AOS PROTOCOL AND PROCEDURE:
INV – AQUATIC MACROINVERTEBRATE SAMPLING

PREPARED BY	ORGANIZATION	DATE
Stephanie Parker	AQU	11/22/2022

APPROVALS	ORGANIZATION	APPROVAL DATE
Kate Thibault	SCI	01/10/2023

RELEASED BY	ORGANIZATION	RELEASE DATE
Tanisha Waters	CM	01/10/2023

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Change Record

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B	02/08/2017	ECO-04359	Update NEON template; Separate and rename snag sampling SOPs; Update sample ID template; Add DNA metabarcode SOP
C	01/19/2018	ECO-03046	Tape jar lids for shipping, remove mini surber, move datasheets to appendix
D	12/19/2018	ECO-05968	Remove stream ponar sampling, update habitat and sampler preference, add contingencies and rules for stream drying, clarify DNA sterilization, lab processing, and storage, reorder sampling sections
E	10/22/2019	ECO-06244	Change lab processing time to 24 hours for DNA samples, individually bag DNA samples, new template
F	03/16/2022	ECO-06781	• Update to reflect change in terminology from relocatable to gradient sites
G	01/10/2023	ECO-06919	• Remove glycerol from preservation • Add alternate hand corer liner method • Clarify rescheduling and samplingImpractical • Add contingent decisions for rivers • Set jar size requirements for sample types • Update labels • Remove sample timing from appendix • Remove part numbers from equipment list for nonspecific items

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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. Many taxa spend their larval life stage in water then emerge to inhabit the terrestrial environment. 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) and from the aquatic environment to terrestrial upon emergence. 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). Community composition, abundance, and diversity are often seasonal as many macroinvertebrate taxa spend only a portion of their life history in the aquatic environment. 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 rivers** 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	NEON EHSS Policy, Program and Management Plan
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: DMP – Data Management
RD[05]	NEON.DOC.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.004257	NEON Standard Operating Procedure (SOP): Decontamination of sensors, field equipment and field vehicles
RD[09]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[10]	NEON.DOC.014050	TOS Protocol and Procedure: BET – Ground Beetle Sampling
RD[11]	NEON.DOC.005224	NEON Protocol and Procedure: Shipping Ecological Samples and Equipment
RD[12]	NEON.DOC.003045	AOS Protocol and Procedure: ALG – Periphyton and Phytoplankton Sampling
RD[13]	NEON.DOC.003039	AOS Protocol and Procedure: APL – Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling
RD[14]	NEON.DOC.003044	AOS Protocol and Procedure: AMC – Aquatic Microbial Sampling
RD[15]	NEON.DOC.001195	AOS Protocol and Procedure: RIP – Riparian Habitat Assessment in Lakes and Non-wadeable Streams
RD[16]	NEON.DOC.001197	AOS Protocol and Procedure: BAT – Bathymetry and Morphology of Lakes and Non-wadeable Streams
RD[17]	NEON.DOC.001194	AOS Protocol and Procedure: ZOO – Zooplankton Sampling in Lakes
RD[18]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[19]	NEON.DOC.003600-NEON.DOC.003618	Aquatic Site Sample Design – NEON Domain ##

2.3 Acronyms

Acronym	Definition
cm	centimeter
DNA	Deoxyribonucleic acid
EMAP	Environmental Monitoring and Assessment Program
FFG	Functional feeding group
ft	Foot
GHS	Globally Harmonized System
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 24**). 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 28**). 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 27**).

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 30**).

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) and are <0.3 m in diameter and 5 m in length.

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 19**).

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 for benthic invertebrates at each aquatic sampling site. These variables, especially diversity and biomass, will be tracked over time 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 (or rivers). Five samples for morphological taxonomy are collected in the dominant habitat type (wadeable stream) or littoral area (lake and river), and three samples for morphological taxonomy are collected in the sub- dominant habitat type (wadeable streams) or pelagic area (lakes and rivers). An additional three samples for DNA metabarcoding will be collected in the dominant habitat type, for a total of eight taxonomy samples plus three DNA samples (11 samples total) 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 on woody debris. In lakes and rivers, 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 C. 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 low-flow 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, normal flow conditions within the watershed. Sampling will not occur directly following a flood in wadeable streams (defined as >3x median discharge; Clausen and Biggs 1997). Should such a flood event occur on or prior to a target collection date, sampling should be delayed at least 5 days 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. Seepage lakes have no true inlet or outlet stream but have littoral sensor sets. In flow-through streams, littoral infrastructure are located in the inlet or outlet stream channel.

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 ecologists **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 Data Quality Plan (AD[05]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Benthic invertebrate sampling in lakes, rivers and wadeable streams occurs three times per year at each site, roughly spring, summer, and autumn (Parker and Utz 2022, Appendix C). Sampling must be initially scheduled within the first 21 days of the 1 month window specified in Appendix C, this helps to allow for rescheduling and weather contingencies. Observe a minimum of two weeks between the sampling date and the start of the next scheduled bout window for Bouts 1 and 2. For Bout 3, allow for 30 days to reschedule missed sampling. Accommodations for local weather conditions (e.g., late ice-off) may be made that cause the sample date to fall later in the bio bout or outside of the pre-determined window. See the Aquatic Site Sampling Design (RD[19]) for your domain for additional details and scheduling preferences. Use NEON's problem reporting system to seek guidance and report sampling efforts that take place outside of the defined sampling window.

Table 1. Sampling frequency for macroinvertebrate procedures on a per SOP per site type basis.

SOP	Site Type	Location	Bout Duration	Bouts Per Year	Bout Interval	Yearly Interval	Remarks
SOP B.2	all aquatic	any	1 day	NA	NA	opportunistic	May collect large or rare specimens that are missed with regular sampling
SOP B.3	lake, river	buoy, inlet, outlet, riparian sections, reach	1 day	3	Minimum of 2 weeks between sampling and start of next bout, or 30 days after the end of Bout 3	annual	DNA samples: Bout 2 send for analysis, Bouts 1 and 3 sent to archive
SOP B.4	stream	reach	1 day	3	Minimum of 2 weeks between sampling and start of next bout or 30 days after the end of Bout 3	annual	DNA samples: Bout 2 send for analysis, Bouts 1 and 3 sent to archive

Scheduling Considerations

1. All samples for a bout must be collected during the same day.
2. **Field Work and Laboratory Processing:** After macroinvertebrate samples are collected, the following points are critical with respect to timing:
   a. Preserve samples in the field within 60 minutes of collection.

b. Keep DNA samples cool (4 °C) during transportation and storage.

c. Replace ETOH in DNA samples within 24 hours of collection.

d. Replace ETOH in taxonomy samples within 72 hours of collection.

e. Store DNA samples at 4 °C until shipping.

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 C).

4.3 Timing for Laboratory Processing and Analysis

Macroinvertebrate samples must have their preservative changed after field collection, and before sending to the external labs. Preservative must be changed within 72 hours of field sampling for taxonomy and within 24 hours for DNA samples. Ethanol may be changed the same day as collection if desired. Samples should ideally be shipped to the external lab within 30 days of collection, though preserved (and refrigerated DNA) samples may be held longer at the domain support facility if necessary.

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/rivers as detailed in this protocol) because of the fluctuating nature of aquatic habitats. Spreading sample collection over multiple days increases the likelihood of variability among samples. Sampling may be rescheduled due to weather or environmental conditions within the bout window (+ 3 days) provided in the Site Sampling Designs (RD[19], Appendix C). An incident ticket must be issued if sampling is rescheduled >3 days past the end of the bout window. A minimum of 2 weeks between sample collection and the start of the next bout window shall be observed, with the exception of specific domains with a limited growing season (e.g., D18).

Table 2. Contingent decisions.

Delay/ Situation	Action	Outcome for Data Products
Hours	Stream, lake, river: If weather conditions deteriorate and conditions become unsafe (e.g. approaching thunderstorm, rapid increase of stream/river water, or the lake/river becomes too windy (>35 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 may be flagged.

	sampling, if not, discard samples, return to the Domain Support Facility and sample at another time.	
5 Days	Stream: If flooding occurs on or prior to the targeted sampling date (>3x median discharge; Clausen and Biggs 1997) or unsafe wading conditions occur (Lane and Fay 1997), wait a minimum of 5 days after water level drops below 3x median discharge 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 may be flagged.
	River: Use the nearest USGS gage to determine whether the river is in “flood stage” ad determined by USGS or NOAA. Flood stage is typically not safe for boating, let alone sampling. Depending on the site, water level may need to be even lower than flood stage to allow for safe boating and sampling. Allow at least 5 days after water levels drop below flood stage to sample.	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data may be flagged.
	Stream: If the channel has dried and enough water (>100 m length) to sample returns to the channel, wait for the channel to be wet for 5 days of recolonization before sampling.	None as long as samples are collected within the pre-determined sampling window. If waiting for water to return causes sampling to occur outside of the sampling window, data may be flagged.
6 Months	Preserved macroinvertebrate samples may be held for up to 6 months at room temperature (INV-tax), 4 °C (INV-dna and INV-arc) in the domain lab if circumstances do not allow shipping to the external lab. If held for longer than 6 months, containers must be topped off with preservative if evaporation has occurred. If space is limited, you may use the follow alternate storage options: • INV-tax at 4 °C • INV-arc at room temperature • INV-dna or INV-arc at -20 °C.	Holding samples past the scheduled shipping date affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected.

4.5 Missed or Incomplete Sampling

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

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

Instances such as those listed above must be documented for scheduling, tracking long-term site suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[18]).

Missed or Incomplete Sampling Terms

- **Canceled Sampling**: Incidence of *scheduled sampling* that did not, and will not, occur. Canceled Sampling is recorded at the same resolution as data that are ordinarily recorded (e.g., each missed macroinvertebrate sample gets its own record).
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Canceled Sampling event – i.e., why sampling did not occur. This field is also used to indicate any individually missed samples within an otherwise successful sampling bout, e.g., if a stream is drying and contingent decisions are used.
- **Biophysical Criteria**: This field name associated with a controlled list of values that is included in the data product to explain changes to the schedule.
- **Rescheduled**: Missed Sampling is rescheduled for another time within the *protocol sampling dates*, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 11**).

Unable to sample
Can sampling be rescheduled?
No
- Submit ServiceNow Incident
- Enter samplingImpractical records
Unsure
- Submit Schedule Change Request
Yes
Rescheduled within Schedule Sampling dates?
Yes
Sample
Was sampling rescheduled?
Yes
No
- Submit ServiceNow Incident
- Enter samplingImpractical records
No
Rescheduled within Protocol Sampling dates?
Yes
Sample
No
- Submit Schedule Change Request
- Enter samplingImpractical records
Schedule Change Request Approved?
Yes
- Sample
- Flag records (biophysicalCriteria)

Figure 11. The documentation to account for a Missed Sampling event depends on the situation for each plot of each bout that is not sampled. Blue rounded boxes represent contingencies, green double line boxes describe the required actions, Orange dotted boxes indicate HQ actions. Required delay and cancellation actions are outlined for each protocol in the 'Scheduled Field Activities – Delays and Cancellations' spreadsheet available on the SSL. Missed Sampling events may also require a Data Quality flag and/or creation of a Site Management record.

