AOS PROTOCOL AND PROCEDURE: MACROINVERTEBRATE SAMPLING IN WADEABLE STREAMS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	10/31/2012	ECO-00680	Initial draft release
B	02/07/2014	ECO-01087	Production release
C	08/29/2014	ECO-02210	Minor updates based on feedback from the field
D	11/03/2014	ECO-02403	Migration to new protocol template
E	05/27/2015	ECO-02665	Minor updates including changes to the equipment list (replace Whirl-paks with jars), addition of sampling contingencies, Hess and mini-Surber sampling options, STREON reach-scale sampling, updates to sample shipping and labeling, and the addition of sampling dates to appendix.
F-OBSOLETE	01/22/2016	ECO-03470	OBSOLETE and superseded by NEON.DOC.003046 AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling

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1 OVERVIEW

1.1 Background

Aquatic invertebrates are a diverse and ubiquitous group of organisms (Hauer and Resh 2006). Streams and rivers harbor most major taxa of aquatic organisms (Hynes 2001), and therefore are excellent study habitats 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 invertebrates are an important part of the aquatic community. Most benthic invertebrates are primary consumers and feed on autotrophic (algae, plants) and heterotrophic (leaf litter, dissolved organic carbon) production. Other benthic invertebrates are predators. In general, benthic invertebrates are important vectors of energy transfer from one trophic level to the next (e.g., from sunlight + nutrients → primary producers → consumers → predators). Macroinvertebrates can be classified into functional feeding groups based on morphological and behavioral mechanisms for food acquisition (Merritt and Cummins 2006). Different taxa are adapted to most efficiently feed on different food sources: scrapers (feed on algae), shredders (feed on leaves), collector-gatherers (feed on organic matter and other material), filter feeders (filter fine particles from the water column), and predators (feed on other invertebrates) are the major functional feeding groups in benthic communities (Figure 1).

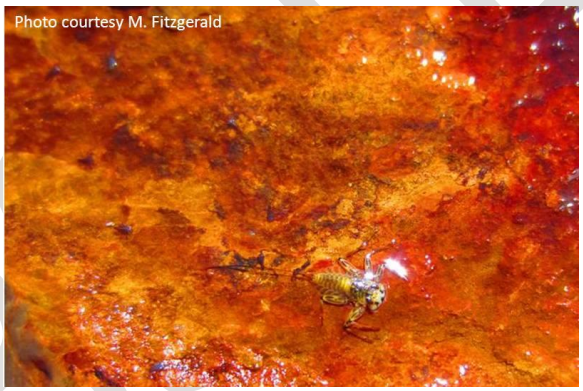

Figure 1. Mayflies (Ephemeroptera: Heptageniidae) are scrapers that are adapted to feed most efficiently on attached periphyton in flowing waters.

Stream benthic invertebrate communities are strongly affected by disturbance, including shifts in nutrient and pollutant concentrations, physical disturbances such as floods, scouring, freezing, drought, and biological disturbance such as predation (Allan 1995). Depending on the type of disturbance (e.g., flooding), recolonization by benthic invertebrates can be relatively rapid in streams. 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 stream, are 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 benthic communities over the time span of the NEON Observatory to determine changes in presence/absence of taxa, taxa diversity and richness, community structure, and species loss will be crucial components of aquatic ecosystem assessment.

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).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

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

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC. 002197	Datasheets for AOS Protocol and Procedure: Macroinvertebrate Sampling in Wadeable Streams
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[09]	NEON.DOC.001151	AOS Protocol and Procedure: Aquatic DNA Barcode
RD[10]	NEON.DOC.001153	AOS Protocol and Procedure: Wadeable Stream Morphology Mapping
RD[11]	NEON.DOC.014050	TOS Protocol and Procedure: Ground Beetle Sampling
RD[12]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[13]	NEON.DOC.000691	AOS Protocol and Procedure: Periphyton and Seston Sampling in Wadeable streams
RD[14]	NEON.DOC.000692	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling in Wadeable Streams
RD[15]	NEON.DOC.001201	AOS Protocol and Procedure: Microbes in Wadeable Streams

2.3 Acronyms

Acronym	Definition
cm	centimeter
DNA	Deoxyribonucleic acid
FFG	Functional feeding group
LTER	Long Term Ecological Research Program
m	meter
µm	micrometer
mL	milliliter
NAWQA	National Water-Quality Assessment (USGS)
NEON	National Ecological Observatory Network
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 (Figure 4). 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.

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 20). Sampler consists of an inner PVC tube and an outer, stainless steel housing for pushing the corer into the substratum.

Hess sampler: Benthic sampler for gravel and pebble bottomed streams (Figure 23). Sampler consists of a stainless steel drum fitted with a flow-through collection 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.

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

Macroinvertebrate: Typically refers to benthic invertebrates that are >500 μm in length.

Modified kicknet: Benthic sampler used for faster and deeper water (runs). The modified kicknet has a 13” x 20” frame with net attached, and a long handle for holding the net in fast current (Figure 22).

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 18).

Pool: Typically the deepest habitats in a stream, pools are characterized by smaller substrate size and relatively slow moving water.

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, swift stream habitat with high turbulence, typically with relatively coarse sediment size

Run: Shallow to deep stream habitat with swift to moderate flow, characterized by low turbulence (i.e., smooth water surface).

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: Submerged woody debris in the stream channel that often provides substratum and refuge for macroinvertebrates

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.

Surber sampler: Benthic sampler for flowing waters. Sampler consists of a 12" x 12" frame and a long net that collects organisms (Figure 12).

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 set up a DNA barcode library (addressed in RD[09]) for benthic invertebrates at each wadeable stream site. These variables, especially diversity and biomass, will be tracked over time and sampled to detect changes in species loss, changes in community structure and function, as well as the introduction and the spread of invasive taxa (presence/absence).

Benthic invertebrates 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 site (NOTE: some NEON sites may be less than 1 km due to permitting restrictions). Five replicate samples are collected in the dominant habitat type (e.g., 5 Surber samples in riffles), and three replicate samples are collected in the second-most dominant habitat type (e.g., 3 core samples in pools) for a total of eight samples on a given sampling date. Field protocols differ depending on the habitat being sampled. However, all samples are collected from the surface of the natural substratum in each habitat. Riffles and runs will often have cobble or pebble substrata, while pools may have silt or sand substrata. The majority of the invertebrate community is likely to colonize riffles and runs. In sandy and silty habitats and in pools, invertebrates are likely to be most diverse in on woody debris. Appropriate site-specific sampling procedures will be determined prior to sampling following NAWQA and EPA protocols (Barbour et al. 1999, Moulton et al. 2002) and presented in site-specific AOS documents as well as in Appendix E.

Macroinvertebrate sampling occurs three times per year. Timing of sampling is site-specific and determined based on historical hydrological and meteorological data. Specific details on sample dates are provided in the NEON Aquatic Sample Strategy Document (RD[07]) and Appendix D. Sample bout 1 is an early-season date, representing a period of rapid biomass accumulation after winter, typically prior

to leaf out or ice-off where applicable. Sample bout 2 targets mid-summer baseflow conditions. Sample bout 3 represents the late growing season (typically autumn) during leaf-fall where applicable. These dates will differ on a site-by-site basis, but should always occur at or near baseflow conditions. Sampling will not occur directly following a flood in the stream (defined as >1.5 x base flow; Biggs et al. 1999). Should such a flood event occur on or prior to a target collection date, sampling should be delayed 3 days-1 week (maximum 2 weeks, dependent on field schedule) to allow for invertebrates to recolonize the substratum (c.f. Brooks and Boulton 1991, Matthaei et al. 1996).

