AOS PROTOCOL AND PROCEDURE: AQUATIC MACROINVERTEBRATE SAMPLING

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See configuration management system for approval history.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by Battelle. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	01/22/2016	ECO-03470	Initial release, supersedes NEON.DOC.000690 and NEON.DOC.001204.
B	02/08/2017	ECO-04359	Update NEON template; Separate and rename snag sampling SOPs; Update sample ID template; Add DNA metabarcode SOP

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1 OVERVIEW

1.1 Background

Aquatic macroinvertebrates are a diverse and ubiquitous group of organisms (Hauer and Resh 2006). Streams and rivers harbor most major taxa of aquatic organisms however some invertebrates occur only in lakes and ponds (Hynes 2001). Because of their presence in nearly all bodies of freshwater, aquatic macroinvertebrates are excellent study organisms to address questions of biodiversity. Aquatic invertebrates are easily sampled, common in all but the most polluted waters, and strongly affected by water quality.

Aquatic macroinvertebrates are an important part of the aquatic community. Most benthic macroinvertebrates are primary consumers and feed on autotrophic (algae, plants) and heterotrophic (leaf litter, dissolved organic carbon) production, while some benthic macroinvertebrates are predators. In general, benthic macroinvertebrates are important vectors of energy transfer from one trophic level to the next (e.g., from sunlight + nutrients → primary producers → consumers → predators). Macroinvertebrates can be classified into functional feeding groups based on morphological and behavioral mechanisms for food acquisition (Merritt and Cummins 2006), including scrapers (feed on algae; Figure 1), shredders (feed on leaf litter), collector-gatherers (feed on organic matter and other material), filter feeders (filter fine particles from the water column), and predators (feed on other macroinvertebrates).

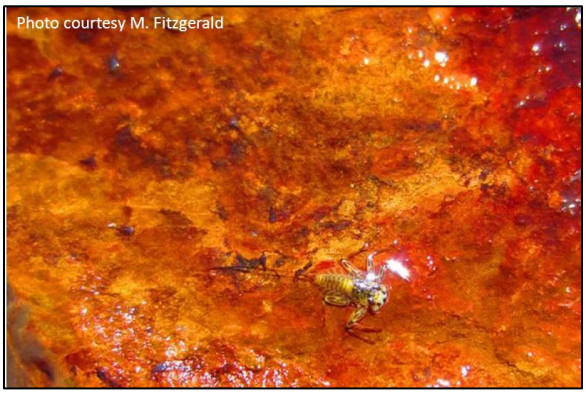

Figure 1. Mayflies (Ephemeroptera: Heptageniidae) are scrapers that are adapted to feed most efficiently on attached periphyton in flowing waters.

Freshwater benthic macroinvertebrate communities are strongly affected by abiotic factors such as disturbance, shifts in nutrient concentrations, pollutants, temperature, and pH, as well as environmental factors such as scouring, freezing, flooding, drought, and biological factors such as vegetation structure and predation (Allan 1995, Wetzel 2001). Such sensitivity to environmental conditions makes benthic invertebrates ideal for use in monitoring programs such as the US Environmental Protection Agency Rapid Bioassessment Protocol (EPA RBP; Barbour et al. 1999) and US Geological Survey National Water-

Quality and Assessment Program (USGS NAWQA; Moulton et al. 2002). Members of the Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) are often thought to be the most sensitive taxa and when present in the aquatic habitat, can be indicators of good to excellent water quality. However other taxa, such as members of the family Chironomidae (midges, non-biting flies) and oligochaetes are ubiquitous and highly tolerant, and can be indicators of poorer water quality. Sampling stream and lake benthic communities over the time span of the NEON Observatory will help researchers determine changes in presence/absence of taxa, taxa diversity and richness, community structure, and species loss.

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

Benthic invertebrate protocols for **wadeable streams** are informed by the US Geological Survey (USGS) National Water Quality Assessment (NAWQA) program (Moulton et al. 2002), the US Environmental Protection Agency (EPA) Rapid Bioassessment Protocols (Barbour et al. 1999), and Arctic Long-Term Ecological Research (LTER) Protocols (Slavik et al. 2004). Benthic invertebrate protocols for **lakes and non-wadeable streams** are based on those of the US Environmental Protection Agency (USEPA) Environmental Monitoring and Assessment Program (EMAP) Program for Surface Waters (Baker et al. 1997), Standard Operating Procedure for Benthic Invertebrate Field Sampling (USEPA 2002), and the USEPA Great River Ecosystems Field Operations Manual (Angradi et al. 2006).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

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

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2, Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.003043	Datasheets for AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[09]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[10]	NEON.DOC.014050	TOS Protocol and Procedure: Ground Beetle Sampling
RD[11]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[12]	NEON.DOC.003045	AOS Protocol and Procedure: Periphyton, Seston, and Phytoplankton Sampling
RD[13]	NEON.DOC.003039	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling
RD[14]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbial Sampling
RD[15]	NEON.DOC.001195	AOS Protocol and Procedure: Riparian Habitat Assessment in Lakes and Non-wadeable Streams
RD[16]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-wadeable Streams
RD[17]	NEON.DOC.001194	AOS Protocol and Procedure: Zooplankton Sampling in Lakes

2.3 Acronyms

Acronym	Definition
cm	centimeter
DNA	Deoxyribonucleic acid
EMAP	Environmental Monitoring and Assessment Program
FFG	Functional feeding group
ft	Foot
GPS	Global Positioning System
hr	Hour
Km	kilometer
LTER	Long Term Ecological Research Program
m	Meter
µm	micrometer
mL	milliliter
mm	millimeter
NAWQA	National Water-Quality Assessment (USGS)
NEON	National Ecological Observatory Network
NLA	National Lakes Assessment
PFD	Personal Flotation Device
qt	Quart
RBP	Rapid Bioassessment Protocol (US EPA)
s	second
USEPA	US Environmental Protection Agency
USGS	US Geological Survey

2.4 Definitions

Autotroph: (Primary producers) Organisms that acquire energy from non-living inorganic sources.

Benthic: The region in or near the sediments or bed of a body of water (e.g., bottom of the stream).

Caddisflies: Aquatic invertebrates in the Phylum Insecta, Order Trichoptera. Larvae of most species build a case of out of sand or small pieces of wood around the soft, larval body (Figure 2), or spin a net to capture suspended particles in the water column.

Figure 2. Caddisflies (Trichoptera) often build cases out of small pebbles, sand, or pieces of small woody debris.

Clams: Benthic mollusks sometimes found in the sediments of streams and lakes, with a shell composed of two hinged shell, thus "bivalves" and similar to mussels. Clams are filter feeders.

Collector-gatherer: (Functional feeding group) Invertebrates that are morphologically adapted to feed on a variety of items, including coarse detritus and periphyton.

Consumers: (Trophic level) Mid-levels of the food chain, organisms that consume primary producers and detritus.

D-frame net: Collecting net with handle. The net frame is shaped like a 'D', allowing the net to be placed against a stream or lake bottom. The D-frame net has a 30 cm wide opening and 243 µm Nitex mesh collection net with handle.

Filter feeder: (Functional feeding group) Invertebrates that are morphologically adapted to feed by straining suspended particles from the water column (Figure 3). In streams, filter feeders are typically attached to the substratum (e.g., blackflies, mussels).

Figure 3. Black fly (Diptera: Simuliidae) larva, an invertebrate that uses fanlike mouthparts to strain particulates from moving water.

Functional feeding group (FFG): Benthic invertebrate groupings based on morphological and behavioral mechanisms for food acquisition (includes scrapers, filter-feeders, collector-gatherers, predators, and shredders).

Hand corer: Benthic sampler for sand- and silt-bottomed streams (Figure 23). Sampler consists of an inner PVC tube and an outer, stainless steel housing for pushing the corer into the substratum. The stainless steel barrel is 5 cm in diameter by 50 cm high with a handle on top.

Hess sampler: Benthic sampler for gravel-bottomed streams (Figure 26). Sampler consists of a stainless steel drum fitted with a flow-through collection net. The drum is 33 cm in diameter by 40 cm high, with a 243 μm Nitex mesh net.

Heterotroph: Organisms that acquire energy from living or non-living sources of organic matter.

Invertebrate: Animal without a backbone. Most stream invertebrates have an exoskeleton, including mussels, snails, crayfish, insects, and zooplankton.

Large woody debris: Downed woody debris submerged in the stream/river that is ≥0.3 m (6 inches) in diameter at the large end and ≥ 5 m long (Angradi et al. 2006).

Leaf litter: Terrestrial leaves that have fallen into the stream channel, where they are colonized by bacteria and fungi.

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

Macroinvertebrate: Typically refers to benthic invertebrates that are >500 μm in length. For the purposes of this protocol, benthic invertebrates >250 μm will be considered macroinvertebrates.

Modified kicknet: Benthic sampler used for faster and deeper water (runs). The modified kicknet has a 33 x 5- cm frame with 243 μm Nitex mesh net attached, and a long handle for holding the net in fast current (Figure 25).

Mollusks: A large phylum of invertebrates, freshwater mollusks often have a soft body surrounded by a shell. This group includes snails, mussels, and clams in freshwaters.

Mussels: Benthic mollusks often found in the sediments of streams and lakes, with a shell composed of two hinged shell, thus “bivalves” (Figure 4). Mussels are filter feeders.

Figure 4. Freshwater mussels are often found in streams. Pictured here, lampmussels (*Lampsilis virescens*) from Alabama.

Petite ponar: Benthic sampler used for soft sediments in deep and slow-moving water. The Ponar consists of 2 steel halves that close when the sampler reaches the sediment, grabbing a 6”x6” area of sediment (Figure 20).

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

Predator: (Functional feeding group and trophic level) Animals that eat other animals. In stream communities, predators include insects, fish, and birds; higher level of the food chain (Figure 5).

Figure 5. A mayfly predator feeding on another aquatic invertebrate.

Primary producers: (Trophic level) Lowest level of the food chain, organisms that use sunlight and nutrients to create energy.

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

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

Scraper: (Functional feeding group) Invertebrates morphologically adapted to feed by scraping algae and periphyton off surfaces (e.g., rocks or plant surfaces; Figure 6).

Figure 6. Mayfly scrapers feeding on a rock surface.

Shredder: (Functional feeding group) Invertebrates morphologically adapted to feed by shredding leaf litter, aquatic plants, and coarse organic matter (Figure 7).

Figure 7. A stonefly shredder feeding on plant detritus in a stream.

Snag (woody snag): Submerged woody debris in the stream channel that often provides substratum and refuge for macroinvertebrates, often associated with other organic matter such as leaf packs (Moulton et al. 2002). For the purposes of this protocol, woody snags are considered to be smaller than large woody debris (LWD).

Snails: Mollusks, members of the class Gastropoda (literally “stomach foot”), snails typically have a coiled shell around a soft body (Figure 8). Aquatic snails are often found in streams or lakes.

Figure 8. *Elimia* is a genus of snail often found in rivers and streams.

Step pool: High-gradient streams (steep) where water cascades over a rock or woody snag, dropping into a pool. Due to the gradient and surrounding geology, this pattern continues down the stream: step (drop)—pool—step—pool—etc. (Figure 9).

Figure 9. Step pools occur in high-gradient streams where there are channel-spanning pools separated by boulder/cobble steps.

Surber sampler: Benthic sampler for flowing waters. A standard Surber sampler consists of a 30 cm x 30 cm frame and a long 243 μm Nitex mesh net that collects organisms (Figure 14). A mini Surber sampler has a 15 cm x 15 cm frame with 243 μm Nitex mesh collection net.

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

Trophic level: Step in the transfer of energy within a food chain or food web.

3 METHOD

The goal of benthic invertebrate sampling is to determine taxa diversity, the number of species present (richness), biomass, and to enable DNA analysis (addressed in RD[09]) for benthic invertebrates at each aquatic sampling site. These variables, especially diversity and biomass, will be tracked over time and sampled to detect changes in species loss, changes in community structure and function, as well as the introduction and the spread of invasive taxa (presence/absence).

Benthic invertebrates at NEON aquatic sites are sampled using a percent-based macrohabitat approach (after Moulton et al. 2002). Habitats sampled focus on riffles, runs, and pools depending on the percent cover of each habitat within each 1 km-long NEON Aquatic **wadeable stream** site (NOTE: some NEON sites may be less than 1 km due to permitting restrictions), and pelagic and littoral samples in **lakes and non-wadeable streams**. Five samples are collected in the dominant habitat type (**wadeable stream**) or littoral area (**lake and non-wadeable stream**), and three samples are collected in the second-most dominant habitat type (**wadeable streams**) or pelagic area (**lakes and non-wadeable streams**) for a total of eight samples on a given sampling date at a site. Field protocols differ depending on the habitat and substrate being sampled. However, all samples are collected from the surface of the natural substratum in each habitat. In **wadeable streams**, the majority of the invertebrate community is likely to colonize riffles and runs, while in sandy and silty habitats and in pools invertebrates are likely to be most diverse in on woody debris. In **lakes and non-wadeable** streams, macroinvertebrates are likely to be most diverse in littoral aquatic plant beds. Appropriate site-specific sampling procedures will be determined prior to sampling and documented in the Site-Specific Sampling Design document. Samples are collected by field personnel then preserved and sent to taxonomists for identification.