To Report Missed or Incomplete Sampling:

1. Canceled or rescheduled sampling must be communicated to Science by a Service Now Incident or Schedule Change Request.
    a. The lead Field Ecologist should consult the Scheduled Field Activities - Delays and Cancellations to best determine when reporting is required (**Figure 11**).

2. **Canceled sampling:** For each missed sample, a **Sampling Impractical** field must be populated in the mobile collection device (**Table 3**).
    a. A minimum of three INV-tax and 3 INV-dna or INV-arc must be collected during each sampling event if following contingent decisions (**Table 5**), else sampling should be re-attempted.
    b. Total number of sample records expected per event (bout): 8
3. For **Rescheduled** sampling events that occur within the biology bout window, sample and record data as normal. Rescheduling within the bout window does not need to be reported to science unless sampling will occur after fish sampling or another disturbance event.
4. **Rescheduled** sampling events that occur outside of the defined AOS biology sampling bout window must be approved by Science in a Schedule Change Request.
5. Sampling events that are **Rescheduled** ±3 days outside the sampling date require an entry in Biophysical Criteria (**Figure 11**).
    a. biophysicalCriteria – An indicator of whether sampling coincided with the intended biophysical conditions (i.e., within the AOS biology sampling bout window)

Table 3. Guidance for responding to delays and cancellations encountered during implementation of the INV-Aquatic Macroinvertebrate Sampling.

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
Aquatic Macroinvertebrate Sampling	> 3 days outside bio bout window or rescheduling after fish (streams only)	IS/OS Schedule Change Request	Submit incident ticket

Table 4. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Field name	Dropdown list options	Description
Sampling Impractical	Location dry	Location dry
Sampling Impractical	Location frozen	Location frozen
Sampling Impractical	Location snow covered	Location snow covered
Sampling Impractical	High water velocity	Water velocity too high to sample in stream or river
Sampling Impractical	Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)
Sampling Impractical	Other	Sampling location inaccessible due to other ecological reason described in the remarks
Biophysical Criteria	OK - no known exceptions	Sampling occurred on schedule, no known issues
Biophysical Criteria	OK - schedule change but conditions met	Sampling occurred not within defined sampling window but reflects the target biophysical conditions

Field name	Dropdown list options	Description
Biophysical Criteria	conditions not met – outside of sampling window	Sampling was conducted outside of the AOS sampling window
Biophysical Criteria	unknown - logistical	Sampling not possible due to logistical considerations
Biophysical Criteria	conditions not met: sampled after fish	Sampling does not reflect the target biophysical conditions, benthic sampling occurred after benthos was disturbed during seasonal fish sampling
Biophysical Criteria	other	Other potential sample timing inconsistencies described in the remarks

Table 5. Contingent decisions for sampling in a wadeable stream.

Situation	Action	Outcome for Data Products	Considerations
<u>**Wadeable stream**</u> site with <500 m aboveground stream length due to stream size or permitting restrictions	Habitat available may be insufficient to accommodate all 8 (or 11) samples without causing harm to the stream. Reduce sampling by collecting 3 taxonomy and 3 DNA samples in the dominant habitat type and 3 samples in the sub-dominant habitat.	Lower resolution for diversity metrics.	If the decision is made to decrease the number of samples collected for this protocol, it should also be reflected in the other wadeable stream biology protocols (RD[12], RD[13], RD[14]).
<u>**Wadeable stream**</u> site with seasonal drying	If the stream experiences seasonal drying such that the chosen habitat types have disappeared, select the next dominant habitat type, or use the sub-dominant habitat if still present. Reduce sampling by collecting samples only in the dominant habitat type that is present at the time of sampling (5 samples if ~200-500 m water available, 3 samples if ~100-200 m water available). Enter “sampling impractical” for the missing habitat type. 3 DNA samples should still be collected during in Bout 2. If enough water is not available during Bouts 1 and 3, prioritize taxonomy samples over DNA samples.	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.
<u>**Wadeable stream**</u> site with seasonal drying	If the stream dries such that that is <100 m of wetted channel, there is not enough habitat left to sample and sampling is considered impractical.	Missing data points	Mark as “sampling impractical” in the field data.

	Wait for water to return or contact Science.		

4.6 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, use NEON's problem reporting system to notify Science. 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 field ecologists for four hours per site, plus travel to and from the site. Lab processing requires one ecologist for one to two hours within 72 hours of field sampling for the preservative swap.

Table 6. Estimated staff and labor hours required for implementation of Macroinvertebrate Collection.

SOP	Estimated time	Suggested staff	Total person hours
SOP A: Preparing for sampling	1 h	1	1 h
SOP B: Determining sampling location and sampler type	1 h first year	2	2 h first year
SOP C: Field sampling (all site and sampler types)	4 h	2	8 h
SOP D: Macroinvertebrate DNA Metabarcode Field Collection	1 h	2	2 h (combined with SOP C)
SOP E: Post-Field Sampling Tasks	1 h	1	1 h
SOP F: Laboratory Sampling and Analysis	1 h	1	1 h
SOP G: Data Entry and Verification	1 h	1	1 h
SOP H: Sample Shipment	1 h	1	1 h

5 SAFETY

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

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Ecologist 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 8 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 ft2/s (0.93 m2/s; Lane and Fay 1997).
2. In lakes and rivers, site-specific hazards may be encountered that necessitate sampling from the boat, without dismounting from the vessel. In addition, use extra caution in waters where alligators are present and maintain a safe distance from hazards.
3. All personnel must be wearing a personal flotation device (PFD) prior to entering a boat.
4. All personnel shall have access to a form of communication with other team members such as a two-way radio.
5. Be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, water release from dams, etc.).

6 PERSONNEL

6.1 Training Requirements

All technicians must complete protocol-specific training as required in the Field Operations Job Instruction Training Plan (AD[04]). Additional protocol-specific required skills and safety training are described here.

All personnel required to operate a boat shall be trained through an approved program. All 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. Personnel must also be trained in safe handling of ethanol as required in the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

6.2 Specialized Skills

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

7 STANDARD OPERATING PROCEDURES

SOP Overview

- SOP A: Preparing for Sampling
- SOP B: Determining Sampling Locations and Sampler Type
- SOP C: Field Sampling
- SOP D: Macroinvertebrate DNA Metabarcode Field Collection
- SOP E: Ending the Sampling Day
- SOP F: Laboratory Sampling and Analysis
- SOP G: Data Entry and Verification
- SOP H: Sample Shipment

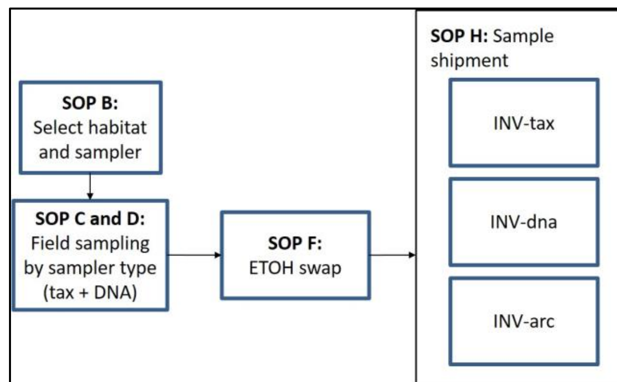

Figure 12. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.

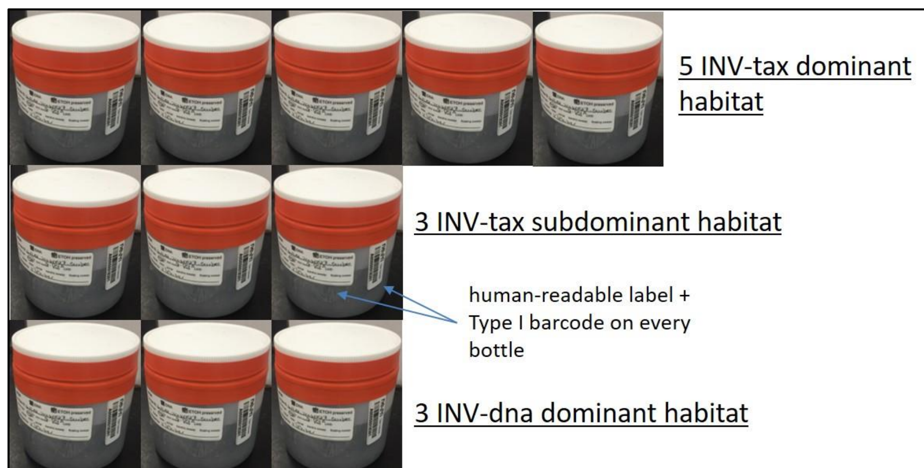

Figure 13. Macroinvertebrate sample suite, including 5 dominant INV-tax, 3 subdominant INV-tax, and 3 dominant INV-dna bottles with human readable and barcode labels. A total of 11 samples are collected per site per bout.

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 and synced 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 Labels and Identifiers

All barcodes need to be applied to dry containers for 30 mins before use. Macroinvertebrate collection uses Type I barcodes (prefix A, plus 11 numbers). Type I barcodes are for all field samples and any non-cryo applications; they have a tolerance from 4C to 105C and still scan.

1. All macroinvertebrate samples will have a weather-resistant, adhesive, human readable label on the outside of the jar (**Figure 14**).

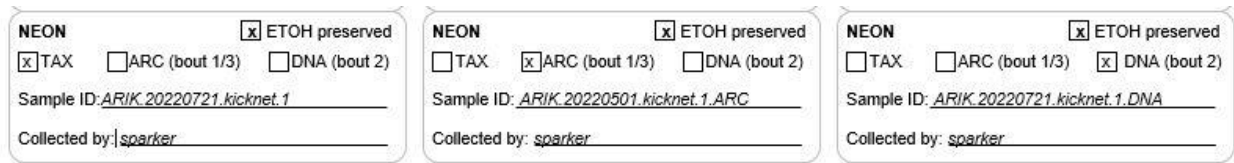

Figure 14. Example of adhesive jar labels for macroinvertebrate sampling. 1) INV-tax, 2) INV-arc, 3) INV-dna

2. INV-tax samples will also have a waterproof human-readable write in the rain label placed inside the jar, printed using a laser printer or written in pencil to withstand ETOH (**Figure 15**).

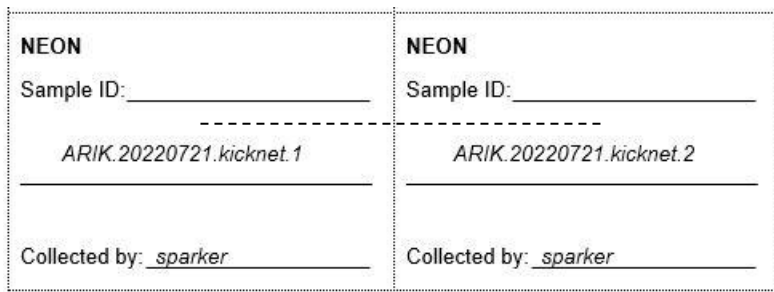

Figure 15. Example of write in the rain paper internal labels for INV-tax samples.

