AOS PROTOCOL AND PROCEDURE: MACROINVERTEBRATE SAMPLING IN LAKES AND NON-WADEABLE STREAMS

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See configuration management system for approval history.

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

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	02/10/2014	ECO-01133	Initial release
B	08/29/2014	ECO-02210	Minor updates based on feedback from the field
C	11/13/2014	ECO-02433	Migration to new protocol template
D	05/27/2015	ECO-02665	Minor updates including changes to the equipment list (replace Whirl-paks with jars), addition of modified kicknet in non-wadeables, updates to sample shipping and labeling, and the addition of sampling dates to appendix.

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1 OVERVIEW

1.1 Background

Aquatic invertebrates are a diverse and ubiquitous group of organisms (Hauer and Resh 2006). Although streams and non-wadeable streams harbor the majority of aquatic invertebrate diversity, 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, present in all but the most polluted waters, and are responsive to changes in water quality. In lakes, the majority of the macroinvertebrate community lives in the lake sediments and in submerged and emergent vegetation, rather than in the water column (Figure 1).

Figure 1. Benthic midges (Diptera: Chironomidae: *Chironomus*) often form tubes on the lake bottom, where they feed on organic matter and detritus as collector/gatherers

Aquatic invertebrates are an important part of aquatic biological communities. Most benthic invertebrates are primary consumers and feed on autotrophic producers (algae, plants) and heterotrophic microbial decomposers (leaf litter, biofilms). Other benthic invertebrates are predators. Benthic invertebrates are typically important vectors of energy transfer from one trophic level to the next in lake and non-wadeable stream communities (e.g., sunlight+nutrients → primary producers → consumers → predators). Different taxa perform various ecological functions in the lake community, including scraping algae off the substrate, shredding leaf litter, filtering particles from the water column, and gathering small pieces of organic matter.

Lake benthic invertebrate communities are strongly affected by abiotic factors such as shifts in nutrient concentrations, pollutants, temperature, and pH, as well as environmental factors such as scouring, freezing, drought, and biological factors such as vegetation structure and predation (Allan 1995, Wetzel 2001). Such sensitivity to environmental conditions makes benthic invertebrates ideal for use in

monitoring programs. Members of the Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) are generally considered the most sensitive taxa and are indicators of good to excellent water quality. However other ubiquitous taxa, such as members of the family Chironomidae (midges, non-biting flies) and oligochaetes, are typically highly tolerant to poor environmental conditions and their dominance in an invertebrate community usually indicates degraded water quality. Large national monitoring programs such as EPA-Rapid Bioassessment Protocol (RBP; Barbour et al. 1999) and USGS-National Water Quality Assessment (NAWQA; Moulton et al. 2002) have used benthic macroinvertebrates to quantify water quality and ecosystem health throughout a broad range of aquatic ecosystems. Collecting data on the benthic communities of lakes and non-wadeable streams over the time span of the NEON Observatory will be crucial components of aquatic ecosystem assessment to 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 lakes and non-wadeable streams (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	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.002193	Datasheets for AOS Protocol and Procedure: Macroinvertebrate Sampling in Lakes and Non-wadeable Streams
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.001154	NEON Aquatic Decontamination Protocol
RD[09]	NEON.DOC.001164	NEON Bathymetric Mapping Protocol
RD[10]	NEON.DOC.001162	NEON Lake Riparian Mapping Protocol
RD[11]	NEON.DOC.014050	TOS Protocol and Procedure: Ground Beetle Sampling
RD[12]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

2.3 Acronyms

Acronym	Definition
EMAP	Environmental Monitoring and Assessment Program
ft	foot
FFG	functional feeding group
GPS	Global Positioning System
hr	hour
km	kilometer
m	meter
µm	micrometer
mL	milliliter
mm	millimeter
NEON	National Ecological Observatory Network
NLA	National Lakes Assessment
PFD	Personal flotation device
qt	quart
USEPA	US Environmental Protection Agency

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

Collector-gatherer: Invertebrates that 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 (Figure 14).

Filter feeder: Invertebrates that feed by straining suspended particles from the water column (Figure 3).

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 how each taxon obtains food (includes scrapers, filter-feeders, collector-gatherers, predators, and shredders).

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

Invertebrate: Animal without a backbone. Lake/non-wadeable stream invertebrates include mussels, snails, crayfish, insects, and zooplankton.

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

Macroinvertebrate: Benthic invertebrates that cannot pass through a 500 μm sieve.

Mollusks: A large phylum (Mollusca) of invertebrates that often have a soft body surrounded by a shell. This group includes snails and mussels.

Mussels: Benthic mollusks often found in the sediments aquatic and marine environments, with a shell composed of two halves (Figure 4). Mussels are filter feeders.

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

Petite Ponar: Benthic sampler used for soft sediments in deep and slow-moving water. The Petite Ponar consists of 2 steel halves that close when the sampler reaches the sediment, "grabbing" a 6"x6" area of sediment (Figure 12).

Predator: (Trophic level) animals that eat other animals. In lake communities, predators include insects, fish, and birds (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 inorganic molecules to create energy.

Scraper: (Functional feeding group) Invertebrates that 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 that 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

Snags: Submerged woody debris in the channel or lake 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 non-wadeable and wadeable streams

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

3 METHOD

The goals of the Aquatic Macroinvertebrate Sampling Protocol in lakes and non-wadeable streams are to quantify biodiversity, number of species present, biomass, and to develop a DNA barcode library for benthic invertebrates at each lake and non-wadeable stream site. These variables, especially biodiversity and biomass, will be tracked over time and data will be collected to enable analyses of changes in species loss, changes in community structure and function, as well as the introduction and the spread of taxa (presence/absence).

Samples are collected following EPA protocols (Barbour et al. 1999 and Angradi et al. 2006), Minnesota EPA National Lake Assessment (NLA) protocol (USEPA 2009), Standard Operative Protocols for the Great Lakes (USEPA 2003), and Protocols for Sampling Macroinvertebrates in Freshwater Wetlands (DiFranco 2006). Shoreline samples are included because invertebrate diversity is often high in the vegetation at these lake- and non-wadeable stream-margin habitats. Samples are collected by field personnel then preserved and sent to taxonomists for identification.

