Ponar®.

6 PERSONNEL

6.1 Training Requirements

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

Personnel are to be trained in sediment chemistry measurements and safe working practices for stream, river, and lake work. All personnel that wade to collect sediments shall review the USFWS Wader Safety Video as required by the NEON safety training program. The safety video is available with CSP2202-OLT Electrofishing Safety. All personnel required to operate a boat shall be trained through the NEON boater safety training program.

6.2 Specialized Skills

Where applicable, personnel will be licensed to operate a boat and be able to safely handle a motor and operate a boat while working.

7 STANDARD OPERATING PROCEDURES

SOP Overview

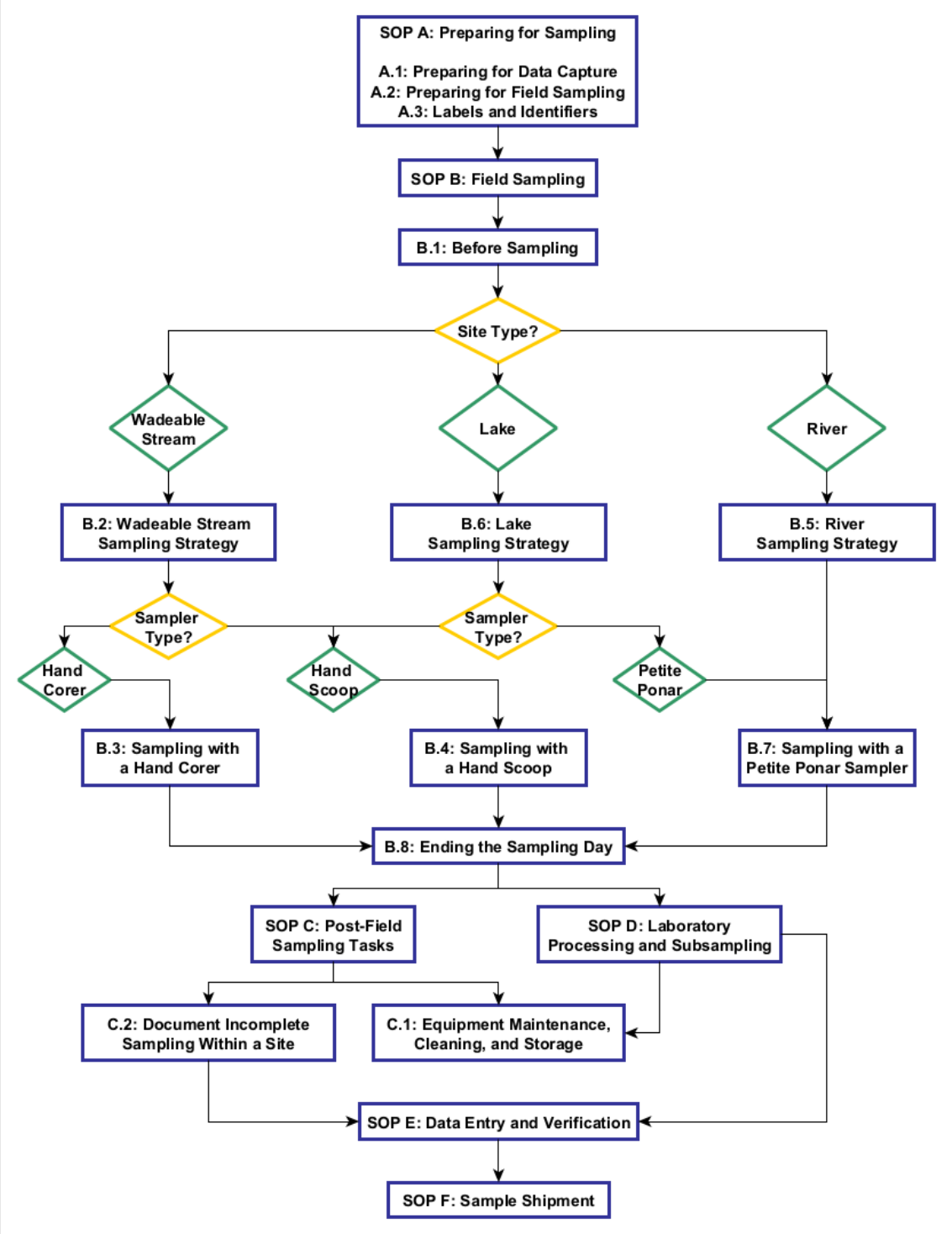

Figure 5. A high-level workflow diagram that visually shows how the separate SOPs are sequentially connected.

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

A NOTE ON SAMPLING EQUIPMENT USE TO IMPLEMENT THE SEDIMENT SAMPLING FOR CHEMICAL AND PHYSICAL PROPERTIES PROTOCOL

This protocol requires careful attention to the maintenance, cleaning, and storage of the equipment used to collect sediment samples. It is important to clean and store all equipment according to the intended use while sample in the field (**SOP C.1**) and to triple rinse all sampling equipment in the field with site water downstream of the sampling reach prior to sampling (**SOP B.1**). There is no single optimal sampler type with which to collect sediment for such a wide breadth of chemical and physical analyses (**Table 2**), plus sediment archiving, which completely eliminates the potential for cross contamination from the sampler or the residues on the equipment from cleaning. This protocol uses stainless-steel sampler types (hand corer, hand scoop, petite Ponar®) for sample collection, glass equipment for sample storage and processing, and a stainless-steel sieve for sediment archive subsampling. One consideration is that stainless-steel equipment will come into contact with sediment to be analyzed for trace elements, which cause some major agencies to discourage use of stainless-steel equipment (USGS 1994, USEPA 2001, UNEP 2006), but not others (IAEA 2003, USACE 2016). While metal (nickel, chromium) contamination in sediment samples has been recorded when new stainless-steel equipment is used, contamination is no longer detectable from the same equipment after multiple uses (USGS 2015). NEON's stainless-steel sampling equipment is used multiple times per year, carefully decontaminated between bouts (**SOP C.1**), and field rinsed with sediment and site water (**SOP B.1**), minimizing potential metal contamination. Another consideration is that samples collected for carbon analyses come into contact with equipment cleaned with methanol. To minimize potential methanol contamination, it is required that all sampling equipment be triple rinsed with site water prior to sampling (**SOP C.1**). Overall, the objective is to minimize the risk of contamination and to keep the equipment and cleaning methods consistent with every sediment sampling bout across NEON.

Ensure all equipment has been cleaned and stored appropriately (SOP C.1).

1. Order all consumable equipment (sample containers, shipping equipment, nitric acid, etc.) far in advance of the bout to ensure timely sampling and decontamination (see **Appendix E** Equipment).
2. Check the sediment chemistry field sampling kit to make sure all supplies are packed and batteries are charged.
3. Check the YSI Pro2030 conductivity calibration and recalibrate if necessary. The conductivity sensor should be calibrated monthly. See Conductivity Sensor User's Manual (ER[01]). Be sure when calibrating and using the conductivity meter that the holes at the top of the sensor are completely covered. (Note: DO will be calibrated every use, at the actual site). Maintain DO Sensor tip and/or refill electrolyte solution in tip on a monthly schedule.
4. Ensure GPS locations of sampling stations are entered into the GPS system.
5. For **wadeable stream** sites, mark the hand corer with 1 cm increments using a waterproof marker to help define the sampling depth.
6. Confirm type of sediment samples that will be collected and take the appropriate sample containers (**Figure 6**) and sampling equipment (**Figure 7**).
7. Mark the composite sample jars with a fill line specific to the bout using a waterproof marker or tape to help indicate when enough sediment has been collected. The fill line should indicate the target volume of bulk sediment, not the total volume of sediment plus overlying water containing suspended sediment.
    a. **Bout 1** target volume = 1 L
    b. **Bout 3** target volume = 1.5 L
8. Prepare a cooler large enough to securely transport composite samples from field site to DSF on ice between 0-6°C.
    a. The cooler should be large enough to fit 2 ≥ 2 L sealed glass jars that are separated from one another by wet ice.

Note: It is very important to thoroughly mix collected sediments in order to minimize the inherent environmental variability within sediments.

Figure 6. Example of amber glass jars with PTFE lined lid used for collecting sediment chemistry analyses and the sediment archive. Sediment for size analyses are collected in a 1 qt plastic sealable bag filled with approximately 8-16 oz (250-500 mL) of sediment and double bagged.

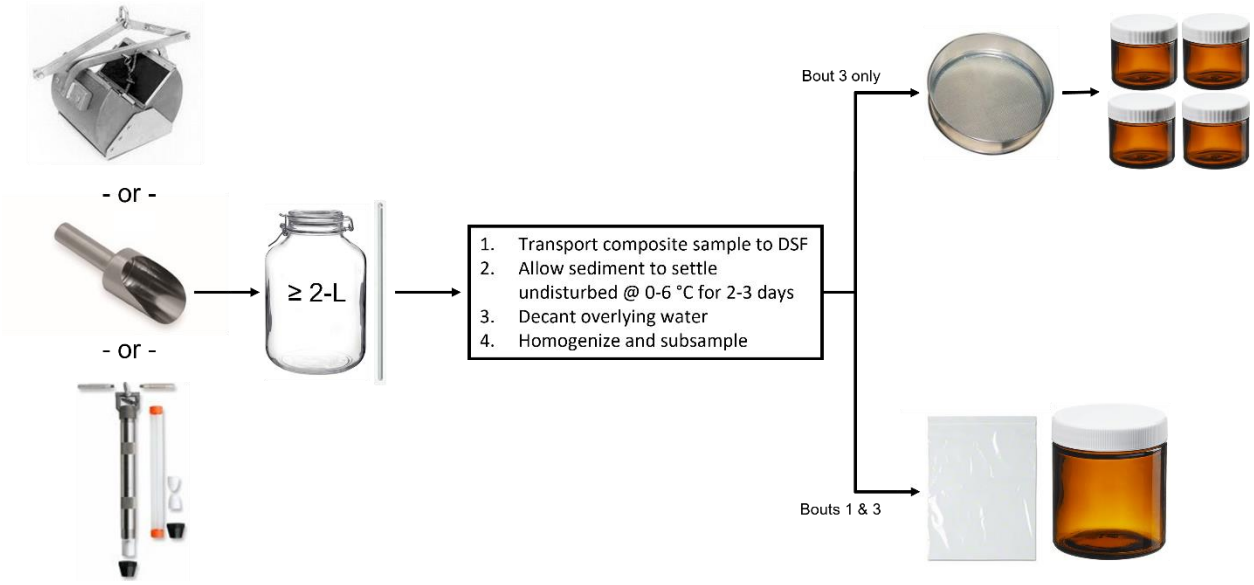

Figure 7. Sampling equipment used to implement the Sediment Sampling for Chemical and Physical Properties protocol.

A.3 Labels and Identifiers

Barcodes and pre-printed labels are useful for minimizing transcription errors and tracking samples from the (DSF) to external locations. **All field samples must use a barcode and all samples being shipped from the DSF must have a barcode** (**Table 6**). All barcodes and pre-printed labels need to be applied to dry containers for 30 mins before use. Type I (prefix A, plus 11 numbers) barcodes are for the sediment chemistry (*.SC*) and sediment size (*.SS*) subsamples that are shipped to the external laboratory; they have a tolerance from 4C to 105C and still scan. Type IV (prefix D, plus 11 numbers) are cryo safe barcodes usable on most cryo samples (rated for liquid nitrogen). Type IV barcodes are used for the sediment archive (*.SA*) subsamples. Labels are waterproof but should be filled out before getting wet to ensure ink is dry.