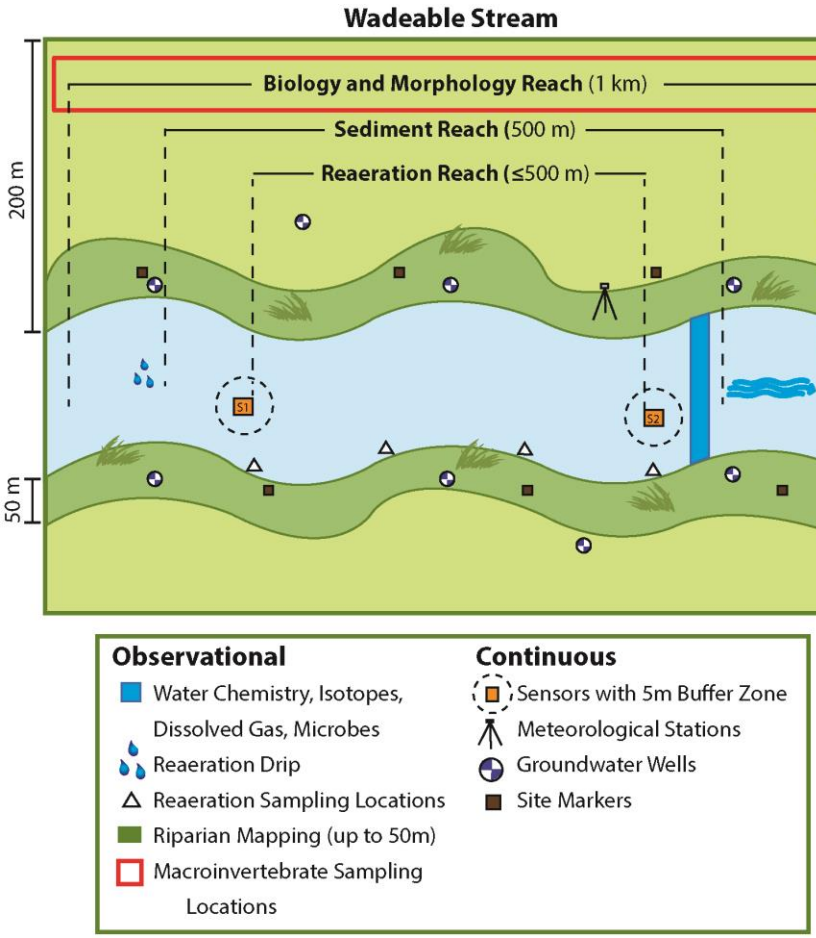

Figure 9. A generic wadeable stream site layout with macroinvertebrate sampling locations.

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

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined

in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

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

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 streamflow, the accumulation of degree days, weather, and riparian phenology (Appendix D).

4.3 Timing for Laboratory Processing and Analysis

Macroinvertebrate samples must have their preservative changed after field collection, and before sending to the macroinvertebrate taxonomist. Preservative must be changed within 12-48 hours of field sampling. Samples must be shipped to the external lab within 30 days of collection. For additional storage and shipping timelines see SOP F.

4.4 Sampling Timing Contingencies

All samples from one sampling bout must be collected within one day (i.e., all 8 samples per stream as detailed in this protocol). A minimum of 2 weeks between sample periods shall be observed.

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If circumstances occur that impede sampling (e.g., wildlife, weather), discard samples and start over the next day that conditions permit.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the streamflow requirements below, continue to collect samples.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If flooding occurs before all samples have been collected for the day, return samples already collected to the stream and start over on the next appropriate day.	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data must be flagged.
	In areas where a Surber sampler is used: If water is above the top of the Surber sampler frame, find a shallower place to sample. If high water is caused by high-flow conditions, wait to sample at baseflow conditions.	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data must be flagged.
3 – 7 Days	If flooding occurs on or prior to the targeted sampling date (>1.5x above baseflow) or unsafe wading conditions occur (Lane and Fay 1997), wait a minimum of 3-7 days after water level drops to near-baseflow conditions (within 25% of baseflow as determined by sensor data) to allow the invertebrate community to recolonize.	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data must be flagged.

4.5 Sampling Specific Concerns

1. Macroinvertebrates often become trapped in the folds of the nets (near the stitching). Check net seams between each sample replicate to ensure that specimens are added to the correct samples, and do not remain in the net.
2. Macroinvertebrates are often lodged in the edges of the sieve. Check the sieve between sample replicates to avoid sample-to-sample specimen contamination.

Samples must be preserved in the field within 1 hour of sampling to prevent predation within the sample, as predatory insects tend to eat smaller insects when put into sample jars

5 SAFETY

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

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

Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 10 ft^2/s (0.93 m^2/s; Lane and Fay 1997). See Section 10 in the NEON Operations Field Safety and Security Plan (AD 01]).

Safety Data Sheets (SDS) shall be readily available and reviewed for all chemicals used during this task.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 2. Equipment list – General equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
RD[10]	R	Site-specific Stream morphology map	Determining sampling locations	1	N
	R	Waders (hip or chest)	Wading	1 pair per person	N
	S	Cooler, 9-28 qt	Field sample storage; use size appropriate to samples being collected	1	N
	S	Clipboard	Recording data	1	N
Consumable items					
RD[06]	R	General AQU Field Metadata Sheet	Recording metadata	1	N
	R	Field data sheets (all-weather copier paper, write in pencil)	Recording data	5 Sheets	N
	R	Pre-printed paper labels (all-weather copier paper, write in pencil)	Labeling samples, inside sample jar	1 Sheet	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Pre-printed 1x2” adhesive labels (	Labeling samples, outside sample jar	1 sheet	N
	R	Pencils	Recording data	4	N

R/S=Required/Suggested

Table 3. Equipment list – Macroinvertebrate Samplers

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
MX100305	R	Surber sampler (243 μm Nitex mesh), 0.093 m^2	Sample collection	Riffles	1	N
MX108298	R	Mini Surber sampler (243 μm Nitex mesh), 0.023 m^2	Sample collection in small streams	Riffles	1	N
MX108051	R	Hess sampler (243 μm Nitex mesh), 0.086 m^2	Sample collection	Riffles	1	N
	R	Kitchen brush w/ nylon bristles	Used with Surber and snag samplers for rock and wood scrubs	All	2	N
MX100303	R	Petite ponar sampler	Sample collection	Pools	1	N
MX100309	R	Hand corer, stainless steel, 20 in	Sample collection	Pools	1	N
MX108199	R	Modified kicknet sampler (243 μm Nitex mesh)	Sample collection	Runs	1	N
MX100304	R	Collapsible quadrat (0.5 x 0.5 m^2)	Used with modified kicknet sampler	Runs	1	N
MX102972	R	Snag sampler (D-frame net without handle, 243 μm Nitex mesh)	Sample collection	Snags	1	N
	R	Bow saw, 21 in	Used with snag sampler	Snags	1	N
	R	Metric ruler	Used with snag sampler	Snags	1	N
Consumable items						

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Petroleum jelly	Used with hand corer to maintain suction	Pools	1	N

R/S=Required/Suggested

Table 4. Equipment list – Macroinvertebrate field elutriation and preservation

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
MX100308	R	Polyethylene wash bottle, unitary (500 mL; Figure 10)	Elutriation, rinsing	2	N
MX100306	R	Sieve, 250 µm	Sample sieving; elutriation	1	N
	R	Plastic bucket, 3 gallon	Substrate scrubbing; elutriation	1	N
	R	HDPE bottles (1 L) or jug (4 L) with lids (clear or amber)	Transporting ethanol to the field	1-4	N
	S	Flexible forceps, featherweight	Collecting clinging insects	1	N
Consumable Items					
	R	Sample jars, HDPE with lid, 16 oz.	Sample container	8	N
	R	Ethanol, 95% non-denatured	Preservative	1-4 L	Y

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	S	Sample jars, HDPE with lid, 32 oz.	Sample container for large samples	5	N
	S	Disposable pipets (polyethylene), 1 mL	Collecting clinging insects	2	N
	S	Latex or Nitrile gloves (pair)	Preventing preservative contact with skin	5	N
	S	Resealable plastic zip-top bags (gallon)	Organizing samples	5	N

R/S=Required/Suggested

Table 5. Equipment list – Laboratory processing: preservative replenishment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Unitary wash bottle, 500 mL, ETOH + glycerol	Adding preservative to sample jars and rinsing filter cup	1	Y
	R	Filter cup (TOS)	Changing preservative	1	N
	R	Safety glasses	Preventing preservative contact with eyes	1 pair	N
MX100306	S	Sieve, 250 µm	Changing preservative	1	N
	S	Featherweight forceps	Picking up insects	1	N
Consumable items					
	R	Ethanol, 95%, undenatured	Preservative	5 L	Y
	R	Glycerol, >99%	Preservative	1 L	Y
	R	250 µm mesh squares for filter cup	Catching insects over the filter cup	8	N
	R	Nitrile gloves	Preventing preservative contact with skin	1 pair	N

R/S=Required/Suggested

Table 6. Equipment list – Shipping supplies

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
(None)					
Consumable Items					
	R	Cardboard box, UN-rated, Group II	Shipping samples to taxonomist	1	N
	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	TBD	N
	R	Heavy duty plastic trash bag	Lining the shipping container	1	
	R	Appropriate labels and forms for limited quantity ground shipments (see AD[03])	Shipping paperwork and external shipping labels	TBD	N
	R	Shipping inventory (RD[12])	Provides sample information to external lab	1	N

R/S=Required/Suggested

Figure 10. Removing the tip of the 500 mL wash bottle helps make rinsing easier and increases water pressure. This tip pulls easily out of the main body of the wash bottle.