Macroinvertebrate sampling occurs three times per year. Timing of sampling is site-specific and determined based on historical hydrological and meteorological data. Specific details on sample dates are provided in the NEON Aquatic Sample Strategy Document (RD[07]) and Appendix D. Sample bout 1 is an early-season date, representing a period of rapid biomass accumulation after winter, typically after ice-off (where applicable) and prior to leaf out. Sample bout 2 targets mid-summer conditions. Sample bout 3 represents the late growing season (typically autumn) during leaf-fall. These dates will differ on a site-by-site basis, but should always occur at or near baseflow conditions in the watershed.

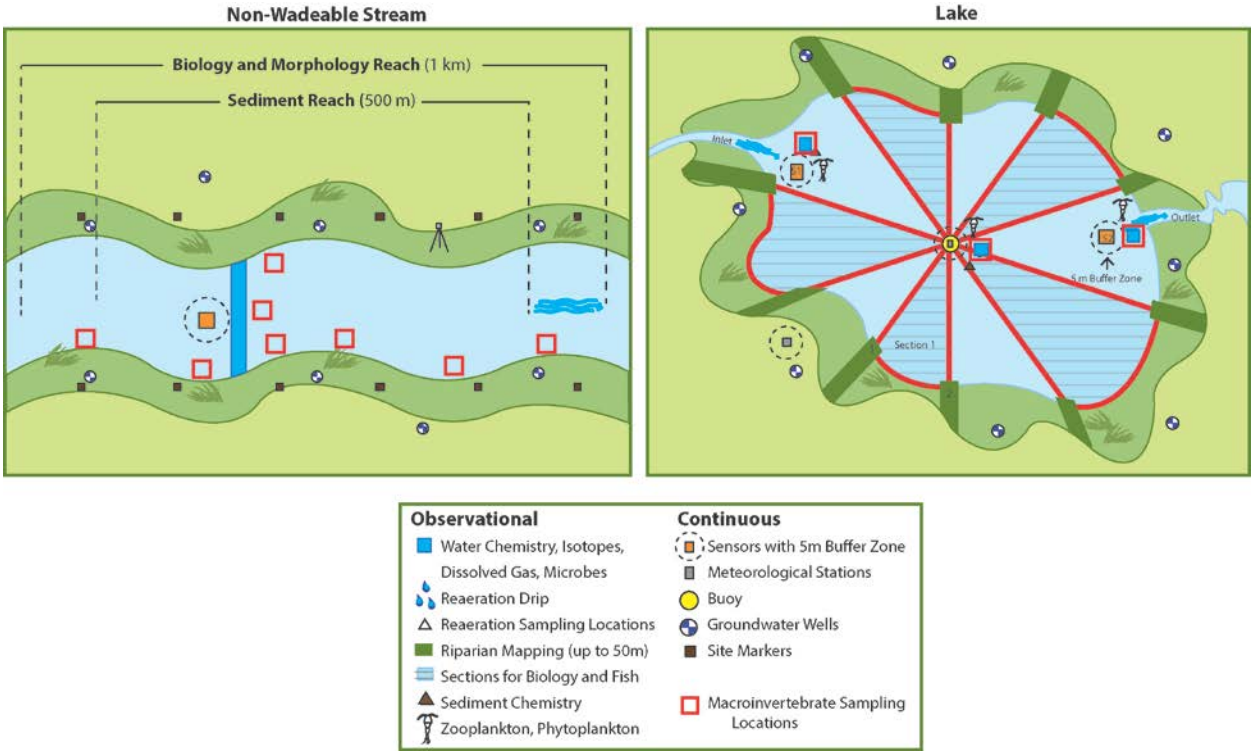


Figure 9. Generic site layouts for lakes and non-wadeable streams with macroinvertebrate sampling locations

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

Lake and non-wadeable macroinvertebrate sampling 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 ice on/ice off (for lake sites), river flow (for non-wadeable stream sites), 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 samples per lake/non-wadeable 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 weather requirements below, continue to collect samples.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If weather conditions deteriorate and the lake/non-wadeable stream becomes too windy (>9 km hr^{-1}) to hold the boat stationary over a sampling point, return to shore and wait in a safe location for 30 minutes. If wind subsides, resume sampling, if not, return to the Domain Support Facility and sample at another time.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If you are able to return to the lake/non-wadeable stream to sample within 24 hours, you may keep samples from the previous day. If you are not able to return within 24 hours, discard any previously collected samples in the lake/non-wadeable stream or at the Domain Support Facility and start over.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
5 or More Days	Samples shall be taken a minimum of 5 days following a major flow even (>25% change in flow within 15 minutes and/or turbidity levels are double the monthly average), allowing for recolonization before sampling occurs.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.

4.5 Sampling Specific Concerns

- 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.
- 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.

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

See Section 10 in the NEON Operations Field Safety and Security Plan (AD 01]) for aquatic-specific field safety requirements. In addition, the following safety requirement must be followed:

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

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	Conditions Used	Quantity	Special Handling
Durable Items						
RD[09]	R	Site-specific Bathymetry Map	Determining sampling locations	All	1	N
RD[10]	R	Site-specific Riparian Map	Determining sampling locations	All	1	N
RD[06]	R	General AQU Field Metadata Sheet	Recording metadata	All	1	N
	S	Clipboard	Recording data	All	1	N
	S	Cooler, 9-28 qt	Field sample storage; use size appropriate to samples being collected	All	1	N
	R	Knee boots or waders (pair)	Boating or wading	All	1 pair per person	N
	R	Handheld GPS unit (with batteries, ± 1 m	Navigating to sampling locations	All	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		accuracy) or Hummingbird				
MX100453	R	Depth finder (see Bathymetry Protocol, RD[09])	Determining depth at sampling location	All	1	N
Consumable Items						
	R	Field data sheets (all-weather copier paper, write in pencil)	Recording data	All	5 Sheets	N
	R	Pre-printed paper labels (all-weather copier paper, write in pencil)	Labeling samples	All	1 Sheet	N
	R	Pre-printed 1x2” adhesive labels	Labeling samples, outside sample jar	All	1 sheet	N
	R	Pencils	Recording data	All	4	N