1. Determine the sampleID based on the sampling location, station ID, date, and subsample (siteID.stationID.YYYYMMDD.sampleType; **Figure 8**).
    a. siteID is the 4-letter site code for NEON aquatic field sites
    b. stationID is either Station "1" (upstream) or Station "2" (downstream) at **wadeable streams** and **rivers**, and Station "IN" (littoral) or Station "C0" (center) at **lakes**.
    c. sampleType is either "SS" (sediment size), "SC" (sediment chemistry), or "SA" (sediment archive).
    d. **NOTE:** Use the same time for all bottles filled at the same sampling station during each sampling event (i.e., the time the sediment was collected from the stream).
2. Attach pre-printed NEON sediment sampling bottle label and use a permanent marker to fill out bottle labels (**Figure 8**). Labels are waterproof but should be filled out before getting

wet to ensure ink will stick to the labels. Mark the correct sample type code (**Figure 8**) on the labels for each bottle.

3. Prepare final sample containers by affixing one Type I adhesive barcode label to each bottle or plastic bag used to contain sediment. Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 min prior).
    a. Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.

Table 6. Details on labeling and data entry for each sample type. A barcode is required for all sample types, including the composite sediment sample.

Sample Type	Data Entry App	Container Type	Required Barcode Used and Quantity	Human-readable Label Type	Location of Barcode
Sediment Chemistry - *.SC*	(AOS) Sediment [PROD]	8 oz (250 mL) amber glass jar	Type I 1 per station (2 per bout)	Weatherproof sample ID label	Side of vial oriented vertically
Sediment Size - *.SS*	(AOS) Sediment [PROD]	1 qt (1 L) sealable plastic bag (double bagged)	Type I 1 per station (2 per bout)	Weatherproof sample ID label	Flat area of inner bag (do not place on outer bag)
Sediment Archive - *.SA*	(AOS) Sediment [PROD]	2 oz (60 mL) amber glass jar	Type IV 4 per station (8 per bout)	Weatherproof sample ID label	Side of vial oriented vertically

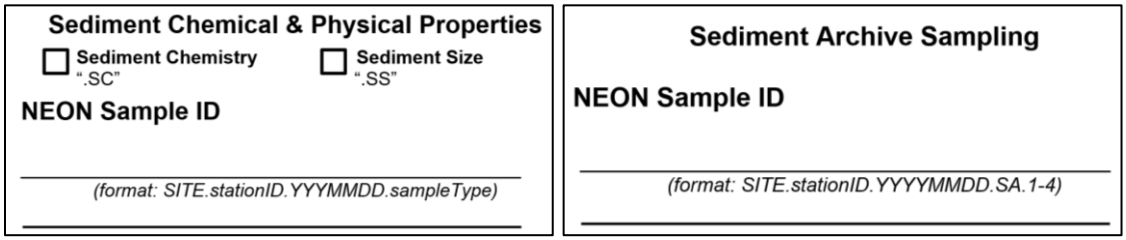

Figure 8. Example of NEON sediment container labels.

Figure 9. Examples of adhesive Type I and Type IV barcode labels.

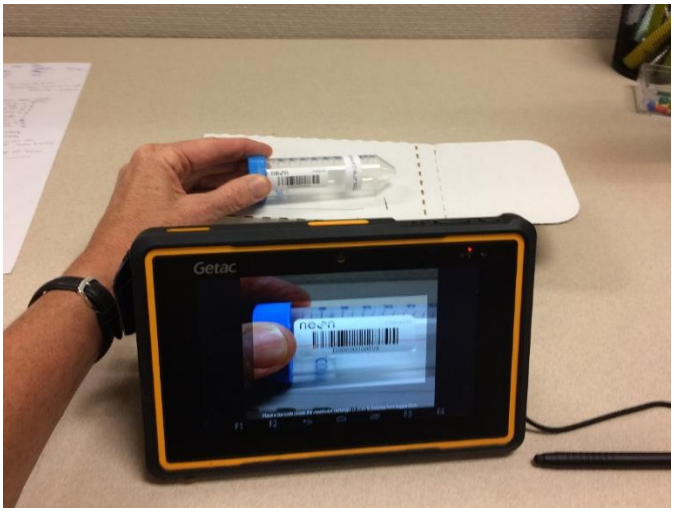

Figure 10. Barcode label scanning.

SOP B Field Sampling

B.1 Before Sampling

1. For each station, identify suitable depositional zones for sampling based on bathymetric (RD[10]) or geomorphology (RD[09] maps if available.
2. Protect the sampling equipment from contamination.
3. Calibrate the handheld YSI Pro2030 for DO before sampling (see instructions in ER[01]).
4. When in the field, rinse all sampling equipment three times with native water prior to use.
    a. Conduct the native water rinse outside (downstream if in a **wadeable stream** or **river**) of the sediment sampling stations.
5. If possible, rinse the sampler types with native sediment prior to use.
    a. Conduct the sediment rinse outside (downstream if in a **wadeable stream** or **river**) of the sediment sampling stations by taking a three "point samples" and immediately discarding them.

Due to the affinity of metals and other nutrients to bind to smaller sized particles (< 2 mm), this size fraction found in depositional zones better represents the potential quantification of sediment chemistry. The zones should not interfere with the biological sampling locations when possible; sediment sampling should occur > 5 m away from in-situ sensors. At sites that are limited by depositional zone areas or sediment substrate size, follow the instructions in **Figure 11**. The priority for these sites is to collect sufficient material for chemical and physical analyses. Indicate if the reach is inaccessible by recording **Sampling Impractical** (dry, frozen, snow, other) and document if the reach condition could affect the data collection (normal flow, segmented pools, partially wetted sediments, low flow, high flow, heavy vegetation, skipped station, other). If multiple reach conditions affect data collection, document the most impactful condition.

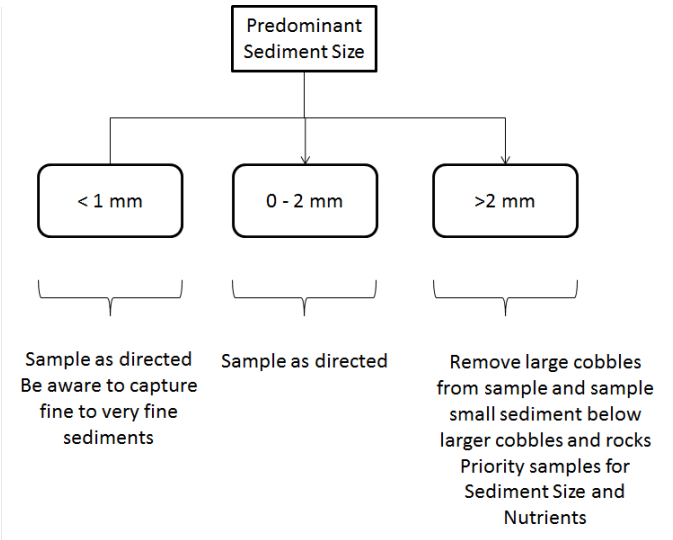

Figure 11. Decision flow chart for sampling sediment that is limited in quantity or type.

B.2 Wadeable Stream Sampling Strategy

Wadeable stream sediment samples shall be collected from 2 sampling stations within the 500 m sediment sampling reach (**Figure 2a**). Each station covers 250 m, or half of the 500 m sediment reach, and include the S1 and S2 sensors. The dividing point between the 2 sediment sampling stations is defined by the mid-way point between the top and bottom of the biology and morphology reach (**Figure 2**a). There are three 3 sampling strategies that can be implemented at sediment sampling stations in a wadeable stream site (**Figure 12**). Only 1 sampling strategy should be implemented throughout a station. The sampling strategies are based on the number, size, and distribution of depositional zones containing fine-grained particulate matter, and are informed by stream morphology data (**Figure 13**, see RD[09]). To determine the optimal sampling strategy for a site, consult the site characterization stream morphology maps, annual rapid habitat assessment surveys, and pebble count data when available.

WADEABLE STREAM SAMPLING STRATEGIES

1. **Optimal Sampling:** 5 - 10 depositional zones, 2 - 5 point samples per zone
   a. Used at most sites where there are ≥ 5 0.5-1 m^2 depositional zones in a 250 m station.
2. **Site-Specific Sampling: Small Zones Sampling:** ≥ 10 depositional zones, 1 point sample per zone
   a. Used at sites where sediment deposition is constrained to small areas with no large depositional zones (e.g., adjacent to debris, between rocks/roots, in small pools).
3. **Site-Specific Sampling: Large Zone Sampling:** 1 depositional zone, ≥ 10 point samples per zone
   a. Used at sites where the majority of the streambed in a 250 m station is composed of connected substrate that is < 2 mm in grain size.

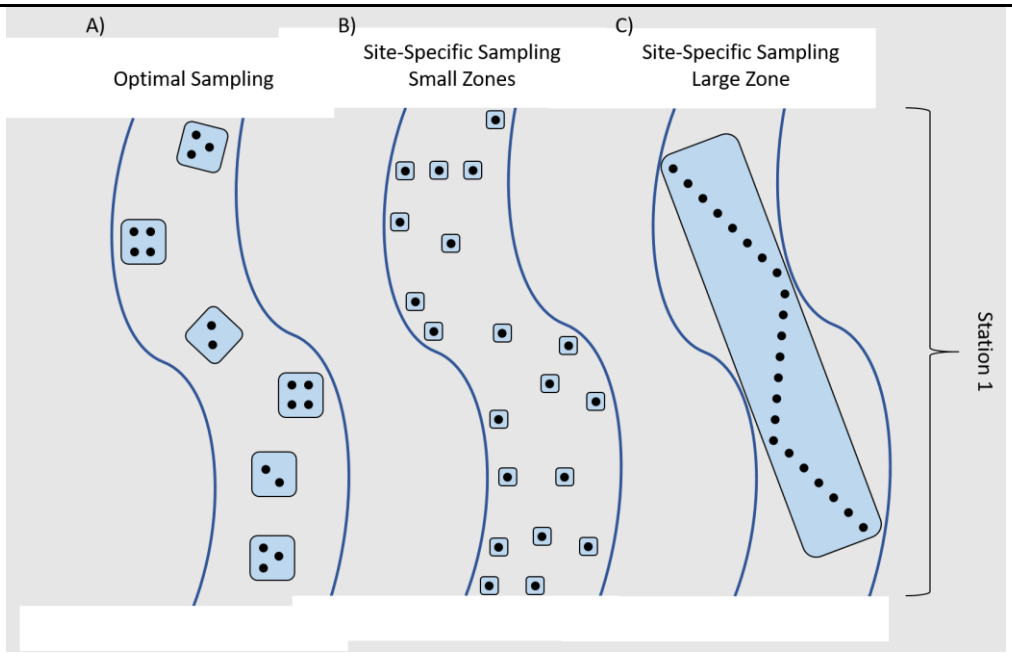

Figure 12. Sediment sampling strategies in wadeable streams based on the number, size, and distribution of depositional zones: A) optimal sampling, B) small zones sampling, and C) large zone sampling.