6.2 Training Requirements

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

Personnel will be trained in field protocols associated with this document, and trained in safe working practices for stream field work.

6.3 Specialized Skills

N/A

6.4 Estimated Time

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

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

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Sampling Equipment Preparation and Checklist

1. Collect and prepare all equipment, including sample jars and labels.

Table 7. Sampling equipment preparation checklist

✓	Item	Action
	Nets and sieves	Check for holes. Repair if necessary. Ensure that both are clean and free of debris and organic matter.
	Hand corer	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.)
	Paper labels	Print on all-weather paper (RD[05], Figure 11).
	Adhesive labels	Print on small adhesive labels (RD[05])
	Ethanol	Fill 1 L HDPE bottles (or 4 L HDPE jug) with 95% ethanol (Stein et al. 2013). Cap tightly. Transport to the field following EHS guidelines. Clearly label bottle.
	General AQU Field Metadata Sheet (RD[06])	Fill out upon every field sampling visit

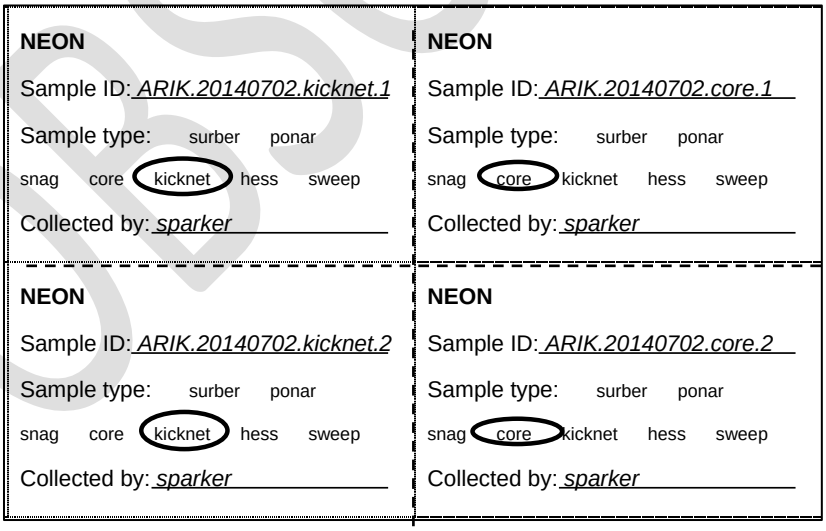

Figure 11. Example of field labels for wadeable stream invertebrate sampling

SOP B Determining Habitat and Sampler Type

B.1 Decision Tree: Determining Habitat to Sample

1. Determine percent cover of habitat types throughout the sampling reach using the Stream Morphology Map for this site (RD[10]) and proceed to Step 2. Habitat suggestions for NEON sites are provided in Appendix E.
    a. The habitat type chosen should be present during all sampling bouts. If the site is a STREON site, the same habitat types should be sampled in both the Aquatic and the STREON reaches.
    b. All 5 (or 3) replicate samples must be taken from the same habitat type on each sampling bout, unless a major event (i.e., a flood) causes significant changes to the stream channel.
    c. Targeted habitat types (see Definitions, Section 2.4):
        1) Riffle
        2) Run
        3) Pool
        4) Snags
2. Does habitat type account for >20% of the reach throughout the year?
    a. If yes, go to Step 3.
    b. If no, ignore this habitat type.
3. Is habitat a shallow (<0.5 m deep) riffle or run with cobble/pebble substratum?
    a. If YES, follow Surber sampling procedure below (7C.1).
    b. If NO, proceed to Step 4.
4. Is habitat a run with cobbles/pebble substratum deeper than 0.5 m?
    a. If YES, follow modified kicknet sampling procedure below (7C.5).
    b. If NO, proceed to Step 5.
5. Does the riffle/run have a silty or sandy bottom?
    a. If YES, proceed to Step 6.
    b. If NO, proceed to Step 8.
6. Does the sand or silt bottomed riffle/run have woody snags? (Must be present at a minimum of least 5 locations throughout the reach with diameter of > 1 cm, underwater, and be colonized by invertebrates.)
    a. If YES, proceed to woody snag sampling procedure below (7C.3).
    b. If NO, proceed to Step 7.
7. Does the sand or silt bottomed riffle lack woody snags?
    a. If YES, proceed to coring procedure (7C.4).
    b. If NO, proceed to Step 8.
8. Is habitat a pool?
    a. If YES, proceed to Step 9.
    b. If NO, proceed to Step 10.
9. Is the pool deeper than 1 m and have a silty or sandy bottom?
    a. If YES, follow Petite ponar sampling procedure (7C.2).
    b. If NO, proceed to Step 10.

10. Is the pool < 1m deep and have a silty, sandy, or cobble bottom?
    a. If YES, follow Hess sampling procedure (7C.6).
    b. If NO, proceed to Step 11.
11. Does the habitat fit any of the above descriptions?
    a. If YES, return to Step 2.
    b. If NO, seek advice from NEON Aquatic Ecologist.

B.2 Habitat and Sampler Selection

1. Do not sample within a 5 m radius of the aquatic instrumentation or STREON baskets.
2. Determine the dominant habitat and second-most dominant habitat based on the Stream Morphology Map (RD[10]).
    a. 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, take 5 kicknet samples and 3 core samples).
    b. If working at a STREON site, use the same habitat types and sampling methods in both the aquatic reach and the STREON reach.
3. Choose the appropriate sampler based on the field conditions and habitat being sampled (see Decision tree, SOP B.1).
4. 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.
5. Spread samples out along the reach. For example, do not collect all replicates for the same substratum in the same riffle, collect replicate samples from 5 different riffles along the reach.
    a. If working in a STREON reach, all sample replicates must be collected between the STREON sensor sets.
6. Do not sample anywhere you or other field technicians have walked in the reach, or locations that appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).
7. The order of preference for sampling habitats is as follows:
    a. Riffles (Surber or Hess)
    b. Runs (Surber, Hess, or modified kicknet)
    c. Snags (D-frame snag net)
    d. Pools (corer, Hess, or petite ponar)
8. Proceed to the protocol for the appropriate sampler type below.

B.3 Contingent decisions

Table 8. Contingent decisions for sampling.

Situation	Action	Outcome for Data Products	Considerations
Wadeable stream site with <200 m aboveground stream length due to stream size or seasonal drying	Habitat available may be insufficient to accommodate all 8 replicate samples without causing harm to the stream. Reduce sampling by collecting samples only in the dominant habitat type (5 samples total).	Lower resolution for diversity metrics.	If the decision is made to decrease the number of samples collected for this protocol, it must also be reflected in the other wadeable stream biology protocols (RD[13], RD[14], RD[15]).

SOP C Field Sampling

C.1 Surber Sampler (Riffles)

At selected small headwater stream sites where habitats are too small to fit a standard Surber sampler (0.093 m^2), a mini Surber sampler (0.023 m^2) may be used in its place. This decision will be made by the site host and NEON aquatic ecologist. All sampling steps below remain the same.