R/S=Required/Suggested

Table 3. Equipment list – Macroinvertebrate sampling

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX100303	R	Petite ponar sampler with rope	Sample collection	Benthic sediments	1	N
MX102972	R	D-frame net with handle (243 µm mesh, 10x1210”x12” mouth)	Sample collection	Littoral aquatic plant beds	1	N
	R	Meter stick or meter tape	Used with D-frame and snag sampler	Littoral aquatic plant beds, Snags	1	N
MX108199	R	Modified tapered kick net with handle (243 µm Nitex mesh)	Sample collection	Littoral snags	1	N
	R	Bow saw, 21 in	Used with snag sampler	Littoral snags	1	N
	R	Metric ruler	Used with snag sampler	Littoral snags	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Kitchen brush w/ nylon bristles	Used with snag sampler for wood scrubs	Littoral snags	2	N
	S	Messenger	Used with petite ponar if jaws do not close	Benthic sediments	1	N
	S	Work gloves (pair)	Used with petite ponar for safe handling of the rope	Benthic sediments	1	N
	S	Long-handled brush w/ nylon bristles	Used with modified kicknet sampler for non-wadeable stream benthic samples	Bedrock-substrata, non-wadeable streams	1	N
Consumable Items						
(none)						

R/S=Required/Suggested

Table 4. Equipment list – Macroinvertebrate field elutriation and preservation

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX100308	R	Polyethylene wash bottle, unitary (500 mL; Figure 13)	Elutriation, rinsing	All	2	N
MX109110	R	Sieve bucket, 242 μm	Sample sieving; elutriation	All	1	N
	R	Plastic bucket, 5 gallon	Sample container; elutriation	All	1	N
	R	1 L HDPE bottles with lid (clear or amber)	Ethanol container	All	4	N
MX100306	S	Sieve, 250 μm	Sample sieving; elutriation	Littoral aquatic plant beds	1	N
	S	Plastic bucket, 3 gallon	Substrate scrubbing; elutriation	All	1	N
	S	Flexible forceps, featherweight	Collecting clinging insects	All	1	N
Consumable Items						
	R	Sample jars, HDPE with lid, 16 oz.	Sample container	Small samples (e.g., sweeps)	8	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Sample jars, HDPE with lid, 32 oz.	Sample container	Large samples (e.g., ponars)	3	N
	R	Ethanol, 95% non-denatured	Preservative	All	4 L	Y
	S	Disposable pipets (polyethylene), 1 mL	Collecting clinging insects	All	2	N
	S	Latex or Nitrile gloves (pair)	Preventing preservative contact with skin	All	5	N
	S	Resealable plastic zip-top bags (gallon)	To organize samples	All	5	N

R/S=Required/Suggested

Table 5. Equipment list – General boating equipment

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
	R	Boat		All	1	Y
	R	Anchor with rope		All	1	N
	R	Oars		All	1	N
	R	Trolling Electric Motor		All	1	Y
	R	Battery (12 volt)		All	1	Y
	R	Safety kit for boat (e.g., flares, bailer, float with rope)		All	1	Y
	R	First Aid Kit		All	1	N
	R	Personal Flotation Devices (PFDs)		All	1 per person	N

R/S=Required/Suggested

Table 6. Equipment list – Laboratory processing: preservative replenishment

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
	R	1 L HDPE bottles with lid (clear or amber)	Preservative container	All	4	N
	R	Unitary wash bottle, 500 mL, ETOH + glycerol	Adding preservative to sample jars and rinsing filter cup	All	1	Y
	R	Filter cup (TOS)	Changing preservative	All	1	N
	R	Safety glasses	Preventing preservative contact with eyes	All	1 pair	N
MX100306	S	Sieve, 250 µm	Changing preservative	All	1	N
	S	Featherweight forceps	Picking up insects	All	1	N
Consumable Items						
	R	Ethanol, 95%, undenatured	Preservative	All	5 L	Y
	R	Glycerol, >99%	Preservative	All	1 L	Y
	R	250 µm mesh squares for filter cup	Catching insects over the filter cup	All	8	N
	R	Nitrile gloves	Preventing preservative contact with skin	All	1 pair	N

R/S=Required/Suggested

Table 7. Equipment list – Shipping supplies

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
Consumable Items						
	R	Cardboard box, UN-rated, Group II	Shipping samples to taxonomist	1	N	N
	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	All	TBD	N
	R	Heavy duty plastic trash bag	Lining the shipping container	1		N
	R	Appropriate labels and forms for limited quantity ground shipments (see AD[03])	Shipping paperwork and external shipping labels	TBD	N	N
	R	Shipping inventory (RD[12])	Provides sample information to external lab	All	1	N

R/S=Required/Suggested

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]).

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

Personnel will be trained in the field protocols associated with this document, and trained in safe working practices for lake- and river-based field work.

6.3 Specialized Skills

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

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

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Sampling Equipment Preparation

- Collect and prepare all equipment, including sample jars and labels.
- Load GPS sampling coordinates on handheld GPS unit.

Table 8. Sampling equipment preparation checklist

✓	Item	Action
	Nets and sieves	Check for holes. Repair if necessary. Assure that both are clean and free of debris and organic matter.
	Paper labels	Print on all-weather paper (RD[05], Figure 10).
	Adhesive labels	Print on small adhesive labels (RD[05])
	Ethanol	Fill 1 L HDPE bottles 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

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

Figure 10. Example of field labels for lake/non-wadeable stream invertebrate sampling

SOP B Determining Sampling Locations and Sampler Type

B.1 Sampling Locations

1. Benthic petite ponars will be taken near aquatic chemistry sampling locations in lakes and downstream of the aquatic sensor set in non-wadeable streams (Section C.1).
   a. If sampling a non-wadeable stream with bedrock substrata, use the modified kicknet method in Section C.6 rather than the ponar sampler.
5. Littoral samples will be collected based on the dominant substratum in the Riparian Habitat Sections (RD[10]) and the lake/non-wadeable stream bathymetry maps (RD[09]).
   a. The habitat type chosen should be present during all sampling bouts.
   b. All 5 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 substrata.
   c. Choose the appropriate sampler based on the field conditions and habitat being sampled (see Decision tree, Section B.2).
   d. The order of preference for sampling substrata is as follows:
      1) Aquatic plant beds or floating mats (D-frame net, Section C.2)
      2) Snags/Large woody debris (Snag sampler, Section C.4)
6. Do not sample anywhere you or other field technicians have recently disturbed (via sampling, walking, driving a boat, etc.) or locations that appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).
7. Proceed to the protocol for the appropriate sampler type below.