Macroinvertebrate sampling occurs three times per year. Timing of sampling is site-specific and determined based on historical hydrological and meteorological data. Specific details on sample dates are provided in the NEON Aquatic Sample Strategy Document (RD[07]) and Appendix D. Sample bout 1 is an early-season date, representing a period of rapid biomass accumulation after winter, typically prior to leaf out or ice-off where applicable. Sample bout 2 targets mid-summer baseflow conditions and sample bout 3 represents the late growing season (typically autumn) during leaf-fall where applicable. These dates will differ on a site-by-site basis, but should always occur at, or near, baseflow conditions within the watershed. Sampling will not occur directly following a flood in **wadeable streams** (defined as >1.5 x base flow; Biggs et al. 1999). Should such a flood event occur on or prior to a target collection date, sampling should be delayed 3 days-1 week (maximum 2 weeks, dependent on field schedule) to allow for invertebrates to recolonize the substratum (c.f. Brooks and Boulton 1991, Matthaei et al. 1996).

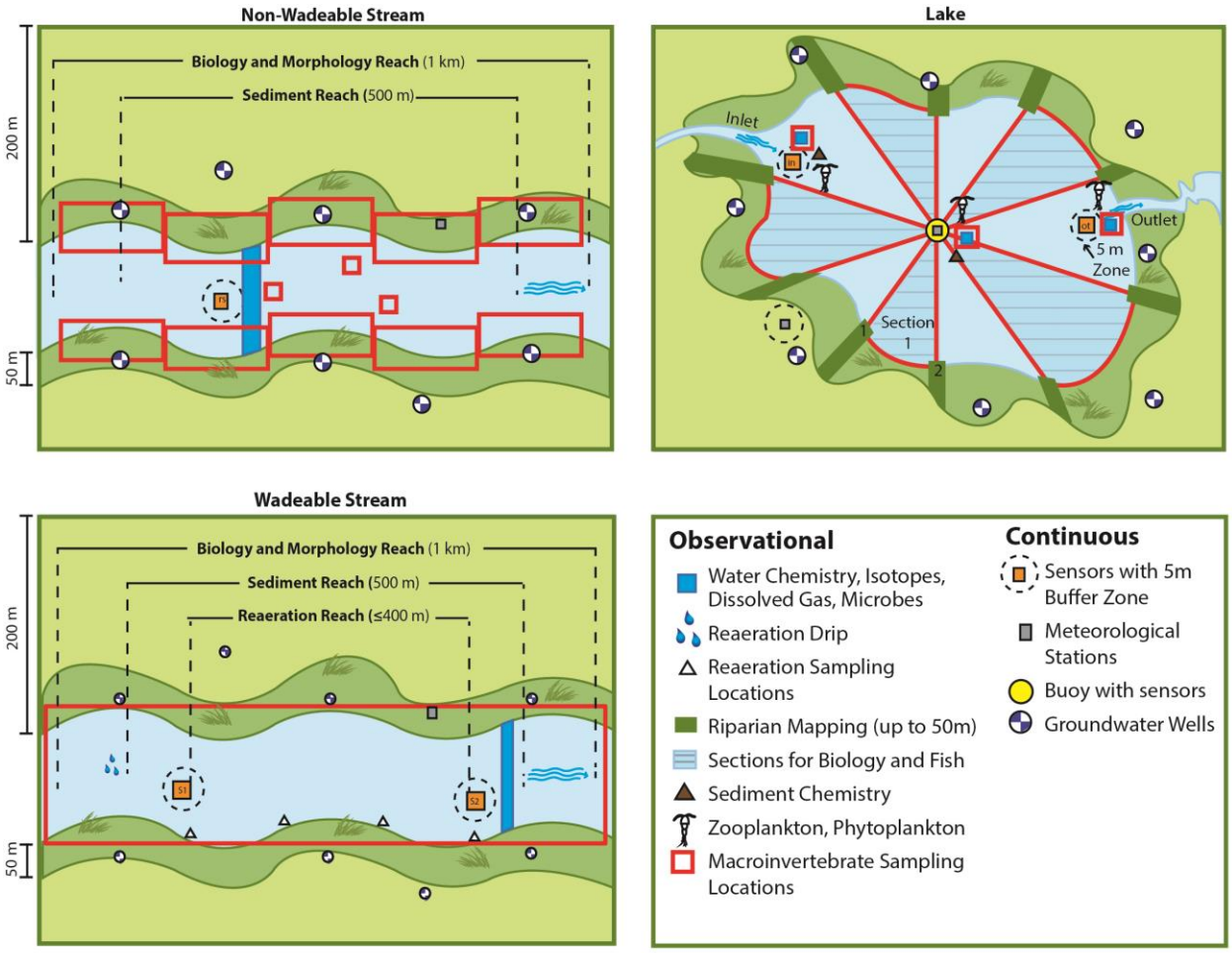

Figure 10. Generic site layouts with macroinvertebrate sampling locations in red.

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

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

Quality assurance will be performed on data collected via these procedures according to the NEON Science Performance QA/QC Plan (AD[05]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Benthic invertebrate sampling in lakes, non-wadeable and wadeable streams occurs three times per year at each site, roughly spring, summer, and autumn. Sampling must be scheduled within the first 21 days of the 1 month window specified in Appendix D with a minimum of two weeks between sampling dates. Accommodations for local weather conditions (e.g., late ice-off) may be made that cause the sample date to fall outside of the pre-determined window. Use NEON's problem reporting system to report sampling efforts that take place outside of the defined sampling window.

4.2 Criteria for Determining Onset and Cessation of Sampling

A range of dates for each site were determined *a priori*, based on historical data including ice on/ice off, streamflow, the accumulation of degree days, weather, and riparian phenology (Appendix D).

4.3 Timing for Laboratory Processing and Analysis

Macroinvertebrate samples must have their preservative changed after field collection, and before sending to the macroinvertebrate taxonomist. Preservative must be changed within 12-72 hours of field sampling. Samples must be shipped to the external lab within 30 days of collection. For additional storage and shipping timelines see SOP G.

4.4 Sample Timing Contingencies

All samples from this protocol in one sampling bout must be collected within one day (i.e., all 8 samples per stream or all samples per lake/non-wadeable streams as detailed in this protocol) because of the fluctuating nature of aquatic habitats. Spreading sample collection over multiple days increases variability among samples. A minimum of 2 weeks between sample periods shall be observed.

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and conditions become unsafe (e.g. approaching thunderstorm, rapid increase of water level in the **wadeable stream**), or the **lake/non-wadeable stream** becomes too windy (>32 km hr^{-1}) and has unsafe wave heights (>1 m) so that the boat cannot be held stationary over a sampling point while at anchor, return to shore and wait in a safe location for 30 minutes. If conditions improve, resume	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.

	sampling, if not, return to the Domain Support Facility and sample at another time.	
	If circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the streamflow or weather requirements below, continue to collect samples after the delay. If conditions do not improve, discard any previously collected samples at the site or at the Domain Support Facility and start over on the next appropriate sampling day.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
5 Days	If flooding occurs on or prior to the targeted sampling date in a **wadeable stream** (>3x median discharge for the preceding year; Clausen and Biggs 1997) or unsafe wading conditions occur (Lane and Fay 1997), wait a minimum of 5 days after water level drops below 3x median discharge and is safely wadeable to allow the invertebrate community to recolonize.	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data must be flagged.
6 Months	Preserved macroinvertebrate samples may be held for up to 6 months at room temperature or 4 °C in the domain lab if circumstances do not allow shipping to the external lab.	Holding samples >30 days affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected and samples do not need to be flagged if held for ≤6 months.

4.5 Criteria for Permanent Reallocation of Sampling Within a Site

Macroinvertebrate sampling will occur on the schedule described above at 8 locations per site in two different habitat types. 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 (relocatable sites). However, circumstances may arise requiring that sampling within a site be shifted from one particular location to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given plot becomes compromised, a problem ticket should be submitted by Field Operations to Science.

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

A common occurrence in macroinvertebrate sampling is loss of habitat due to channel drying or permitting restrictions. Contingent decisions in Table 2 should be followed if the wetted area of sampling is decreased, and a problem ticket should be submitted. If water returns to the reach, full sampling should resume. Shifting of the stream channel is expected and does not necessarily compromise sampling.

Table 2. Contingent decisions for sampling in a wadeable stream.

Situation	Action	Outcome for Data Products	Considerations
Wadeable stream site with <500 m aboveground stream length due to stream size or permitting restrictions	Habitat available may be insufficient to accommodate all 8 samples without causing harm to the stream. Reduce sampling by collecting samples only in the dominant habitat type (5 samples total). Enter "sampling impractical" for the missing habitat type.	Lower resolution for diversity metrics.	If the decision is made to decrease the number of samples collected for this protocol, it must also be reflected in the other wadeable stream biology protocols (RD[12], RD[13], RD[14]).
Wadeable stream site with seasonal drying	If the stream experiences seasonal drying such that the chosen habitat types have disappeared, select the next dominant habitat type in Figure 13, or use the sub-dominant habitat if still present.	Less standardization of the dataset.	Habitat types sampled should be present throughout the year. The decision to switch habitat types should only occur during periods of extreme drying such that typical habitat types are no longer present at the site.

4.6 Sampling Specific Concerns

1. Macroinvertebrates often become trapped in the folds of the nets (near the stitching). Check net seams between each sample to ensure that specimens are added to the correct samples, and do not remain in the net.
2. Macroinvertebrates are often lodged in the edges of the sieve. Check the sieve between samples to avoid sample-to-sample specimen contamination.
3. Samples must be preserved in the field within 1 hour of sampling to prevent predation within the sample, as predatory insects tend to eat smaller insects when put into sample jars.

4.7 Safety

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

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

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

See Section 10 in the NEON Operations Field Safety and Security Plan (AD[02]) for aquatic-specific field safety requirements. In addition, the following safety requirements must be followed:

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

5 PERSONNEL AND EQUIPMENT

5.1 Equipment

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

Table 3. Equipment list – General equipment

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
RD[09], RD[15], RD[16]	R	Site-specific Rapid Habitat Assessment or Riparian Sampling Locations	Determining sampling locations	All	1	N
	S	Clipboard	Recording data	All	1	N
	S	Cooler, 9-28 qt	Field sample storage; use size appropriate to samples being collected	All	1	N
	R	Waders (hip or chest) or knee boots	Wading	All	1 pair/ person	N
	R	Personal flotation device (PFD)	Boating safety	Lakes/non-wadeables	1 per person	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Handheld GPS unit (with batteries, ±4 m accuracy) or Humminbird	Navigating to sampling locations	Lakes/non-wadeables	1	N
MX100453	R	Depth finder (see Bathymetry Protocol, RD[17])	Determining depth at sampling location	Lakes/non-wadeables	1	N
Consumable items						
RD[06]	R	General AQU Field Metadata Sheet	Recording metadata	All	1	N
RD[05]	R	Field data sheets (all-weather copier paper, write in pencil)	Recording data	All	5 sheets	N
	R	Pre-printed paper labels (all-weather copier paper, write in pencil)	Labeling samples, inside sample jar	All	1 sheet	N
	R	Pre-printed 1x2” adhesive labels (write in permanent marker)	Labeling samples, outside sample jar	All	1 sheet	N
	R	Pencils	Recording data	All	4	N

R/S=Required/Suggested

Table 4. Equipment list – Macroinvertebrate Samplers

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special
Durable items						
MX100305	R	Surber sampler (243 μm Nitex mesh), 0.093 m^2	Sample collection in riffles	Wadeables	1	N
MX108298	R	Mini Surber sampler (243 μm Nitex mesh), 0.023 m^2	Sample collection in small streams	Wadeables	1	N
MX108051	R	Hess sampler (243 μm Nitex mesh), 0.086 m^2	Sample collection in riffles, runs, shallow pools	Wadeables	1	N
MX100303	R	Petite ponar sampler with rope	Sample collection in soft sediments	Lakes/Non-wadeables	1	N
	S	Messenger	Used with petite ponar	Lakes/Non-wadeables	1	N
MX100309	R	Hand corer, stainless steel, 20 in	Sample collection in sandy or silty habitats (streams)	Wadeables	1	N
MX108199	R	Modified kicknet sampler (rectangular, 243 μm Nitex mesh)	Sample collection in runs (streams) and large woody debris	Wadeables, Non-wadeables	1	N
MX100304	R	Collapsible quadrat (0.5 x 0.5 m^2)	Used with modified kicknet sampler in streams	Wadeables	1	N
MX102972	R	D-frame net (243 μm Nitex mesh)	Sample collection for woody snags and littoral (lakes/rivers)	All	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Bow saw, 21 in or loppers	Used with D-frame snag sampler in streams	Wadeables, Non-wadeables	1	N
	S	Work Gloves (pair)	Used with petite ponar for safe handling of ponar rope	Lakes/Non-wadeables	1	N
	R	Meter stick, meter tape, or metric ruler	Used with snag and LWD samplers (streams and lakes/rivers)	All	1	N
	R	Kitchen brush with nylon bristles	Used with Surber, Hess, snag, and LWD samplers for rock and wood scrubs	All	2	N
	R	Deck brush with polypropylene bristles, 60 inch handle	Used with LWD/modified kicknet sampler	Non-wadeables	1	N
	S	Putty knife	Scrape plant material off cobbles	Wadeables	1	N
Consumable items						
	S	Petroleum jelly	Used with hand corer to maintain suction	Wadeables	1	N