3. Adhesive barcode labels should be applied to dry, room temperature macroinvertebrate jars in advance of their use in the field, at least 30 minutes prior (**Figure 16**).

a. Barcode labels should be aligned lengthwise along the bottle as the scanner will not work on a curved surface (i.e., labels wrapped horizontally around the bottle will not scan; **Figure 13**).

b. Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.

Figure 16. Example of adhesive barcode labels, Type I. These large-size, field-tolerant barcodes have a prefix of 'A' followed by 11 numbers.

4. Sample numbering points below are suggestions. It is **required** that the sample numbers and IDs between the mobile app and the physical label match.
    a. Sample numbers are used to make the sample IDs unique. Numbers are selected by the field technician in the mobile app, and automatically populate into the sample ID (e.g., ARIK.20190827.SURBER.1, with “1” being the sample number)
    b. Lakes/rivers
        i. Ponar samples should be numbered 1-3
        ii. Littoral samples should be numbered using the riparian section number (1,3,5,7,9 or 2,4,6,8,10)
    c. Streams
        i. Dominant habitat type (1st sampler) samples are typically numbered 1-5
        ii. Subdominant habitat type (2nd sampler) samples are typically numbered 1-3
        iii. If all samples at the site are collected using the same sampler type (for example, riffles and runs both use a surber), number samples 1-8.

Table 7. Sample types and barcodes used.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required ?	Bar-code Qty
INV-tax	Preserved macroinvertebrate sample	MAYF.20161027.surber.1 MAYF.20161027.core.1 MAYF.20161027.kicknet.1, etc.	(AOS) Macroinvertebrate Field Data [PROD]	HDPE jar, various sizes	Type I	Yes	1 per jar, 8 per bout
INV-dna	Preserved macroinvertebrate DNA sample	MAYF.20161027.surber.1.dna MAYF.20161027.core.1.dna MAYF.20161027.kicknet.1.dna, etc.	(AOS) Macroinvertebrate Field Data [PROD]	HDPE jar, various sizes	Type I	Yes	1 per jar, 3 per bout

A.3 Macroinvertebrate Preparation and Field Sampling

1. Collect and prepare all equipment, including sample jars and labels: paper (inside jar, **Figure 15**) and adhesive/barcode (outside jar) (RD[05], **Figure 14**).
2. Once you know the approximate size of samples at your site, consider the size of jar you are using to collect samples. Use the smallest jar you can where you can still have a ~1:1 ratio of sample:ETOH. This saves space and cost in in shipping.
3. Check nets and sieves for holes, repair if necessary. Ensure that both are clean and free of debris and organic matter.
4. If collecting DNA samples, decontaminate all equipment used in DNA sample collection with 10% bleach following normal decontamination procedures (Section D.3 in RD[08]) prior to field collection. This bleach cleaning differs from normal decontamination as it is intended to remove any DNA existing on the equipment from storage that could contaminate the samples collected in the field.
5. If using a hand corer or liner 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.
6. Fill 1 L HDPE bottles (or 4 L HDPE jug) with 95% ethanol (Stein et al. 2013). Cap tightly. Clearly label bottle (suggest using Globally Harmonized System (GHS) labels) and transport to the field following EHS guidelines (AD[03]).
7. Fill out general aquatic field metadata mobile app upon every field sampling visit. If other protocols are done in the same day, one record for field metadata for the day is sufficient.

SOP B Determining Sampling Locations and Sampler Type

Data for field sampling are entered in the (AOS) Macroinvertebrate Field [PROD] mobile application. Instructions for the use of this application can be found in the Sampling Support Library in the document "Manual for Fulcrum Application: (AOS) Macroinvertebrate [PROD]".

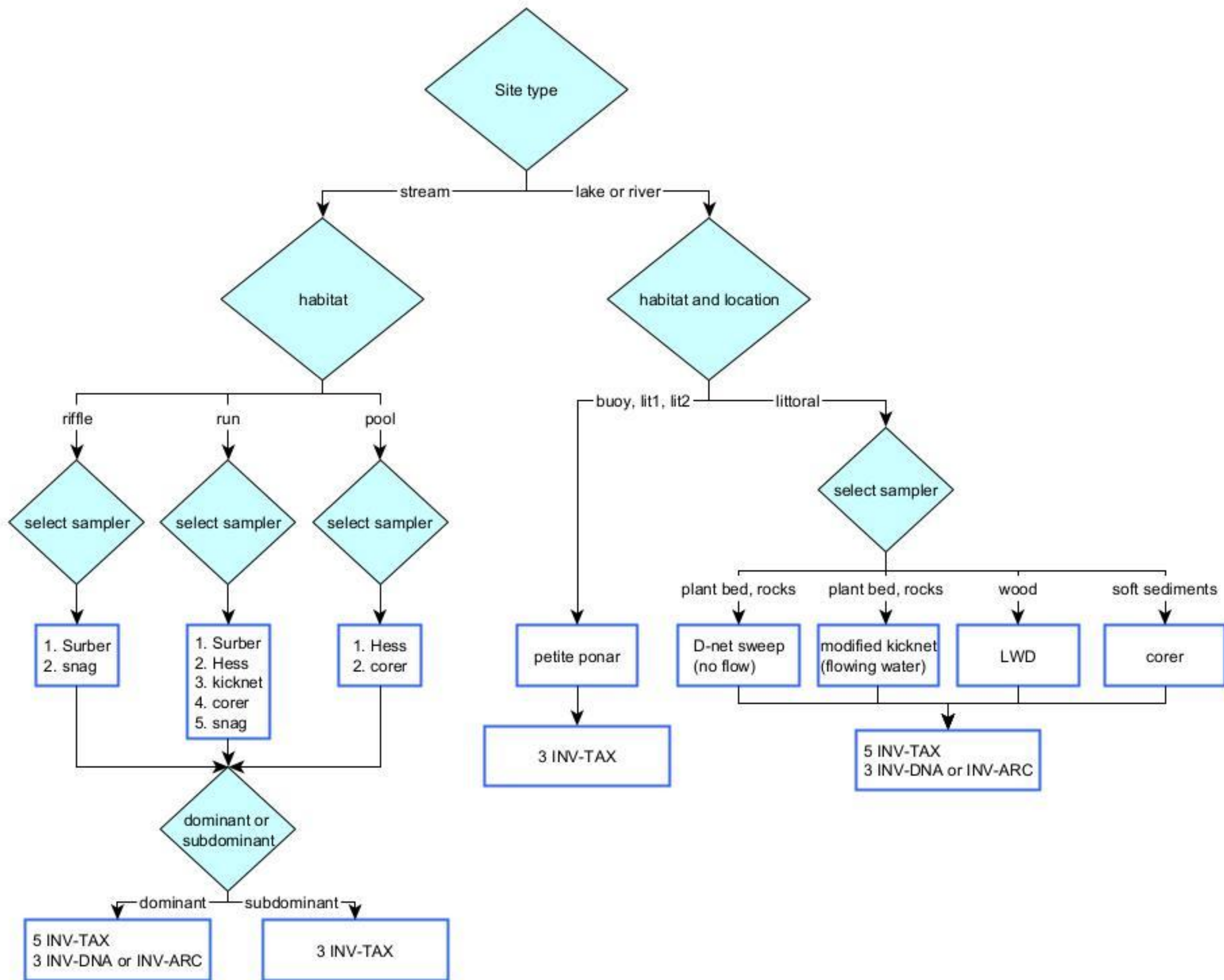

Figure 17. Workflow for habitat and sampler selection (SOP B) for macroinvertebrate collection.

B.1 Spatially and Temporally Linked Protocols

Field Metadata and Gauge Height

- Data are entered into the (AOS) Field Metadata and Gauge Height [PROD] app at the start and end of every aquatic field day.

B.2 Unique and Rare Specimen Collection

1. If you discover taxa during sampling for this or other protocols that you believe are not captured during regular macroinvertebrate sampling (e.g., bryozoan, sponges), you may collect a grab sample for identification by the external lab.
2. Do not collect mussels at sites where there is a permitting restriction. Never collect endangered species.
3. Preserve sample in ETOH as outlined below for taxonomy samples.
4. Contact Science with a sample ID, sampling date, location, and remarks about the sample so data can be manually entered into the data collection app. Once the metadata are entered, you will be able to add this to a shipping manifest and ship on the regular schedule.

B.3 Lakes and Rivers

1. Do not sample within a 5 m radius of the aquatic instrumentation.
2. Recommendation: collect littoral (benthic) samples before ponar samples as the equipment will be cleaner for DNA sample collection in littoral areas before the sieve and bucket get muddy.
3. Benthic sampling
   a. **Lakes**: Benthic petite ponar samples (Section C.6) will be collected near aquatic chemistry sampling locations in lakes: at the buoy, inlet, and outlet. If conditions near the infrastructure are not conducive to ponar sampling (e.g., heavy plant cover), move to a location where the sampler will work properly and note in the data remarks.
   b. **Rivers**: Benthic petite ponar samples (Section C.6) will be collected downstream of the buoy (≥5 m), two other deep-water locations within the 1 km reach.
      i. If sampling a **river** with rocky substrata, use the modified kicknet method in Section C.3.
4. Littoral sampling
   a. Samples will be collected in 5 of 10 pre-defined riparian sections (RD[09]) based on the dominant substratum (e.g., submerged plants) in each section.
      i. A section is the littoral area between the riparian sampling coordinates.
      ii. Choose either even or odd sections for the sampling day.
      iii. Move to the next riparian section if the appropriate substratum type is not present in the chosen section. This may mean that section number are not all even or all odd.
      iv. D09 PRLA has 12 sections rather than 10, refer to Appendix E for section selection guidance.
   b. The substratum type chosen should be present during all sampling bouts and account for at least 20% of the littoral area, ensuring that there is enough of the same substrata in which to collect all samples.

c. All 5 taxonomy samples (+ 3 DNA/ARC samples) must be collected from the same substratum 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 substratum availability through NEON's problem tracking system.

d. Choose the appropriate sampler based on the field conditions and substrata being sampled.

e. The order of preference for sampling substrata is as follows, habitat suggestions for each NEON site can be found in Appendix D:

  i. D-frame sweep (SOP C.8): For littoral areas with plant growth and/or no flow (e.g., lakes).

  ii. Large woody debris (SOP C.5): For submerged large woody debris (>0.3 m diameter and 5 m length) substrata in littoral areas with flowing water (rivers only) where the petite ponar is not appropriate. Water can be over the top of the net during sampling.

  iii. Hand corer (SOP C.2): For littoral areas with sand or silt substrate and little or no plant growth. Can be submerged completely during sampling.

4. Do not sample anywhere you or other people/animals 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.4 Wadeable Streams

1. Do not sample within a 5 m radius of the aquatic instrumentation.
2. Determine the dominant habitat and sub-dominant habitat based on the Stream Morphology Map (RD[09]) or rapid habitat assessment (RD[09], SOP F) for the site. Habitat suggestions for each NEON sites are provided in Appendix D.