B.2 Decision Tree

1. Determine percent cover of substratum types in the lake or non-wadeable stream littoral areas using the Lake/Non-wadeable stream Morphology Map (RD[09]). Habitat suggestions for NEON sites are provided in Appendix E.
   a. Targeted habitat types (see Definitions, Section 2.4):
      1) Benthic sediments (ponar or modified kicknet)
      2) Aquatic plant beds (D-frame)
      3) Large woody debris/snags (snag net/modified kicknet)
2. Pelagic sampling
   a. Take 3 samples in benthic sediments at water chemistry sampling locations (in non-wadeable streams, there is only 1 water chemistry location, so two additional sampling locations will be established in the procedures below) and proceed to Step 2b.
   b. Follow petite Ponar sampling procedure (Section C.1).
3. Littoral sampling
   a. Does the substratum type account for >20% of the available habitat throughout the year?
      1) If yes, got to Step 3b.
      2) If no, ignore this substratum type and proceed to Step3c.

b. Does the lake or non-wadeable stream bottom have submerged or emergent aquatic plant beds? (Must be present in a minimum of 5 riparian sections.)
   1) If yes, follow D-net Sweep Samples sample procedure (Section C.2).
   2) If no, proceed to Step 3c.
c. Does the lake or non-wadeable stream have large woody debris or snags? (Must be present at a minimum of 5 locations with a diameter or > 1 cm, and be colonized by invertebrates.)
d. If YES, follow Snag Samples procedure (Section C.4).
e. If NO, seek advice from NEON Aquatic Ecologist.

SOP C Field Sampling

C.1 Petite Ponar Sampling

1. Sampling in three locations per lake or non-wadeable stream:
   f. Lakes (near water chemistry sampling locations):
      1) Deepest point in the lake, determined by bathymetric site map and GPS coordinates (location = “c0”)
      2) Near the lake inlet (location = “in”)
      3) Near the lake outlet (location = “ot”)
   g. Non-wadeable streams 50-100 m below aquatic sensor set):
      1) Thalweg
      2) Half the distance between the thalweg and the right bank
      3) Half the distance between the thalweg and the left bank
4. Navigate boat to the sampling location.
5. Gently lower an anchor so as not to suspend the sediments.
   a. Allow ~5 minutes for sediments to settle after lowering the anchor, you can use this time to prepare the sampling equipment.
   b. If you are sampling at a buoyed station with a sufficient anchor to hold the boat, you may tie up to the buoy to avoid further disturbing the sediments.
6. Record GPS coordinate (datum WGS84) and depth (m) of sampling location (using depth finder) on the field data sheet (Figure 11, RD[05]).

NEON Aquatic Benthic Invertebrate Collection

Lakes and Non-wadeable Streams

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

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

Local time (HH:MM): *13:00* **Sampling protocol & Rev.:** *NEON.DOC.001204vA*

Ponar locations

Location ID	Latitude	Longitude	Sample type	Replicate
buoy	*29.676473°*	*-82.009089°*	ponar	1
inlet	*29.675629°*	*-82.008206°*	ponar	2
outlet	*29.676423°*	*-82.007893°*	ponar	3

Location, Section ID	Habitat	Sample type	Replicate	Sample ID	Habitat percent	Substratum size class	Ponar depth (m)	Snag length (cm)	Snag diameter (cm)
buoy	*pelagic*	*ponar*	*1*	*BARC.20140702.ponar.1*	*NA*	*NA*	*2.10*	*NA*	*NA*
inlet	*pelagic*	*ponar*	*2*	*BARC.20140702.ponar.2*	*NA*	*NA*	*1.40*	*NA*	*NA*
outlet	*pelagic*	*ponar*	*3*	*BARC.20140702.ponar.3*	*NA*	*NA*	*1.20*	*NA*	*NA*
Section1	*littoral*	*sweep*	*1*	*BARC.20140702.swep.1*	*45%*	*sand*	*NA*	*NA*	*NA*
Section3	*littoral*	*sweep*	*2*	*BARC.20140702.swep.2*	*45%*	*sand*	*NA*	*NA*	*NA*
Section5	*littoral*	*sweep*	*3*	*BARC.20140702.swep.3*	*45%*	*sand*	*NA*	*NA*	*NA*
Section7	*littoral*	*sweep*	*4*	*BARC.20140702.swep.4*	*45%*	*sand*	*NA*	*NA*	*NA*
Section9	*littoral*	*sweep*	*5*	*BARC.20140702.swep.5*	*45%*	*sand*	*NA*	*NA*	*NA*

Figure 11. Example of field data sheet for lake/non-wadeable stream invertebrate sampling

7. Sample near the bow of the boat, away from the motor and anchor, so as not to interfere with the ponar or disturb the sediments.
8. Release the safety-pin on the ponar sampler and open the bottom of the ponar (Figure 12). Place the pinch-pin (with spring) in the ponar and hold the ponar by the rope at the top.
   a. **CAUTION**: keep hands away from the open jaws and maintain tension on the rope so as not to trip the trigger mechanism.
   b. The sampler will stay in the open position as long as there is tension on the rope. The ponar will close automatically when the tension is released, that is, when the sampler hits the sediments.