R/S=Required/Suggested

Table 5. Equipment list – Macroinvertebrate field elutriation and preservation

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
MX100308	R	Polyethylene wash bottle, unitary (500 mL; Figure 19)	Rinsing sieve, bucket	2	N
MX100306	R	Sieve, 250 µm	Sample sieving; elutriation	1	N
MX109110	S	Sieve bucket, 242 µm	Sample sieving; elutriation in **lakes/non-wadeable streams**	1	N
	R	Plastic bucket, 3 or 5 gallon	Substrate scrubbing (streams), elutriation, collecting waste water on boat (lake/river)	1-2	N
MX102975	S	Flexible forceps, featherweight	Collecting clinging insects	1	N
	R	HDPE bottles (1 L) or jug (4 L) with lids (clear or amber)	Transporting ethanol to the field	1-4	N
Consumable Items					
MX110492 MX108207 MX109111	R	Sample jars, HDPE with screw-top lid, 6, 16, or 32 oz.	Sample container, technicians choose sizes appropriate for site	8	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	S	Disposable pipets (polyethylene), 3 mL	Collecting clinging insects	2	N
	R	Ethanol, 95% non-denatured	Preservative	1-4 L	Y
	S	Latex or Nitrile gloves (pair)	Preventing preservative contact with skin	5	N
	S	Resealable plastic zip-top bags (gallon)	Organizing samples	5	N

R/S=Required/Suggested

Table 6. Equipment list – Laboratory processing: preservative replenishment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Unitary wash bottle, 500 mL, ETOH + glycerol	Adding preservative to sample jars and rinsing filter cup	1	Y
MX100306	S	Sieve, 250 μm	Changing preservative	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
RD[10] and MX107766	R	Filter cup (500 mL HDPE bottle and 243 µm Nitex mesh square, see TOS beetle protocol)	Changing preservative	1	N
MX102975	S	Featherweight forceps	Picking up insects	1	N
	R	Safety glasses	Preventing preservative contact with eyes	1 pair	N
Consumable items					
	R	Ethanol, 95%, undenatured	Preservative	5 L	Y
	R	Glycerol, >99%	Preservative	1 L	Y
MX107766	R	243 µm Nitex mesh cloth, cut into squares for filter cup	Catching insects over the filter cup	8	N
	R	Nitrile gloves	Preventing preservative contact with skin	1 pair	N

R/S=Required/Suggested

Table 7. Equipment list – Shipping supplies

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
Consumable Items					
	R	Cardboard box, UN-rated, Group II	Shipping samples to taxonomist	1	N
	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	TBD	N
	R	Heavy duty plastic trash bag	Lining the shipping container	1	
AD[03]	R	Appropriate labels and forms for limited quantity ground shipments	Shipping paperwork and external shipping labels	TBD	N
RD[11]	R	Shipping inventory	Provides sample information to external lab	1	N

R/S=Required/Suggested

5.2 Training Requirements

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

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

Personnel will be trained in field protocols associated with this document, and trained in safe working practices for aquatic field work. Technicians must also be trained in safe handling of ethanol (AD[03]).

5.3 Specialized Skills

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

5.4 Estimated Time

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

Field sampling requires two technicians for four hours per site, plus travel to and from the site. Lab processing requires one technician for one to two hours within 12-48 hours of field sampling.

6 STANDARD OPERATING PROCEDURES

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 Macroinvertebrate Field Sampling

1. Collect and prepare all equipment, including sample jars and labels: paper (inside jar) and adhesive (outside jar) (RD[05], Figure 11).
2. **Figure 11**Load GPS sampling coordinates on handheld GPS unit.
3. Check nets and sieves for holes, repair if necessary. Ensure that both are clean and free of debris and organic matter.
4. If using a hand corer in **wadeable streams**, mark 5 and 10 cm from the bottom of the barrel with permanent marker. The contents of the nosepiece are not contained in the final sample.
5. Fill 1 L HDPE bottles (or 4 L HDPE jug) with 95% ethanol (Stein et al. 2013). Cap tightly. Clearly label bottle and transport to the field following EHS guidelines.
6. Fill out general aquatic field metadata sheet (RD[06]) upon every field sampling visit.

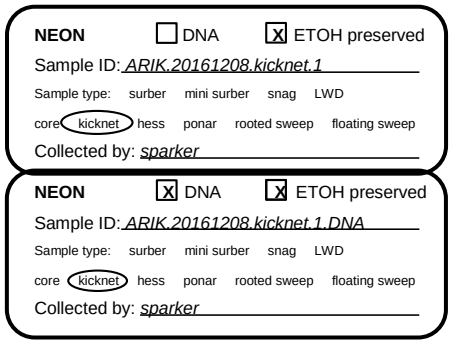

Figure 11. Example of adhesive jar labels for macroinvertebrate sampling.

7. Sample IDs will be generated by the mobile app as follows (Table 8). Sample IDs written on the physical sample label must match the sample ID generated by the mobile app.

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

Sampler type	Site type	Field app populates as:	Example Sample ID
Surber	Stream	surber	MAYF.20161027.surber.1
Core	Stream	core	MAYF.20161027.core.1
Hess	Stream	hess	MAYF.20161027.hess.1
Modified kicknet	Stream	kicknet	MAYF.20161027.kicknet.1
Petite Ponar	Stream, lake, river	ponar	CRAM.20161027.ponar.1
Snag, D-frame	Stream, River	snag	MAYF.20161027.snag.1
Mini Surber	Stream	miniSurber	MAYF.20161027.miniSurber.1
Benthic Sweep, D-frame	Lake, River	benthicSweep	CRAM.20161027.benthicSweep.1
Floating Sweep, D-frame	Lake, River	floatingSweep	CRAM.20161027.floatingSweep.1
LWD, Modified kicknet	Stream, River	lwd	TOMB.20161027.lwd.1

SOP B Determining Sampling Locations and Sampler Type

NEON aquatic macroinvertebrate sampling is intended to be quantitative. Although different sampler types may be used (see SOPs below) depending on the habitat and substrate at a site, the data that are produced are quantitative and thus will be comparable after ingest and processing.

B.1 Lakes and Non-wadeable Streams

1. Do not sample within a 5 m radius of the aquatic instrumentation.
2. Benthic sampling
   a. **Lakes**: Benthic petite ponar samples (Section C.2) will be collected near aquatic chemistry sampling locations in lakes: at the buoy, inlet, and outlet.
   b. **Non-wadeable streams**: Benthic petite ponar samples (Section C.2) will be collected downstream of the sensor set (≥5 m), river right, and river left.
      i. If sampling a **non-wadeable stream** with rocky substrata, use the modified kicknet method in Section C.6 (Figure 12).
3. Littoral sampling
   a. Samples will be collected in 5 of 10 pre-defined riparian sections (RD[09]) based on the dominant substratum in each section.
      i. A section is the littoral area between the riparian sampling coordinates.
      ii. Move to the next riparian section if the appropriate substratum type is not present in the chosen section.
   b. The habitat type chosen should be present during all sampling bouts and account for at least 20% of the littoral area, ensuring that there is enough habitat in which to collect all samples.
   c. All 5 samples must be taken from the same habitat type on each sampling bout, unless a major event (i.e., a flood) causes significant changes to the substrata.
      i. Document changes in sampler and habitat availability through NEON's problem tracking system.

d. Choose the appropriate sampler based on the field conditions and habitat being sampled.
e. The order of preference for sampling substrata is as follows (Figure 12), habitat suggestions for NEON sites in Appendix E:
   i. Aquatic plant beds, living or dead, or floating mats (D-frame net, Section C.8)
   ii. Snags and Large woody debris (Snag and Large Woody Debris Sampling, Section C.3)

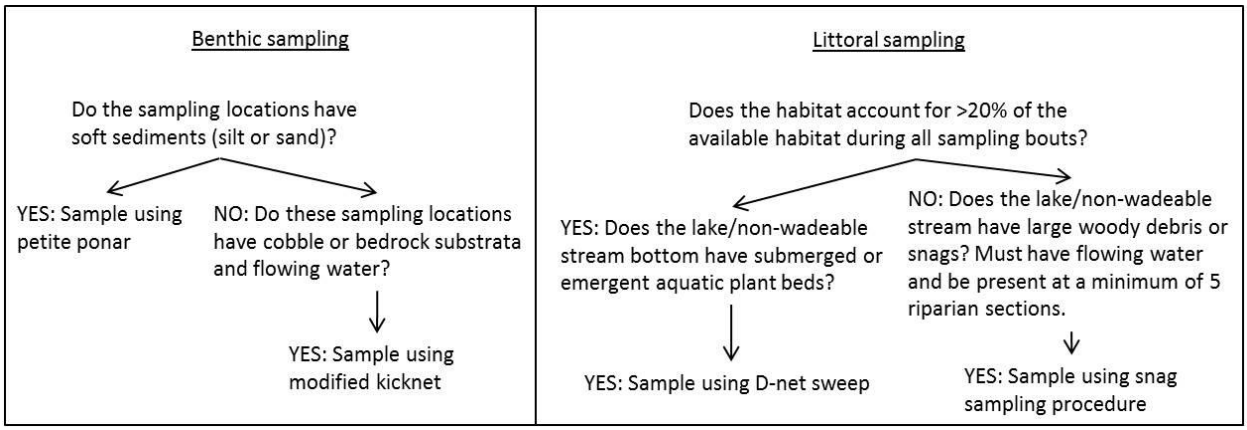

Figure 12. Lake and non-wadeable stream decision tree for choosing the appropriate sampler.

4. Do not sample anywhere you or other field technicians have recently disturbed (via sampling, walking, driving a boat, etc.) or locations that appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).

B.2 Wadeable Streams

1. Do not sample within a 5 m radius of the aquatic instrumentation.
2. Determine the dominant habitat and second-most dominant habitat based on the Stream Morphology Map (RD[09]) or rapid habitat assessment (RD[09], SOP F) for the site. Habitat suggestions for NEON sites are provided in Appendix E.
   a. The habitat types chosen should be present during all sampling bouts, and should account for at least 20% of the habitat area in the permitted reach as determined by the Rapid Habitat Assessment (RD[09]). The 20% cutoff ensures that there is enough habitat in which to collect all samples.
   b. All 5 dominant samples must be collected from the same habitat type on each sampling bout and all 3 subdominant samples must be collected from the sample habitat type on each sampling bout, unless a major event (i.e., a flood or dewatering of the stream) causes significant changes to the stream channel and the habitat type is no longer present.
   c. If there is only one clear habitat at the site, sample two different types of substrata using the sampling methods below (e.g., in a slow-moving run, collect 5 kicknet samples and 3 core samples).
   d. Targeted habitat types in order of sampling preference (see Definitions, Section 2.4; Figure 13):

i. Riffles: Surber (SOP C.1) or Hess (SOP C.7)
ii. Runs: Surber (SOP C.1), Hess (SOP C.7), or modified kicknet (SOP C.6)
iii. Snags: D-frame snag net (SOP C.3)
iv. Pools: hand corer (SOP C.4) or Hess (SOP C.7)

3. Start sampling at the bottom of the reach, working upstream so as not to stir up sediments in the water column which can decrease visibility and cause invertebrates to drift.
4. Spread samples out along the reach. For example, do not collect all samples for the same substratum in the same riffle, collect samples from 5 different riffles along the reach.
5. Do not sample anywhere you or other field technicians have walked in the reach, or locations that appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).

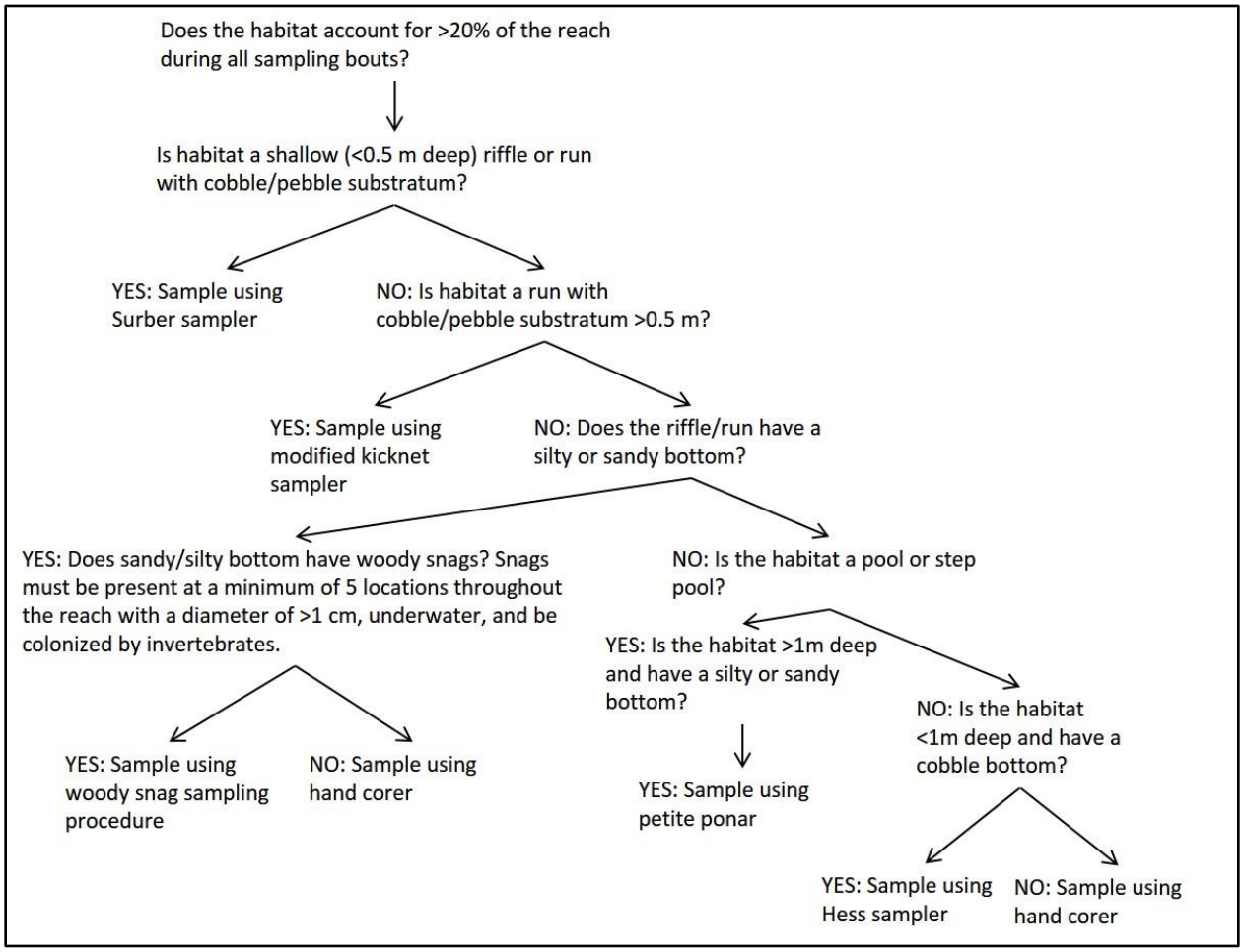

Figure 13. Wadeable stream decision tree for choosing the appropriate sampler.