  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 (and 3 DNA samples) must be collected from the same habitat and substratum type using the same sampler on each sampling bout from year to year, and all 3 subdominant samples must be collected from the sample habitat type on each sampling bout from year to year, 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 (see Contingent Decisions, **Table 2**).

    i. If the habitats sampled in the previous year(s) no longer represent the dominant and secondary habitats, or are no longer present, contact science.

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 and samplers in order of sampling preference (see Definitions, Section 2.4):

   i. Riffles

      1. Surber (SOP C.1): Requires water level lower than top of frame and gravel substrate or larger

      2. Snag (modified D-frame, SOP C.5): For riffles with significant woody debris.

   ii. Runs

      1. Surber (SOP C.1): Requires water level lower than top of frame and gravel substrate or larger.

      2. Hess (SOP C.4): Requires water level lower than top of frame, gravel substrate or larger, best used where flow is fairly slow

      3. Modified kicknet (SOPC.3): For deeper water, water can be over top of frame. Least ideal option because it is semi-quantitative, but commonly used in runs.

      4. Hand corer (SOP C.2): For runs with sand or silt substrate.

      5. Snag (modified D-frame, SOP C.5): For runs with significant woody debris.

   iii. Pools and step pools (if the step pool has significant flow, treat as the appropriate riffle or run category above)

      1. Hess (SOP C.4): Requires water level lower than top of frame, gravel substrate or larger.

      2. Hand corer (SOP C.2): For pools with sand or silt substrate. Most common sampler used in NEON pools.

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. Try to spread sampling out over the entire 1000 m reach (or 500 m at MCDI).

5. Do not sample anywhere you or other people/animals 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.).

SOP C Field Sampling

NEON aquatic macroinvertebrate sampling is intended to be quantitative. Although different sampler types may be used (**Figure 18**) depending on the habitat and substrate at a site, the data produced are quantitative and thus will be comparable after ingest and processing.

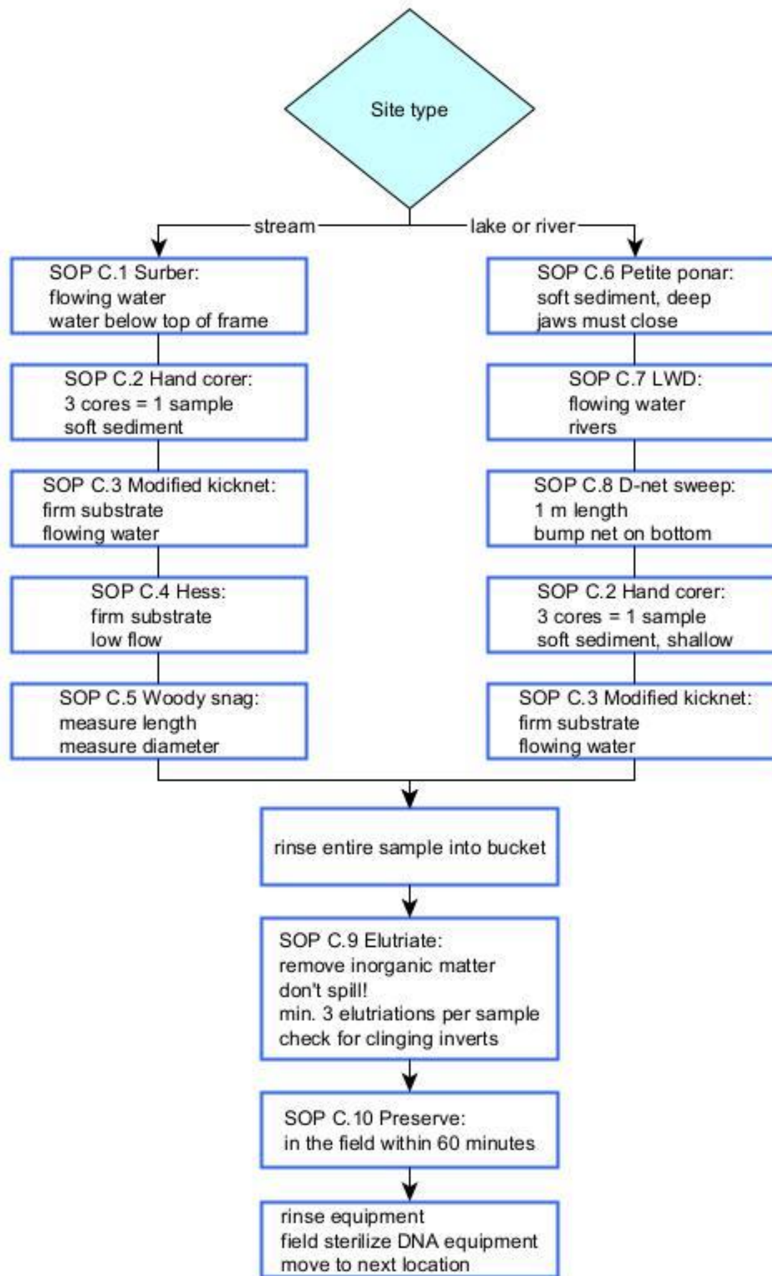

Figure 18. Workflow for macroinvertebrate collection with key points for each sampler type (SOP C).

STREAMS

C.1 Surber Sampler

In **wadeable streams**, the Surber sampler is used in shallow riffle and run habitats.

1. Ensure that all samples are collected from areas 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 or run 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 19**).
    a. Periodically check that the screws on the arms and the frame are secure as these tend to loosen and fall out over time.
4. Carry the 3 or 5 gallon bucket, the nylon brush 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 or 5 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 19**).

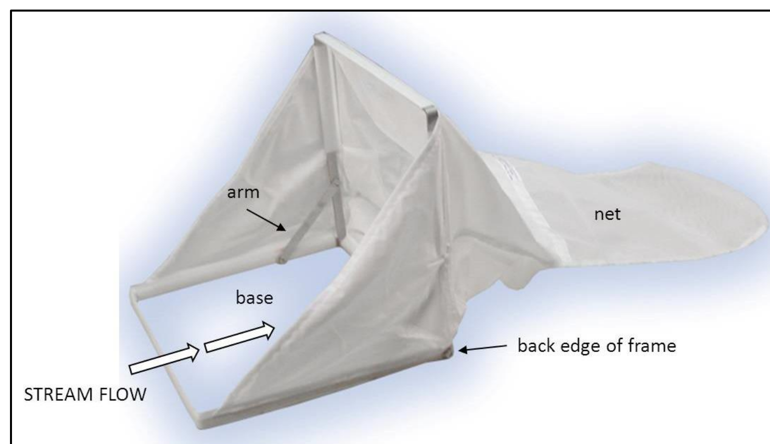

Figure 19. Diagram of Surber sampler set-up. The inside of the base is the sample area of the stream bottom (0.09 m2).

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 in the mobile app.
   b. Always stand downstream of the frame so you do not kick additional material into the sample.
   c. Hold Surber in place using a hand or brace the frame 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).
9. Remove top layer of cobbles one at a time from the area inside the base of the Surber frame (**Figure 21**).
   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 or 5 gallon bucket (**Figure 20**).
   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 21**).

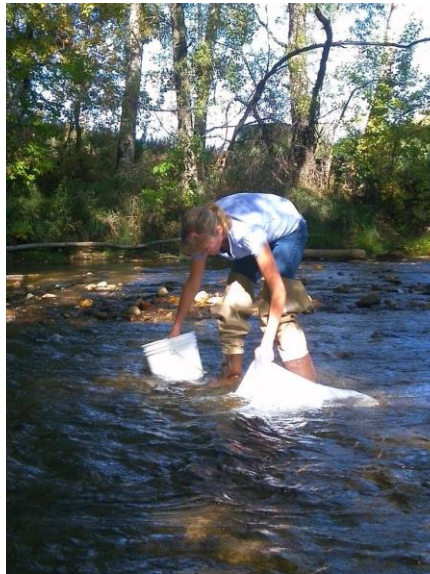

Figure 20. Hold the Surber net on the stream bottom, and keeps the bucket nearby for depositing rocks from the base of the Surber.

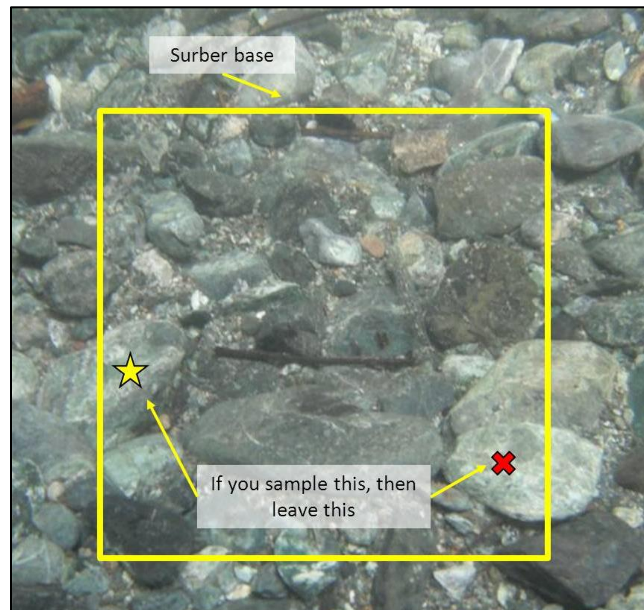

Figure 21. Cobble selection at Surber edges. The yellow square represents the bottom of the Surber sampler.

10. After the top layer of cobbles has been removed and placed in the bucket, disturb the remaining sediments (~10 cm deep in the remaining sediments; 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 the 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 22**).
    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 to the side or downstream of the sample area. Do not resample cobbles.

d. Repeat until all cobbles in the bucket have been scrubbed and discarded.

Figure 22. Scrub rocks from the Surber sample in 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.

i. Immediately release any fish or other vertebrates captured in the net. You may write a remark in the mobile app if you know what species was released.

b. Depending on site-specific permits, mussels collected in the sampler may also need to be released. Make a remark in the data collection app if this occurs, and the species or common name if you know the type(s) of mussels collected. You may also collect a photo to share with Science or local contacts for identification.

c. Use 500 mL wash bottle filled with stream water to rinse any residual organic matter or insects from the net (**Figure 23**). Check for clinging insects.

Figure 23. 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 or 5 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 stream taxonomy**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat.
    b. **Wadeable stream DNA**: 3 samples in the dominant habitat type

C.2 Hand Corer

The hand corer is used in **wadeable streams** in pools or runs with sand or silt substrata. The hand corer may also be used in **lake** or **river** littoral zones with sand or silt substrata. Field ecologists may choose to use the metal hand corer alone, the metal hand corer with the insert, or the insert alone. The hand corer and/or insert may be fully submerged during sampling. Each hand corer sample represents a composite of 3 core samples.