1. Ensure that all replicate samples are taken from riffles of similar depth and flow (within approximately 20%). Ensure that water level is below the top of the Surber frame.
2. Choose a location in the riffle that has flowing water and appears to be underwater most of the time (i.e., not underwater only at high flow). Avoid locations that are on top of, or just downstream of, large boulders (e.g., boulders that affect the flow of water at your location). Ensure that all substrata within the Surber frame are underwater.
3. Set up the Surber sampler by extending the base and clipping the arms into the screws on the base of the Surber frame (Figure 12).
4. Carry the 3 gallon bucket and Surber sampler with you to the sample location in the stream.
5. Approach sample location from downstream so as not to step on the cobbles that you want to sample.
6. Fill 3 gallon bucket ¼ - ½ full with stream water so that it is weighted when resting on the stream bottom or manually hold bucket in place. Place the bucket on the stream bottom in a shallow part of the riffle (or on the shore) **that you can still reach** from the Surber location.
7. Orient the Surber so the opening of the net is facing into the stream flow (Figure 12).

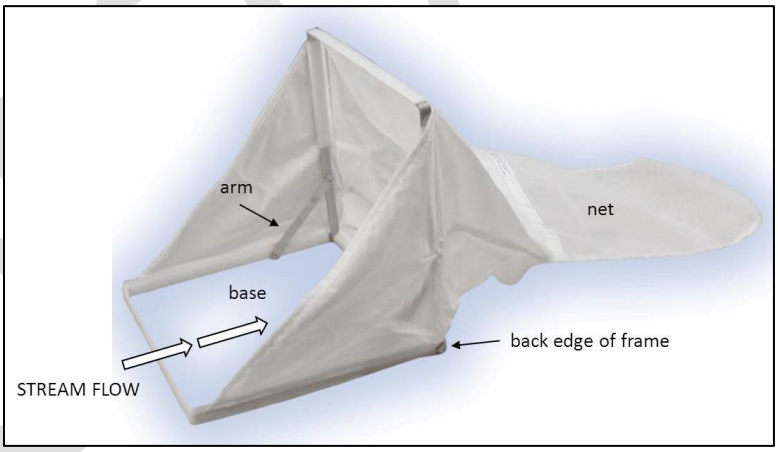

Figure 12. Diagram of Surber sampler set-up. The inside of the base is the sample area of the stream bottom (0.09 m^2)

8. Place the back edge of the frame on the stream bottom first, gently lowering the rest of the base onto the substratum. Once base is placed on the stream bottom, do not move the sampler.
   a. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 13).
   b. Record the distance from the STREON dripper if sampling at a STREON site (RD[05]).

NEON Aquatic Benthic Invertebrate Collection

Wadeable Streams

Site (4-letter code): *ARIK*	**Recorded by:** *sparker*
Date (YYYYMMDD): *20140712*	**Collected by:** *kgoodman*
Local time (HH:MM): *0930*	**Sampling protocol & Rev.:** *NEON.DOC.000690vB*

Location ID	Habitat	Sample type	Replicate	Sample ID	Habitat percent	Substratum size class	Mean core depth (m)	Snag length (cm)	Snag diameter (cm)
DS sensors	*run*	*kicknet*	*1*	*ARIK.20140712.kicknet.1*	*75%*	*sand*			
DS sensors	*run*	*kicknet*	*2*	*ARIK.20140712.kicknet.2*	*75%*	*sand*			
sensor reach	*run*	*kicknet*	*3*	*ARIK.20140712.kicknet.3*	*75%*	*sand*			
US sensors	*run*	*kicknet*	*4*	*ARIK.20140712.kicknet.4*	*75%*	*sand*			
US sensors	*run*	*kicknet*	*5*	*ARIK.20140712.kicknet.5*	*75%*	*sand*			
DS sensors	*pool*	*core*	*1*	*ARIK.20140712.core.1*	*20%*	*silt*	*0.15*		
DS sensors	*pool*	*core*	*2*	*ARIK.20140712.core.2*	*20%*	*silt*	*0.10*		
US sensors	*pool*	*core*	*3*	*ARIK.20140712.core.3*	*20%*	*silt*	*0.08*		

Figure 13. Example of field data sheet for wadeable stream invertebrate sampling

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

Figure 14. A field technician holds the Surber net on the stream bottom, and keeps the bucket nearby for depositing rocks from the base of the Surber

   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 15).

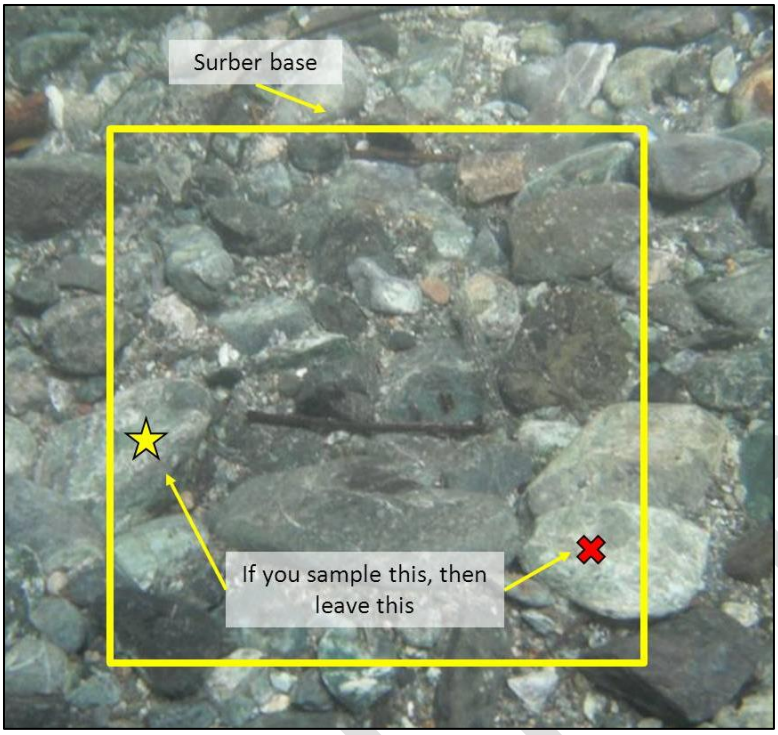

Figure 15. Cobble selection at Surber edges

10. After the top layer of cobbles has been removed and placed in the bucket, disturb the remaining sediments (~10 cm deep; Cuffney et al. 1993) with your hand or kitchen brush so that all detritus flows into the Surber net for 1 minute.
11. After all suspended material has been washed into the net, lift the Surber sampler up off the substratum.
12. Dip the net into the stream a few times to rinse the sides of the net, always directing flow towards the back of the net. This allows material caught in the net to concentrate at the bottom of the net. Be sure to keep the net opening above the water surface so as not to accidentally lose or collect additional material in the sample.
13. Return to the processing location on the stream bank with the bucket of cobbles and the Surber sampler.
14. Set the Surber at the side of the stream so that the frame is on shore, and the end of the net (and all material collected) is sitting in water (to keep insects alive).
15. Scrub each cobble in the bucket using the kitchen brush (Figure 16). Scrape off all aquatic plants using the pot scrubber side of the brush. Brush all invertebrates into the bucket (Figure 17).
    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.

Figure 16. Example of nylon kitchen brush with pot scrubber

Figure 17. A field technician scrubs rocks from the Surber sample into the 3 gallon bucket

b. Rinse the cobble in the bucket. Visually inspect the cobble for organic material (e.g., moss) and clinging invertebrates (e.g., caddisflies and snails) before discarding.
c. Discard cleaned cobble onto the stream bank.
d. Repeat until all cobbles in the bucket have been scrubbed and discarded.

16. Invert Surber net (turn it inside-out) into bucket of water. Swirl net around in bucket water to remove all material from the net. Use your hands to brush material from the net into the bucket.
    a. Immediately release any fish or other vertebrates captured in the net.
    b. Depending on site-specific permits, mussels collected in the sampler may also need to be released.

c. Use 500 mL wash bottle filled with stream water to rinse any residual organic matter or insects from the net. Watch for clinging insects.