SOP C Field Sampling

C.1 Surber Sampler

In **wadeable streams**, the Surber sampler is used in shallow riffle and run habitats. At selected small headwater stream sites where habitats are too small to fit a standard Surber sampler (0.093 m^2), a mini Surber sampler (0.023 m^2) may be used in its place. The decision to use the mini Surber will be made by the site host and NEON aquatic ecologist. All sampling steps below remain the same for either sampler.

1. Ensure that all samples are taken from riffles of similar depth and flow (within approximately 20%) in the **wadeable stream**. Ensure that water level is below the top of the Surber frame (best practice for sampling using a Surber).
2. Choose a location in the riffle that has flowing water and appears to be underwater most of the time (i.e., not underwater only at high flow). Avoid locations that are on top of, or just downstream of, large boulders (e.g., boulders that affect the flow of water at your location). Ensure that all substrata within the Surber frame are underwater.
3. Set up the Surber sampler by extending the base and clipping the arms into the screws on the base of the Surber frame (Figure 14).
4. Carry the 3 gallon bucket and Surber sampler with you to the sample location in the stream.
5. Approach sample location from downstream so as not to step on the cobbles that you want to sample.
6. Fill 3 gallon bucket ¼ - ½ full with stream water so that it is weighted when resting on the stream bottom or manually hold bucket in place. Place the bucket on the stream bottom in a shallow part of the riffle (or on the shore) **that you can still reach** from the Surber location.
7. Orient the Surber so the opening of the net is facing into the stream flow (Figure 14).

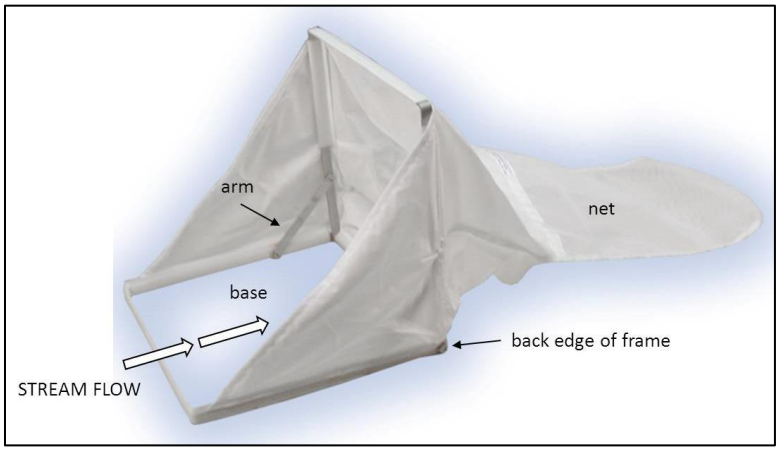

Figure 14. Diagram of Surber sampler set-up. The inside of the base is the sample area of the stream bottom (0.09 m2).

8. Place the back edge of the frame on the stream bottom first so that any material dislodged will flow into the net. Gently lower the rest of the base onto the substratum. Once base is placed on the stream bottom, do not move the sampler.
   a. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 15).
   b. Always stand downstream of the frame so you do not kick additional material into the sample.
   c. Hold Surber in place using hand or brace against your leg or knee. Avoid stepping on the bottom frame as that can affect the flow of water into the net.
   d. Ensure that nothing obstructs the flow of water into and out of the net (i.e., the net should not be kinked or partially out of the water).

NEON Aquatic Benthic Invertebrate Collection

Site ID: *BARC* | **Sampling protocol:** *NEON.DOC.003046* **Rev.:** *B*
Date: *2016-10-03* | **Recorded by:** *jstewart@battelleecology.org*
Local time: *13:00* | **Collected by:** *sparker@Field-ops.org*

Named location	Sampling impractical	Habitat type	Sample type	Sample number	Substratum size class	Sample ID	DNA sample?	*Ponar depth (m)	*Snag length (cm)	*Snag diameter (cm)
in		pelagic	petite ponar	1	silt	BARC.20161003.ponar.1		2.10		
ot		pelagic	petite ponar	2	sand	BARC.20161003.ponar.2		1.40		
c0		pelagic	petite ponar	3	sand	BARC.20161003.ponar.3		1.20		
02-Riparian		littoral	benthic sweep	2	sand	BARC.20161003.sweep.2				
02-Riparian		littoral	benthic sweep	2	sand	BARC.20161003.sweep.2.DNA	y			
04-Riparian		littoral	benthic sweep	4	sand	BARC.20161003.sweep.4				
04-Riparian		littoral	benthic sweep	4	sand	BARC.20161003.sweep.4.DNA	y			
06-Riparian		littoral	benthic sweep	6	sand	BARC.20161003.sweep.6				
06-Riparian		littoral	benthic sweep	6	sand	BARC.20161003.sweep.6.DNA	y			
08-Riparian		littoral	benthic sweep	8	silt	BARC.20161003.sweep.8				
08-Riparian		littoral	benthic sweep	8	silt	BARC.20161003.sweep.8.DNA	y			
10-Riparian		littoral	benthic sweep	10	silt	BARC.20161003.sweep.10				
10-Riparian		littoral	benthic sweep	10	silt	BARC.20161003.sweep.10.DNA	y			

Figure 15. Example of field data sheet for invertebrate sampling at a lake site.

9. Remove cobbles one at a time from the area inside the base of the Surber frame (Figure 14).
   a. Hold each cobble near the net opening and lightly brush debris, insects, etc. from all surfaces of the cobble with your hands. The stream flow will rinse this material into the Surber net.
   b. Place cobbles in 3 gallon bucket (Figure 16).
   c. Continue until the top layer of cobbles has been removed from the net.

d. For cobbles that are partially inside and outside of the frame: for every 1 cobble that you select that is partially outside of the frame, leave one cobble that is partially outside of the frame in the substratum (Figure 17).

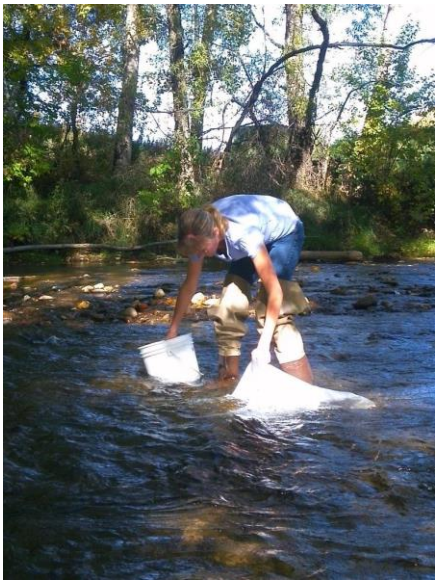

Figure 16. A field technician holds the Surber net on the stream bottom, and keeps the bucket nearby for depositing rocks from the base of the Surber.

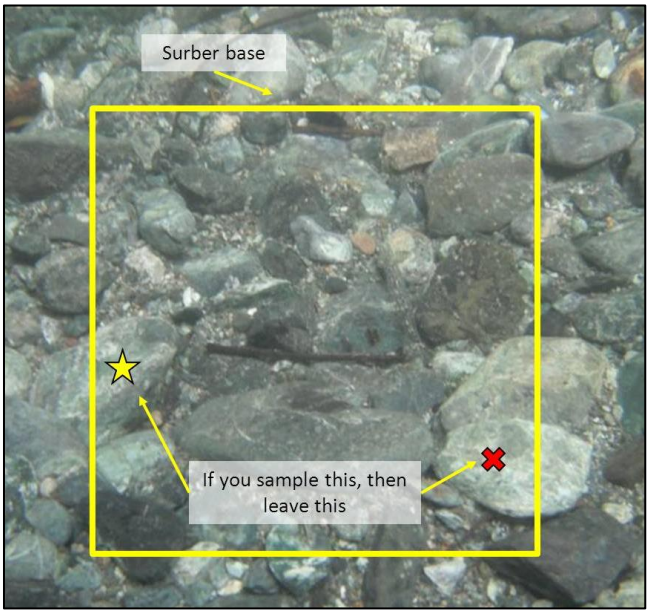

Figure 17. Cobble selection at Surber edges. The yellow square represents the bottom of the Surber sampler.

10. After the top layer of cobbles has been removed and placed in the bucket, disturb the remaining sediments (~10 cm deep; Cuffney et al. 1993) with your hand or kitchen brush so that all detritus flows into the Surber net for 1 minute.
11. After all suspended material has been washed into the net, lift the Surber sampler up off the substratum.
12. Dip the net into the stream a few times to rinse the sides of the net, always directing flow towards the back of the net. This allows material caught in the net to concentrate at the bottom of the net. Be sure to keep the net opening above the water surface so as not to accidentally lose or collect additional material in the sample.
13. Return to the processing location on the stream bank with the bucket of cobbles and the Surber sampler.
14. Set the Surber at the side of the stream so that the frame is on shore, and the end of the net (and all material collected) is sitting in water (to keep insects alive).
15. Scrub each cobble in the bucket using the kitchen brush. Scrape off all aquatic plants using the pot scrubber side of the nylon kitchen brush or a putty knife. Brush all invertebrates into the bucket (Figure 18).
    a. Scrub gently but firmly to remove insects, but not so vigorously as to damage insects. They are often identified by fragile anatomical structures such as external gills.
    b. Rinse the cobble in the bucket. Visually inspect the cobble for organic material (e.g., moss) and clinging invertebrates (e.g., caddisflies and snails) before discarding.
    c. Discard cleaned cobble onto the stream bank.
    d. Repeat until all cobbles in the bucket have been scrubbed and discarded.

Figure 18. A field technician scrubs rocks from the Surber sample into the 3 gallon bucket

16. Invert Surber net (turn it inside-out) into bucket of water. Swirl net around in bucket water to remove all material from the net. Use your hands to brush material from the net into the bucket.
    a. Immediately release any fish or other vertebrates captured in the net.
    b. Depending on site-specific permits, mussels collected in the sampler may also need to be released.
    c. Use 500 mL wash bottle filled with stream water to rinse any residual organic matter or insects from the net (Figure 19). Check for clinging insects.

Figure 19. Removing the tip of the 500 mL wash bottle helps makes rinsing easier and increases water pressure. This tip pulls easily out of the main body of the wash bottle.

17. Once the Surber net is empty, all of the sample material will be suspended in water in the 3 gallon bucket. Proceed to Section C.9: Sample Elutriation and Preservation.
18. After elutriation and preservation, move to next location and repeat the above steps until you have the desired number of samples.
    a. **Wadeable streams**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat.

C.2 Petite Ponar

The petite ponar is primarily used for benthic sampling in **lakes** and **non-wadeable streams**. In **wadeable streams**, the petite ponar may be used in pools with soft sediments that are too deep to use the hand corer safely.

1. Navigate to sampling location:
    a. For **wadeable streams**; sample in pools of similar depth and flow that have soft substratum, and are > 1 m deep.
        i. 5 locations for dominant habitat, 3 locations for secondary habitat.
    b. For **lake and non-wadeable stream** sample in three locations:
        i. **Lakes** (near water chemistry sampling locations):
            1) Deepest point in the lake, determined by bathymetric site map and GPS coordinates (location = c0)

2) Near the lake inlet (location = in)
3) Near the lake outlet (location = ot)

i. **Non-wadeable streams** (near water chemistry sampling location and 2 other haphazardly chosen locations):
1) Sensor/buoy location (location = c0)
2) Choose two other locations representative of the sensor location. Locations should be ±10 m of the river depth at the buoy and a minimum of 50 m apart from each other.

2. In **wadeable streams** stand just downstream of the location or on the bank near where you want to sample. Do not disturb the sediments where you plan to sample.
   a. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 15; RD[05]).
3. In **lakes and non-wadeable** streams, gently lower an anchor at the bow and allow boat to float back with wind or current to sampling location. Drop a second anchor at the stern to hold boat in place.
   a. Allow ~5 minutes for sediments to settle after lowering the anchor, you can use this time to prepare the sampling equipment.
   b. Using a bow anchor rope 2 times the water depth will minimize disturbance of the sediment at the sampling location.
   c. Sample on the side of the boat, away from the motor and anchor, so as not to interfere with the ponar or disturb the sediments
4. Release the safety-pin on the ponar sampler and open the bottom of the ponar (Figure 20). Place the pinch-pin (with spring) in the ponar and hold the ponar by the rope at the top. The sampler should stay in the open position as long as there is tension on the rope. The ponar will close automatically when the tension is released (i.e., the sampler hits sediment).