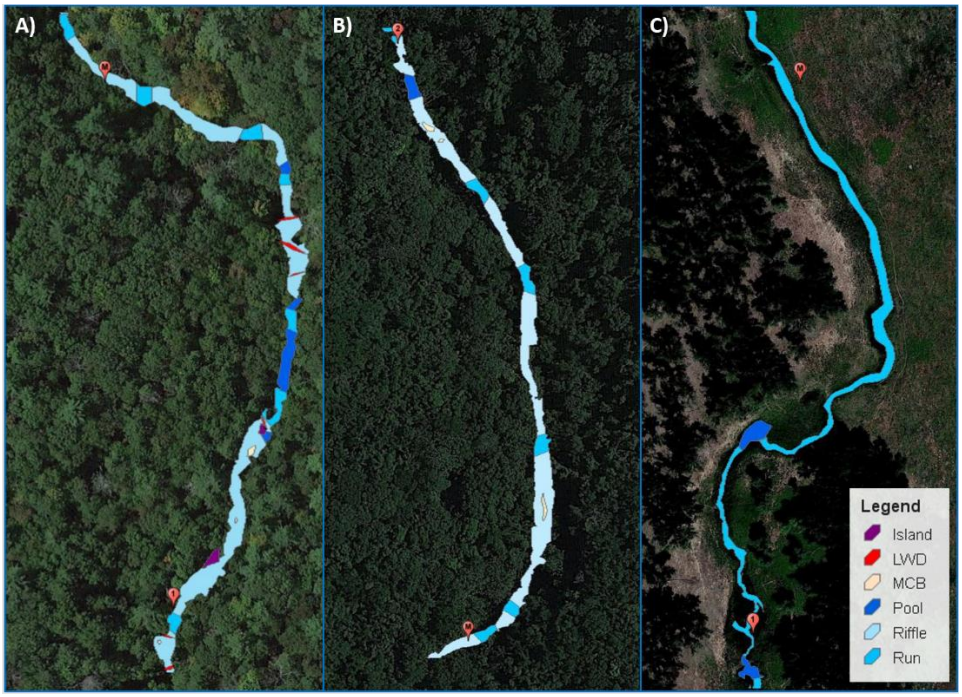

Figure 13. Examples of how NEON stream morphology maps can be used to inform sediment sampling strategy for a station in a wadeable stream (reach between red pins): A) Hop Brook (D01, MA) Station 1 contains many habitat type transitions and LWD, conditions that favor large depositional zones, so Optimal Sampling is preferred; B) Rio Cupeyes (D04, PR) Station 2 is majorly composed of riffle habitat, which is not conducive to forming large depositional zones, so Small Zones Sampling is preferred; C) Arikaree River (D10, CO) Station 1 is majorly composed of run habitat that forms a uniform sandy streambed, so Large Zone Sampling is preferred.

Per Station:

1. Select the optimal sampling strategy.
2. Start work at the most downstream station (Station 2) and zone, working from downstream to upstream in order to minimize sediment disruption. If sediment is disrupted, wait until the area is cleared before sampling.
3. Before sampling, complete the (AOS) Field Metadata [PROD] Fulcrum App.
4. Carry all sampling equipment in the stream (corer, scoop, mobile device, YSI, GPS, stir rod, composite sample jar, all other required material) to minimize the amount of movement between shore and depositional zones and, thus, sediment disturbance.

Per Depositional Zone:

1. Measure dissolved oxygen (mg/L), dissolved oxygen saturation (%), temperature (°C) and specific conductivity (µS/cm) with the YSI Pro2030 and record in the mobile device.
    a. Measure water quality using the YSI by holding it ~10 cm above the depositional zone in the water column.

b. For zones < 10 cm deep, measure water quality half-way between the water surface and the depositional zone.
c. Recording water quality in the mobile app by sampling strategy:
    i. **Optimal Sampling:** Record once at the zone-level. Data will auto-populate at the point-level.
    ii. **Small Zones Sampling:** Record once at the zone-level. Data will auto-populate at the point-level (one point per zone).
    iii. **Large Zone Sampling:** Record once per point at the point-level.

2. Collect GPS coordinates.
    a. For zones ≤ 5 m^2, collect coordinates from one center location.
    b. For zones > 5 m^2, collect coordinates from the downstream and upstream edges of the zone.
3. Record the dominant habitat type.
4. Record which sampler type will be used.
    a. See **Figure 3** for a decision tree on the optimal sampler type for depositional zones in a wadeable stream.
        i. Hand Corer (**SOP B.3**)
        ii. Hand Scoop (**SOP B.4**)
    b. Use the same sampler type throughout a depositional zone

Per Point Sample:

1. Using a foldable measuring stick, measure and record water depth.
2. Sample the sediment.
    a. **NOTE:** Regardless of sampler type, the approximate volume of sediment extracted per point sample should be **≤ 2 oz (~60 mL)**.
        i. This volume is approximately the volume of sediment extracted using the hand corer up to 3 cm deep into the sediment.
    b. When using the hand scoop, DO NOT extract > 2 oz of sediment per point sample.

Tip: To get a target volume of 1 L bulk sediment (> 1 L total volume with overlying water, a minimum of 17 point samples must be extracted across the length of the station. To get a target volume of 1.5 L bulk sediment, a minimum of 25 point samples must be extracted. Taking more than the minimum number of point samples is encouraged.

B.3 Sampling with a Hand Corer

The Wildco® hand corer **Figure 14** is the optimal sampler type in depositional zones that are found in deep (> 30 cm) and/or fast flowing water (USGS 1994). The hand corer works best when sampling straight into the sediment. If depositional sediment is obstructed by debris, rocks, roots, etc., do not use the hand corer to extract. At depositional zones in deep, fast flowing water that are not obstructed, the hand corer should always the first sampler type attempted, as it is optimal for sample quality (USGS

1994). If the hand corer is unable to properly extract sediment due to the sediment being too large or unconsolidated, the hand scoop becomes the optimal sampler type. Consult **Figure 3** for a sampler type decision flowchart.

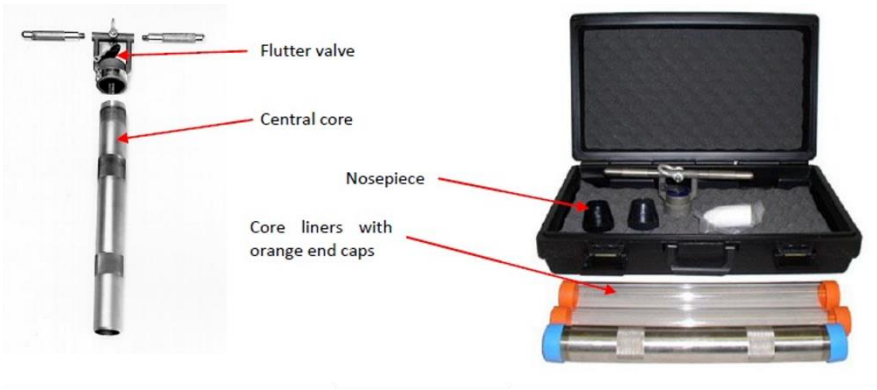

Figure 14. Hand corer for use in wadeable streams.

To sample sediment using the Wildco® hand corer (see ER[02] for instructions):

1. Put on nitrile gloves.
2. Assemble the hand corer
    a. Do not insert the plastic core liner or use the plastic nosepiece. All samples are to be collected using the stainless-steel core tube (see note in **SOP A.2**).
3. Triple rinse the hand corer with native water (**SOP B.1.4** and native sediment (**SOP B.1.5**) prior to use.
4. To operate the hand corer:
    a. Get in position for sampling over the targeted depositional sediment
        i. To ensure the extraction of fine (< 2 mm grain size) surficial (< 3 cm deep) sediment, avoid any disturbance to the bottom area to be sampled.
    b. Release the flutter valve.
    c. Aim the corer vertically and insert sampler into the sediment.
        i. The corer can be inserted > 3 cm into the sediment to ensure surficial sediment is retained
    d. Ensure the upper flutter valve is closed and lift the corer to the sediment surface maintaining it as vertical as possible. It may require using native water to keep the flutter valve wet for a good seal.
5. Cap the end of the corer before the sampler breaks the surface of the water to prevent the sample from washing out. Cap with the stainless steel core cap or other appropriate glass or stainless steel equipment (i.e., glass petri dish, beaker).
6. Discard all but the topmost 3 cm of sediment.
7. Inspect for adequate fine material

a. If not appropriate (e.g., contains mostly pebbles, detritus, organic material), discard the point sample downstream of the depositional zone and resample

8. Deposit the point sample in the composite sample jar and proceed to next point sample or depositional zone.
9. Clean the hand corer with the scrub brush and triple rinse the hand corer with native water downstream of the depositional zone.

B.4 Sampling with a Hand Scoop

A hand scoop (**Figure 15**) is the optimal sampler type in depositional zones that are found in shallow (< 30 cm), slow flowing water (USGS 1994). If depositional sediment is obstructed by debris, rocks, roots, etc., the hand scoop is the optimal sampler type. Consult **Figure 3** for a sampler type decision flowchart.

Note: Sample quality is jeopardized when the hand scoop is used in suboptimal conditions because fine-grained sediment can easily be lost in the water column from an open-mouth hand scoop. When using the hand scoop, take great care to protect fine sediment from being washed away as the sample is brought to the water surface.

Figure 15. Scoop samplers are used for collecting sediment in wadeable streams and lakes.

To sample sediment using the hand scoop:

1. Put on nitrile gloves.
2. To operate the hand scoop:
    a. Remove the top layer (approximately 1-3 cm) of fine sediment carefully by gently scooping in the upstream direction.
        i. DO NOT extract > 2 oz (~60 mL) total per point sample with the hand scoop.
    b. Bring the sample to the water surface taking care that no material is lost downstream as the scoop moves up the water column.
    c. Slowly pour off most of the overlying water over one of the BACK corners of the scoop.
        i. Make sure that the top layer of fine sediment is not discarded.
        ii. If suspended fine sediment is being poured off, stop pouring off immediately.
3. Inspect for adequate fine material.

a. If not appropriate (e.g., contains mostly pebbles, detritus, organic material), discard the point sample downstream of the depositional zone and resample.
b. Do not sample large (> 4 mm) material. If pebbles, cobbles, detritus, or biota are present in the sample, gently remove using stainless-steel featherweight forceps.

4. Deposit the sediment in the composite jar and proceed to next point sample or zone.
5. Clean the hand scoop between each point sample by triple rinsing with native water downstream of the depositional zone.

B.5 River Sampling Strategy

Non-wadeable stream sediment samples shall be collected from two previously identified sediment-sampling stations within the reach (**Figure 2b**). The station divide is defined by the mid-way location between the top and the bottom of the aquatic reach. At each of the 2 sampling stations, identify at least 5 depositional zones containing fine-grained particulate matter (**Figure 16**). Identification of depositional zones can be aided by using the most recent bathymetric map (**Figure 17**; see RD[10]). The optimal sampling strategy at a river site is to collect sediment along the inside of a bend in the river. The most recent depositional sediment will be found on the inside of a river bend where the water velocity and energy regime are lowest (Johannesson and Parker 1989). To ensure the collection of a composite sample representative of the most recent depositional sediment in a station, collect 1 Ponar® grab sample from ≥ 5 depositional zones that span the length of the station (**Figure 16**).

Per Station:

1. Start work at the most downstream station (Station 2) and zone, working from downstream to upstream in order to minimize sediment disruption. If sediment is disrupted, wait until the area is cleared before sampling.
2. Before sampling, complete the (AOS) Field Metadata [PROD] Fulcrum App.

Per Depositional Zone (1 Point Sample per Depositional Zone):

1. Position the boat at the appropriate location and lower the anchor gently to not suspend any sediments. Contamination is minimized by anchoring the boat downstream of the sampling site and using an anchor line 3x as long as the depth of the non-wadeable stream.
2. Measure dissolved oxygen (mg/L), dissolved oxygen saturation (%), temperature (°C) and specific conductivity (µS/cm) with the YSI Pro2030 and record in the mobile device.
    a. Measure ~50 cm above the depositional zone in the water column.
    b. Record once at the zone-level. Data will auto-populate at the point level
3. Collect GPS coordinates from one center location (the Ponar® drop point).
4. Record the dominant habitat type and sampler type (petite Ponar®, **SOP B.7**).
5. Using a foldable measuring stick, weighted measuring tape, or handheld depth reader, measure and record water depth.
6. Sample the sediment. To allow ≥ 5 Ponar® grabs per station, the approximate volume of bulk sediment extracted per point sample should be **4-6 oz (~120-180 mL)**.