17. Once the Surber net is empty, all of the sample material will be suspended in water in the 3 gallon bucket. Proceed to Section 7C.7 Sample Elutriation and Preservation.
18. After elutriation and preservation, move to next location and repeat Steps 5-17 until you have the desired number of samples (5 samples if this is the dominant habitat type, 3 samples if this is the second-most dominant habitat).

C.2 Petite Ponar (Deep Pools)

1. Find pools of similar depth and flow that have soft substratum, and are > 1 m deep.
2. Release the safety-pin on the ponar sampler and open the bottom of the ponar (Figure 18). Place the pinch-in (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).

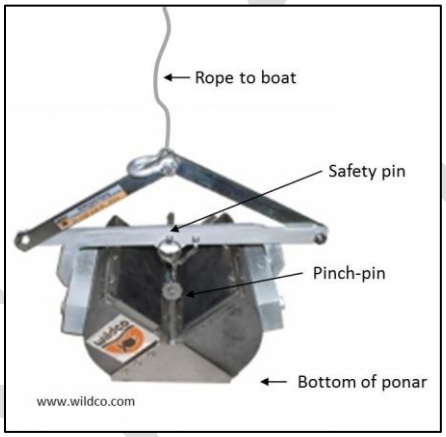

Figure 18. Example of a petite ponar setup

3. Stand just downstream or on the bank near where you want to sample. Do not disturb the sediments where you plan to sample.
   a. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 13; RD[05]).
   b. Record the distance downstream from the STREON dripper if sampling at a STREON site (RD[05]).
4. Hold the open ponar above the water surface above the point where you want to sample.
5. Drop the sampler in the water. When the ponar hits the sediments and the lines goes slack, pull up on the rope to close the jaws.
6. Pull the ponar up to the surface using the rope.
7. 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 at the stream bank and return to Step 1 in a different location.

8. If the ponar is properly closed, place the sampler over the bucket and open the jaws to release the sample.
9. Rinse the inside of the ponar into the bucket using the 500 mL wash bottle.
10. Once ponar is empty and clean, all of your sample material will be in the 3 gallon bucket.
11. Carefully add water to the bucket to until bucket is about ½ full. Proceed to Section 7C.7 Sample Elutriation and Preservation.
12. After elutriation and preservation, rinse sampler in the stream, move to the next sampling location and repeat Steps 1-11 until you have the desired number of samples (5 samples if this is the dominant habitat type, 3 samples if this is the second-most dominant habitat).

C.3 Snag Sampling (Coarse Wood Debris)

1. Sample in areas of similar flow and depth along the sample reach (Figure 19).
   a. Snags must be submerged and appear to be underwater most of the time (i.e., not underwater only at high flow).
   b. Snags may include leaf litter packs, overhanging branches, or submerged coarse woody debris, but the litter packs/debris must be present throughout the year (not just seasonally).
   c. Note the dominant substratum size class below the snag at the sampling location on the field data sheet (Figure 13; RD[05]).
   d. Record the distance downstream from the STREON dripper if sampling at a STREON site (RD[05]).

Figure 19. Sites such as D08 Mayfield Creek are well-suited to snag sampling with their sandy/silty substrate and abundance submerged coarse woody debris

2. Place the 243 μm mesh bag around the end of the snag with the bag opening facing upstream so invertebrates will not escape.
3. Remove the snag section covered by the bag by sawing with the bow saw or breaking the snag at the end of the bag.

a. If you are unable to remove the snag or need to leave it in the stream for other sampling, you may scrub into the net without breaking off the piece as long as the stream flows into the net to collect any organisms that are brushed off the snag.

4. Fill 3 gallon bucket ¼ - ½ full with stream water.
5. Place the snag in the bucket of stream water.
6. 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.
7. Use the 500 mL wash bottle filled with stream water to rinse any residual organic matter or insects from the net.

   a. Watch for clinging insects.
   b. Set net aside.
8. Brush insects off snag surface using the nylon kitchen brush.
9. Rinse snag surface into bucket using the 500 mL wash bottle.
10. Measure length and width of snag and record on field data sheet (RD[05]).
11. Visually inspect snag and remove any remaining insects using flexible forceps or disposable pipet.
12. Once the net is empty and the snag is clean, all of your sample material will be suspended in water in the bucket. Proceed to Section 7C.7 Sample Elutriation and Preservation.
13. After elutriation and preservation, rinse net in stream and move to next sampling location and repeat Steps 1-12 until you have the desired number of samples (5 samples if this is the dominant habitat type, 3 samples if this is the second-most dominant habitat).

C.4 Hand Corer (Sandy/Silty Bottomed Streams and Shallow Pools)

1. Sample in areas of similar flow and depth along the sample reach.
   a. Sample in areas with soft sediments (sand, silt, clay).
2. Rinse the corer assembly to be sure that all screw threads are clean of silt and sediment.
3. Assemble the hand corer (Figure 20).

   a. Screw the corer head onto the top of the stainless steel core tube (Figure 20b). **NOTE**: Petroleum jelly may be applied to the threads to aid sealing.
   b. Insert the white eggshell core-catcher into the bottom of the clear PVC liner tube (Figure 20b).

      1) **NOTE**: Cores are usually more stable without the PVC liner tube. The corer may be used with or without the liner tube, this decision may be made by the technician at the time of sampling.
   c. Insert the PVC liner tube (if using the liner tube) into the stainless steel core tube (Figure 20b).
   d. Screw the stainless steel nosepiece onto the bottom of the core tube (Figure 20c).
   e. Petroleum jelly may be applied to the flutter valve to help create suction in the corer.

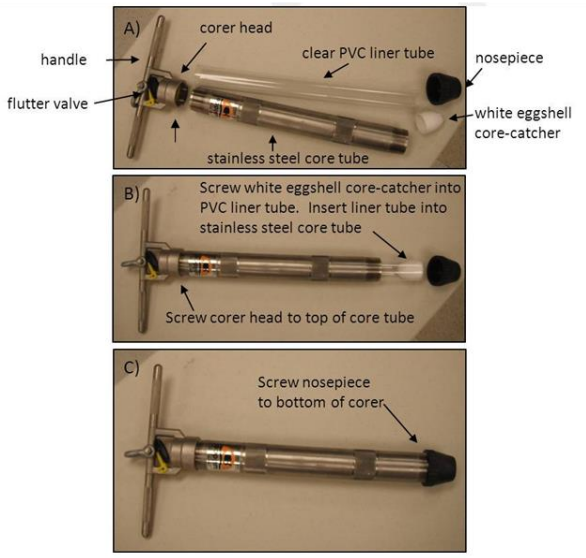

Figure 20. Hand-corer assembly: A) Disassembled corer. B) Partially assembled corer. C) Assembled corer. Note: the nosepiece may be stainless steel (not black Lexan as in photo) in some domains

4. Approach sample site from downstream so as not to step in the location where you want to sample.
5. Hold the core sampler firmly by the handle (Figure 20a).
6. Push the core sampler through the water and into the sediments in one smooth, continuous movement (Figure 21).
    a. Depending on the sediment type, it may take a lot of force to push the corer into the sediments.
    b. Do not hammer or pound the corer into the sediments.
    c. Keep shaft of corer at a 90^{o} angle with the sediments.
    d. Note the dominant substratum size class at the sampling location in the field data sheet (Figure 13; RD[05]).
    e. Record the distance downstream from the STREON dripper if sampling at a STREON site (RD[05]).