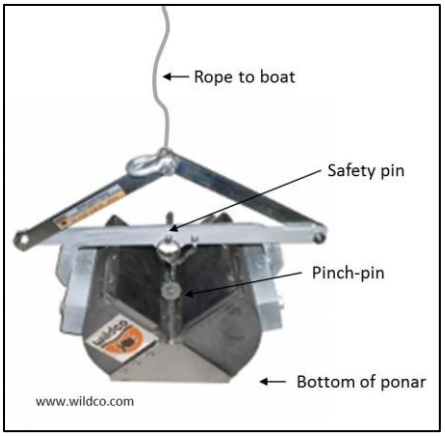

Figure 20. Example of a petite ponar setup

5. Hold the open ponar above the water surface above the point where you want to sample.
6. Slowly lower the ponar in the water and maintain rope tension.
7. When the ponar hits the sediments and the lines goes slack, pull up on the rope to close the jaws.

8. If the jaws do not close when the sampler hits the **lake/non-wadeable stream** bottom, attach a messenger to the line and drop onto the ponar to trigger the jaws to close.
9. Pull the ponar up to the surface using the rope.
10. Check to see if the ponar properly closed. If it did not close or there is an object (such as a stick) holding the jaws open, dump the sample away from your next sampling location and return to Step 5 in a different location (at least 2 m away from original location in lakes and non-wadeable streams).
11. If the ponar is properly closed, place the sampler over the bucket and open the jaws to release the sample.

   a. NOTE: Ponar sampling in areas of heavy macrophyte growth may cause the ponar to close improperly. Sample in areas of lighter or no plant growth if possible.
12. Rinse the inside of the ponar into the bucket using the 500 mL wash bottle.
    a. If in a **lake**, rinse the inside of the ponar into the bucket using the 500 mL wash bottle filled with filtered (through the 250 μm sieve) **lake** water to exclude zooplankton.
    b. If in a **non-wadeable stream** and zooplankton is apparent in rinsewater to the naked eye, use 250 mm filtered water as detailed in the previous step.
13. Once ponar is empty and clean, all of your sample material will be in the 3 gallon bucket.
14. Carefully add water to the bucket to until bucket is about ½ full.
    a. In a **lake**, use >250 μm filtered water to remove zooplankton. You may either use the 250 μm sieve or 243 μm Nitex mesh (used for preservative change in lab) to filter water.
15. Proceed to Section C.9 Sample Elutriation and Preservation.
16. After elutriation and preservation, rinse sampler with native water, move to the next sampling location and repeat the steps above until you have the desired number of samples.
    a. **Wadeable streams**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat
    b. **Lakes and non-wadeable streams**: 3 samples

C.3 Woody Snag Sampling (small woody debris)

1. Woody snag sampling should be used where snag/woody debris fits inside a D-frame net for sampling. This method is considered quantitative because all of the sample material fits inside the net. Sample in areas of similar flow and depth for each sample.
    a. Snags must be submerged and appear to be underwater most of the time (i.e., not underwater only at high flow).
    b. Snags must account for >20% of habitat throughout the sampling area.
    c. Snags may include leaf litter packs, overhanging branches, submerged wood (< 0.3 m (6 inches) in diameter and < 5 m long. If using leaf litter packs/debris must be present throughout the year (not just seasonally).
    d. Note the dominant substratum size class below the snag at the sampling location on the field data sheet (Figure 15; RD[05]).

Figure 21. Sites such as D08 Mayfield Creek are well-suited to snag sampling with their sandy/silty substrate and abundance submerged large woody debris.

2. Two technicians are needed to collect the sample. Place the 243 µm mesh bag (D-frame net) around the end of the snag with the bag opening facing upstream so invertebrates will not escape.
    a. If there is slow- or no flow at the sampling location, try to angle the net toward the water surface to prevent macroinvertebrates from swimming away.
3. The second technician should quickly saw or lop the snag near the end of the sampling net (near the mouth of the D-frame net).
4. Remove the snag section covered by the bag by sawing with the bow saw or breaking the snag at the end of the bag.
    a. If you are unable to remove the snag or need to leave it in the stream for other sampling, you may scrub into the net without breaking off the piece as long as the stream flows into the net to collect any organisms that are brushed off the snag. This step requires flow to be present at the sampling location.
5. Fill 3 or 5 gallon bucket ¼ - ½ full with native water.
6. **Wadeable stream**: Remove snag from net and place in the bucket of stream water.
7. Invert net (turn it inside-out) into the bucket of water. Swirl net around in bucket water to remove all material from the net. Use your hands to brush material from the net into the bucket.
8. Use the 500 mL wash bottle filled with native water to rinse any residual organic matter or insects from the net. Check for organisms clinging to the sides of the net or in the seams.
9. **Wadeable stream**: Brush insects off snag surface using the nylon kitchen brush.
    a. Rinse snag surface into bucket using the 500 mL wash bottle.
    b. Measure length and width of snag and record on field data sheet (RD[05]).
    c. Visually inspect snag and remove any remaining insects using flexible forceps or disposable pipet.
10. All sample material should now be in the 3 or 5 gallon bucket.
11. Fill out field sheet with location, date, and type of samples (Figure 15).

12. Proceed to Section C.9 Sample Elutriation and Preservation.
13. After elutriation and preservation, rinse net in stream, move to next sampling location and repeat the steps above until you have the desired number of samples
    a. **Wadeable streams**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat

C.4 Large Woody Debris Sampling

Large woody debris (LWD) sampling is intended for logs that are too large to cut off and sample via the snag method (SOP C.3). This sampling method is considered semi-quantitative because the entire substrate that is sampled does not fit inside the net.

1. Sample in areas of similar flow for each sample.
    a. LWD must be submerged and appear to be underwater most of the time (i.e., not underwater only at high flow).
    b. LWD must account for >20% of habitat throughout the sampling area.
    c. LWD is typically characterized by trees that have fallen into the stream/river from the bank, or have been carried downstream by the current and become lodged against the stream/river bottom.
    d. In a **non-wadeable stream**, choose either 5-even or 5-odd riparian sections for sampling
        i. Choose sections by looking at the time: if the minute is even, sample even sections. If the minute is odd, sample odd sections.
        ii. If there is no LWD in the section chosen, proceed to the next section. You may sample 2 snags from the same section if necessary, as long as they are >5 m apart.
        iii. The sample number on the sample labels should correspond to the Riparian Habitat Section numbers.
    e. Note the dominant substratum size class below the snag at the sampling location on the field data sheet (Figure 15; RD[05]).
2. Ensure that the LWD is submerged to at least 0.6 m (2 ft) deep, in flowing water (**non-wadeable stream** only), and has a diameter of ≥0.15 m (6 inches). The snag should either break the water surface or come within 0.3 m (1 ft) or the water surface for ease of sampling (Angradi et al. 2006).
    a. Navigate boat to first LWD, approaching slowly from downstream.
    b. Carefully drop and anchor the boat or have the boat driver should hold the boat in place in the current using the boat motor.
    c. Place the modified kick-net (13” x 20” opening) on top of the LWD with the opening facing up-current, just below where the snag breaks the water surface. Make sure the frame of the net is touching the LWD surface
    d. Using the long-handled deck brush, scrub ~1 m of snag so that organisms and debris wash into the sampling net (Figure 22). Scrub the top and sides of the snag.
        i. As this is a semi-quantitative method, not all organisms will be washed into the net.

e. Measure length and width of snag sampled and record on Field Data Sheet (RD[05]).
f. At the end of the sweep, turn the net opening toward the water surface and lift out of the water to prevent organisms from escaping.
g. Dip net into the water a few times to rinse the sides of the net, always directing flow towards the back of the net. This allows material to concentrate at the bottom of the net. Be sure to keep the net opening above the water surface so as not to accidentally lose or collect additional material in the sample.
h. Pull the net into the boat.

Figure 22. From Angradi et al. 2006, example of snag sampling in a large river. While the boat driver holds the boat in position, one technician holds the modified kicknet against the snag, while a second technician scrubs the snag with a long-handled deck brush.

3. Fill 3 or 5 gallon bucket ¼ - ½ full with native water.
4. Invert net (turn it inside-out) into the bucket of water. Swirl net around in bucket water to remove all material from the net. Use your hands to brush material from the net into the bucket.
5. Use the 500 mL wash bottle filled with native water to rinse any residual organic matter or insects from the net. Check for organisms clinging to the sides of the net or in the seams.
6. All sample material should now be in the 3 or 5 gallon bucket.
7. Fill out field sheet with location, date, and type of samples (Figure 15).
8. Proceed to Section C.9 Sample Elutriation and Preservation.
9. After elutriation and preservation, rinse net in stream, move to next sampling location and repeat the steps above until you have the desired number of samples
   a. **Wadeable streams**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat
   b. **Non-wadeable stream**: 5 samples

C.5 Hand Corer

The hand corer is used in **wadeable streams** in pools or runs with sand or silt substrata.

1. Sample in areas of similar flow and depth along the **wadeable stream** sample reach.
   a. Sample only soft sediments (sand, silt, clay).
2. Rinse the corer assembly to be sure that all screw threads are clean of silt and sediment.
3. Assemble the hand corer (Figure 23).
   a. Screw the corer head onto the top of the stainless steel core tube (Figure 23). **NOTE**: Petroleum jelly may be applied to the threads to aid sealing.
   b. Cores are usually more stable without the PVC liner tube. The corer may be used with or without the liner tube and core catcher, this decision may be made by the technician at the time of sampling.
   c. Screw the stainless steel nosepiece onto the bottom of the core tube (Figure 23).
   d. Petroleum jelly may be applied to the flutter valve to help create suction in the corer.

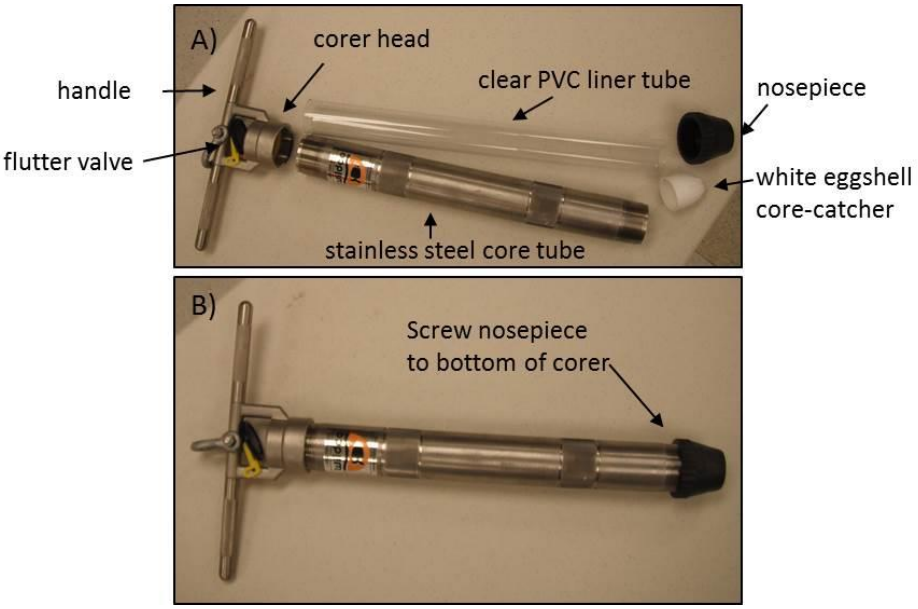

Figure 23. Hand-corer assembly: A) Disassembled corer. Use of the liner tube and core catcher are optional. B) Assembled corer. Note: the nosepiece may be stainless steel (not black Lexan as in photo) in some domains

4. Approach sample location from downstream so as not to step in the location where you want to sample.
5. Hold the core sampler firmly by the handle (Figure 23).
6. Push the core sampler through the water and into the sediments in one smooth, continuous movement (Figure 24). Allow the flutter valve to remain open.
   a. Depending on the sediment type, it may take a lot of force to push the corer into the sediments.
   b. Do not hammer or pound the corer into the sediments.

c. Keep barrel of corer at a 90° angle with the sediments.
d. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 15; RD[05]).

Figure 24. Push the hand corer through the water and into the sediments at a 90˚ angle

7. Push corer approximately 5-10 cm into the substratum while keeping the blue flutter valve open.
   a. Pre-measure 5 cm and 10 cm on the outside of the corer before sampling.
8. If the corer is not completely submerged, wet the blue flutter valve (or apply petroleum jelly) on the top of the corer head and close by hand (Figure 23).
   a. The valve must be kept wet to maintain a good seal and prevent loss of sample when the corer is extracted. Petroleum jelly may help keep the flutter valve sealed.
   b. If the corer is submerged, allow air to escape from the flutter valve. When the corer is pulled out, the flutter valve should close by itself.
9. Holding the flutter valve closed, **slowly** pull the corer straight up and out of the sediments.
   a. If the corer is pulled up too fast, you may lose the contents.
   b. You may also use a spatula or your hand to cap the bottom of the core before pulling it out of the sediments if maintaining suction is a problem at the site
10. Continue to hold the flutter valve closed and lift the entire core sampler clear of the water, keeping the corer vertical so as not to spill the sample.
    a. If the sample spills before reaching the bucket, you may rinse the corer and start over at Step 3 approximately 0.5 m away from the original location, as long as it has not been disturbed.
    b. If some, but not all, of the sample spills and is not deposited in the bucket, discard the entire sample and start over.

i. Use a secondary container to deposit each core so that if one core fails, the entire composite sample is not lost.