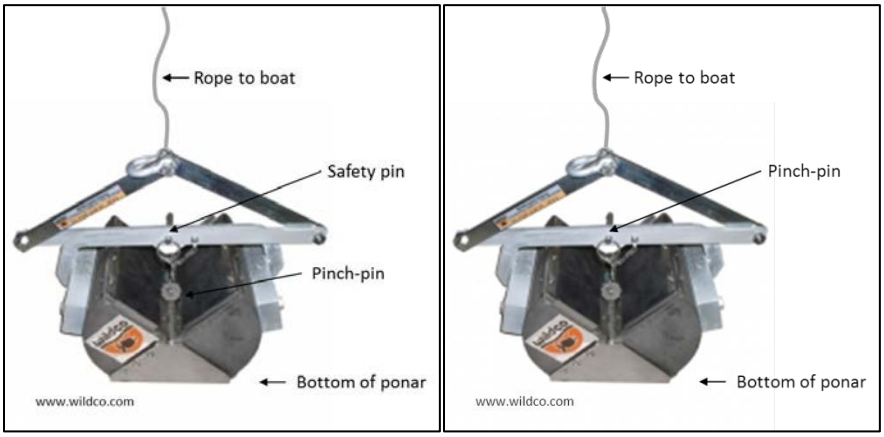


Figure 12. Example of petite ponar setup

9. Hold the open ponar by the rope above the water surface above the point in the lake/non-wadeable stream bottom where you want to sample.
10. Slowly lower the ponar in the water and maintain rope tension. Ensure that the free end of the rope is not snagged on anything in the boat.
11. When the ponar hits the sediments and the line goes slack, the pinch-pin is released causing the jaws to close.
    a. 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.
12. With the jaws closed, pull the ponar slowly up to the surface using the rope.
13. Check to see if the ponar properly closed (e.g., sticks or other debris are not jammed between the jaws). If it did not close or there is an object (such as a stick) holding the jaws open, discard the remaining sample into the water. Return to Step 1 and sample in a new, undisturbed location (at least 2 m away from original location).
    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.
14. If the ponar is properly closed, place the sampler inside the 3 gallon bucket and open the jaws to release the sample.
15. Rinse the inside of the ponar into the bucket using the 500 mL wash bottle filled with filtered (through the 250 μm sieve) lake/non-wadeable stream water (Figure 13).

Figure 13. 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

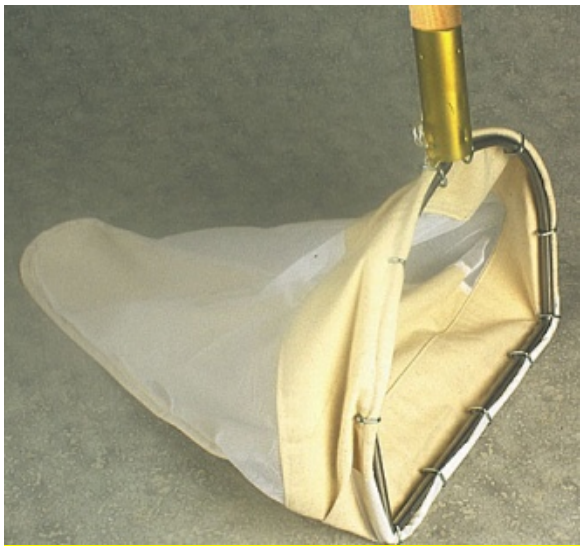
16. Once the ponar is empty and rinsed, close the jaws carefully and set aside. All of your sample material should now be in the 3 gallon bucket.
17. Carefully add lake/non-wadeable stream water to the bucket until bucket is about ½ full. You may use the boat bailer or carefully submerge a portion of the bucket at the lake/non-wadeable stream surface.
    a. Filter rinsewater through sieve first to remove >250 µm zooplankton.
18. Proceed to Section C.6 Sample Elutriation and Preservation.
19. After elutriation and preservation, rinse sampler with native water and move to next location. Repeat Steps 1-18 until you have 3 replicate samples.

C.2 D-net Sweep Samples (Semi-quantitative)

1. Sampling locations will correspond to the 10 habitat stations set forth in the Riparian Site Location Protocol (RD[10], RD[06]). This divides the lake/non-wadeable stream margins into 10 sections.
2. Choose either the 5-even or 5-odd habitat sections for sampling.
    a. Choose sections by looking at the time. If the hour is even, sample even sections. If the hour is odd, sample odd sections.
    b. The sample replicate on the sample labels should correspond to the Riparian Habitat Section numbers.
3. Navigate boat to an aquatic plant bed in the first sampling section. Ensure that you are able to reach the lake/non-wadeable stream bottom with the sampling net.
4. 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.
    a. If permits allow and the area of the lake or non-wadeable stream is shallow enough to wade, you may step out of the boat and sample by wading (permits will be provided by NEON EHS).

b. If permits do not allow getting out of the boat, sample carefully from the bow of the boat.

5. Attach handle to D-frame net (Figure 14).



6. **Figure 14.** D-frame net, used for sweep samples in vegetation and silty habitats. Sweep the D-frame net through the vegetation over a 1 m distance (DiFranco 2006, USEPA 2011).
   a. To determine the sweep length, measure 1 m on the net handle (typically 1 m = the wooden part of the handle). Using this as a reference, place feet 1 m apart on the lake/river bottom and sweep from foot to foot
   b. The net should remain submerged during the entire sweep, and the bottom of the net should brush the sediments during the entire sweep (Figure 15. **Field** technicians demonstrate the sweep technique in a littoral area. The D-frame net must remain submerged and close to the substrate throughout the entire sweep.).
   c. For rooted aquatic plants: bump bottom of net frame against the sediment 3 times (beginning, middle, end) to collect benthic organisms.
   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.
   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.

Figure 15. Field technicians demonstrate the 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 or any rooted plants that are trapped in the net, hold the net in the water with the mouth above the water line. Agitate plant material in water in the net for 1 minute. Rinse plant material with 500 mL wash bottle if necessary. Remove and discard plant material while keeping macroinvertebrate sample in the net (FDEP 2011).
8. Dip D-frame net into the water a few times to rinse the sides of the net, always directing flow towards the back of the net. This allows material to concentrate at the bottom of the net. **Be sure to keep the net opening above the water** surface so as not to accidentally lose or collect additional material in the sample.
9. Pull the net into the boat.
10. Fill the 3 gallon bucket ¼ – ½ full with lake/non-wadeable stream water.
    a. Filter rinsewater through sieve 0r 250 µm Nitex mesh first to remove >250 µm zooplankton.
11. Invert net (turn it inside-out) into bucket of rinsewater. Swirl net around in rinsewater to remove all material from the net. Use your hands to brush material from the net into the bucket.
12. Use 500 mL wash bottle filled with filtered lake/non-wadeable stream water to rinse any residual organic matter 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 gallon bucket
13. Fill out field sheet with location, date, and type of samples (Figure 13).