Figure 21. Push the hand corer through the water and into the sediments at a 90˚ angle

7. Push corer approximately 5-10 cm into the substratum while keeping the blue flutter valve open.
    a. Pre-measure 5 cm and 10 cm on the outside of the corer before sampling.
8. If the corer is not completely submerged, wet the blue flutter valve on the top of the corer head and close by hand (Figure 20a).
    a. The valve must be kept wet to maintain a good seal and prevent loss of sample when the corer is extracted. Petroleum jelly may help keep the flutter valve sealed.
    b. If the corer is submerged, allow air to escape from the flutter valve. When the corer is pulled out, the flutter valve should close by itself.
9. Holding the flutter valve closed, **slowly** pull the corer straight up and out of the sediments.
    a. If the corer is pulled up too fast, you may lose the contents.
10. Continue to hold the flutter valve closed and lift the entire core sampler clear of the water, keeping the sampler vertical so as not to spill the sample.
    a. If the sample spills before reaching the bucket, you may rinse the corer and start over at Step 3 approximately 0.5 m away from the original location, as long as it has not been disturbed.
    b. If some, but not all, of the sample spills and is not deposited in the bucket, discard the entire sample and start over.
11. Hold the core sampler over the 3 gallon bucket and unscrew the nosepiece, allowing the sample to drop into the bucket.
12. Fill the 500 mL wash bottle with stream water and rinse any residual organic matter or insects from the corer into the bucket.
    a. Watch for clinging insects.
13. Once the corer is empty, all of your sample material will be suspended in water in the 3 gallon bucket.

14. Repeat Steps 3-13 two more times, until there is a composite of 3 cores in the 3 gallon bucket. Proceed to Section 7C.7 Sample Elutriation and Preservation.
    a. 3 cores = 1 composite sample
15. After elutriation and preservation, clean core in stream water and move to next location and repeat Steps 1-14 until you have the desired number of composite samples (5 samples if this is the dominant habitat type, 3 samples if this is the second-most dominant habitat).

C.5 Modified Kicknet Sampler (Runs)

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

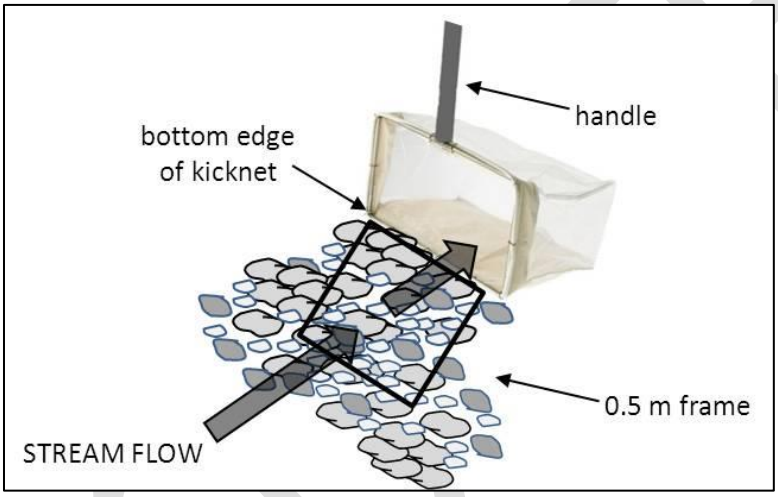

Figure 22. Example of modified kicknet setup

3. Approach sample site from downstream so as not to step in 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 22).
6. Place 0.5 m^2 quadrat just upstream (1-5 cm) of the kicknet edge.
    a. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 13; RD[05]).
    b. Record the distance downstream from the STREON dripper if sampling at a STREON site (RD[05]).
7. If you can reach the stream bottom with your hands, pick up each cobble and brush the surface with a kitchen brush so that the stream current carries all insects/organic matter into the net.
    a. 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 15).
    b. Once a cobble is cleaned, discard to the side or downstream of the sample area. Do not resample cobbles.

8. 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.
9. Sweep the kicknet toward the water surface, taking care to keep the opening facing upstream so no contents will spill.
10. 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.
11. Take the net to a processing location on the stream bank.
12. Half-fill 3 gallon bucket with stream water.
13. 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.
14. Use 500 mL wash bottle to rinse any residual organic matter or insects from the net.
    a. Watch for clinging insects.
15. Once the net is empty, all of the sample material will be suspended in water in the 3 gallon bucket. Proceed to Section 7C.7 Sample Elutriation and Preservation.
16. After elutriation and preservation, clean net in stream water and move to next location and repeat Steps 3-15 until you have the desired number of samples (5 samples if this is the dominant habitat type, 3 samples if this is the second-most dominant habitat).

C.6 Hess Sampler (Riffles, Runs, or Pools with Small Substratum)

1. Ensure that all replicate samples are taken from areas of similar depth and flow (within approximately 20%). Ensure that water level is below the top of the Hess frame.
2. Choose a location that appears to be underwater most of the time (i.e., not underwater only at high flow). Avoid locations that are on top of, or just downstream of, large boulders (e.g., boulders that affect the flow of water at your location).
3. Carry the 3 gallon bucket and Hess sampler with you to the sample location.
4. Fill 3 gallon bucket ¼ - ½ full with stream water so that it is weighted when resting on the stream bottom or manually hold bucket in place. Place the bucket on the stream bottom in a shallow part of the riffle (or on the shore) **that you can still reach** from the sampling location.
5. Position the Hess frame securely on the stream bottom, with the screened opening facing upstream and the net facing downstream (Figure 23).
    a. Hold the sampler in position by applying pressure with your knees by a second field technician.
    b. Note the dominant substratum size class at the sampling location on the field data sheet (Figure 13).
    c. Record the distance downstream from the STREON dripper if sampling at a STREON site (RD[05]).

Figure 23. Hess sampler with collection net and dolphin bucket

6. Reach into the sampler and remove any larger substrata (i.e., cobbles) and place in the 3 gallon bucket.
7. After the top layer of cobbles has been removed and placed in the bucket, disturb the remaining sediments (~10 cm deep; Cuffney et al. 1993) with your hand or kitchen brush so that all detritus flows into the Surber net (typically ~30-60 seconds).
    a. If there is insufficient stream flow to wash sample and detritus into the net, create flow with your hands until the water inside the Hess sampler appears clear.
8. Dip the net into the stream a few times to rinse the sides of the net, always directing flow towards the back of the net. 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 (Figure 16). Scrape off all aquatic plants using the pot scrubber side of the brush. Brush all invertebrates into the bucket (Figure 17).
    a. Scrub gently but firmly to remove insects, but not so vigorously as to damage insects. They are often identified by fragile anatomical structures such as external gills.
    b. Rinse the cobble in the bucket. Visually inspect the cobble for organic material (e.g., moss) and clinging insects (e.g., caddisflies) before discarding.
    c. Discard cleaned cobbles on the stream bank.
    d. Repeat until all cobbles in the bucket have been scrubbed and discarded.
12. Rinse all material in the net into the dolphin bucket.
13. Holding the end of the net and dolphin bucket over the 3 gallon bucket, unscrew the dolphin bucket and rinse its contents into the 3 gallon bucket.
    a. Invert net into 3 gallon bucket and rinse any remaining insects to the sample.
14. Once the Hess net is empty, all of the sample material will be suspended in water in the 3 gallon bucket. Proceed to Section 7C.7 Sample Elutriation and Preservation.
15. After elutriation and preservation, clean net in stream water and move to the next location and repeat Steps 5-14 until you have the desired number of samples (5 samples if this is the dominant habitat type, 3 samples if this is the second-most dominant habitat).

C.7 Sample Elutriation and Preservation

1. Gently swirl contents in bucket to create a “whirlpool” and suspend organic material. This process (Steps 1-4) is called elutriation (Figure 24-1).

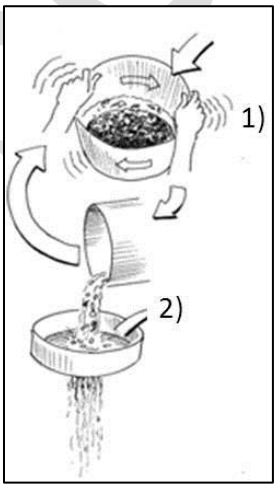

Figure 24. Process of elutriation (Dates and Byrne 1997)

2. Carefully pour contents of bucket into 250 μm sieve (Figure 24-2). Some material will be retained in the bucket. (If the sieve fills up with organic matter, you may pick clumps out of the sieve and place in sample jar (Figure 25).