11. Quickly hold the core sampler over the 3 gallon bucket and release the flutter valve, allowing the sample to drop into the bucket.
    a. Depending on suction in the corer, the sample may drop into the bucket soon after it is removed from the water.
12. Fill the 500 mL wash bottle with stream water and rinse any residual organic matter or insects from inside the corer into the bucket.
    a. Check for clinging insects.
13. Once the corer is empty, all of your sample material will be suspended in water in the 3 gallon bucket.
14. Repeat Steps 3-13 two more times, until there is a composite of 3 cores in the 3 gallon bucket. Proceed to Section C.9 Sample Elutriation and Preservation.
    a. 3 cores = 1 composite sample
15. After elutriation and preservation, clean core in stream water and move to next location and repeat the steps above until you have the desired number of composite samples.
    a. **Wadeable streams**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat

C.6 Modified Kicknet Sampler

The modified kicknet may be used for deep runs in **wadeable streams**, or benthic sampling in **non-wadeable streams** with cobble or bedrock substrata in place of the petite ponar.

1. Choose runs (or deep riffles) that are similar in flow and depth.
2. Make sure net is firmly attached to handle.

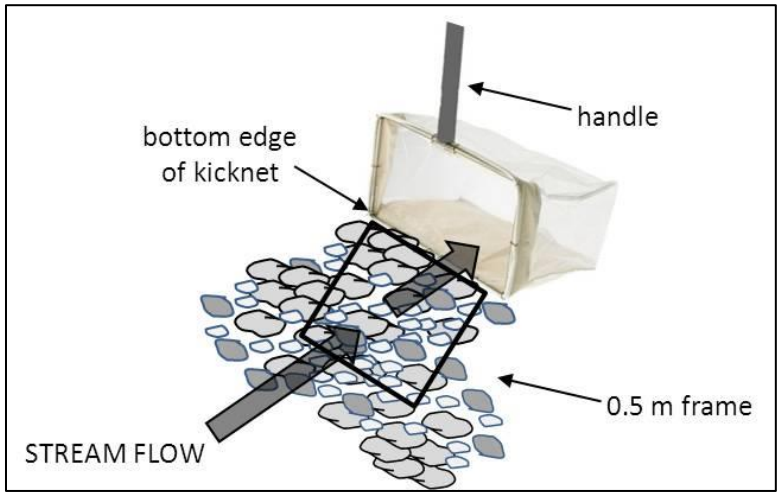

Figure 25. Example of modified kicknet setup.

3. Approach sample location from downstream so as not to disturb the location where you want to sample.
4. Orient the net so the opening of the net is facing into the stream flow.
5. Place the bottom edge of the frame on the stream bottom first, hold in place. Once the net edge is placed on the stream bottom, do not move the net (Figure 25).
6. Disturb the substrata ~0.25 m^2 upstream of the kicknet.
    a. **Wadeable stream**: Place 0.25 m^2 quadrat just upstream (1-5 cm) of the kicknet edge.
        i. If you can reach the stream bottom of the wadeable stream with your hands, pick up each cobble and brush the surface with a kitchen brush so that the stream current carries all insects/organic matter into the net.
            1) For cobbles that are partially inside and outside of the frame: for every 1 cobble that you select that is partially outside of the frame, leave one cobble that is partially outside of the frame in the substratum (Figure 25).
            2) Once a cobble is cleaned, discard to the side or downstream of the sample area. Do not resample cobbles.
        ii. If you cannot reach the stream bottom, step into the quadrat. Use feet to disturb the sediments by kicking back and forth for 60 seconds inside the quadrat, so all organic matter flows into the net.
    b. **Non-wadeable streams**: If water is too deep for wading, disturb substrata using a long-handled brush so all organic matter flows into the net and estimate the area of the 0.25 m^2 quadrat.
7. Sweep the kicknet toward the water surface, taking care to keep the opening facing upstream so no contents will spill.
8. Dip the net into the stream a few times to rinse the sides of the net, always directing flow towards the back of the net. This allows material caught in the net to concentrate at the bottom of the net. Be sure to keep the net opening above the water surface so as not to accidentally lose or collect additional material in the sample.
9. Take the net to a processing location on the stream bank.
10. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 15; RD[05]).
11. Half-fill 3 or 5 gallon bucket with native water.
12. Invert net (turn it inside-out) into bucket of water. Swirl net around in bucket water to remove all material from the net. Use your hands to brush material from the net into the bucket.
13. Use 500 mL wash bottle to rinse any residual organic matter or insects from the net.
    a. Check for clinging insects.
14. Once the net is empty, all of the sample material will be suspended in water in the 3-5 gallon bucket. Proceed to Section C.9 Sample Elutriation and Preservation.
15. After elutriation and preservation, clean net in stream water and move to next location and repeat the steps above until you have the desired number of samples.

a. **Wadeable streams**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat
b. **Non-wadeable stream**: 3 samples (replaces petite ponar samples)

C.7 Hess Sampler

In **wadeable streams**, the Hess sampler should be used in shallow riffles, runs or pools where the other samplers above will not work due substratum type or stream flow.

1. Ensure that all samples are taken from areas of similar depth and flow (within approximately 20%). Water level must be below the top of the Hess frame.
2. Choose a location that appears to be underwater most of the time (i.e., not underwater only at high flow). Avoid locations that are on top of, or just downstream of, large boulders (e.g., boulders that affect the flow of water at your location).
3. Carry the 3 gallon bucket and Hess sampler with you to the sample location.
4. Fill 3 gallon bucket ¼ - ½ full with stream water so that it is weighted when resting on the stream bottom or manually hold bucket in place. Place the bucket on the stream bottom in a shallow part of the riffle (or on the shore) **that you can still reach** from the sampling location.
5. Position the Hess frame securely on the stream bottom, with the screened opening facing upstream and the net facing downstream (Figure 26)
   a. Hold the sampler in position by applying pressure with your knees to the back of the sampler, or have a second field technician hold the sampler.
   b. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 15).

Figure 26. Hess sampler with collection net and dolphin bucket

6. Reach into the sampler and remove any large substrata (i.e., cobbles) and place in the 3 gallon bucket.
7. After the top layer of cobbles has been removed and placed in the bucket, disturb the remaining sediments (~10 cm deep; Cuffney et al. 1993) with your hand or kitchen brush so that all detritus flows into the Hess net (typically ~30-60 seconds).
   a. If there is insufficient stream flow to wash sample and detritus into the net, create flow with your hands until the water inside the Hess sampler appears clear.

8. Dip the net into the stream a few times to rinse the sides of the net, always directing flow towards the back of the net and dolphin bucket. This allows material caught in the net to concentrate at the bottom of the net. Be sure to keep the net opening above the water surface so as not to accidentally lose or collect additional material in the sample.
9. Return to the processing location on the stream bank with the bucket of rocks and the Hess sampler.
10. Set the Hess at the side of the stream so that the frame is on shore, and the end of the net (and all material collected) is sitting in water (to keep insects alive).
11. Scrub each cobble in the bucket using the kitchen brush. Scrape off all aquatic plants using the pot scrubber side of the brush. Brush all invertebrates into the bucket.
    a. Scrub gently but firmly to remove insects, but not so vigorously as to damage insects. They are often identified by fragile anatomical structures such as external gills.
    b. Rinse the cobble in the bucket. Visually inspect the cobble for organic material (e.g., moss) and clinging insects (e.g., caddisflies) before discarding.
    c. Discard cleaned cobbles on the stream bank.
    d. Repeat until all cobbles in the bucket have been scrubbed and discarded.
12. Rinse all material in the net into the dolphin bucket.
13. Holding the end of the net and dolphin bucket over the 3 gallon bucket, unscrew the dolphin bucket and rinse its contents into the 3 gallon bucket.
    a. Invert net into 3 gallon bucket and rinse any remaining insects to the sample.
14. Once the Hess net is empty, all of the sample material will be suspended in water in the 3 gallon bucket. Proceed to Section C.9: Sample Elutriation and Preservation.
15. After elutriation and preservation, clean net in stream water and move to the next location and repeat Steps 5-14 until you have the desired number of samples
    a. **Wadeable streams**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat

C.8 D-net Measured Sweep Samples (Semi-quantitative)

In **lakes** and **non-wadeable streams**, the D-net sweep samples are the preferred sampling method for littoral samples.

1. Sampling locations will correspond to the 10 habitat stations set forth in the Riparian Habitat Assessment Protocol (RD[15]). This divides the **lake/non-wadeable stream** margins into 10 sections (Figure 27).

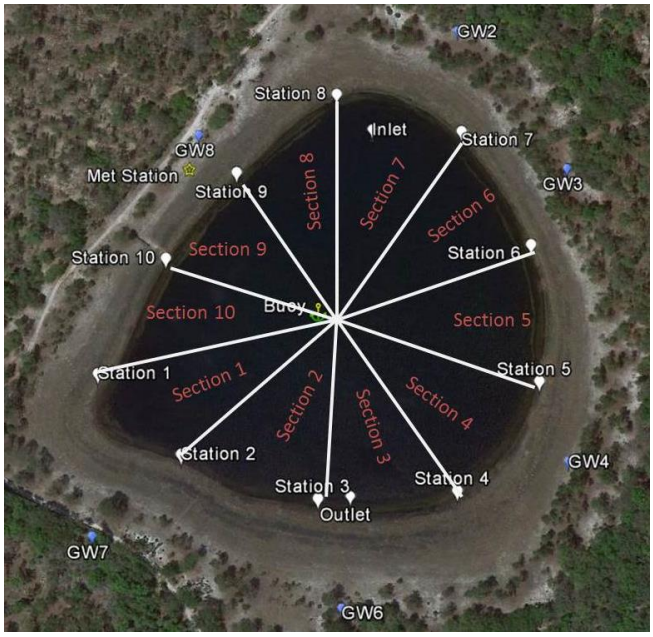

Figure 27. Example of Riparian Stations splitting the lake into sections at D04 Lake Barco.

2. Choose either the 5-even or 5-odd habitat sections for sampling.
   a. Choose sections by looking at the time. If the minute is even, sample even sections. If the minute is odd, sample odd sections.
   b. The sample number on the sample labels should correspond to the Riparian Habitat Section numbers.
   c. Sample in rooted plant habitat <1m deep if possible. Choose similar depth and habitat to sample in each section.
      i. If the lake has no rooted plant habitat, sample floating vegetation mats.
      ii. Senescent or dead plant material is acceptable for sampling.
   d. You may sample anywhere along the shoreline of the section in order to find the preferred sampling habitat. If no preferred habitat is available, skip to the next section, then continue with the previously selected sections.
3. Navigate boat to an aquatic plant bed in the chosen sampling section. Ensure that you are able to reach the **lake/non-wadeable stream** bottom with the sampling net.
4. If permits allow and the area of the **lake or non-wadeable stream** is shallow enough to wade, step out of the boat and sample by wading (permits will be provided by NEON Permitting). If permits are not available, sample near the bow of the boat, away from the motor so the motor will not interfere with the sweep net or disturb the sediments.
5. Attach handle to D-frame net (Figure 28).

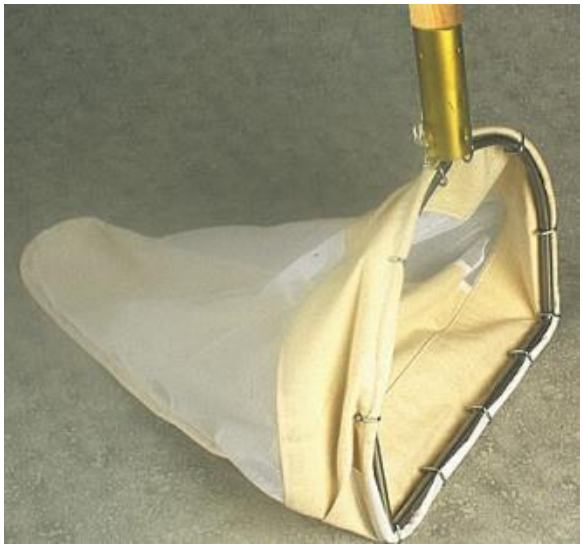

Figure 28. D-frame net, used for sweep samples in vegetation and silty habitats.

6. Sweep the D-frame net through the vegetation across a 1 m distance (DiFranco 2006, USEPA 2011).
   a. To determine the sweep length, measure 1 m on the net handle (typically 1 m = the wooden part of the handle). Using this as a reference, place feet 1 m apart on the **lake/non-wadeable stream bottom** and sweep from foot to foot
   b. The net should remain submerged during the entire sweep, and the bottom of the net should brush the sediments during the entire sweep (Figure 29).
   c. For rooted aquatic plants: bump or jab the bottom of net frame against the sediment several times (> 3 times) to collect benthic organisms while keeping the net frame close to, but not scraping, the bottom.
   d. For floating vegetation mats: scoop all vegetation into the net.
   e. Keep net in motion to prevent organisms from swimming out of the net. Conduct sweep quickly, entire sweep should last ~3 seconds.
   f. At the end of the sweep, turn the net opening toward the water surface and lift out of the water to prevent organisms from escaping.
   g. Retain all plants that are completely in the net in the sample. If plants are hanging on the net frame, break the plants and include the portions that are in the net with the sample, discard those that are hanging outside the net.
   h. Rinse net by dipping in the water, keeping the mouth of the net above the water line so as not to introduce additional organisms to the sample.

Figure 29. D-frame sweep technique in a littoral area; the D-frame net must remain submerged and close to the substrate throughout the entire sweep.