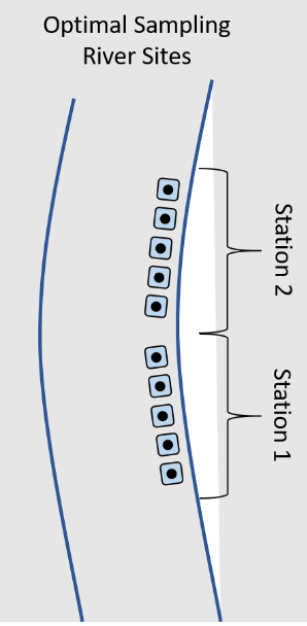

Figure 16. Optimal sediment sampling strategy in rivers based on flow and energy regimes that determine depositional zones.

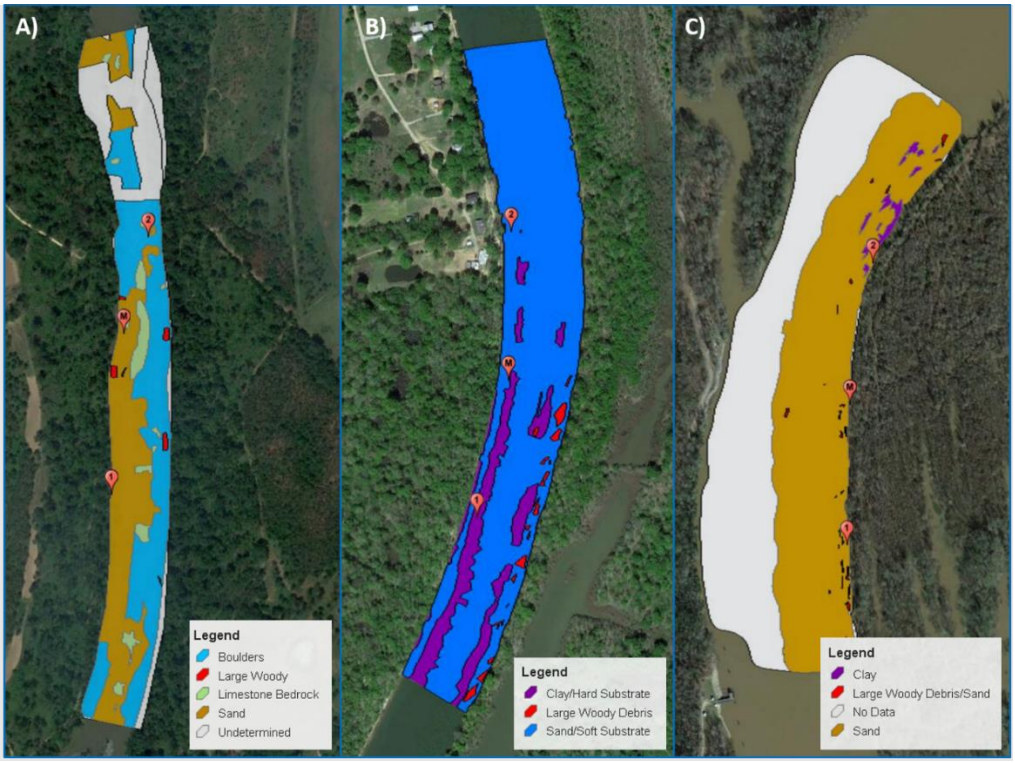

Figure 17. Examples of how NEON bathymetric maps can be used to inform the distribution of fine sediment in a station in a river (reach between red pins). Note the sediment sampling reaches are located in a sinuous stretch of river. The river sites shown are A) Flint River (D03, GA), B) Black Warrior River (D08, AL), and C) Tombigbee River (D08, AL).

B.6 Lake Sampling Strategy

Lake sediment samples will be collected from two stations: Station C0 (central; near the buoy) near the deepest part of the lake which represents the maximum long-term accumulation of sediment. Station IN includes in-shore sediments in the lake littoral zone, which represents an area of shorter-term sediment transport and deposition (**Figure 2c,d**). The sampling zones must be 5 m from aquatic sensors (littoral sensors and buoy) but no more than 10 m beyond the sensor exclusion zone (**Figure 18**). The optimal sampling strategy at a lake site is station-dependent. While the physical composition of deep lakebed sediment can be relatively homogenous, sediment chemical properties can contain a high degree of spatial heterogeneity (Vogel et al. 2010, Yong et al. 2012). Therefore, at Station C0, 2-5 depositional zones should be sampled to capture spatial heterogeneity in sediment biogeochemistry (**Figure 19**). Staff should avoid conducting > 2 Ponar® grab samples per depositional zone because the impact of the petite Ponar® will disturb surrounding sediment in the immediate vicinity of the drop point. The optimal distribution of point samples in a Station C0 depositional zone is 2 point samples collected from opposite corners of the boat (e.g., 1 off the port bow, 1 off the starboard quarter). Staff should also collect sediment from 2-5 depositional zone at Station IN (**Figure 19**), but the number of point samples per depositional zones can be > 2, especially if the scoop sampler is being used for collection. Either the petite Ponar® or hand scoop can be used to collect littoral sediment in Station IN depending on the depth of the littoral zone, distribution of fine sediment (i.e., evenly distributed, patches between rocks/roots) and the safety, logistics, or permissions for working outside the boat.

Per Station:

1. Before sampling, complete the (AOS) Field Metadata [PROD] Fulcrum App.
2. Navigate to either the buoy or littoral infrastructure of the lake using the GPS coordinates and the site map provided.

Per Depositional Zone:

1. Position the boat at the appropriate location and lower the anchor gently to not suspend any sediments.
2. Measure dissolved oxygen (mg/L), dissolved oxygen saturation (%), temperature (°C) and specific conductivity (µS/cm) with the YSI Pro2030 and record in the mobile device.
    a. **Station C0:** Measure ~50 cm above the depositional zone in the water column.
    b. **Station IN:** Measure ~10 cm above the depositional zone in the water column.
        i. Water quality in shallow littoral zones should be homogenous, so only 1 measurement is necessary per depositional zone, regardless of size.
    c. Record once at the zone-level. Data will auto-populate at the point level.
3. Collect GPS coordinates.
    a. **Station C0:** Collect from one center location (the Ponar® drop point).
    c. **Station IN:** For zones ≤ 5 m^2, collect coordinates from one center location. For zones > 5 m^2, collect coordinates from the downstream and upstream edges of the zone.

4. Record the dominant habitat type.
5. Record which sampler will be used.
    a. **Station C0:** Should always be petite Ponar® (**SOP B.7**).
    b. **Station IN:** See **Figure 3** for a decision tree on the optimal sampler type for depositional zones in the lake littoral zone.
        i. Petite Ponar® (**SOP B.7**).
        ii. Hand scoop (**SOP B.4**).
        iii. Use the same sampler type throughout a depositional zone.

Per Point Sample:

1. Using a foldable measuring stick, weighted measuring tape, or handheld depth reader, measure and record water depth.
2. Sample the sediment.
    a. **Station C0:** To allow for 2-4 Ponar® grabs in the station across at least 2 depositional zones, the approximate volume of bulk sediment extracted per point sample should be **6-8 oz (~180-250 mL)**.
    b. **Station IN:** When using the hand scoop, DO NOT extract **> 2 oz (~60 mL)** of sediment per point sample. When using the petite Ponar®, the approximate volume of bulk sediment extracted per point sample should be **4-6 oz (~120-180 mL)**.

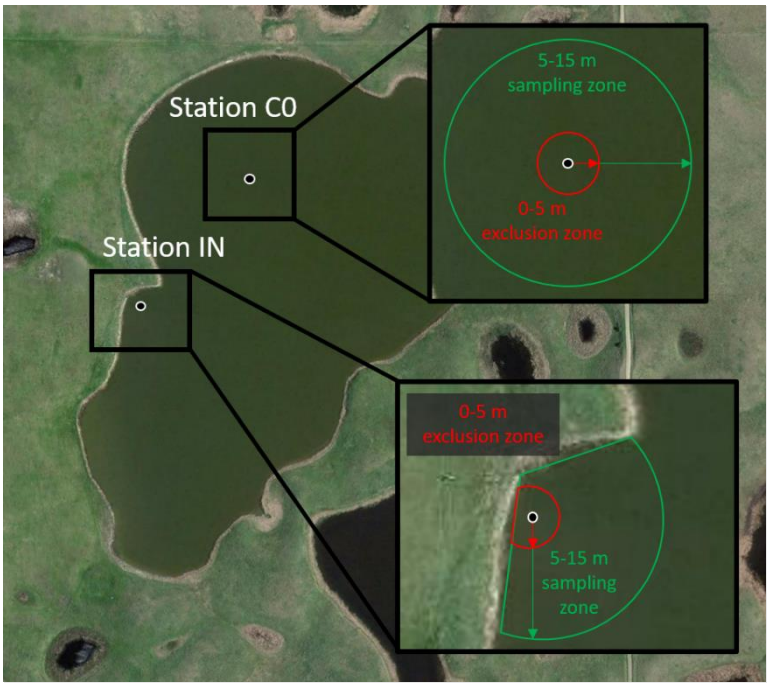

Figure 18. Example of how stations and sampling zones are determined at NEON lake sites, showing Station C0 (lake center), Station IN (littoral), 5 m exclusion zone from NEON sensor infrastructure (red circles), and 10 m sampling zone outside exclusion zone (5-15 m from sensor, green circles). The lake site shown is Prairie Pothole (D09, ND).

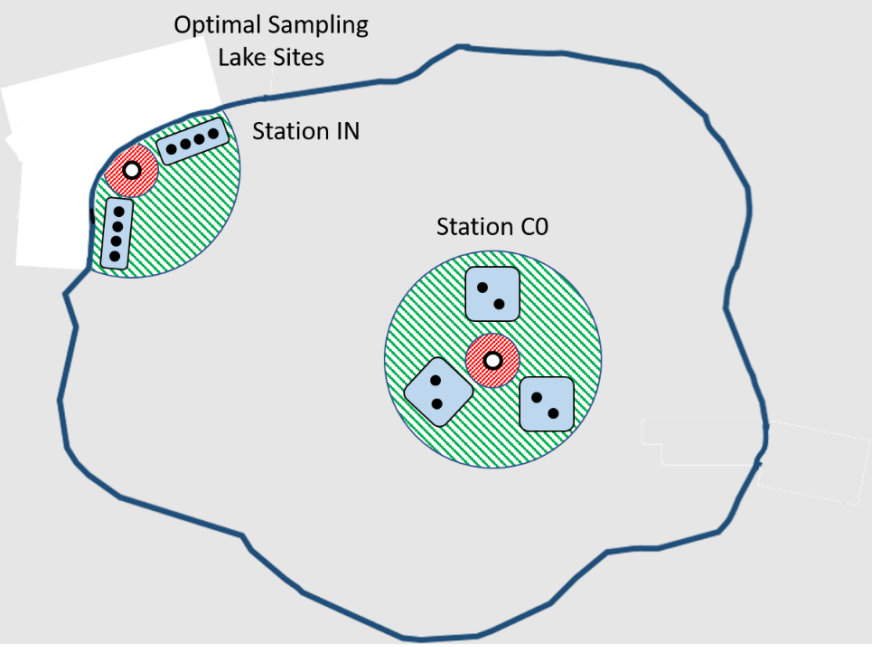

Figure 19. Station-specific optimal sampling strategies in lakes.

B.7 Sampling with a Petite Ponar® Sampler

The Wildco® petite Ponar® (**Figure 20**) is the optimal sampler type in deep depositional zones that are found in lakes and rivers (EPA, 1994).

Figure 20. Petite Ponar® sampler for use in lakes and non-wadeable streams.