1. Sample in areas of similar flow and depth along the **wadeable stream** or **river** sample reach or similar depth in a **lake** or **river**. Three core samples will be combined to create a composite sample at each location.
    a. Sample only soft sediments (sand, silt, clay). Larger sediment (i.e. gravel) will not maintain suction in the corer barrel during sampling.
2. Select sampler to use. Field ecologists may use the metal hand corer, metal hand corer + plastic liner tub, or the liner tube alone. Wear nitrile gloves for handling sediments.
3. Metal hand corer: Rinse the corer assembly to be sure that all screw threads are clean of silt and sediment.
    a. Assemble the hand corer (**Figure 24**).

i. Screw the corer head onto the top of the stainless steel core tube (**Figure 24**). Petroleum jelly may be applied to the threads to aid sealing.

ii. 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 field ecologist at the time of sampling.

iii. Screw the stainless steel nosepiece onto the bottom of the core tube (**Figure 24**).

iv. Petroleum jelly may be applied to the flutter valve to help create suction in the corer.

v. Pre-measure 5-10 cm on barrel and mark with permanent marker or tape prior to sampling.

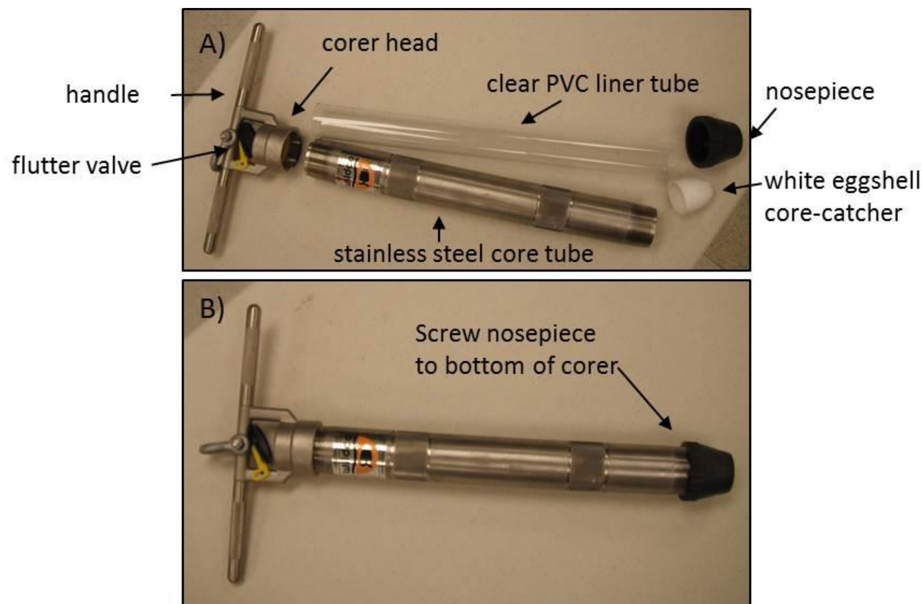

Figure 24. Hand-corer assembly: A) Disassembled corer. Use of the liner tube and core catcher are optional. B) Assembled corer. Note: the nosepiece may be stainless steel (not black Lexan as in photo) in some domains

b. Approach sample location from downstream so as not to step in the location where you want to sample.

c. Hold the core sampler firmly by the handle (**Figure 24**).

d. Push the core sampler through the water and into the sediments in one smooth, continuous movement (**Figure 25**). Allow the flutter valve to remain open.

e. Depending on the sediment type, it may take a lot of force to push the corer into the sediments.

   i. Do not hammer or pound the corer into the sediments.

   ii. Keep barrel of corer at a 90° angle with the sediments.

   iii. Note the dominant substratum size class at the sampling location in the mobile app.

Figure 25. Push the hand corer through the water and into the sediments at a 90˚ angle

f. Push corer approximately 5-10 cm into the substratum while keeping the blue flutter valve open.

g. 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 24**).

i. 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.

ii. If the corer is submerged, allow air to escape from the flutter valve. When the corer is pulled up, the flutter valve should close by itself.

h. Holding the flutter valve closed, **slowly** pull the corer straight up and out of the sediments.

i. If the corer is pulled up too fast, you may lose the contents.

ii. 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.

iii. Hands must be gloved for DNA or ARC samples.

i. 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.

4. Liner tube alone: Rinse the clear liner tub and 2 orange end caps

a. Mark the desired sediment sampling depths on the core liner prior to getting the liner wet (**Figure 26**)

b. Approach sample location from downstream so as not to step in the location where you want to sample.

c. Hold the liner tube near the middle to top of tube.

d. Push the liner tube through the water and into the sediments in one smooth, continuous movement (**Figure 26**). Keep the end caps off.

e. Depending on the sediment type, it may take a lot of force to push the corer into the sediments.
    i. Do not hammer or pound the liner tube into the sediments.
    ii. Keep the liner tube at a 90o angle with the sediments.
    iii. Note the dominant substratum size class at the sampling location in the mobile app.

f. Push liner tube approximately 5-10 cm into the substratum while keeping the top end cap off.

g. Cap the top of the tube with an orange end cap.

h. Slide 1 gloved hand or a spatula under the bottom of the corer and lift it out of the water in one smooth movement. The top end cap should help maintain suction.

i. Continue to hold a gloved hand or spatula under the liner tube and lift the entire core sampler clear of the water, keeping the corer vertical so as not to spill the sample.

Figure 26. Steps for using the core liner tube alone. 1) Insert the liner tube into the sediments vertically; 2) Cap the liner; 3) Stop the bottom and pull the liner tube out of the sediments; 4) Deposit sample material into the bucket.

5. If the sample spills before reaching the bucket, you may rinse the corer or liner tube and start over at Step 33.a approximately 0.5 m away from the original location, as long as it has not been disturbed.
    a. If some, but not all, of the sample spills and is not deposited in the bucket, discard the entire sample and start over.
    b. Use a secondary container to deposit each core so that if one core fails, the entire composite sample is not lost.
6. Quickly hold the core sampler over the 3 or 5 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.
7. 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.
8. Once the corer is empty, all of your sample material will be suspended in water in the 3 or 5 gallon bucket.
9. Repeat Steps 3.a-8 two more times, until there is a composite of 3 cores in the 3 or 5 gallon bucket. Proceed to Section C.9 Sample Elutriation and Preservation.
    a. 3 cores = 1 composite sample
10. After elutriation and preservation, clean corer in stream water and move to next location and repeat the steps above until you have the desired number of composite samples.
    a. **Wadeable stream taxonomy**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat
    b. **Wadeable stream DNA**: 3 samples in the dominant habitat type
    c. **Lake or river taxonomy**: 5 samples in the littoral zone
    d. **Lake or river DNA**: 3 samples in the littoral habitat

C.3 Modified Kicknet Sampler (Semi-quantitative)

The modified kicknet may be used for deep runs in **wadeable streams**, or benthic sampling in **rivers**. Water may flow over the top of the net as this is a semi-quantitative sampling method.

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

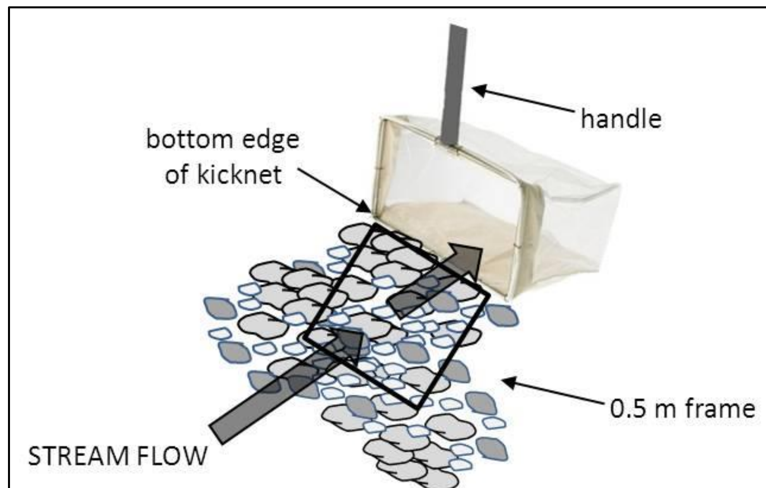

Figure 27. Example of modified kicknet setup.

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 27**).
6. Disturb the substrata within the ~0.25 m^2 quadrat 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 inside the quadrat with your hands:
         1) 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.
         2) 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 21**).
         3) Once a cobble is cleaned, discard to the side or downstream of the sample area. Do not resample cobbles.
         4) Follow by using feet to disturb the sediments by kicking back and forth for 60 seconds inside the quadrat, so all organic matter flows into the net.
      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. **Rivers**: 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 as you will not be able to place and hold the 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 in the mobile app.
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 stream taxonomy**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat
    b. **Wadeable stream DNA**: 3 samples in the dominant habitat type
    c. **River taxonomy**: 3 samples if replacing petite ponar samples, 5 samples if littoral sampling
    d. **River DNA**: 3 samples in the littoral habitat

C.4 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. The Hess is best used in areas with firm substrate (gravel or larger) with very little flow.

1. Ensure that all samples are collected 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 or 5 gallon bucket, Hess sampler, and kitchen brush with you to the sample location.
4. Fill 3-5 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 28**)
    a. Hold the sampler in position by applying pressure with your knees to the back of the sampler, or have a second person hold the sampler.
    b. Note the dominant substratum size class at the sampling location in the mobile app.

Figure 28. 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 or 5 gallon bucket.
7. After the top layer of cobbles has been removed and placed in the bucket, disturb the remaining sediments (~10 cm deep in the remaining sediments; 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 inside the sampler 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 to the side or downstream of the sample area. Do not resample cobbles.
    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 or 5 gallon bucket, unscrew the dolphin bucket and rinse its contents into the 3 gallon bucket.
    a. Invert net into 3 or 5 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 or 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 the next location and repeat the steps above until you have the desired number of samples
    a. **Wadeable stream taxonomy**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat
    b. **Wadeable stream DNA**: 3 samples in the dominant habitat type

C.5 Woody Snag Sampling (small woody debris, semi-quantitative)

Woody snag sampling is used in **wadeable streams**, primarily in riffles and runs with a silty or sandy bottom.

1. Woody snag sampling should be used where snag/woody debris fits inside a D-frame net for sampling. This method is considered semi-quantitative because although all of the sample material fits inside the net, we are estimating surface area of the snag. 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 in the mobile app.

Figure 29. 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 people are needed to collect the sample. Place the 243 µm mesh bag (D-frame net) around the end of the snag with the net 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 person 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 net by sawing with the bow saw or breaking the snag at the end of the net.
   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 so organisms are not lost from the net.
5. Fill 3 or 5 gallon bucket ¼ - ½ full with native water.
6. 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. 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 in the mobile app.

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 the mobile app with location, date, and type of samples.
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 stream taxonomy**: 5 samples for the dominant habitat type, 3 samples for the secondary habitat

    b. **Wadeable stream DNA**: 3 samples in the dominant habitat type

LAKES AND RIVERS

C.6 Petite Ponar

The petite ponar is primarily used for benthic sampling in **lakes** and **rivers**.

1. Navigate to sampling location:

    a. **Lakes** (3 samples):

        i. Deepest point in the lake, determined by bathymetric site map and GPS coordinates (location = c0)

        ii. Near the lake inlet (location = lit1 or in)

        iii. Near the lake outlet (location = lit2 or ot)

        iv. Please note that you can move away from the inlet or outlet sensor locations if conditions are inappropriate (e.g., too much plant cover, hard substrata) for sampling due to changes in water level or plant growth. If this occurs, please note approximate distance from the infrastructure in the date remarks.

    b. **Rivers** (3 samples, near water chemistry sampling location and 2 other haphazardly chosen locations):

        i. Sensor/buoy location (location = c0)

        ii. 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 **lakes and river**s, 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
    d. Measure the depth to bottom and record in the mobile app.
    e. Rinse the ponar with lake/river water prior to sampling at a new location.