7. For floating aquatic plants only, hold the net in the water with the mouth above the water line. Agitate the plant material with your hands, in water in the net for 1 minute. Rinse plant material with 500 mL wash bottle if necessary. Inspect vegetation to ensure that you have removed invertebrates. Remove and discard plant material while keeping macroinvertebrate sample in the net (FDEP 2011).
    a. Keep in mind that this is a quantitative sampling approach, so do not discard material that contains macroinvertebrates.
8. With the macroinvertebrate sample in the net, dip D-frame net into the water a few times to rinse the sides of the net, always directing flow towards the back of the net. This allows material to concentrate at the bottom of the net. **Be sure to keep the net opening above the water** surface so as not to accidentally lose or collect additional material in the sample.
9. Pull the net into the boat.
10. Fill the 3 gallon bucket ¼ – ½ full with 250 µm filtered **lake** water to exclude most zooplankton.
11. Invert net (turn it inside-out) into bucket of filtered rinsewater. Swirl net around in rinsewater to remove all material from the net. Use your hands to brush material from the net into the bucket.
12. Use 500 mL wash bottle filled with 250 µm filtered **lake** water to rinse any residual organic matter, plants, or organisms from the net into the bucket. Check for organisms clinging to the sides of the net or in the seams.
    a. All sample material should now be in the 3 or 5 gallon bucket.
13. Fill out field data sheet and label with location, date, and type of sample (Figure 15).
14. Complete Sample Elutriation and Preservation (SOP C.9).

15. After elutriation and preservation, rinse net in native water and move to the next location, repeating the steps above until you have the desired number of samples.
    a. **Lakes and non-wadeable streams**: 5 samples

C.9 Sample Elutriation and Preservation

1. Samples only need to be elutriated if they have inorganic material that you do not want to include with the sample. If the sample is all organic material, include all material in the sample and proceed to preservation (Step 15).
2. Gently swirl contents in bucket to create a "whirlpool" and suspend organic material. This process is called elutriation (Figure 30).

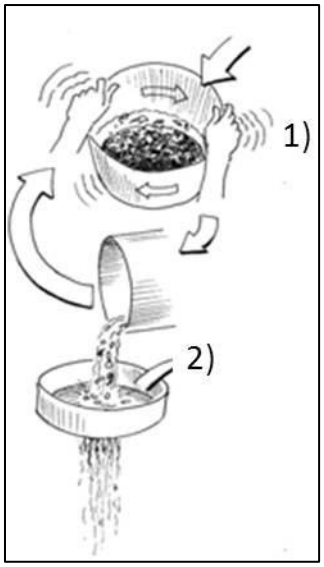

Figure 30. Process of elutriation (Dates and Byrne 1997).

3. Carefully pour contents of bucket into 250 µm sieve or sieve bucket (Figure 31). If you are in a boat, hold the sieve over a 5 gallon bucket to capture the waste water. Some material will be retained in the original bucket.
    a. The sieve bucket should be used for petite ponar samples for thick, fine sediments.
    b. If the sieve fills up with organic matter, you may pick clumps out of the sieve and place in sample jar.
    c. You may use your fingers or shake the sieve or sieve bucket to stir the sample and move fine material through the sieve.
    d. You may use low-pressure water to wash sediment through sieve mesh. High pressure will damage organisms.
    e. Sieving the sample in small portions may help prevent clogging.
    f. Continue rinsing to reduce the sediment in the sample, especially for petite ponar samples. Reduce samples to ≤750 mL if possible.

Figure 31. A field technician carefully pours water from the 3 gallon bucket into the sieve.

4. Partially refill bucket with water (bucket opening should be facing upstream) without losing any of the organic matter contents of the bucket.
   a. Use 250 µm filtered water in **lakes** to exclude zooplankton. You may use the 250 µm sieve or 243 µm Nitex mesh (used to change preservative in the lab) for filtering rinsewater.
5. Repeat elutriation (Steps 2-4) until the water appears relatively clean. There may be some sand or silt in the bottom of the bucket. Elutriate a **minimum** of 3 times per sample.
   a. Elutriating ~3-5 times (until you no longer see insects in the swirled water in the bucket) is typically sufficient to remove insects from the inorganic matter.
   b. At sites with fine sediments (i.e., petite ponar samples in lakes), elutriation may be particularly difficult. If the sieve bucket, dunk the bottom of the sieve bucket directly into the lake water, without adding new water to the top of the bucket, to facilitate sediment rinsing.
6. Check sides of bucket for clinging invertebrates. Check sand/inorganic material at the bottom of the bucket for caddisflies (rock cases) or other heavy invertebrates (e.g., snails), place in sample container using forceps or fingers. Retain any plant material in the sample jar.
   a. Place any invertebrates from this inspection in the sieve with the rest of the sample.
   b. Mussels may need to be returned to the stream depending on permitting requirements.
7. Discard inorganic material remaining in the bucket, and rinse bucket with native water. Set bucket aside.
8. The entire sample should now be in the sieve or sieve bucket.
9. Add a waterproof paper label (write in pencil) inside the sample jar **AND** a small adhesive label to the outside of the sample jar with the sample ID for use by the external taxonomy lab. Write in

pencil as ethanol will erase permanent marker. Make sure the label sample ID matches the sample ID generated by the data ingest.

a. Sample ID format: *SITE.DATE.sampleType.sampleNumber* (Table 8)
b. Example: *ARIK.20140620.kicknet.3*

10. Fill the 500 mL wash bottle with native water. Use 250 µm filtered water in **lakes** to exclude additional floating invertebrates.
11. Rinse sieve screen and edges with wash bottle and tap sieve until organic material is collected at one edge of the sieve (Figure 32).

Figure 32. A field technician rinses the sample from the sieve into the open container with the 500 mL wash bottle.

12. Open the sample jar and carefully rinse (using the 500 mL wash bottle) sample from the sieve into the sample jar. You can also use your fingers to move the sample from the sieve into the sample jar.
    a. Use as little water as possible. Be sure to rinse sieve and fingers into the sample jar to thoroughly remove all material.
    b. If there is too much rinse-water, you may re-sieve sample by dumping contents of sample jar back into the sieve, and repeating Steps 11-12.
13. If sample is too large for one sample jar, you may use multiple jars. Each jar must be properly labeled with site and date information, as well as "Sample 1 of 2" or "Sample 2 of 2".
14. Check sieve for clinging insects that may not have been washed into the sample jar.
15. **Carefully** add preservative. Preserving immediately prevents damage to insects in the samples.
    a. Wear latex gloves when preserving samples.
    b. Preserve samples in a well-ventilated location (e.g., outdoors).

c. Add enough ethanol preservative to the sample to preserve in a 1:1 sample material: preservative ratio (sample material includes water in the jar).

16. Close the sample jar tightly.

C.10 Sample Preservation

Preserve samples using 95% ethanol streamside/lakeside or at the field vehicle (see details above in each sampling section) to prevent predation within the samples to a final concentration of ~70% ethanol.

C.11 Ending the Sampling Day

1. Refresh the sampling kit
   a. Replace sample jars and resealable bags.
   b. Print new field labels and field data sheets.
   c. Refill/restock preservative containers.
2. Equipment maintenance, cleaning and storage
   a. Decontaminate all equipment that has come in contact with site water according to the NEON Aquatic Decontamination Protocol (RD[08]).
   b. Dry all equipment thoroughly between sites and before storage.
   c. Check all nets for holes and patch if necessary.

SOP D Macroinvertebrate DNA Metabarcode Field Collection

Macroinvertebrate DNA samples are collected during each sampling bout, three times per year. Samples collected specifically for DNA metabarcoding will be analogs to three of the samples already being collected in the dominant habitat type for morphological taxonomy. This sampling approach uses the community metabarcoding approach, where replicate samples from the site are preserved in high-concentration ethanol for sequencing analysis.

D.1 Sterilize equipment

All equipment must be cleaned and sterilized prior to sample collection at the site to prevent contamination of DNA from the person collecting the sample, DNA from another site, or DNA from the environment outside of the site (Laramie et al. 2015).

1. Cleaning equipment:
   a. Wearing nitrile gloves, clean all nets, waders, and other equipment using a 10% bleach solution rinsed with DI (Jane et al. 2014). Note that this is a higher concentration that is usually used in the Aquatic Decontamination Protocol (RD[08]).
2. Wearing clean nitrile gloves, place equipment and consumables in a clean bag so as not to contaminate it on the way to the field site.

D.2 Field collection

1. Fill out and place an adhesive label on the sample jar. Circle “DNA” on label. **Do not** use a paper label inside the sample jar for this protocol.
2. Collect an additional sample at 3 of the 5 dominant habitat sampling locations chosen above (SOP B). Use the same sampler already chosen to use for taxonomy samples at each of those locations.
3. Prior to sampling, prime the sampler by rinsing well with local source water.
4. Collect samples according to SOP B for the appropriate habitat type and sampler.
5. Choose “DNA” on the field datasheet, and the appropriate habitat and sampler metadata.
6. Sample ID = *SITE.YYYYMMDD.sampleType.sampleNumber.DNA*
7. After sample collection, use forceps or clean-gloved hands to pick out large organic matter, leaving the macroinvertebrates in the sample.
8. After the entire sample is in the sample jar, carefully drain the some of the extra water off the top. You may want to use the sieve to help drain without losing macroinvertebrates from the sample.
9. Add 95% ETOH to sample jar to completely cover sample (Stein et al. 2012). ETOH concentration should be as close to 95% as you can get (as little extra water as possible.
10. Return sample to domain lab and store in the dark at 4 ^{o}C and proceed to Section E.3.

SOP E Laboratory Sampling and Analysis

E.1 Preparation

3. Fill 500 mL wash bottle with 475 mL 95% undenatured ethanol and 25 mL 99% glycerol (final concentration: 5% glycerol; Stein et al. 2013). Clearly label bottle.

E.2 Processing Samples – Taxonomy

1. Wearing safety glasses and nitrile gloves, open field-preserved sample under fume hood.
2. Carefully decant liquid over a clean 250 μm sieve or filter cup similar to that used in the TOS Beetle Protocol (RD[10]) over an ETOH waste container. Take care not to lose any sample material.
    a. Rinse down sides of sample jar with ETOH/glycerol wash bottle so no material remains near the top of the sample jar.
    b. If using sieve, check for any macroinvertebrates or other organic material that may have come from the sample. Using featherweight forceps, place this material back in the sample jar.
    c. If using the TOS beetle filter cup, rinse down the sides of the cup with ETOH, and place the filter mesh and all material on it back into the sample jar. Use 250 μm mesh, not the TOS mesh.
3. Check that sample label is still readable and inside the sample jar. Check that the external adhesive label is on the outside of the sample jar.
4. Re-preserve sample with 95% ethanol/5% glycerol solution. Fill jar with liquid to prevent sample damage during shipping.
5. Close the sample jar tightly.
6. Carefully clean sieve or filter cup before decanting the next sample.
7. Continue until preservative in all samples has been replaced and proceed to Sample Shipping (SOP G).

E.3 Processing Samples – DNA

1. Change ethanol between 24 and 48 hours in the domain lab.
2. **Do not add glycerol** to DNA samples.
3. Wearing safety glasses and nitrile gloves, open field-preserved sample under fume hood.
4. Follow Section E.2 to change out the ethanol in the sample, with the exception of adding glycerol.
5. Replace ethanol, preserving as close to 95% as possible.
    a. Container does not need to be filled to the top with liquid.
6. Close the sample jar tightly, place in -20 ^{o}C and dark until shipping. Proceed to SOP G.

E.4 Sample Storage

Samples may be stored at the domain support facility at room temperature or 4 ^{o}C until shipping. For shipping instructions see SOP G.

E.5 Ending the Processing Day

1. Equipment maintenance, cleaning and storage
   a. Carefully clean and dry sieve to remove all organic debris.
   b. Dry all equipment thoroughly before storage.

SOP F Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription error 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 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

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

SOP G Sample Shipment

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

1. Keep morphological taxonomy samples and DNA samples separate.
2. Place sealed sample jars into one or several gallon-sized resealable zip-top bags, grouped by site. Sample jars are acceptable "inner containers" required for shipping.
2. Line a Group II cardboard box with a heavy-duty trash bag.
3. Place all sample jars right-side up inside the trash bag, inside the Group II cardboard shipping box. Add Grade 2 Vermiculite in the trash bag liner as needed to take up excess space in container and cushion samples.
4. Navigate to the "Shipping Information for External Facilities" document on CLA's NEON intranet site. Check whether items such as permits or cover letters are required in the shipment. Check this document often as instructions are subject to change.
5. Prepare a shipping inventory detailing the contents of the shipment, using the protocol-specific templates found on CLA's NEON intranet site (RD[11]. Include a printed copy of the shipping inventory in the shipment box in a zip-top bag.
6. Save the inventory with the following naming convention: "DXX_MOD_ShippingInventory_YYYYMMDD_XofX"
    a. Example: "D05_INV_ShippingInventory_20161202_1of2"
7. Complete packing slip, address shipment, and ship ground to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.
    a. Follow instructions for shipping ETOH in limited quantity ground shipments in AD[03].
    b. DNA samples do not need to be shipped on ice.
8. Email an electronic copy of the shipping manifest and tracking number to the email addresses listed in the CLA "Shipping Information for External Facilities" document, including the NEON CLA email address.

G.1 Handling Hazardous Material

Follow procedures for shipping ethanol in limited quantity ground shipments in AD[03].

G.2 Supplies/Containers

See section SOP G Steps 1-8 and Table 7 for specific shipping materials.