To sample sediment using the Wildco® petite Ponar® (see ER[04] for instructions):

1. Put on nitrile gloves.
2. Triple rinse the sampler with native water.
3. Place two buckets in the boat that will be used to collect excess sediment from the Ponar® to minimize contamination of successive grabs.
    a. Dump bucket: use one bucket to discard sediment from the Ponar® that was not sampled into the composite sample jar. Use of the dump bucket will ensure

discarded sediment will not reenter the station during sampling. The bucket can be emptied back into the lake/river once all sampling at the station is complete

b. Rinse bucket: rinse the Ponar® by either submerging it into the rinse bucket filled with native water or pouring native water over it into the rinse bucket.
    i. River sites with sustained flow can simply rinse the Ponar® in the water column as the residual sediment will be transported downstream, thus minimizing contamination risk.

4. Set the grab sampling device with the jaws cocked open (**Figure 20**). Replace the straight safety pin with the spring-loaded pin for deployment.
    a. **NOTE:** great care should be taken while handling the device while it is set; accidental closure can cause serious injuries.
5. Ensure that the rope is securely fastened to the sampler, and that the other end of the rope is tied to the boat.
6. Lower the sampler until it is resting on the sediment (its own weight is adequate to penetrate soft sediment). Be sure that the sampler does not descend too fast to avoid suspending the fine sediment and so that the sampler only collects the top 3 cm of material.
    a. At this point, the slackening of the line activates the mechanism to close the jaws.
    b. It is also recommended to use a weighted "messenger" to ensure the jaws close.
        i. Attach the messenger to the rope and push it down to trigger closure of the sampler.
        ii. Make sure the rope is taut to ensure the messenger does not lose its effectiveness.
7. Retrieve the sampler slowly and steadily to minimize the effect of turbulence that can cause loss of fine material.
8. From the top of the Ponar®, scoop sediments out from the center taking care to avoid removing material that is touching the metal internal surface of the Ponar®. Place this material in the composite sample jar.
    a. Do not sample large (> 4 mm) material. If pebbles, cobbles, detritus, or biota are present in the sample, gently remove using stainless-steel featherweight forceps.
9. Between each point sample:
    a. Discard all excess sediment into the dump bucket.
    b. Rinse the Ponar® using the rinse bucket.
10. Between each depositional zone:
    a. Clean the Ponar® with the scrub brush.
    b. Triple rinse the Ponar® in the water column.
    c. Make sure any visible residual sediment is removed from the sampler.

<u>NOTE</u>: If the jaws were not closed completely enough to trap a sufficient volume of sediment, the sample must be discarded. Discard the sample into a bucket if the second collection attempt is made from the same general area. Dump the unwanted sample back into the water only after all samples have been successfully collected in a station.

B.8 Ending the Sampling Day

When sample collection is complete, do the following before ending the sampling day:

1. Immediately record observations regarding the appearance of the sediment (i.e., texture, color, odor, presence of biota, presence of detritus).
2. Gently place the composite samples in a cooler with ice immediately upon returning to shore.
    a. Separate the composite jars with ice or other material to avoid breakage during transport.
3. Dispose of any excess sediment not collected in the composite samples near the shore, but away from depositional zones.
4. Decontaminate all equipment according to RD[08] before using at another site. For equipment-specific cleaning, see **SOP C.1**.

SOP C Post-Field Sampling Tasks

C.1 Equipment Maintenance, Cleaning, and Storage

1. Ensure all containers, tools, and equipment used for sediment sample collection are cleaned prior to storage or before initiating sampling:
    a. Rinse equipment and containers to remove obvious residual sediments (dump rinsate into an appropriate waste container; large sediments could clog lab sink drains over time).
    b. Prepare a tub with 0.2% phosphate-free detergent.
    c. Wash containers/equipment in the detergent bath.
    d. Soak the containers/equipment for at least 30 min.
    e. Rinse thoroughly with de-ionized water three times using new water each time.
    f. For all glass equipment, proceed to **SOP C.1.2**.
    g. For all stainless-steel equipment, proceed to **SOP C.1.3**.
    h. For all remaining equipment, allow to air dry.
2. Additional cleaning instruction for **glass** equipment:
    a. After the detergent soak described above, rinse with the containers/equipment with 5% high purity nitric acid (HNO_3).
        i. Here is an example for making 1.0 L of 5% HNO_3 with 69.1% nitric solution. Mix 72.36 mL HNO_3 with 927.64 mL tap water. **ALWAYS add acid to water!**
        ii. VERY IMPORTANT, consult the Chemical Hygiene Plan and Biosafety Manual (AD[03]) for disposal of acid waste or other hazardous chemicals.
    b. Rinse three times with DI water and allow to air dry.
3. Additional cleaning instruction for **stainless-steel** equipment (including all sampler types and the stainless-steel sieve):
    a. After the detergent soak described above, rinse containers and equipment with methanol from the Teflon wash bottle.
    b. Do not rinse with DI water.
    c. Allow methanol rinsed equipment to air dry.
4. Long-term storage:
    a. For most sampling equipment, once dry, place aluminum foil over any openings of glass or stainless-steel equipment
    b. Once dry, store the hand corer in the carrying case provided by the manufacturer. DO NOT store wet!
    c. Store the petite Ponar® with safety pin inserted and aluminum foil over the top opening. DO NOT store wet!
    d. Once decontaminated, avoid exposing any sampling equipment to plastic. For example, do not store equipment in plastic bags or buckets.

C.2 Document Incomplete Sampling Within a Site

Sediment Chemistry sampling is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in **Section 4.1** and **Appendix C**. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (gradient sites). However, sampling may be shifted from one location to another when sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced.

There are two main pathways by which sampling can be compromised. First, sampling locations can become inappropriately suited to answer meaningful biological questions – e.g., a terrestrial sampling plot is compromised after road-building activities, or a stream moves after a flood and the location is no longer within the stream channel. Second, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

If sampling at a given plot is not possible during a given bout a problem ticket should be submitted by Field Science staff.

To document locations not sampled during the current bout:

1. Review Fulcrum records to determine which locations were scheduled for sampling but were not sampled.
2. Create an incident with the following naming convention to document the missed sampling: 'AOS/TOS Sampling Incomplete: MOD – [Root Cause Description]
    a. Example: 'TOS Sampling Incomplete: CDW – Could not access plot due to permanently closed road'
3. Staff scientists review incident tickets periodically to determine whether a sampling location is compromised.

SOP D Laboratory Processing and Subsampling

D.1 Settle and Decant.

All composite samples are to be decanted of overlying water prior to subsampling to ensure the sediment that is shipped to the external laboratory and NEON biorepository contain the least amount of water possible. Little-to-no overlying water maximizes the amount of analyzable sediment in a sample jar, minimizes the chance of breakage during transport and storage/archiving, and decreases laboratory processing time.

1. Upon returning to the DSF, immediately place the composite sample jars in cold storage (0-6 °C). DO NOT FREEZE.
    a. Composite sample jars should be place in a location that will minimize disturbance during settling (i.e., will not need to be moved around to access other material in cold storage).
2. Enter the date and time the composite sample jars were placed in cold storage to begin settling in the mobile app.

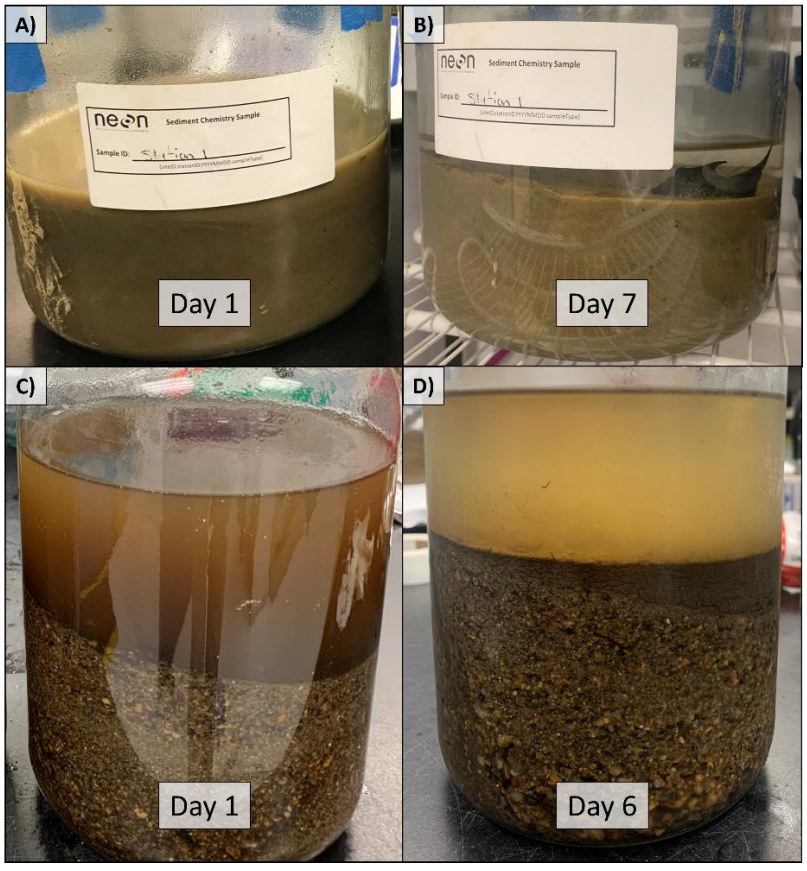

Figure 21. Images of sediment settling out of the overlying water in a composite sample jar. Images A and B are from a river site (BLWA) with loamy sediment that took 7 days to settle. Images C and D are from a stream site (HOPB) with sandy sediment that took 6 days to settle.

3. Allow sediment to settle.
    a. Sediment should settle for a **minimum of 2-3 d** (USGS 1994).
    b. At some sites, particularly those with very fine silty sediment, composite samples may take longer than 2-3 d to settle. In this case, sediment can be left undisturbed to settle for a **maximum of 9 d** before processing.
    c. When all fine sediment has settled in the composite sample jar (overlying water is visibly clear, **Figure 21**), the sample is ready to decant.
4. Gently remove the composite sample jar from cold storage in such a way that does not resuspend the top layer of sediment and place on the lab bench.
    a. To hold the decanted water, either place the composite sample jar near a sink, or place a tub next to the composite sample jar on the lab bench.
5. Allow the composite sample to sit for 5-10 min so any sediment that may have resuspended during removal from cold storage can re-settle.
6. Record the date and time of decanting in the mobile app.
7. Put on nitrile gloves.
8. Decant the overlying water from the composite sample jar to a tub/sink using a peristaltic pump (**Figure 22**).
    a. The peristaltic pump should be fitted with peristaltic tubing connected to ¼ in Inner Diameter (I.D.) C-Flex tubing on either end.
    b. Place a **CLEAN** end of the tubing no more than 2 cm deep in the overlying water (**Figure 22b**) so as to avoid perturbation of the sediment and minimize the area of tubing exposed to the overlying water.
    c. Place the other end of the pump tubing into a sink or waste tub.
    d. **SLOWLY** pump overlying water out of the sample jar into a waste tub/sink.

Figure 22. Decanting from the composite sample jar into a sink using the peristaltic pump.

NOTE: Keep the tubing at a constant 1-2 cm deep in the overlying water so as to not perturb the sediment by being too deep.

e. If any sediment is resuspended while pumping, stop decanting and allow the sediment to settle for 5-10 min.
f. Decant until there is ~1 cm of overlying water in the composite sample jar.

9. Discard the decanted water.
10. Repeat steps 1-9 for the composite sample from the other station. Rinse the tubing with DI water before decanting the next composite sample.

D.2 Subsampling for the External Laboratory

The samples that are shipped to the external laboratory include the sediment chemistry (*.SC*) and sediment size (*.SS*) subsamples. All barcodes and pre-printed labels need to be applied to dry containers for 30 min before use (**SOP A.3**). Type I barcodes (prefix A, plus 11 numbers) are required for both the *.SC* and *.SS* subsamples.