3. Release the safety pin on the ponar sampler and open the bottom of the ponar (**Figure 30**). 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).

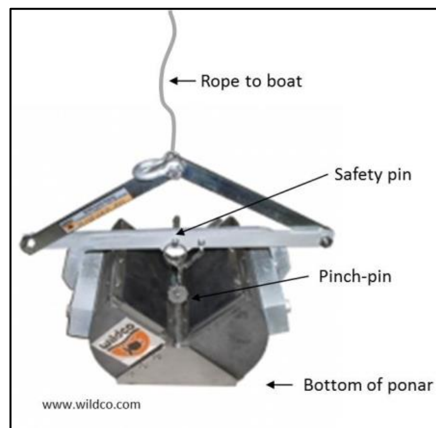

Figure 30. Example of a petite ponar setup.

4. Hold the open ponar above the water surface above the point where you want to sample.
5. Slowly lower the ponar in the water and maintain rope tension.

6. When the ponar hits the sediments and the lines goes slack, pull up on the rope to close the jaws.
7. If the jaws do not close when the sampler hits the **lake/river** bottom, attach a messenger to the line and drop onto the ponar to trigger the jaws to close.
8. Pull the ponar up to the surface using the rope.
9. 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 4 in a different location (at least 2 m away from original location in lakes and rivers).

10. 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.

11. Rinse the inside of the ponar into the bucket using the 500 mL wash bottle.
    a. Be aware that the sediments may splash up and out of the bucket. Try to prevent this as much as possible as this represents sample loss.
    b. 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 of DI. If using DI, be sure that bottles are clearly labeled.
    c. If in a **river** and zooplankton is apparent in rinse water to the naked eye, use 250 µm filtered water as detailed in the previous step or DI water.
12. Once ponar is empty and clean, all of your sample material will be in the 3 or 5 gallon bucket.
13. 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.
14. Proceed to Section C.9 Sample Elutriation and Preservation.
15. 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. **Lakes and rivers**: 3 samples

C.7 Large Woody Debris Sampling (Semi-quantitative)

Large woody debris (LWD) sampling is intended for logs in **rivers** that are too large to cut off and sample via the snag method (SOP C.5). 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 river from the bank, or have been carried downstream by the current and become lodged against the river bottom.
    d. In a **river**, 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.

iii. The sample number on the sample labels should correspond to the Riparian Habitat Section numbers. The mobile app will generate the sample ID using the riparian section number you have entered.

iv. Corresponding taxonomy and DNA samples should be collected from the same riparian section. If you are unable to collect both from the same section, notify Science through the problem reporting system.

e. Note the dominant substratum size class below the snag at the sampling location in the mobile app.

2. Ensure that the LWD is submerged to at least 0.6 m (2 ft) deep, in flowing water (**rivers** 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 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 31). 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.

ii. It is preferred that there is flow at the location, however if there is little to no flow at the location, create flow with hands or a paddle to wash invertebrates into the net.

e. The length is assumed to be 1 m and the width is assumed to be the width of the modified kicknet, you do not need to record length or width.

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 31. From Angradi et al. 2006, example of snag sampling in a large river. While the boat driver holds the boat in position, one person holds the modified kicknet against the snag, while a second 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 the mobile app with location, date, and type of samples.
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. **River taxonomy**: 5 samples in the littoral habitat
   b. **River DNA**: 3 samples in the littoral habitat

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

In **lakes** and **rivers**, 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/river** margins into 10 sections (**Figure 32**).

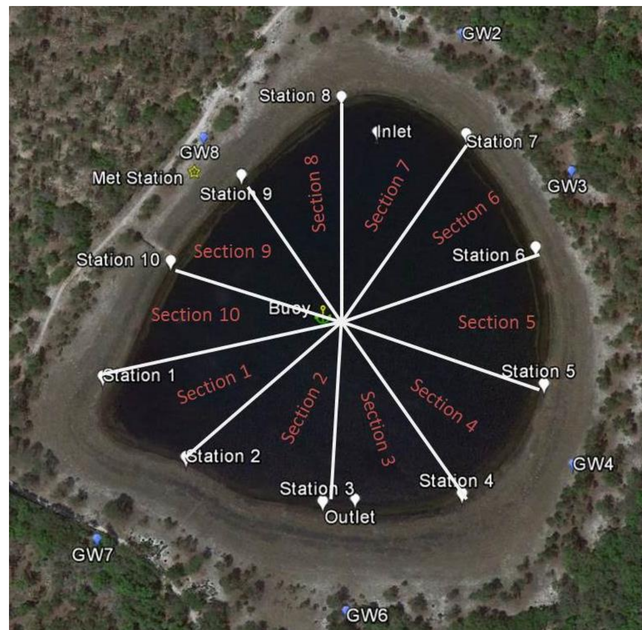

Figure 32. Example of Riparian Stations splitting the lake into sections at D03 Lake Barco.

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. The mobile app will generate the sample ID using the riparian section number you have entered.
   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/river** bottom with the sampling net.
4. If permits allow and the area of the **lake or river** 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 33**).

Figure 33. D-frame net, used for sweep samples in vegetation and silty habitats.

6. Sweep the D-frame net through the vegetation one time across a 1 m distance in one direction (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/river bottom** and sweep from foot to foot. Distance is estimated if sampling from the boat.
   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 34**).
   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 34. 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 or 5 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 rinse water. Swirl net around in rinse water 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 mobile application and label with location, date, and type of sample.
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. **Lake or river taxonomy**: 5 samples in the littoral habitat
    b. **Lake or river DNA**: 3 samples in the littoral habitat

ALL 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 35**).

Figure 35. Process of elutriation (Dates and Byrne 1997).

3. Carefully pour contents of bucket into 250 µm sieve or sieve bucket (**Figure 36**). If you are in a boat, hold the sieve over a 5 gallon bucket to capture the waste water or hold over the side of the boat (note: if you spill the material in the sieve over the side of the boat, you will need to collect a new sample). Some material will be retained in the original bucket.
    a. Be careful not to slosh sample water over the edge of the sieve. All material must be contained within the sieve to maintain a quantitative sample. If the sieve spills, collect a new sample.

b. The sieve bucket should be used for petite ponar samples for thick, fine sediments.

c. If the sieve fills up with organic matter, you may pick clumps out of the sieve and place in sample jar.

d. You may use your fingers or shake the sieve or sieve bucket to stir the sample and move fine material through the sieve.

e. You may use low-pressure water to wash sediment through sieve mesh. High pressure will damage organisms.

f. Sieving the sample in small portions may help prevent clogging.

g. Continue rinsing to reduce the sediment in the sample, especially for petite ponar samples. Reduce samples to ≤750 mL if possible.

Figure 36. 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 **<u>lakes</u>** to exclude zooplankton. You may use the 250 μm sieve or 243 μm Nitex mesh (used to change preservative in the lab) for filtering rinse water.

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 using 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 small adhesive label and barcode 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. For taxonomy samples, also add a waterproof paper label (write in pencil) inside the sample jar.
   a. Sample ID format: *SITE.DATE.sampleType.sampleNumber* (
   b. **Table 7**)
   c. Example: *ARIK.20140620.kicknet.3*
   d. Paper labels are added inside the jar because ETOH can degrade the external labels during shipping.
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 37**).

Figure 37. Rinse 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 or a clean funnel to move the sample from the sieve into the sample jar.
    a. Use as little water as possible. The more water used, the more ETOH is needed. Be sure to rinse sieve and fingers into the sample jar to thoroughly remove all material. You may also use an ETOH squirt bottle to rinse sample into the jar, be sure to label any ETOH bottle used clearly.
    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".

    a. Use a Type I barcode label on sample jar 1 and band or tape the two jars together, or place both containers in one zip-top bag. The external lab will re-combine both into one sample.
14. Check sieve for clinging insects that may not have been washed into the sample jar.
15. **Carefully** add preservative. Preserving immediately prevents predation and damage to insects in the samples.
    a. Wear latex gloves when preserving samples if your domain's chemical hygiene plans calls for it. Always wear eye protection (e.g., sunglasses).
    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). Ideal preservation is 70% ethanol, but using 1:1 as an eyeball estimate is usually a good start for the field preservation.

16. Record data in the mobile app.

a. Scan the barcode label with the tablet or hand-held scanner (**Figure 38**).

b. Record all relevant metadata for each sample in the mobile app.

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

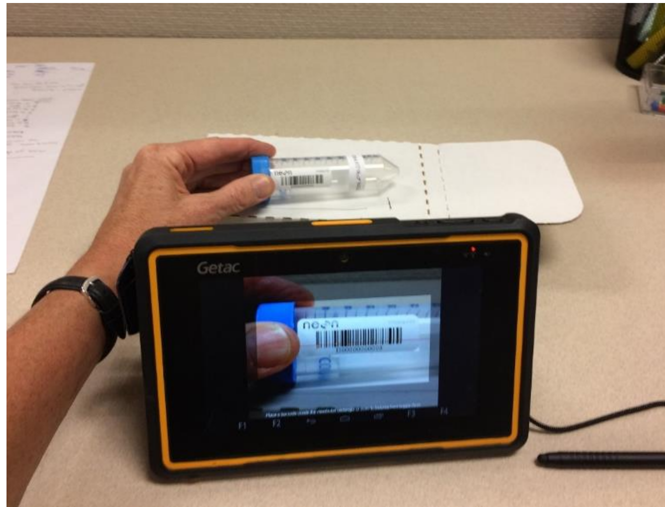

Figure 38. Barcode label scanning.

17. Close the sample jar tightly.

C.10 Sample Preservation

Preserve samples within 60 minutes 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.

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 (or littoral) 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). Equipment is difficult to sterilize in the field, so thorough rinsing of all equipment between samples is necessary.