G.3 Timelines and Conditions

Taxonomy samples: Shipping should occur within one week of sampling if possible, however samples may be held for up to 30 days at the domain support facility if necessary. Samples may be stored at the domain support facility room temperature or 4 ^{o}C until shipping. Samples are shipped ground at ambient temperature.

DNA samples: Shipping should occur within one week of sampling if possible, however samples may be held for up to 30 days in the dark at -20 ^{o}C at the domain support facility if necessary. Samples may be held for a maximum of 6 months if batch shipping is requested by the external lab. Samples are shipped overnight at ambient temperature.

G.4 Grouping/Splitting Samples

Group samples by site per bout in plastic bags. Samples from multiple sites may be sent in the same shipment. Macroinvertebrate samples may be shipped with zooplankton samples (RD[17]) if going to the same external lab.

G.5 Return of Materials or Containers

Include return shipping label if any shipping materials need to be returned to the domain support facility (e.g., cooler). Sample jars will not be returned.

G.6 Shipping Inventory

Shipments are to have a hardcopy of the per sample tab of the shipping inventory (RD[11]) sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. Also include the shipment tracking number in the email. The shipping inventory must accurately document the physical samples inside the shipping container.

G.7 Laboratory Contact Information and Shipping/Receipt Days

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

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 9. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.003043	Datasheets for AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002494	Datasheets for AOS Shipping Inventory

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

APPENDIX B QUICK REFERENCES

B.1 Steps for Macroinvertebrate Sampling

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

Step 2 – Prepare internal (below) and external sample labels (2" x 4").

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

Step 4 – Determine habitat sampling locations from the Rapid Habitat Assessment (RD[09]).

Step 5 – Determine sampler type based on the habitats present and the order of preference for sampling habitats.

Wadeable streams

1. Riffles (Surber or Hess)
2. Runs (Surber, Hess, or modified kicknet)
3. Snags (D-frame snag net)
4. Pools (corer, Hess, or petite ponar)

Lakes/Non-wadeable streams – Benthic

1. Petite ponar
2. Modified Kicknet

Lakes/Non-wadeable streams – Littoral

1. Aquatic plant beds or floating mats (D-frame net), dead vegetation is acceptable.
2. Snags/large woody debris (Modified kicknet snag sampler)

Step 6 – Collect taxonomy samples (**Wadeable Streams**: 5 per dominant habitat type, 3 per second-most dominant habitat type; **Lakes/Non-wadeable streams**: 3 in benthic sediments, 5 in the dominant littoral habitat type with the appropriate sampler). Collect DNA samples (**Wadeable Streams**: 3 per dominant habitat type; **Lakes/Non-wadeable streams**: 3 per dominant littoral habitat type (with samples sampler as taxonomy).

Step 7 – Elutriate and pour sample over sieve or sieve bucket.

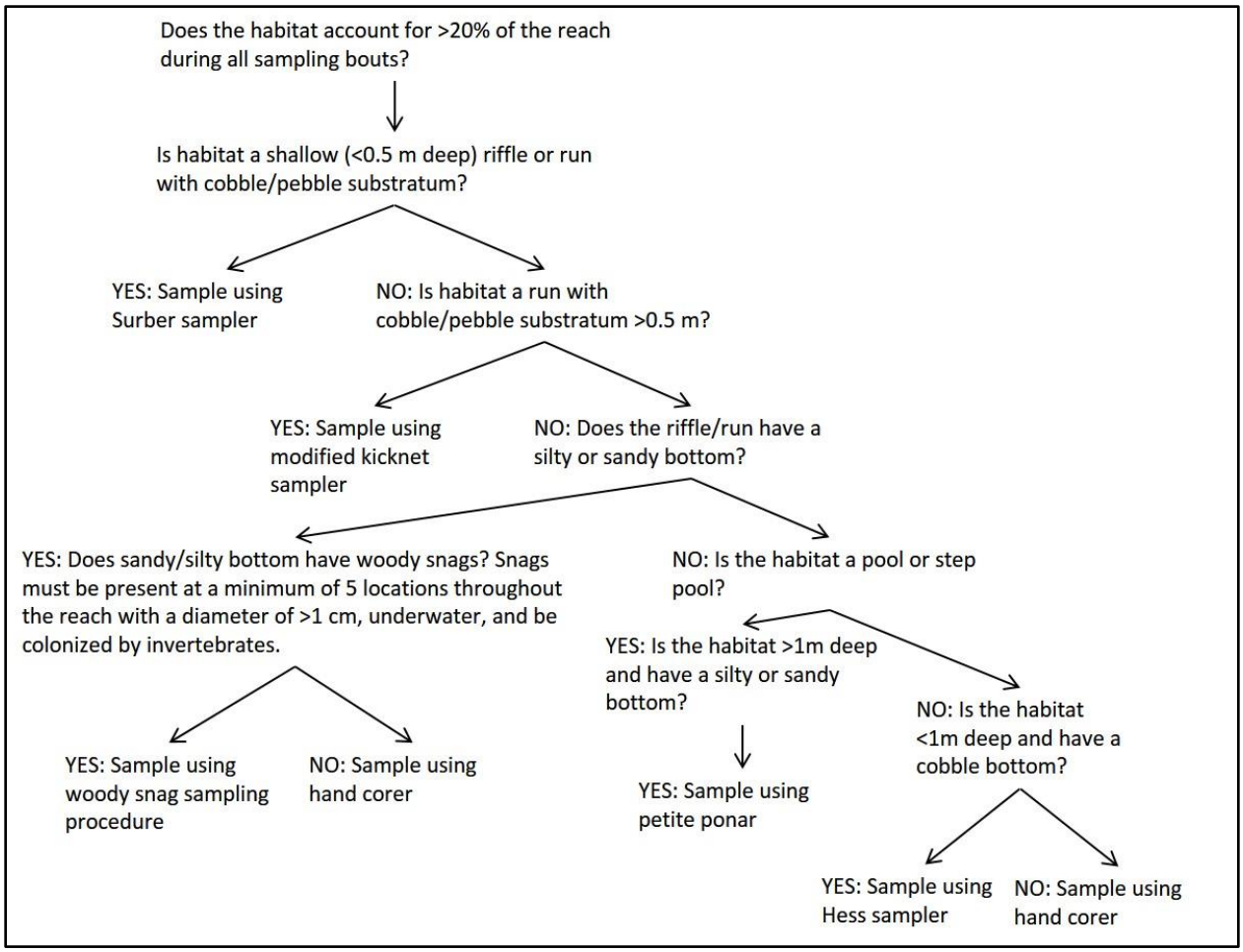
Step 8 – Transfer sample from sieve to sample jars

Step 9 – Preserve with 95% ethanol to a final concentration of ~70% ethanol in the field.

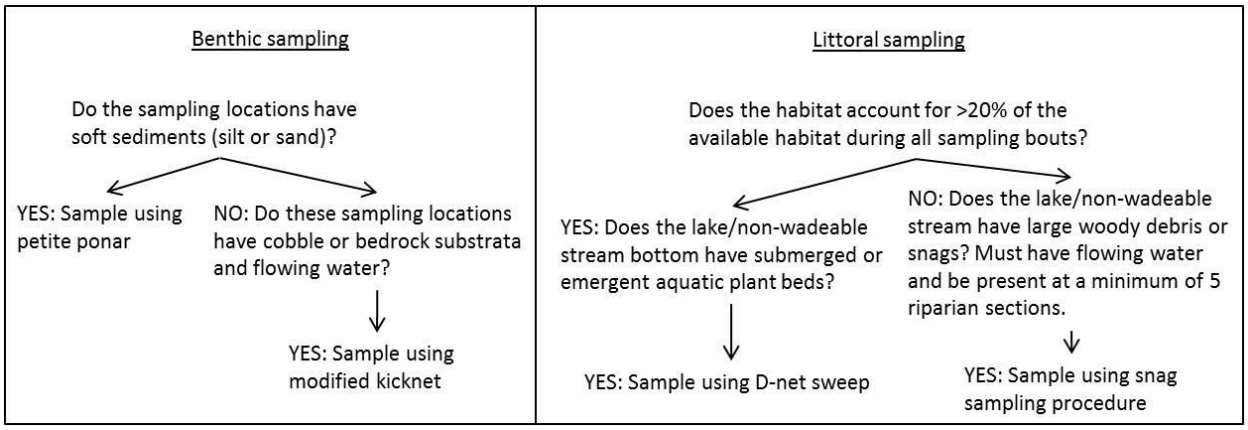
Step 10 – Taxonomy: Change preservative and add glycerol at the Domain Support Facility within 12-48 hours of sample collection. DNA: Change preservative (no glycerol) at the Domain Support Facility within 12-48 hours of sample collection.

Step 11 – Store appropriately and ship samples to external facility.

B.2 Determining Habitat to Sample in Wadeable Streams



B.3 Decision Tree for Lakes/Non-wadeable Streams



APPENDIX C REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare all equipment, including sample jars and labels.
- ☑ Pre-print waterproof labels and small adhesive labels.
- ☑ Check for holes in nets and sieve, assure that both are clean and free of debris.
- ☑ Bleach clean all equipment between sites for DNA sampling.
- ☑ Fill 1 L HDPE bottles with 95% undenatured ethanol. Cap tightly and clearly label bottle. Transport to the field following EHS guidelines.

Sample collection: Be sure to…

- ☑ Determine the habitats to sample based on the Stream Morphology or rapid habitat assessment, Bathymetry and Riparian Maps.
- ☑ Choose the appropriate sampler.
- ☑ Start sampling at the bottom of the reach in flowing water, working upstream so as not to decrease visibility and cause invertebrates to drift (**wadeable streams**).
- ☑ Spread samples out along the reach or among riparian sections.
- ☑ Do not sample anywhere you or other field technicians have walked in the reach, recently sampled, or locations that appear recently disturbed.
- ☑ **Lakes/Non-wadeable streams**: Collect 3 samples from benthic sediments and 5 samples from the dominant littoral habitat for taxonomy. Collect 3 samples from the dominant littoral habitat for DNA.
- ☑ **Wadeable Streams**: Collect 5 samples from the dominant habitat type and 3 samples from the secondary habitat type for taxonomy. Collect 3 samples from the dominant habitat type for DNA.

Sample preservation: Be sure to…

- ☑ Preserve the samples streamside/lakeside or at the vehicle to reduce predation.
- ☑ Change the preservative (and add glycerol for taxonomy only) within 12-48 hours of field sampling.
- ☑ Fill jar with ETOH to prevent destruction of organisms during shipping.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for biological sampling bouts. General guidelines for developing these dates are presented in the NEON Aquatic Sample Strategy Document (RD[07]). Also see the Site Specific Sampling Strategy Document on NEON's FOPS intranet site.

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

APPENDIX E SITE-SPECIFIC INFORMATION: HABITAT AND SAMPLER RECOMMENDATIONS

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

Domain	Site	Habitat 1 (5 reps)	Habitat 2 (3 reps)
D01	Hop Brook	Riffles (Surber)	Pools (corer)
D02	Lewis Run	Runs (core)	Riffles (Surber)
D02	Posey Creek	Riffles (Surber)	Pools (Hess)
D03	Flint River	Littoral (D-net sweep rooted)	Benthic (modified kicknet)
D03	Lake Barco	Littoral (D-net sweep rooted)	Benthic (petite ponar)
D03	Lake Suggs	Littoral (D-net sweep floating)	Benthic (petite ponar)
D04	Rio Guilarte	Riffles (Surber)	Pools (Hess)
D04	Rio Cupeyes	Riffles (Surber)	Runs (Surber)
D05	Crampton Lake	Littoral (D-net sweep rooted)	Benthic (petite ponar)
D05	Little Rock Lake	Littoral (D-net sweep rooted)	Benthic (petite ponar)
D06	Kings Creek	Riffle/run (modified kicknet)	Pools (corer)
D06	McDiffett Creek	tbd	tbd
D07	Leconte Creek	Riffles (Surber)	Pools (corer)
D07	Walker Branch	Riffles (Surber)	Runs (Surber)
D08	Mayfield Creek	Runs/riffles (snags)	Runs (core)
D08	Black Warrior River	Littoral (snag)	Benthic (petite ponar)
D08	Tombigbee River	Littoral (snag)	Benthic (petite ponar)
D09	Prairie Lake	Littoral (D-net sweep rooted)	Benthic (petite ponar)
D09	Prairie Pothole	Littoral (D-net sweep rooted)	Benthic (petite ponar)
D10	Arikaree River	Runs (modified kicknet)	Pools/Runs (core)
D11	Pringle Creek	Runs (core)	Riffles (Surber)
D11	Blue River	Runs (modified kicknet)	Riffles (Hess)
D12	Blacktail Deer Creek	Riffles (Surber)	Riffles/Runs (snags)
D13	Como Creek	Riffles (Surber)	Runs (modified kicknet)
D13	West St. Louis Creek	Riffles (Surber)	Pools (core)
D14	Sycamore Creek	Riffles (Surber)	Runs/Pools (core)
D15	Red Butte Creek	Riffles (Surber)	Runs (modified kicknet)
D16	McRae Creek	Riffles (Surber)	Step pools (snags)
D16	Martha Creek	Riffles (Surber)	Pools (snags)
D17	Teakettle 2 Creek	tbd	tbd
D17	Upper Big Creek	tbd	tbd
D18	Oksrukuyik Creek	Runs (modified kicknet)	Riffles (Surber)
D18	Toolik Lake	Littoral (D-net sweep rooted)	Benthic (petite ponar)
D19	Caribou Creek	Runs (modified kicknet)	Riffles (Surber)