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

Figure 25. A field technician carefully pours water from the 3 gallon bucket into the sieve

3. Partially refill bucket with water (bucket opening should be facing upstream) without losing any of the organic matter contents of the bucket.
4. Repeat elutriation (Steps 1-3) 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 bucket) is typically sufficient to remove insects from the inorganic matter.
5. 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). Retain any plant material in the sample.
   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.
6. Discard inorganic material remaining in the bucket, and rinse bucket with stream water. Set bucket aside.
7. The entire sample should now be in the sieve. Complete a sample label using a pencil (RD[05]) and place inside a sample jar.
   a. Sample ID format: SITE.DATE.sample type.replicate
      1) Example: ARIK.20140620.kicknet.3
8. Fill the 500 mL wash bottle with stream water.
9. Rinse sieve screen and edges with wash bottle and tap sieve until organic material is collected at one edge of the sieve (Figure 26).

Figure 26. A field technician rinses the sample from the sieve into the open container with the 500 mL wash bottle

10. Open the sample jar and carefully rinse (using the 500 mL wash bottle) sample from the sieve into the sample jar. You can also use your fingers to move the sample from the sieve into the sample jar (Figure 26).
    a. Use as little water as possible. Be sure to rinse sieve and fingers into the sample jar to thoroughly remove all material.
    b. If there is too much rinse-water, you may re-sieve sample by dumping contents of sample jar back into the sieve, and repeating Steps 9-10.
11. 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. Do not use zip-top bags in place of Whirl-paks® or sample jars as they do not seal properly.
12. Check sieve for clinging insects that may not have been washed into the sample jar.
13. **Carefully** add preservative. Preserving immediately prevents damage to insects in the samples.
    a. Wear latex gloves when preserving samples.
    b. Preserve samples in a well-ventilated location (e.g., outdoors).
    c. Add enough ethanol preservative to the sample to preserve in a 1:1 sample material:preservative ratio.
14. Close the sample jar tightly.
15. Add a small adhesive label to the outside of the sample jar with the sample ID for use by the external taxonomy lab. Write in pencil as ethanol will erase permanent marker.

C.8 Sample Preservation

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

C.9 Ending the Sampling Day

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

SOP D Laboratory Sampling and Analysis

D.1 Equipment Checklist

Table 9. Laboratory equipment preparation checklist

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

D.2 Processing Samples

1. Wearing safety glasses and nitrile gloves, open field-preserved sample under fume hood.
2. Carefully decant liquid over a clean 250 μm sieve or filter cup similar to that used in the TOS Beetle Protocol (RD[11]) over an ETOH waste container. Take care not to lose any sample material.
    a. Rinse down sides of sample jar bag with ETOH/glycerol wash bottle so no material remains near the top of the sample jar.
    b. If using sieve, check for any macroinvertebrates or other organic material that may have come from the sample. Using featherweight forceps, place this material back in the sample bag.
    c. If using the TOS beetle filter cup, rinse down the sides of the cup with ETOH, and place the filter mesh and all material on it back into the sample jar.
3. Check that sample label is still readable and inside the sample jar. Check that the external adhesive label is on the outside of the sample jar.
4. Re-preserve sample with 95% ethanol/5% glycerol solution in a 1:1 sample material:preservative ratio.
5. Close the sample jar tightly.
6. Carefully clean sieve before decanting the next sample.
7. Continue until preservative in all samples has been replaced and proceed to Sample Shipping (SOP F).

D.3 Analyzing Samples

N/A

D.4 Sample Storage

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

D.5 Ending the Processing Day

1. Equipment maintenance, cleaning and storage

a. Carefully clean and dry sieve to remove all organic debris.
b. Dry all equipment thoroughly before storage.

SOP E Data Entry and Verification

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

Enter all data from the field datasheets into Excel workbooks “aquatic field metadata data entry” and “inv_strDomainFieldData_YYYYMMDD_in_DXX”.

SOP F Sample Shipment

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

Shipments are to include a hardcopy of the "per sample" tab of the shipping inventory as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. The shipment tracking number (shipment ID) must be included in the electronic version of the shipping inventory as well as the email, but is not necessary on the hard copy.

F.1 Macroinvertebrate Sample Shipping

1. Place sealed sample jars into one or several gallon-sized resealable zip-top bags, grouped by site. Sample jars are acceptable "inner containers" required for shipping.
2. Line a Group II cardboard box with a heavy-duty trash bag.
3. Place all sample jars right-side up inside the trash bag, inside the Group II cardboard shipping box. Add Grade 2 Vermiculite in the trash bag liner as needed to take up excess space in container and cushion samples.
4. Include shipping inventory/manifest in an additional zip-top bag.
5. Follow instructions for shipping ETOH in limited quantity ground shipments in AD[03].

F.2 Handling Hazardous Material

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

F.3 Supplies/Containers

See section F.1 and Table 6 for specific shipping materials.

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.

F.4 Conditions

Samples may be stored at the domain support facility room temperature or 4 ^{o}C until shipping. Samples are shipped ground at ambient temperature.

F.5 Grouping/Splitting Samples

Group samples by site per bout in plastic bags. Samples from multiple sites may be sent in the same shipment.

F.6 Return of Materials or Containers

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

F.7 Shipping Inventory

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

F.8 Laboratory Contact Information and Shipping/Receipt Days

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

8 REFERENCES

Allan, J. D. 1995. Stream Ecology: Structure and Function of Running Waters. Kluwer Academic Publishers, Boston, MA. 388 pages.

Barbour, M. T., Gerritsen, B. D. Snyder, and B. Stribling. 1999. Benthic Macroinvertebrate Protocols. Pages 7.1-7.34 *in* Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2nd Edition. EPA 841-B-99-002. U.S. Environmental Protections Agency, Office of Water, Washington, D.C.

Brooks, S. S. and A. J. Boulton. 1991. Recolonization dynamics of benthic macroinvertebrates after artificial and natural disturbances in an Australian temporary stream. Australian Journal of Marine and Freshwater Research **42:**295-308.

Cuffney, T. F., M. E. Gurtz, and M. R. Meador. 1993. Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program. U.S. Geological Survey Open-File Report 93-406. Raleigh, NC.

Dates, G. and J. Byrne. 1997. Living Waters: Using Benthic Macroinvertebrates and Habitat to Assess Your River's Health. River Watch Network, Montpelier, VT.

Hauer, F. R. and V. H. Resh. 2006. Macroinvertebrates. Pages 435-463 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in Stream Ecology, Second Edition. Academic Press, Boston, MA.

Hynes, H. B. N. 2001. The Ecology of Running Waters. The Blackburn Press, Caldwell, NJ. 555 pages.

Lane, S. L. and R. G. Fay. 1997. Safety in Field Activities: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chapter A9. Accessed 11 April 2013, at http://pubs.water.usgs.gov/twri9A9/.

Matthaei, C. D., U. Uhlinger, E. I. Meyer, and A. Frutiger. 1996. Recolonization by benthic invertebrates after experimental disturbance in a Swiss prealpine river. Freshwater Biology **35**: 233-248.

Merritt, R. W. and K. W. Cummins. 2006. Trophic Relationships of Macroinvertebrates. Pages 585-609 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in Stream Ecology, Second Edition. Academic Press, Boston, MA.

Moulton, S. R., II, J. G. Kennen, R. M. Goldstein, and J. A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program. Open-File Report 02-150. U.S. Geological Survey, Reston, VA.

Slavik, K., B. J. Peterson, L. A. Deegan, W. B. Bowden, A. E. Hershey, and J. E. Hobbie. 2004. Long-term responses of the Kuparuk River ecosystem to phosphorus fertilization. Ecology **85**:939-954.

Stein, E. D., B. P. White, R. D. Mazor, P. E. Miller, and E. M. Pilgrim. 2013. Evaluating ethanol-based sample preservation to facilitate use of DNA barcoding in routine freshwater biomonitoring programs using benthic macroinvertebrates. PLoS ONE **8**: e51273.