Once the composite sample has been decanted to ~1 cm of overlying water:

1. Put on nitrile gloves.
2. Thoroughly homogenize the composite sample using a glass stir rod or stainless-steel spatula.
    a. If the composite sample jar is too deep to adequately homogenize with a glass stir rod, pour the sediment into a glass bowl that has been previously decontaminated, triple rinsed with DI water, and properly stored (**SOP C.1**).
3. Subsample by gently transferring sediment from the composite sample jar or glass bowl to the appropriate sample container using a glass spoon or stainless-steel scoopula.
    a. **Sediment Chemistry (*.SC*):**
        i. Subsample into an 8 oz (250 mL) certified clean amber glass jar that has been pre-labeled with both human- and machine-readable labels.
        ii. Fill the sample jar to ~1 cm of the jar rim.
    b. **Sediment Size (*.SS*):**
        i. Subsample into a 1 qt (1 L) sealable, heavy duty plastic freezer bag that has been pre-labeled with both human- and machine-readable labels.
        ii. Fill the bag with 8-16 oz (250-500 mL) of sediment, depending on the water content of the sediment.
        iii. Double bag the sample in a second 1 qt sealable, heavy duty plastic freezer bag for shipping.
4. Store the samples between 0-6 °C until shipment (DO NOT FREEZE).
5. Repeat steps 1-4 for the composite sample from the other station. Triple rinse all equipment with DI water before subsampling the next composite sample.
6. Proceed to next step:

a. **Bout 1:** No archive samples are collected, so proceed to **SOP C.1**.
b. **Bout 3:** Archive samples are collected, so proceed to **SOP D.3**.

D.3 Subsampling for the NEON Biorepository

During Bout 3 (fall), an additional 4 subsamples are collected for long-term archiving at the NEON biorepository. Each of these subsamples contain 2 oz (60 mL) of sediment and are archived for the purpose of allowing the user community to conduct physical and/or chemical analyses beyond that which NEON provides. Sediment for the analysis of chemical and physical properties are prioritized over archive sediment, so collect sediment archive (*.SA*) subsamples only after sediment chemistry (*.SC*) and sediment size *(.SS*) subsamples are collected. All barcodes and pre-printed labels need to be applied to dry containers for 30 mins before use (**SOP A.3**). Type IV barcodes (prefix D, plus 11 numbers) are required for all *.SA* subsamples.

For the purpose of archiving the maximum amount of fine sediment (< 2 mm) possible, the first step after collecting subsamples for the external laboratory is to sieve the remaining composite sediment through a 2,000 µm (2 mm) stainless-steel sieve. **DO NOT use a brass sieve**.

1. Put on nitrile gloves.
2. If the remaining composite sample is in the glass bowl, transfer the sediment back to the composite jar because the glass bowl will be used to capture sediment from the sieve.
    a. Triple rinse the glass bowl with DI before proceeding.
3. Place the stainless-steel sieve over the glass bowl.
4. Gently work aliquots of sediment through the sieve using a glass stir-rod, stainless-steel spatula, or other glass/stainless-steel tool. **DO NOT USE WATER**.
5. When all the fine sediment from an aliquot has passed through the sieve, discard the > 2 mm portion of sediment and proceed to the next aliquot.
6. Repeat steps 4-5 until all the remaining composite sample has been sieved.
7. Thoroughly homogenize the < 2 mm fraction of sediment in the glass bowl using a glass stir rod or stainless-steel spatula.
8. Subsample by gently transferring sediment from the glass bowl to the appropriate sample container using a glass spoon or stainless-steel scoopula.
    a. **Sediment Archive (*.SA*):**
        i. Subsample 4 2 oz (60 mL) certified clean amber glass jar that has been pre-labeled with both human- and machine-readable labels.
        ii. Fill each sample jar to the bottom of the cap threads. The samples will be stored frozen (-20 °C) at the biorepository, so do not overfill and risk breakage.
9. Store the samples frozen at -20 °C until shipment.
    a. **Tip:** To minimize the risk of sample jar breakage due to sample expansion, loosely place the cap of the sample jar on top of the sample until the sediment within completely freezes (24-48 h), then secure the cap.

10. Repeat steps 1-9 for the composite sample from the other station. Rinse all equipment with DI water before subsampling the next composite sample.
11. Proceed to **SOP C.1**.

SOP E Data Entry and Verification

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

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 d of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 d of collection or the end of a sampling bout (where applicable). See RD[4] for complete instructions regarding manual data transcription.

SOP F Sample Shipment

For detailed shipping information see Shipping Ecological Samples and Equipment (RD[12]).

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APPENDIX A QUICK REFERENCES

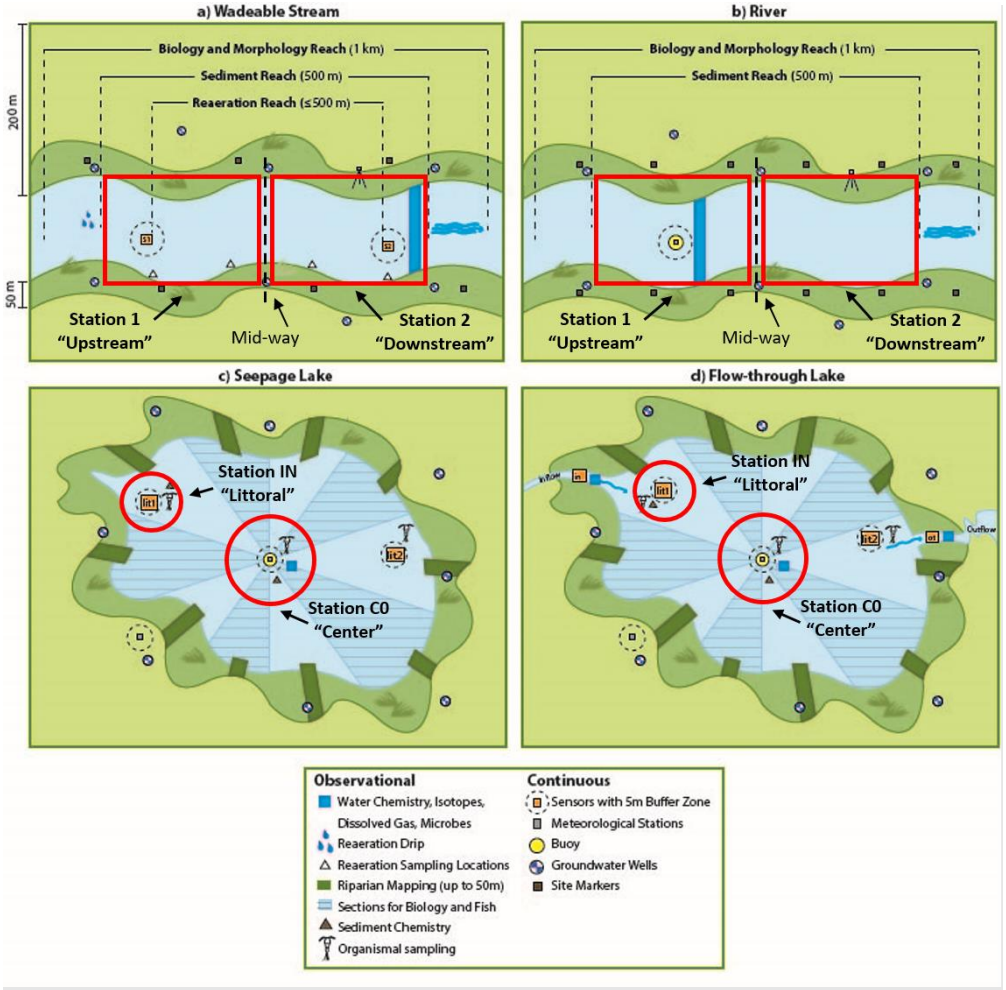
A.1 The following datasheets and mobile device applications are associated with this protocol:

Table 7. Datasheets and mobile applications associated with this protocol.

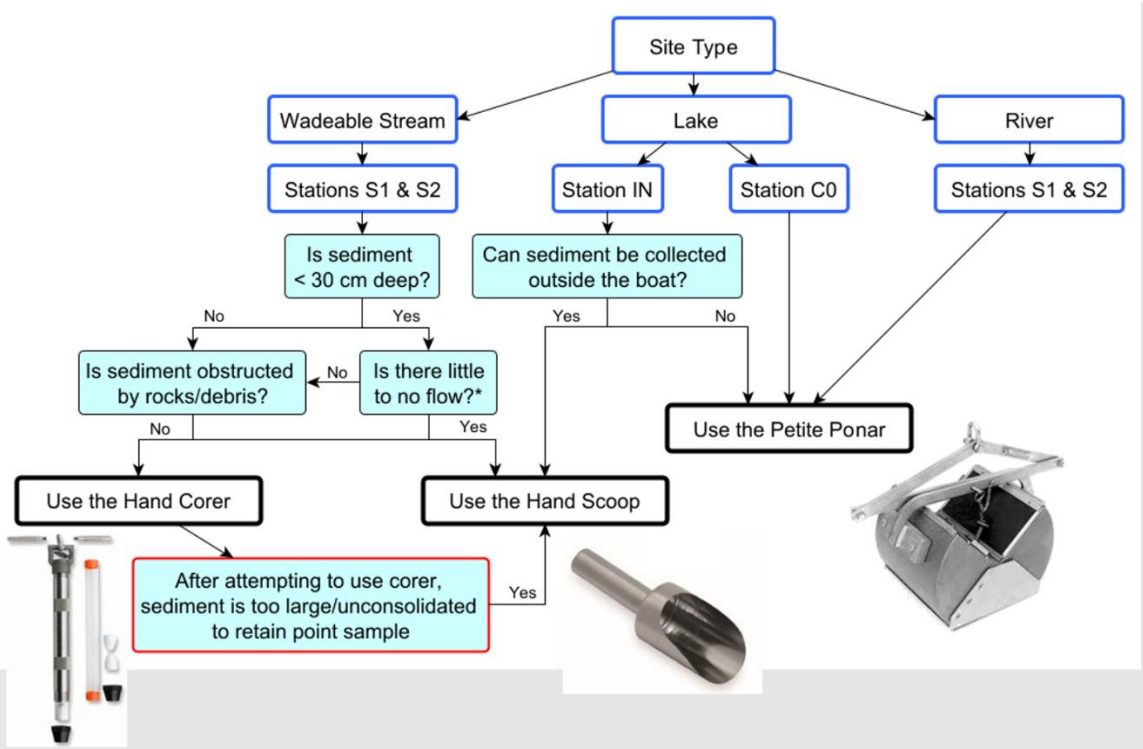
NEON Doc. #	Title	Mobile Application
NEON.DOC.005326	Datasheets for AOS Protocol and Procedure: Sediment Sampling for Chemical and Physical Properties	(AOS) Sediment [PROD]
NEON.DOC.001646	General AQU Field Metadata Sheet	(AOS) Field Metadata [PROD]
NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory	Shipping Application [PROD]