1. Cleaning equipment:
    a. Wearing nitrile gloves, clean all nets, waders, and other equipment that comes in contact with samples during macroinvertebrate sampling using a 10% bleach solution. Follow with a DI or tap water rinse to remove the residual bleach (Jane et al. 2014). Note that this is a higher concentration what is usually used in the Decontamination Protocol (RD[08]).
        i. Field equipment to decontaminate with stronger solution:
            1. Sampling net(s)
            2. Sieve
            3. Bucket
            4. Brush
            5. Forceps
            6. Boots/waders
            7. Sample jars if they are being reused from other sampling, or come uncapped from the manufacturer
        ii. Lab equipment to decontaminate
            1. Filter cup
            2. Filter mesh
            3. Sieve (if using)
            4. Pipette (if using)
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 human-readable label and barcode label on the sample jar. Circle "DNA" on label. **Do not** use a paper label inside the sample jar for this protocol because this could introduce contamination.
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. Be sure to thoroughly rinse all equipment that comes in contact with the DNA samples with stream water between samples.
   a. Choose 3 riparian sections or habitat units that 1) have enough habitat to support two samples and 2) are spread out along the stream/river reach or lakeshore (ideally samples 1, 3, and 5).
   b. If following contingent decisions, still collect 3 samples. Three is the minimum number of samples you can collect for the data to be statistically relevant to users
3. Wear nitrile gloves while sampling for DNA. You can re-use and clean gloves by wiping with an ethanol wipe or spritzing with ETOH.
4. Prior to sampling, prime the sampler by rinsing well with local source water to remove any residual debris or organisms.
   a. You do not need to decontaminate between samples with bleach or ETOH within the same day at a site because it is difficult and largely ineffective to sterilize nets in the field. Rinse well with local water, tap water, or DI.
   b. Clean nitrile gloves with ETOH spray and or wipes between samples, or use new gloves for each sample.
5. Collect samples according to SOP B for the appropriate habitat type and sampler.
   a. If it appears that a sample has no macroinvertebrates in it (e.g., D-frame sweep samples, snag, or LWD samples), recollect the sample at an adjacent location.
   b. It is particularly important to pay attention to the elutriation step. Samples will be homogenized in a blender at the external facility, and inorganic matter (pebbles, sand) will interfere with homogenization.
6. Choose "DNA" in the mobile app, and the appropriate habitat and sampler metadata.
7. Sample ID = *SITE.YYYYMMDD.sampleType.sampleNumber.DNA*
8. After sample collection, use forceps or clean-gloved hands to pick out large organic matter, leaving the macroinvertebrates in the sample.
   a. Pick large plant material out of the sample only if you are sure that it is free of macroinvertebrates. The external lab will homogenize these samples in a blender, so minimizing extra material in the sample helps keep lab costs down.

   b. Samples will be homogenized in a blender at the external facility. Make sure there are no large sticks or other material in the sample that could interfere with homogenization.

9. 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.
10. Add 95% ETOH to sample jar to completely cover sample (Stein et al. 2013). ETOH concentration should be as close to 95% as you can get (as little extra water as possible. You do not need to fill the jar to the top with ETOH.
11. Return sample to domain lab and store in the dark at 4 °C and proceed to Section F.3.

SOP E 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. Make sure barcode labels are available.
   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 Decontamination Protocol (RD[08]) within 48 hours of returning from the field. If sampling occurs on Friday, decontamination can occur the following Monday.
   b. Stainless steel sieves tend to rust. Clean and dry thoroughly prior to storage. To minimize rust, you may also do a methanol wipe to prevent corrosion.
      i. Put on nitrile gloves.
      ii. Wipe sieve with a methanol-wetted Kimwipe® to reduce methanol waste.
      iii. DO NOT rinse with DI water after methanol rise and wipe.
      iv. Allow methanol-rinsed equipment to air dry prior to storage.
   c. Dry all equipment thoroughly between sites and before storage.
   d. Check all nets for holes and patch if necessary.
3. Data QA/QC
   a. Required checks
      i. Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample jars.
      ii. Check that the barcode labels in in the mobile application(s) match the barcode labels adhered to the samples. At a minimum, check the last few numbers of the barcode.
   b. Nice to check
      i. Site ID, collect date, sampling protocol version

SOP F Laboratory Sampling and Analysis

Figure 39. An expanded diagram of the lab workflow for the macroinvertebrate lab SOP.

F.1 Preparation

1. Fill 500 mL wash bottle with 475 mL 95% undenatured ethanol for taxonomy preservation. Clearly label bottle for ETOH.

Table 8. Storage conditions, containers, and lab processing timing for INV samples. Note that if you store at -20 °C, use a Type II barcode.

Sample type	Time to preservative swap	Storage conditions	Container
INV-TAX	Arrival at DSF-72 hours	ambient (dark & ETOH-safe) or refrigerated (4 °C)	HPDE widemouth straight-sided jar with screw top, appropriate size for sample
INV-DNA	Arrival at DSF -24 hours	refrigerated (4 °C) or frozen (-20 °C)	16 oz. widemouth straight-sided HDPE; may use 32 oz. (if needed for large samples) and individually bag each jar – jars must have ≥3" diameter mouth
INV-ARC	Arrival at DSF -24 hours	ambient (dark & ETOH-safe), refrigerated (4 °C), or frozen (-20 °C)	Smallest Nalgene that fits the sample, please downsize during ETOH swap if possible to save storage space at the biorepository. A Nalgene is required because it has a better seal for long-term storage. Apply new labels if needed.

F.2 Processing Samples – Taxonomy

1. Replace preservative between returning to the DSF and 72 hours of field collection in the domain lab (**Table 8**).
2. Wearing safety glasses and nitrile gloves, open field-preserved sample under fume hood.
3. 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 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. You may either include the mesh and all samples material on it in the sample jar, or rinse sample material from the mesh into the jar and discard the mesh. Use 250 μm mesh, not the TOS mesh as it is a different mesh size.
   d. Alternatively, you may make a filter lid using cut out jar or Nalgene lid. Place the 250 μm Nitex square over the top of the sample container, and screw the modified lid firmly on top. Invert the jar over the waste container, and rinse sample material from the mesh lid back into the sample jar. This may work better at sites that have light or no sediment in samples.

4. Check that sample label is still readable and inside the sample jar. Check that the external adhesive label/barcode label is on the outside of the sample jar.
5. Re-preserve sample with 95% ethanol. Fill jar with liquid to prevent sample damage during shipping.
6. Close the sample jar tightly.
7. Carefully clean sieve or filter cup before decanting the next sample.
8. Continue until preservative in all samples has been replaced and proceed to Sample Shipping (SOP H).

F.3 Processing Samples – DNA and ARC

Preservative in DNA and ARC samples must be changed between returning to the DSF and 24 hours to preserve the integrity of the DNA. This process replaces the water in the organisms with ethanol. This is also a good time to transfer to new jars if needed.

1. Change ethanol within 24 hours in the domain lab. This helps to ensure that DNA is preserved quickly and will be in good condition for analysis.
2. Decontaminate the filter apparatus, nylon mesh, and any equipment that comes in contact with the DNA sample during processing, or keep a separate, decontaminated filter apparatus for DNA.
3. Wearing safety glasses and nitrile gloves, open field-preserved sample under fume hood.
4. Follow Section F.2 to change out the ethanol in the sample.
    a. Clean and decontaminate any equipment or consumables that come in contact with the sample material with 10% bleach prior to the sampling day, and rinse with 10% bleach followed by DI or tap water between samples.
5. Replace ethanol, preserving as close to 95% as possible.
    a. The DNA container does not need to be filled to the top with liquid since these specimens do not need to remain intact for processing. The jar opening must be at least 3” diameter for lab processing, change jars at this point if needed.
    b. The ARC container should be the smallest Nalgene that fits the sample, and should be filled to the top with ETOH because researchers may used these archived samples for a range of uses.
6. Close the sample jar tightly, place in 4 °C and dark until shipping.
7. ETOH wipe and sterilize filter apparatus or any equipment that comes in contact with the DNA sample material between samples.
8. Carefully clean and dry all equipment prior to storage.

F.4 Sample Storage

Taxonomy samples may be stored at the domain support facility at room temperature or 4 °C until shipping. For shipping instructions see SOP H.

DNA samples from may be stored at 4 °C or frozen at -20 °C until shipping

SOP G 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. Adhesive barcode labels will be used and scanned into the mobile application. 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. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL).

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 H Sample Shipment

Information included in this SOP conveys science-based handling and packaging instructions for the sample types included in this protocol. For shipping instructions, see RD[11].

1. Keep morphological taxonomy samples and DNA samples separate.
   a. Bout 2 DNA samples will be shipped to an external lab for analysis.
   b. Bout 1 and Bout 3 DNA samples will be shipped directly to the archive facility.
2. Seal jar lids with electrical tape prior to shipping. This helps prevent leaks and prevent ethanol fumes from turning labels black during shipment.
   a. Tip: Assigning a different color of electrical tape to each site in the domain helps keep samples organized.
3. Bag samples
   a. INV-tax: 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.
   b. INV-dna: Individually bag each DNA sample in a zip-top bag to prevent contamination of DNA from sample to sample during shipment.
4. See RD[11] for further shipping instructions.

H.1 Handling Hazardous Material

Follow procedures for shipping ethanol in limited quantity ground shipments in AD[03] and RD[11].

H.2 Supplies/Containers

See section SOP H Steps 1-3 and Appendix F specific shipping materials.

H.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: Samples from Bout 2 must be held at 4 ^{o}C and will be shipped to the external facility for analysis on a CLA-defined schedule. Samples from Bouts 1 and 3 will be shipped directly to the archive facility on a CLA-defined schedule. Samples may be held for a maximum of 6 months if batch shipping is requested by the external lab. Samples are shipped ground at ambient temperature.

H.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.

H.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.

H.6 Shipping and Chain of Custody

The mobile shipping applications are used to create chain of custody documents. Shipments are to include a hardcopy of the shipping manifest (RD[11]) in each box. An electronic shipping manifest is also emailed to the receiving laboratory and NEON Collections and Laboratory Analysis at the time of shipment (created by the shipping application). The shipping manifest must accurately document the physical samples inside the shipping container.

H.7 Laboratory Contact Information and Shipping/Receipt Days

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

8 REFERENCES

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APPENDIX A QUICK REFERENCES

A.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), external sample labels (2” x 4”), and barcode labels.

Step 3 – Ensure the General AQU Field Metadata and Gauge Height (RD[06]) app 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, Hess, or snag)
2. Runs (Surber, Hess, modified kicknet, hand corer, or snag)
3. Pools and step pools (Hess or hand corer)

Lakes/Rivers – Benthic

1. Petite ponar
2. Modified kicknet

Lakes/Rivers – Benthic

1. Aquatic plant beds or floating mats (D-frame net), dead vegetation is acceptable
2. Snags/large woody debris (Modified kicknet snag sampler – **rivers** only)
3. Littoral – firm substrate (modified kicknet)
4. Littoral – soft substrate (hand corer)

Step 6 – Collect taxonomy samples (**Wadeable Streams**: 5 per dominant habitat type, 3 per subdominant dominant habitat type; **Lakes/Rivers**: 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/Rivers**: 3 per dominant littoral habitat type (with same 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 at the Domain Support Facility within 72 hours of sample collection. DNA: Change preservative at the Domain Support Facility within 24 hours of sample collection.

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

APPENDIX B REMINDERS

Before heading into the field:

- Collect and prepare all equipment, including sample jars and labels.
- Pre-print waterproof labels, small adhesive labels, and barcode 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:

- When making contingent decisions (**Table 5**), at minimum collect a suite of 3 samples using the same sampler and habitat type in order to do statistics on the data. Do not collect fewer than 3 samples.
- Determine the habitats to sample based on the Stream Morphology, Rapid Habitat Assessment, Bathymetry and Riparian Maps, or previous sampling bouts.
- Choose the appropriate sampler and follow depth and substrate rules for 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 people/animals have walked in the reach, recently sampled, or locations that appear recently disturbed.
- **Lakes/Rivers**: 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 sub-dominant habitat for taxonomy. Collect 3 samples from the dominant habitat for DNA.
- Macroinvertebrates often become trapped in the folds of the nets. Check net seams between each sample to ensure that specimens are added to the correct samples, and do not remain in the net.
- Macroinvertebrates are often lodged in the edges of the sieve. Check the sieve between samples to avoid sample-to-sample contamination.