C.3 Proceed to Section C.6 Modified Kicknet Sampler (Non-wadeable streams)

The modified kicknet sampler is to be used in place of the ponar sampler at non-wadeable stream sites with bedrock substrata (Appendix E).

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

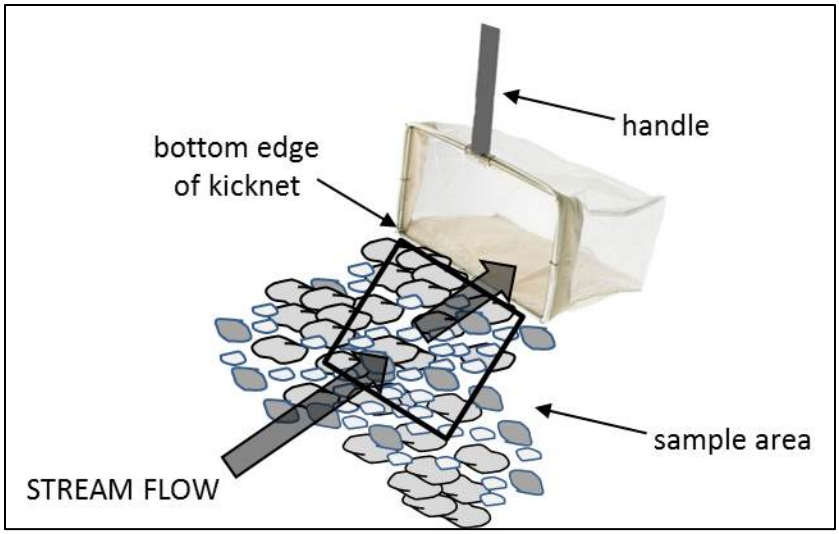


Figure 16. Example of modified kicknet setup.

3. Approach sample site 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 16).
6. Disturb the substrata ~0.25 m^2 upstream of the kicknet.
   a. If water is shallow enough for wading, substrata may be disturbed by wading, 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.
   b. If water is too deep for wading, disturb substrata using a long-handled brush so all organic matter flows into the net.
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. Half-fill 3 or 5 gallon bucket with stream water.

10. 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.
11. Use 500 mL wash bottle to rinse any residual organic matter or insects from the net.
    a. Watch for clinging insects.
12. Once the net is empty, all of the sample material will be suspended in water in the 3 gallon bucket. Proceed to Section C.7 Sample Elutriation and Preservation.
13. After elutriation and preservation, clean net in stream water and move to next location and repeat Steps 35-44 until you have collected 3 samples.
14. Sample Elutriation and Preservation.
15. After elutriation and preservation, rinse net in native water and move to the next location, repeating Steps 1-13 until you have 5 replicate samples.

C.4 Snag samples (Qualitative)

1. Ensure that snags account for >20% of habitat throughout the sampling area (this habitat type is most likely to occur in non-wadeable streams).
2. Choose either the 5-even or 5-odd riparian sections for sampling.
   a. Choose sections by looking at the time: if the hour is even, sample even sections. If the hour is odd, sample odd sections.
   b. If there is no snag in the section chosen, proceed to the next section. You may sample 2 snags from the same section if necessary, as long as they are >5 m apart.
3. The sample replicate on the sample labels should correspond to the Riparian Habitat Section numbers.
4. Navigate boat to first snag, approaching slowly from downstream (if non-wadeable site).
5. Ensure that the snag is submerged to at least 0.6 m (2 ft) deep, in flowing water (non-wadeable stream only), and has a diameter of ≥0.15 m (6 inches). The snag should either break the water surface or come within 0.3 m (1 ft) or the water surface for ease of sampling (Angradi et al. 2006).
6. Carefully drop and anchor or have the boat driver should hold the boat in place in the current (non-wadeable streams) using the boat motor.
7. Place the modified kick-net (13” x 20” opening) against the snag with the opening facing up-current, just below where the snag breaks the water surface.
8. Using the nylon kitchen brush, scrub ~1 m of snag so that organisms and debris wash into the sampling net.
   a. Scrub the top and sides of the snag.
   b. As this is a qualitative method, not all organisms will be washed into the net.
9. Measure length and width of snag sampled and record on Field Data Sheet (RD[05]).
10. 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.
11. 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.
12. Pull the net into the boat.
13. Fill the 3 gallon bucket ¼ – ½ full with lake/non-wadeable stream water.
    a. Filter rinsewater through sieve first to remove >250 µm zooplankton.
14. Invert net (turn it inside-out) into bucket of rinsewater. Swirl net around in rinsewater to remove all material from the net. Use your hands to brush material from the net into the bucket.
15. Use 500 mL wash bottle filled with filtered lake/non-wadeable stream water to rinse any residual organic matter 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 gallon bucket.

16. Carefully add lake/non-wadeable stream water to the bucket until bucket is about ½ full. You may use the boat bailer or carefully submerge a portion of the bucket at the lake/non-wadeable stream surface.
17. Fill out field sheet with location, date, and type of samples (Figure 11).

C.5 Proceed to Section C.6, Modified Kicknet Sampler (Non-wadeable streams)

The modified kicknet sampler is to be used in place of the ponar sampler at non-wadeable stream sites with bedrock substrata (Appendix E).