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

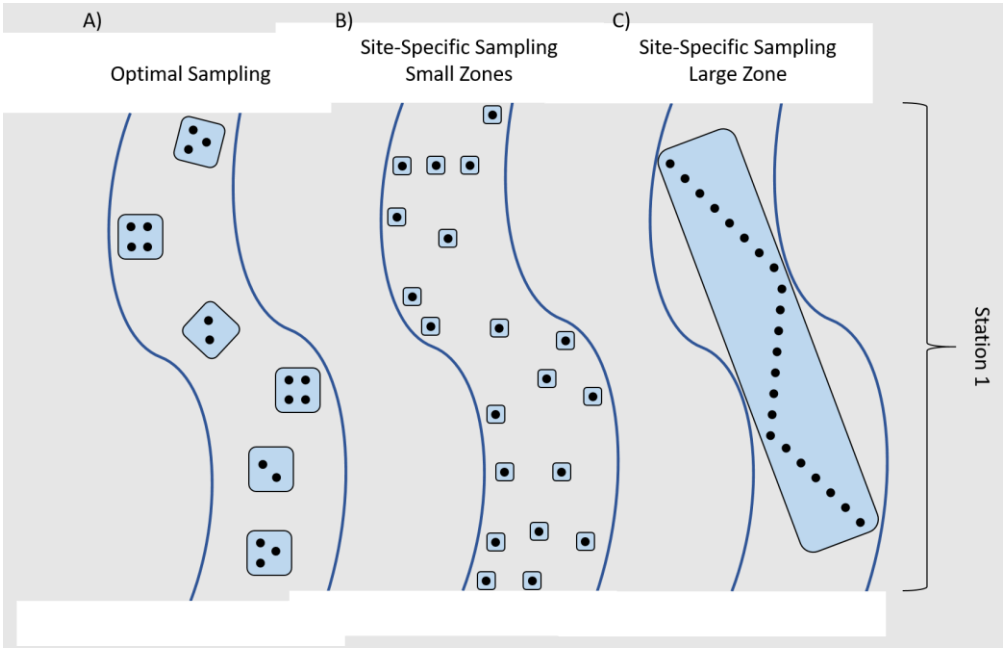
A.2 Generic Site Layouts Showing Sediment Sampling Stations



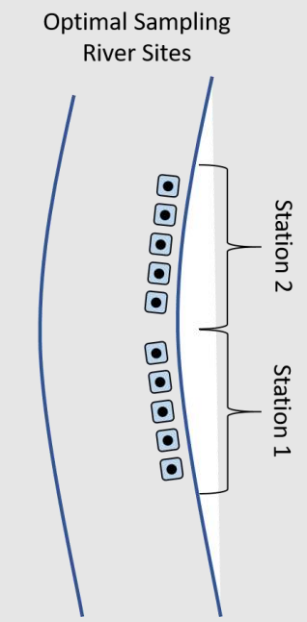
A.3 Decision Tree to Determine Optimal Sediment Sampler Type



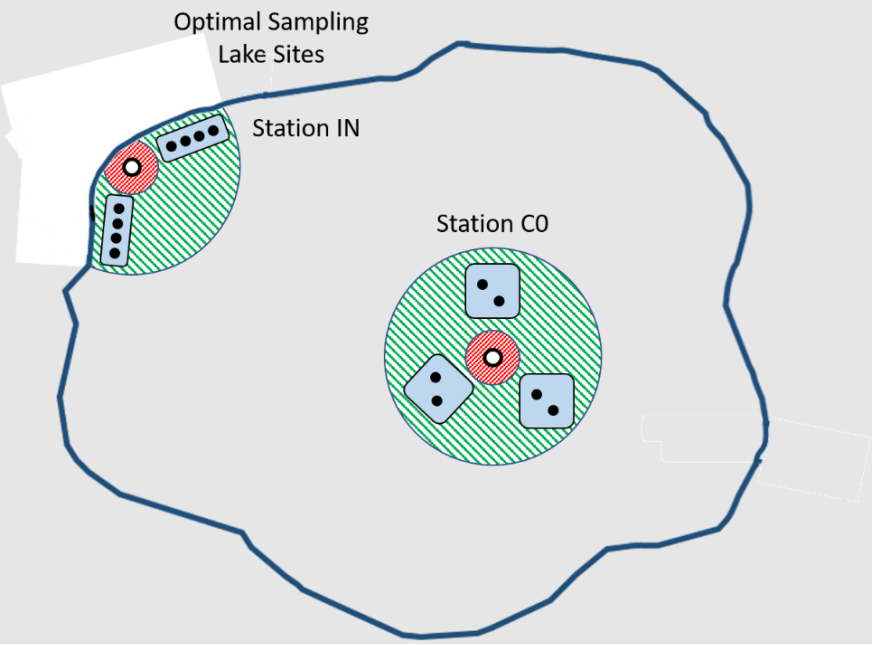
A.4 Diagram of Sample Collection Zones in a Wadeable Stream



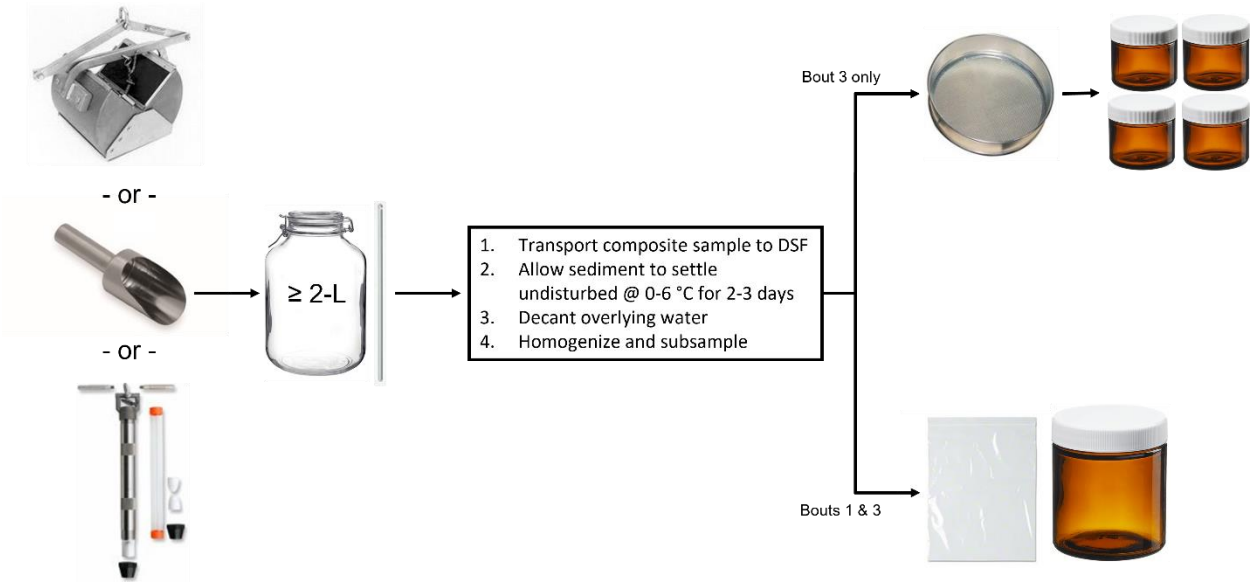
A.5 Diagram of Sample Collection Zones in a River



A.6 Diagram of Sample Collection Zones in a Lake



A.7 Flowchart of Sample Collection



A.8 Steps for Preparing Sample Bottles and Bags

Step 1: Determine the sampleID based on the sampling location, station ID, date, and subsample.

NOTE: Use the same time for all bottles filled at the same sampling station during each sampling event.

Step 2: Attach NEON sediment sampling bottle label and use a permanent marker to fill out labels.

Step 3: Affix one Type I barcode label to each container that will be shipped to the external laboratory.

Step 4: Affix one Type IV barcode to each container that will be shipped to the NEON Biorepository.

A.9 Steps for Sampling in a Wadeable Stream

Per Station:

Step 1: Select the optimal sampling strategy.

Step 2: Start work at the most downstream station and zone, working from downstream to upstream.

Step 3: Before sampling, complete the (AOS) Field Metadata [PROD] Fulcrum App.

Step 4: Carry equipment in the stream to minimize movement between shore and depositional zones.

Per Depositional Zone:

Step 1: Measure water quality with the YSI Pro2030 and record in the mobile device.

Step 3: Collect GPS coordinates.

Step 4: Record the dominant habitat type.

Step 5: Record which sampler type will be used.

Per Point Sample:

Step 1: Using a foldable measuring stick, measure and record water depth.

Step 2: Put on nitrile gloves

Step 3: Sample the sediment. Regardless of sampler type, sample volume should be **≤ 2 oz (~60 mL)**.

A.10 Steps for Sampling in a River

Per Station:

Step 1: Start work at the most downstream station and zone, working from downstream to upstream.

Step 2: Before sampling, complete the (AOS) Field Metadata [PROD] Fulcrum App.

Per Depositional Zone (1 Point Sample per Depositional Zone):

Step 1: Position the boat and lower the anchor gently to not suspend any sediments.

Step 2: Measure water quality with the YSI Pro2030 and record in the mobile device.

Step 3: Collect GPS coordinates.

Step 4: Record the dominant habitat type.

Step 5: Record which sampler type will be used.

Step 5: Using a foldable measuring stick, measure and record water depth.

Step 6: Put on nitrile gloves.

Step 7: Sample the sediment. Point sample volume should be **4-6 oz (~120-180 mL)**.

A.11 Steps for Sampling in a Lake

Per Station:

Step 1: Before sampling, complete the (AOS) Field Metadata [PROD] Fulcrum App.

Step 2: Locate the deepest part of the lake using the GPS coordinates and the site map provided.

Per Depositional Zone:

Step 1: Position the boat and lower the anchor gently to not suspend any sediments.

Step 2: Measure water quality with the YSI Pro2030 and record in the mobile device.

Step 3: Collect GPS coordinates.

Step 4: Record the dominant habitat type.

Step 5: Record which sampler type will be used.

Per Point Sample:

Step 1: Using a foldable measuring stick, measure and record water depth.

Step 2: Put on nitrile gloves.

Step 3: Sample the sediment.

Step 2a: Station C0: each point sample should be **6-8 oz (~180-250 mL)**.

Step 2b: Station IN: point sample should be **≤ 2 oz (~60 mL)** (hand scoop) or **4-6 oz (~120-180 mL)** (petite Ponar®).

A.12 Steps for Settling and Decanting during Laboratory Processing

Step 1: Upon returning to the DSF, immediately place the composite sample jars in cold storage (0-6 °C).

Step 1a: Composite sample jars should be place in a location that will minimize disturbance.

Step 2: Enter the date and time the composite sample jars were placed in cold storage.

Step 3: Allow sediment to settle.

Step 3a: Sediment should settle for a **minimum of 2-3 d** (USGS, 1994).

Step 3b: Sediment can be left undisturbed to settle for a **maximum of 9 d** before processing.

Step 3c: When all fine sediment has settled, the sample is ready to decant.

Step 4: Gently remove the composite sample jar from cold storage and place on the lab bench.

Step 4a: Place the composite sample jar near a sink or a tub next to the composite sample.

Step 5: Allow the composite sample to sit for 5-10 min.

Step 6: Record the date and time of decanting in the mobile app.

Step 7: Put on nitrile gloves.

Step 8: Decant the overlying water from the sample jar to a waste tub/sink using a peristaltic pump.

Step 8a: The pump should be fitted with peristaltic tubing connected to ¼ in (I.D.) C-Flex tubing.

Step 8b: Place a **CLEAN** end of the tubing no more than 2 cm deep in the overlying water.

Step 8c: Place the other end of the pump tubing into a sink or waste tub.

Step 8d: SLOWLY pump overlying water out of the sample jar into a waste tub/sink.

Step 8e: Keep the tubing at a constant 1-2 cm in the overlying water to not perturb sediment.

Step 8f: If sediment is resuspended, stop decanting and allow sediment to settle for 5-10 min.

Step 8g: Decant until there is ~1 cm of overlying water in the composite sample jar.

Step 9: Discard the decanted water.

A.13 Steps for Subsampling for the External Laboratory

Step 1: Put on nitrile gloves.

Step 2: Thoroughly homogenize the composite sample.

Step 3: Subsample by transferring sediment to the appropriate sample container.

Step 3a: Sediment Chemistry (*.SC*): Subsample into 8 oz (250 mL) certified clean amber glass jar.

Step 3b: Sediment Size (*.SS*): Subsample into a 1 qt (1 L) sealable, heavy duty plastic freezer bag.

Step 4: Store the samples between 0-6 °C until shipment (DO NOT FREEZE).

Step 5: Proceed to next step:

Step 5a: Bout 1: No archive samples are collected, so proceed to SOP C.1.

Step 5b: Bout 3: Archive samples are collected, so proceed to SOP D.3.