Sample preservation:

- 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 in sample jars.
- Change ethanol within 72 hours of field sampling for INV-tax, and replace ETOH within 24 hours for INV-dna and INV-arc.
- Fill jar with ETOH to prevent destruction of organisms during shipping.

Data QA/QC:

Required checks

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

Nice to check

- Site ID, collect date, sampling protocol version

APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for biological sampling bouts are based on the NEON temporal sampling strategy (Parker and Utz 2022). Refer to the Aquatic Site Sampling Design for your domain (RD[19]) for bout window start and end dates.

APPENDIX D SITE-SPECIFIC INFORMATION: HABITAT AND SAMPLER RECOMMENDATIONS

Sampler types should be consistent at a location from year to year.

Domain	Site		Habitat 1 (5 reps)	Habitat 2 (3 reps)
D01	Hop Brook	HOPB	Riffle (Surber)	Pool (corer)
D02	Lewis Run	LEWI	Run (core)	Riffle (Surber)
D02	Posey Creek	POSE	Riffle (Surber)	Pool (Hess)
D03	Flint River	FLNT	Littoral (LWD)	Benthic (petite ponar)
D03	Lake Barco	BARC	Littoral (D-net benthic sweep)	Benthic (petite ponar)
D03	Lake Suggs	SUGG	Littoral (D-net floating sweep)	Benthic (petite ponar)
D04	Rio Guilarte	GUIL	Riffle (Surber)	Pools (Hess)
D04	Rio Cupeyes	CUPE	Riffle (Surber)	Run (Surber)
D05	Crampton Lake	CRAM	Littoral (D-net benthic sweep)	Benthic (petite ponar)
D05	Little Rock Lake	LIRO	Littoral (D-net benthic sweep)	Benthic (petite ponar)
D06	Kings Creek	KING	Riffle/run (Surber)	Pools (corer)
D06	McDiffett Creek	MCDI	Riffle (Surber)	NA
D07	Leconte Creek	LECO	Riffle (Surber)	Pool (corer)
D07	Walker Branch	WALK	Riffle (Surber)	Run (Surber)
D08	Mayfield Creek	MAYF	Riffle/run (snags)	Run (core)
D08	Black Warrior River	BLWA	Littoral (LWD)	Benthic (petite ponar)
D08	Tombigbee River	TOMB	Littoral (LWD)	Benthic (petite ponar)
D09	Prairie Lake	PRLA	Littoral (D-net benthic sweep)	Benthic (petite ponar)
D09	Prairie Pothole	PRPO	Littoral (D-net benthic sweep)	Benthic (petite ponar)
D10	Arikaree River	ARIK	Run (modified kicknet)	Pool (core)
D11	Pringle Creek	PRIN	Run (core)	Riffle (Surber)
D11	Blue River	BLUE	Run (modified kicknet)	Riffle (Surber)
D12	Blacktail Deer Creek	BLDE	Riffle (Surber)	Run (Surber)
D13	Como Creek	COMO	Riffle (Surber)	Run (modified kicknet)
D13	West St. Louis Creek	WLOU	Riffle (Surber)	Pool (core)
D14	Sycamore Creek	SYCA	Run (Surber)	Pool (core)
D15	Red Butte Creek	REDB	Step pool (Hess)	Run (Hess)
D16	McRae Creek	MCRA	Step pool/Riffle (Surber)	Step pool/Pool (Surber)
D16	Martha Creek	MART	Riffle (Surber)	Pool (Hess)
D17	Teakettle 2 Creek	TECR	Riffle-cobble (Surber)	Riffle-bedrock (Surber)
D17	Upper Big Creek	BIGC	Run (Surber)	Riffle (Surber)
D18	Oksrukuyik Creek	OKSR	Run (modified kicknet)	Riffle (Surber)
D18	Toolik Lake	TOOK	Littoral (D-net benthic sweep)	Benthic (petite ponar)
D19	Caribou Creek	CARI	Run (modified kicknet)	Riffle (Surber)

APPENDIX E D09 PRLA RIPARIAN SECTION SELECTION FOR LITTORAL BENTHIC SAMPLES

Riparian sections 11 and 12 were added to D09 PRLA in 2020 due to a permitting change. The table below details the 5 riparian sections to select for littoral benthic macroinvertebrate sampling for each bout. After year 4 in the table, start back at the top. Years are noted in the "Sampling date" column as an example

Site	Year	Bout	Sampling date	Section selection for littoral sampling
PRLA	1	1	2020 & 2024 Bout1 (odd-1)	3,5,7,9,11
PRLA	1	2	2020 & 2024 Bout2 (even-2)	4,6,8,10,12
PRLA	1	3	2020 & 2024 Bout3 (odd-3)	1,5,7,9,11
PRLA	2	1	2021 & 2025 Bout1 (even-4)	2,6,8,10,12
PRLA	2	2	2021 & 2025 Bout2 (odd-5)	1,3,7,9,11
PRLA	2	3	2021 & 2025 Bout3 (even-6)	2,4,8,10,12
PRLA	3	1	2022 & 2026 Bout1 (odd-7)	1,3,5,9,11
PRLA	3	2	2022 & 2026 Bout2 (even-8)	2,4,6,10,12
PRLA	3	3	2022 & 2026 Bout3 (odd-9)	1,3,5,7,11
PRLA	4	1	2023 & 2027 Bout1 (even-10)	2,4,6,8,12
PRLA	4	2	2023 & 2027 Bout2 (odd-11)	1,3,5,7,9
PRLA	4	3	2023 & 2027 Bout3 (even-12)	2,4,6,8,10

APPENDIX F EQUIPMENT

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

Table 9. Equipment list – General equipment.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
RD[09], RD[15], RD[16]	N	Site-specific Rapid Habitat Assessment or Riparian Sampling Locations	Determining sampling locations	1
	N	Mobile data entry tablet	Field data entry	1
	N	Cooler, 9-28 qt	Field sample storage; use size appropriate to samples being collected	1
Grainger, W.W. Forestry Suppliers, Inc. Cabela's	N	Waders (hip or chest) or knee boots	Wading	1 pair/ person
	N	Personal flotation device (PFD)	Boating safety in lakes/ rivers	1 per person
	N	Handheld GPS unit (with batteries, ±4 m accuracy) or Humminbird	Navigating to sampling locations in lakes/rivers	1
	N	Depth finder (see Bathymetry Protocol, RD[17])	Determining depth at sampling location in lakes/rivers	1
	N	General AQU Field Metadata Sheet and field data sheets (all-weather copier paper, write in pencil)	Recording metadata in case tablet fails	1
	N	Pre-printed paper labels (all-weather copier paper, write in pencil)	Labeling samples, inside sample jar	1 sheet
	N	Pre-printed 1” x 2 5/8” adhesive labels (write in permanent marker)	Labeling samples, outside sample jar	1 sheet
Fisher Scientific Company; BPA4084	Y	Methanol (Spectranalyzed™), Fisher Chemical™	Drying metal sieves to prevent rust	As needed

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
	N	Adhesive barcode labels (Type I)	Labeling sample bottles with barcode-readable	1 sheet
	N	Pencils	Recording data	4

Table 10. Equipment list – Macroinvertebrate Samplers.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
	N	Surber sampler (243 um Nitex mesh), 0.093 m^2	Sample collection in riffles and runs	1
	N	Hess sampler (243 um Nitex mesh), 0.086 m^2	Sample collection in riffles, runs, shallow pools	1
	N	Petite ponar sampler with rope	Sample collection in soft sediments in lakes/rivers	1
	N	Messenger	Used with petite ponar in lakes/rivers	1
	N	Hand corer, stainless steel, 20 in with plastic insert	Sample collection in sandy or silty habitats (streams)	1
	N	Modified kicknet sampler (rectangular, 243 um Nitex mesh)	Sample collection in runs (streams) and large woody debris	1
	N	Collapsible quadrat (0.5 x 0.5 m^2)	Used with modified kicknet sampler in streams	1
	N	D-frame net (243 um Nitex mesh)	Sample collection for woody snags and littoral (lakes/rivers)	1
	N	Bow saw, 21 in or loppers	Used with D-frame snag sampler in streams	1
	N	Work Gloves (pair)	Used with petite ponar for safe handling of ponar rope	1
	N	Meter stick, meter tape, or metric ruler	Used with snag and LWD samplers (streams and lakes/rivers)	1
	N	Kitchen brush with nylon bristles	Used with Surber, Hess, snag, and LWD samplers for rock and wood scrubs	2

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
	N	Deck brush with polypropylene bristles, 60 inch handle	Used with LWD/modified kicknet sampler in rivers	1
	N	Putty knife	Scrape plant material off cobbles in streams	1
	N	Petroleum jelly	Used with hand corer to maintain suction in streams	1
	N	Sterile 70% ethanol wipes (e.g., www.soscleanroom.com item TX3044P pre-wetted wipe OR TX3215 dry wipe)	Field-sterilization for gloves for DNA samples	3
	N	ETOH spray bottle (with 95% ETOH)	Field sterilization for gloves for DNA samples	1

Table 11. Equipment list – Macroinvertebrate field elutriation and preservation.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
	N	Polyethylene wash bottle, unitary (500 mL; Figure 23)	Rinsing sieve, bucket	2
	N	Sieve, 250 um	Sample sieving; elutriation	1
	N	Sieve bucket, 242 um	Sample sieving; elutriation in **lakes/rivers**	1
	N	Plastic bucket, 3 or 5 gallon	Substrate scrubbing (streams), elutriation, collecting waste water on boat (lake/river)	1-2
	N	Flexible forceps, featherweight	Collecting clinging insects	1
	N	Small funnel	Help transfer sample to jar	1
	N	HDPE bottles (1 L) or jug (4 L) with lids (clear or amber)	Transporting ethanol to the field	1-4
	N	Sample jars, HDPE with screw-top lid, 6, 16, or 32 oz.	Sample container, field staff choose sizes appropriate for site	11
	N	Nalgene bottles, widemouth, with screw top lid (250 mL to 1 L)	Sample container for INV-arc, field staff choose sizes appropriate for site	3

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

Table 12. Equipment list – Laboratory processing: preservative replenishment.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
	N	Unitary wash bottle, 500 mL, ETOH	Adding preservative to sample jars and rinsing filter cup	1
	N	Sieve, 250 um	Changing preservative	1
	N	Filter cup (500 mL HDPE bottle and 243 um Nitex mesh square, see TOS beetle protocol)	Changing preservative	1
	N	Featherweight forceps	Picking up insects	1
	N	Safety glasses	Preventing preservative contact with eyes	1 pair
	N	Ethanol, 95%, undenatured	Preservative	5 L
	N	243 um Nitex mesh cloth, cut into squares for filter cup	Catching insects over the filter cup	8
	N	Nitrile gloves	Preventing preservative contact with skin	1 pair