18. Choose runs that are similar in flow and depth.
19. Make sure net is firmly attached to handle.

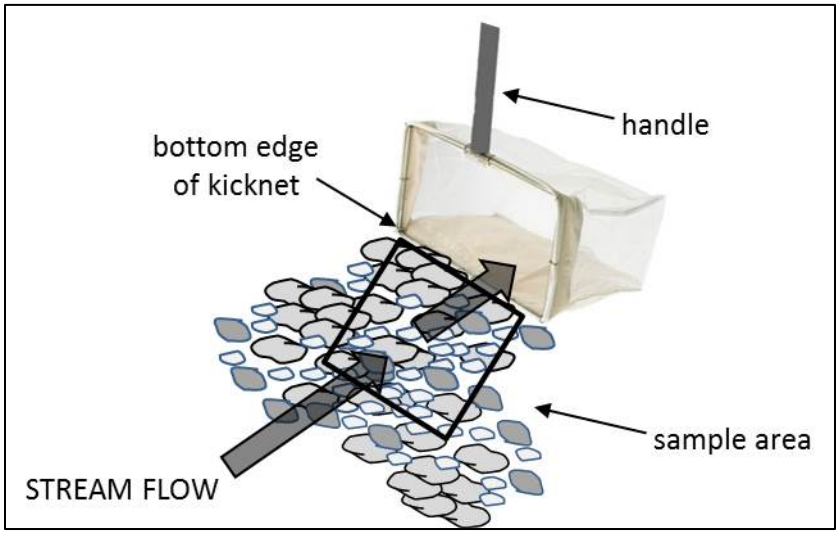


Figure 16. Example of modified kicknet setup.

20. Approach sample site from downstream so as not to disturb the location where you want to sample.
21. Orient the net so the opening of the net is facing into the stream flow.
22. 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 16).
23. Disturb the substrata ~0.25 m^2 upstream of the kicknet.
    a. If water is shallow enough for wading, substrata may be disturbed by wading, 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.
    b. If water is too deep for wading, disturb substrata using a long-handled brush so all organic matter flows into the net.

24. Sweep the kicknet toward the water surface, taking care to keep the opening facing upstream so no contents will spill.
25. 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.
26. Half-fill 3 or 5 gallon bucket with stream water.
27. 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.
28. Use 500 mL wash bottle to rinse any residual organic matter or insects from the net.
    a. Watch for clinging insects.
29. Once the net is empty, all of the sample material will be suspended in water in the 3 gallon bucket. Proceed to Section C.7 Sample Elutriation and Preservation.
30. After elutriation and preservation, clean net in stream water and move to next location and repeat Steps 35-44 until you have collected 3 samples.
31. Sample Elutriation and Preservation.
32. After elutriation and preservation, rinse net in native water and move to the next location, repeating Steps 1-17 until you have 5 replicate samples.

C.6 Modified Kicknet Sampler (Non-wadeable streams)

The modified kicknet sampler is to be used in place of the ponar sampler at non-wadeable stream sites with bedrock substrata (Appendix E).

33. Choose runs that are similar in flow and depth.
34. Make sure net is firmly attached to handle.

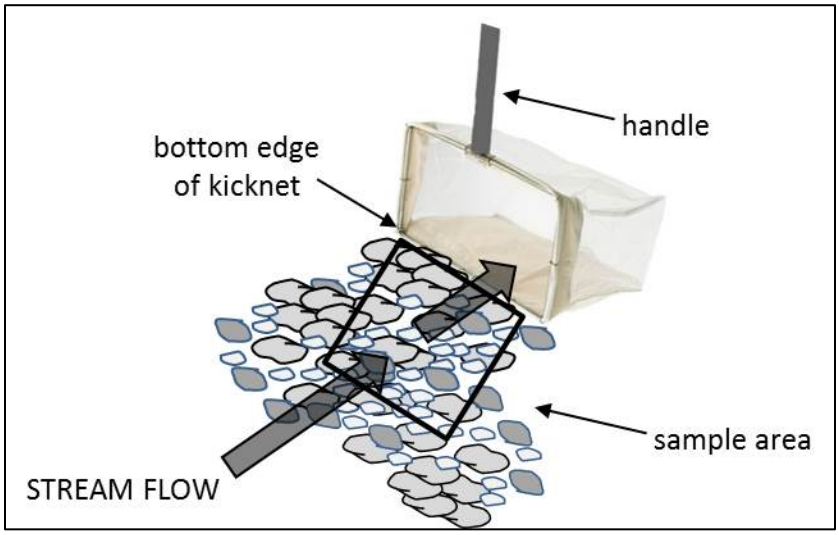


Figure 16. Example of modified kicknet setup.

35. Approach sample site from downstream so as not to disturb the location where you want to sample.
36. Orient the net so the opening of the net is facing into the stream flow.
37. 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 16).
38. Disturb the substrata ~0.25 m^2 upstream of the kicknet.
    a. If water is shallow enough for wading, substrata may be disturbed by wading, 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.
    b. If water is too deep for wading, disturb substrata using a long-handled brush so all organic matter flows into the net.
39. Sweep the kicknet toward the water surface, taking care to keep the opening facing upstream so no contents will spill.
40. 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.
41. Half-fill 3 or 5 gallon bucket with stream water.
42. 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.
43. Use 500 mL wash bottle to rinse any residual organic matter or insects from the net.
    a. Watch for clinging insects.
44. Once the net is empty, all of the sample material will be suspended in water in the 3 gallon bucket. Proceed to Section C.7 Sample Elutriation and Preservation.
45. After elutriation and preservation, clean net in stream water and move to next location and repeat Steps 35-44 until you have collected 3 samples.

C.7 Sample Elutriation and Preservation

1. Gently swirl contents in bucket to create a “whirlpool” and suspend organic material. This process (Steps 1-46) is called elutriation (Figure 17-1).

Figure 17. Process of elutriation (Dates and Byrne 1997)

2. Hold sieve or sieve bucket over large 5 gallon bucket (waste bucket; Figure 18).
3. Carefully pour suspended contents of the 3 gallon bucket through a 250 µm sieve or 242 um sieve bucket (Figure 17-2) with waste water going into the 5 gallon waste bucket. Some sediment will be retained in the 3 gallon sample bucket (Figure 18).
   a. The sieve bucket should be used for ponar samples for thick, fine sediments.
   b. You may use your fingers or shake the sieve or sieve bucket to stir the sample and move fine material through the sieve.
   c. You may use low-pressure water to wash sediment through sieve mesh. High pressure will damage organisms.
   d. Sieving the sample in small portions may help prevent clogging.
   e. Continue rinsing to reduce the sediment in the sample. Reduce samples to ≤750 mL if possible.