A.14 Steps for Subsampling for the NEON Biorepository

Step 1: Put on nitrile gloves.

Step 3: Place the stainless-steel sieve over the glass bowl.

Step 4: Work aliquots of sediment through the sieve.

Step 5: When all fine sediment from aliquot has passed through the sieve, discard the > 2 mm portion.

Step 6: Repeat steps 4-5 until all the remaining composite sample has been sieved.

Step 7: Thoroughly homogenize the < 2 mm fraction of sediment in the glass bowl.

Step 8: Subsample by transferring sediment from the glass bowl to the appropriate sample container.

Step 8a: Sediment Archive (*.SA*): Subsample 4 2 oz (60 mL) certified clean amber glass jar.

Step 9: Store the samples frozen at -20 °C until shipment.

Step 10: Proceed to SOP C.1: Equipment Maintenance, Cleaning, and Storage.

APPENDIX B REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare decontaminated equipment including labels.
- ☑ Pre-print labels (and barcode labels when available) on waterproof paper.
- ☑ Fill out the labels before they get wet.
- ☑ Apply labels to all sample containers before starting the bout.
- ☑ Ensure the YSI Pro2030 conductivity sensor is calibrated.

Sample collection: Be sure to…

- ☑ Calibrate the YSI Pro2030 for DO before sampling.
- ☑ Triple rinse all sampling equipment with native water before sampling.
- ☑ Triple rinse the sampler types with native sediment before sampling.
- ☑ When using the hand scoop or hand corer at all site types, never extract > 2 oz sediment.
- ☑ When using the petite Ponar®, never extract > 4-6 oz at river sites and lake Station IN, and ever extract > 6-8 oz at lake Station C0.
- ☑ Take great care to protect fine sediment from being washed away when using the hand scoop as the sample is brought to the water surface.
- ☑ Take great care when handling the petite Ponar® when it is set because accidental closure can cause serious injury.

Equipment maintenance, cleaning, and storage: Be sure to…

- ☑ Always add acid to water when mixing the nitric acid solution.
- ☑ Follow additional decontamination instruction for all glass and stainless-steel equipment beyond the detergent soak.
- ☑ Not store stainless-steel equipment wet.

Laboratory processing: Be sure too…

- ☑ Not freeze the composite sample nor any of the subsamples before shipping.
- ☑ Allow the composite sample to settle undisturbed for 2-9 d.
- ☑ Enter the dates and times in the mobile app for when 1) the composite sample was placed in cold storage, and 2) when decanting began.
- ☑ Have a length of Tygon® tubing that is long enough to create a stable siphon for decanting.

Subsampling for the external lab and NEON biorepository: Be sure to…

- ☑ Thoroughly homogenize the composite sample prior to subsampling.
- ☑ Sieve the sediment archive (*.SA*) subsamples through a 2 mm stainless-steel sieve prior to subsampling.

APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are estimated from satellite MODIS-EVI phenology data averaged from 2005-2014 (Didan 2015). Dates presented here are only a guide and are derived according to the logic presented in Section 4.2. Because individual years may vary widely from the average dates provided below, it is essential that domain staff monitor real-time conditions to determine when to start (and stop) sampling.

Table 8. Site-specific bout windows for spring (Bout 1) and fall (Bout 3) bouts.

Domain	Site	Bout 1	Bout 3
D01	Hop Brook	11Apr-9May	3Oct-31Oct
D02	Lewis Run	19Mar-16Apr	18Oct-15Nov
D02	Posey Creek	19Mar-16Apr	18Oct-15Nov
D03	Flint River	18Feb-24Mar	4Oct-7Nov
D03	Lake Barco	6Feb-12Mar	26Oct-29Nov
D03	Lake Suggs	6Feb-12Mar	26Oct-29Nov
D04	Rio Guilarte	26Jan-23Feb	9Nov-7Dec
D04	Rio Cupeyes	24Jan-21Feb	10Nov-8Dec
D05	Crampton Lake	17Apr-21May	10Sep-14Oct
D05	Little Rock Lake	17Apr-21May	10Sep-14Oct
D06	Kings Creek	23Mar-20Apr	3Oct-31Oct
D06	McDiffett Creek	23Mar-20Apr	3Oct-31Oct
D07	Leconte Creek	15Mar-12Apr	12Oct-9Nov
D07	Walker Branch	9Mar-6Apr	19Oct-16Nov
D08	Mayfield Creek	5Mar-2Apr	31Oct-28Nov
D08	Black Warrior River	16Feb-22Mar	28Oct-1Dec
D08	Tombigbee River	19Feb-25Mar	30Oct-3Dec
D09	Prairie Lake	15Apr-19May	8Sep-12Oct
D09	Prairie Pothole	17Apr-21May	8Sep-12Oct
D10	Arikaree River	21Mar-18Apr	20Sep-18Oct
D11	Pringle Creek	17Feb-17Mar	23Oct-20Nov
D11	Blue River	7Mar-4-Apr	12Oct-9Nov
D12	Blacktail Deer Creek	1May-29May	30Aug-27Sep
D13	Como Creek	20May-17Jun	30Aug-27Sep
D13	West St. Louis Creek	2May-30May	3Sep-1Oct
D14	Sycamore Creek	12Jan-11Feb	3Jun-3Jul
D15	Red Butte Creek	29Mar-26Apr	29Sep-27Oct

D16	McRae Creek	10Apr-8May	23Sep-21Oct
D16	Martha Creek	6Apr-4May	22Sep-20Oct
D17	Teakettle 2 Creek	10Apr-8May	25Sep-23Oct
D17	Upper Big Creek	2Apr-30Apr	28Sep-26Oct
D18	Oksrukuyik Creek	21May-18Jun	7Aug-4Sep
D18	Toolik Lake	16Jun-20Jul	31Aug-4Oct
D19	Caribou Creek	2May-30May	18Aug-15Sep

APPENDIX D SITE-SPECIFIC INFORMATION

See the Site Specific Sampling Strategy Document on the FOPS AOS intranet site.

APPENDIX E 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 9. Equipment list – Supplies needed to perform sediment sampling in the field, processing in the lab, and shipment to external facility.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Decontamination				
Fisher Scientific Company/ 435826	Y	Decon™ Contrex™ CF Cation- and Phosphate-Free Liquid Detergent	Decontaminating equipment	As needed
Fisher Scientific Company/ A200212	Y	Nitric Acid (Certified ACS Plus), Fisher Chemical™	Decontaminating equipment	As needed
Fisher Scientific Company/ BPA4084	Y	Methanol (Spectranalyzed™), Fisher Chemical™	Decontaminating equipment	As needed
	N	Gloves, nitrile	Safety and avoiding contamination	Multiple
	N	Safety goggles	Safety when working with nitric acid	1
	N	Lab coat	Safety when working with nitric acid	1
Grainger, W.W./ 6CHG5	Y	Reynolds Foil Roll, Aluminum, Heavy Duty, 500 ft Roll Length, 12 in Width, Silver	Storing equipment and avoiding contamination	1
Fisher Scientific Company/ 0340912E	Y	Thermo Scientific™ Nalgene™ Wash Bottles made with Teflon™ FEP	Decontaminating equipment with methanol	1
Boat and Related Equipment				
	N	Boat	Locomotion	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Anchor with rope	Anchoring boat	1
	N	Oars	Locomotion	2
	N	Trolling Electric Motor or gas motor	Locomotion	1
	N	Battery (12 volt)	Locomotion	1
	N	Safety kit for boat (e.g., flares, bailer, float with rope)	Safety	1
	N	Personal Flotation Devices (PFDs)	Safety	1 Per Person
	N	Bucket	Waste buckets	2
Field Sampling and Laboratory Processing				
Grainger, W.W./ 1YPC3	Y	Scoop, 8 oz, 304 Stainless Steel	Collecting sediment	1
Forestry Suppliers, Inc./ 77258	Y	Wildco® Hand Core Sediment Sampler	Collecting sediment	1
Forestry Suppliers, Inc./ 77928	Y	Wildco® Petite Ponar® Stainless Steel Grab	Collecting sediment	1
	N	Glass Bowl 4 qt	Collecting sediment/ Laboratory processing	1
	N	Glass jar, ≥ 2 L, sealable	Composite sample jar	2
	N	Gloves, nitrile	Avoiding contamination	Multiple
	N	C-Flex® tubing, ¼" I.D. and 3/8" O.D.	Pumping overlying water from composite jar to sink/waste tub	1
Masterflex® L/S® Easy-Load® pump head	Y	Pump Assembly • Easy-load peristaltic pump head	Pumping overlying water from composite jar to sink/waste tub	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
		• 18-V drill pump (power source for pump head) • Tubing connectors		
	N	18-V drill battery charger	Pumping overlying water from composite jar to sink/waste tub	1
	N	U bolt	Keeping the drill in the "on" position to pump stream water continuously	1
	N	Glass stir rod	Collecting sediment/ Laboratory processing	1
	N	Stainless-steel micro-spatula	Collecting sediment/ Laboratory processing	1
	N	Stainless-steel scoopula-spatula	Collecting sediment/ Laboratory processing	1
BioQuip Products Inc./(4748 or 4750)	Y	Stainless-steel flexible forceps, featherweight	Removing large debris from samples	1
Forestry suppliers, Inc./ 71112	Y	Measuring stick, foldable, plastic	Measuring the water depth at sampling locations	1
	N	Field documentation forms and field books	Documenting notes in the field	3
	N	Brush, scrub, soft nonmetallic	Cleaning samplers	1
Fisher Scientific Company/ 15177622	Y	Handheld YSI Pro 2030	Measuring water quality	1
Fisher Scientific Company/ 0488110G	Y	Fisherbrand™ U.S. Standard Stainless Steel Sieve – 2 mm	Sieving sediment for archiving	1
	N	Cooler	Transport composite samples	1+

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Grainger, W.W./ 11C657	Y	First Aid Kit	Safety	1
	N	Mobile field data recording device (Tablet)	Recording data	1
	N	Boots and/or hip waders	Safe wading	2
	N	GPS	Navigation/ Collecting coordinates	1
Sample Containers, Labels, and Shipping Equipment				
Fisher Scientific Company/ 05721102	Y	Thermo Scientific™ I-Chem™ Wide-Mouth Amber Glass Jars 250 mL (8 oz), Certified, PTFE lid	Sample container for sediment chemistry (*.SC*) subsample	2
	N	1 qt-sized sealable, heavy duty freezer bag	Double bagged sample container for sediment size (*.SS*) subsample	4
Fisher Scientific Company/ 0571914	Y	Thermo Scientific™ I-Chem™ Wide-Mouth Amber Glass Jars 60 mL (2 oz), Certified, PTFE lid	Sample container for sediment archive (*.SA*) subsample	8 (Bout 3 only)
ULINE/ (S-13392 or S-12683)	Y	Insulted shipping kit, suggested: 12 x 12 x 11 ½" for 1 site 16 ¾ x 16 ¾ x 15" for > 1 site	Shipping samples to external laboratory and NEON Biorepository	1 (Bout 1) 2 (Bout 3)
	N	Permanent markers	Labelling samples	3
	N	Bottle labels	Labelling sample with human-readable label	4 (Bout 1) 12 (Bout 3)
	N	Adhesive barcode labels (Type I)	Labelling sample with machine-readable label	4
	N	Adhesive barcode labels (Type IV)	Labelling sample with machine-readable label	8 (Bout 3 only)
	N	Packing material	Filling up extra space and adding absorbent material	As needed

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Resealable plastic bags (gallon and quart size)	Separately enclosing the shipping labels, ice packs, and samples	As needed
	N	Ice or ice packs (< or = 0°C packs)	Keeping the samples cool (water ice is preferable if logistically feasible)	As needed
	N	Clear packing tape, roll	Labeling shipment	1
	N	Shipping labels	Labeling shipment and cooler return	2