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 10. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002197	Datasheets for AOS Protocol and Procedure: Macroinvertebrate Sampling in Wadeable Streams
NEON.DOC.001646	General AQU Field Metadata Sheet

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

APPENDIX B QUICK REFERENCES

B.1 Steps for Macroinvertebrate Sampling

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

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

NEON Sample ID: *ARIK.20140702.kicknet.1* Sample type: surber ponar snag core (kicknet) hess sweep Collected by: *sparker*	**NEON** Sample ID: *ARIK.20140702.core.1* Sample type: surber ponar snag core (kicknet) hess sweep Collected by: *sparker*
NEON Sample ID: *ARIK.20140702.kicknet.2* Sample type: surber ponar snag core (kicknet) hess sweep Collected by: *sparker*	**NEON** Sample ID: *ARIK.20140702.core.2* Sample type: surber ponar snag core (kicknet) hess sweep Collected by: *sparker*

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

Step 4 – Determine habitat sampling locations from the Stream Morphology Map (RD[10]).

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

Step 6 – Collect samples: 5 per dominant habitat type, 3 per second-most dominant habitat type.

Step 7 – Elutriate and pour sample over sieve.

Step 8 – Transfer sample from sieve to sample jars

Step 9 – Preserve with 95% ethanol.

Step 10 – Change preservative at the Domain Support Facility within 12-48 hours of sample collection.

Step 11 – Ship samples to external facility.

B.2 Order of Preference for Sampling Habitats

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

B.3 Determining Habitat to Sample

1. Determine percent cover of habitat types throughout the sampling reach using the Stream Morphology Map for this site (RD[10]) and proceed to Step 2. Habitat suggestions for NEON sites are provided in Appendix E.
    a. Targeted habitat types (see Definitions, Section 2.4):
        1) Riffle
        2) Run
        3) Pool
        4) Snags
2. Does habitat type account for >20% of the reach?
    a. If yes, go to Step 3.
    b. If no, ignore this habitat type.
3. Is habitat a shallow (<0.5 m deep) riffle or run with cobble/pebble substratum?
    a. If YES, follow Surber sampling procedure below (7C.1).
    b. If NO, proceed to Step 4.
4. Is habitat a run with cobbles/pebble substratum deeper than 0.5 m?
    a. If YES, follow modified kicknet sampling procedure below (7C.5).
    b. If NO, proceed to Step 5.
5. Does the riffle/run have a silty or sandy bottom?
    a. If YES, proceed to Step 6.
    b. If NO, proceed to Step 8.
6. Does the sand or silt bottomed riffle/run have woody snags? (Must be present at a minimum of least 5 locations throughout the reach with diameter of > 1 cm, underwater, and be colonized by invertebrates.)
    a. If YES, proceed to woody snag sampling procedure below (7C.3).
    b. If NO, proceed to Step 7.
7. Does the sand or silt bottomed riffle lack woody snags?
    a. If YES, proceed to coring procedure (7C.4).
    b. If NO, proceed to Step 8.
8. Is habitat a pool?
    a. If YES, proceed to Step 9.
    b. If NO, proceed to Step 10.
9. Is the pool deeper than 1 m and have a silty or sandy bottom?
    a. If YES, follow Petite ponar sampling procedure (7C.2).
    b. If NO, proceed to Step 10.

10. Is the pool < 1m deep and have a silty, sandy, or cobbly bottom?
    a. If YES, follow Hess sampling procedure (7C.6).
    b. If NO, proceed to Step 11.
11. Does the habitat fit any of the above descriptions?
    a. If YES, return to Step 2.
    b. If NO, seek advice from NEON Aquatic Ecologist.

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

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

Sample collection: Be sure to…

- ☑ Determine the dominant habitat and second-most dominant habitat based on the Stream Morphology Map.
- ☑ Choose the appropriate sampler.
- ☑ Start sampling at the bottom of the reach, working upstream so as not to decrease visibility and cause invertebrates to drift.
- ☑ Spread replicate samples out along the reach.
- ☑ Do not sample anywhere you or other field technicians have walked in the reach, or locations that appear recently disturbed.

Sample preservation: Be sure to…

- ☑ Preserve the samples streamside or at the vehicle to reduce predation.
- ☑ Change the preservative within 12-48 hours of field sampling.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

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

Domain	Site	Bout 1	Bout 2	Bout 3
D01	West Branch Bigelow Brook*	11Apr-9May	9Jul-6Aug	3Oct-31Oct
D01	Sawmill Brook	8Apr-6May	9Jul-6Aug	9Oct-6Nov
D02	Mill Run*	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D02	Posey Creek	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D04	Rio Guilarte	26Jan-23Feb	21Jun-19Jul	9Nov-7Dec
D04	Rio Cupeyes	24Jan-21Feb	21Jun-19Jul	10Nov-8Dec
D05	Pickerel Creek*	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D06	Kings Creek	23Mar-20Apr	3Jul-31Jul	3Oct-31Oct
D07	Leconte Creek	15Mar-12Apr	30Jun-28Jul	12Oct-9Nov
D07	Walker Branch	9Mar-6Apr	1Jul-29Jul	19Oct-16Nov
D08	Mayfield Creek	5Mar-2Apr	29Jun-27Jul	31Oct-28Nov
D10	Arikaree River	21Mar-18Apr	4Jul-1Aug	20Sep-18Oct
D11	Pringle Creek	17Feb-17Mar	29Jun-27Jul	23Oct-20Nov
D12	Bozeman Creek	11Apr-9May	7Jul-4Aug	6Sep-4Oct
D12	Blacktail Deer Creek	1May-29May	13Jul-10Aug	30Aug-27Sep
D13	Como Creek	20May-17Jun	14Jul-11Aug	30Aug-27Sep
D13	West St. Louis Creek	2May-30May	5Jul-2Aug	3Sep-1Oct
D14	Sycamore Creek	17Feb-17Mar	29Jun-27Jul	21Oct-18Nov
D15	Red Butte Creek	29Mar-26Apr	6Jul-3Aug	29Sep-27Oct
D16	McRae Creek	10Apr-8May	11Jul-8Aug	23Sep-21Oct
D16	Planting Creek	6Apr-4May	5Jul-2Aug	22Sep-20Oct
D17	Convict Creek*	31Mar-29Apr	8Jul-5Aug	15Sep-13Oct
D17	Providence Creek*	19Mar-16Apr	1Jul-29Jul	25Sep-23Oct
D18	Oksrukuyik Creek	21May-18Jun	29Jun-27Jul	7Aug-4Sep
D19	Caribou Creek	2May-30May	26Jun-24Jul	18Aug-15Sep

*soft sites as of November 2014

APPENDIX E SITE-SPECIFIC INFORMATION: HABITAT AND SAMPLER RECOMMENDATIONS FOR WADEABLE STREAMS

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

Domain	Site	Habitat 1	Habitat 2
D01	West Branch Bigelow Brook	Pools (corer)	Pools (snag)
D01	Sawmill Brook	Riffles (Surber)	Pools (corer)
D02	Mill Run	to be determined	to be determined
D02	Posey Creek	Riffles (Surber)	Pools (Hess)
D04	Rio Guilarte	Riffles (Surber)	Pools (corer)
D04	Rio Cupeyes	Riffles (Surber)	Pools (corer)
D05	Pickerel Creek	Riffles (Surber)	Pools (corer)
D06	Kings Creek	*	*
D07	Leconte Creek	*	*
D07	Walker Branch	*	*
D08	Mayfield Creek	Runs/riffles (snags)	Runs (core)
D10	Arikaree River	Runs (modified kicknet)	Pools/Runs (core)
D11	Pringle Creek	*	*
D12	Bozeman Creek	Riffles (Surber)	Riffles/Runs (snags)
D12	Blacktail Deer Creek	Riffles (Surber)	Riffles/Runs (snags)
D13	Como Creek	Riffles (Surber)	*
D13	West St. Louis Creek	Riffles (Surber)	Pools (core)
D14	Sycamore Creek	Riffles (Surber)	Runs/Pools (core)
D15	Red Butte Creek	Riffles (Surber)	*
D16	McRae Creek	Riffles (Surber)	Step pools (snags)
D16	Planting Creek	Riffles (Surber)	Pools (snags)
D17	Convict Creek	Riffles (Surber)	*
D17	Providence Creek	*	*
D18	Oksrukuyik Creek	Riffles (Surber)	Pools (petite ponar/core)
D19	Caribou Creek	Riffles (Surber)	*