Figure 18. A field technician carefully pours water from the 3 gallon bucket into the sieve, with the waste water going into the 5-gallon bucket

4. Partially refill bucket with sieve-filtered water without losing any of the organic matter contents of the bucket.
46. Repeat Steps 1-4 until the water poured out of the 3 gallon bucket appears relatively clear. There may be sand or silt in the bottom of the 3 gallon 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 separate organisms and organic matter from the inorganic matter.
5. Check sides of the 3 gallon bucket for clinging invertebrates. Check sand/inorganic material at the bottom of the bucket for caddisflies (rock cases, Figure 2) or other heavy invertebrates (e.g., mussels and snails, Figure 4). Retain any plant material in the sample.
    a. Place any invertebrates from this inspection in the sieve with the rest of the sample.
6. Discard inorganic material remaining in the 3 gallon bucket, and rinse bucket with lake or non-wadeable stream water. Set bucket aside. Discard waste water from 5 gallon bucket into the lake/non-wadeable stream.
7. The sample should now be in the sieve. Complete a sample label using pencil (Figure 10, RD[05]) and place the inside a sample jar.
    a. Sample ID format: SITE.DATE.sample type.replicate.
        1) Example: BARC.20140702.ponar.1
8. Fill the 500 mL wash bottle with sieve-filtered lake/non-wadeable 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 or sieve bucket (Figure 19).

Figure 19. A field technician rinses the sample from the sieve into the open sample 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 use your fingers to move the sample from the sieve into the sample jar (Figure 19).
    a. Use as little water as possible. Be sure to rinse sieve and fingers into the sample jar thoroughly to 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 bag, 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 sample jars as they do not seal properly.

12. Check sieve for organisms that may not have been washed into the sample jar. Any organisms found should be placed in 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 waterproof paper label (write in pencil) inside the sample jar **AND** a small adhesive label to the outside of the sample jar with the sample ID for use by the external taxonomy lab. Write in pencil as ethanol will erase permanent marker.

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

47. 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.
48. 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 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. Re-seal sample jar bag.
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_lakDomainFieldData_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. 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 7 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.

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 10. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002193	Datasheets for AOS Protocol and Procedure: Macroinvertebrate Sampling in Lakes and Non-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: *BARC.20140702.ponar.1* Sample type: surber (ponar) snag core kicknet hess sweep Collected by: *sparker*	**NEON** Sample ID: *BARC.20140702.sweep.1* Sample type: surber ponar snag core kicknet hess (sweep) Collected by: *sparker*
NEON Sample ID: *BARC.20140702.ponar.2* Sample type: surber (ponar) snag core kicknet hess sweep Collected by: *sparker*	**NEON** Sample ID: *BARC.20140702.sweep.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 Bathymetry (RD[09]) and Riparian Maps (RD[10]).

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

Step 6 – Collect samples: 3 in benthic sediments with the petite ponar or modified kicknet, 5 in the dominant littoral habitat type with the appropriate sampler.

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

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 Determining Sampling Locations

1. Benthic petite ponars or modified kicknet samples will be taken near aquatic chemistry sampling locations in lakes and downstream of the aquatic sensor set in non-wadeable streams (Section C.1).
2. Littoral samples will be collected based on the dominant substratum in the Riparian Habitat Sections (RD[10]) and the lake/non-wadeable stream bathymetry maps (RD[09]).
   a. Choose the appropriate sampler based on the field conditions and habitat being sampled (see Decision tree, Section B.2).
   b. The order of preference for sampling substrata is as follows:
      1) Aquatic plant beds or floating mats (D-frame net, Section C.2)
      2) Snags/Large woody debris (Snag sampler, Section C.4)
3. Do not sample anywhere you or other field technicians have recently disturbed (via sampling, walking, driving a boat, etc.) or locations that appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).

B.3 Decision Tree

1. Determine percent cover of substratum types in the lake or non-wadeable stream littoral areas using the Lake/Non-wadeable stream Morphology Map (RD[09]). Habitat suggestions for NEON sites are provided in Appendix E.
   a. Targeted habitat types (see Definitions, Section 2.4):
      1) Benthic sediments (ponar or modified kicknet)
      2) Aquatic plant beds (D-frame)
      3) Large woody debris/snags (snag net/modified kicknet)
2. Pelagic sampling
   a. Take 3 samples in benthic sediments at water chemistry sampling locations (in non-wadeable streams, there is only 1 water chemistry location, so two additional sampling locations will be established in the procedures below) and proceed to Step 2b.
   b. Follow petite Ponar sampling procedure (Section C.1).
3. Littoral sampling
   a. Does the substratum type account for >20% of the available habitat throughout the year?
      1) If yes, got to Step 3b.
      2) If no, ignore this substratum type and proceed to Step3c.
   b. Does the lake or non-wadeable stream bottom have submerged or emergent aquatic plant beds? (Must be present in a minimum of 5 riparian sections.)
      1) If yes, follow D-net Sweep Samples sample procedure (Section C.2).
      2) If no, proceed to Step 3c.
   c. Does the lake or non-wadeable stream have large woody debris or snags? (Must be present at a minimum of 5 locations with a diameter or > 1 cm, and be colonized by invertebrates.)
   d. If YES, follow Snag Samples procedure (Section C.4).
   e. 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 labels on waterproof paper.
- ☑ 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 based on the Bathymetry (RD[09]) and Riparian Maps (RD[10]).
- ☑ Choose the appropriate sampler.
- ☑ Take 3 samples from benthic sediments and 5 samples from the dominant riparian habitat.

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 lakes and non-wadeable streams . Also see the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

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

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

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

Domain	Site	Pelagic	Littoral
D03	Ichawaynochaway Creek	Modified kicknet	D-net sweep rooted
D03	Lake Barco	Ponar	D-net sweep rooted
D03	Lake Suggs	Ponar	D-net sweep floating
D05	Crampton Lake	Ponar	D-net sweep rooted
D06	McDowell Creek	Ponar	D-net sweep rooted
D08	Black Warrior River	Ponar	Snag
D08	Tombigbee River	Ponar	Snag
D09	Prairie Lake	Ponar	D-net sweep rooted
D09	Prairie Pothole	Ponar	D-net sweep rooted
D11	South Pond at Klemme	Ponar	D-net sweep rooted
D18	Toolik Lake	Ponar	D-net sweep