AOS PROTOCOL AND PROCEDURE: AQUATIC MICROBIAL SAMPLING

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	01/21/2016	ECO-03455	Initial release, amalgamation of NEON.DOC.001200 and NEON.DOC.001201 and updated lake sampling depth guidelines.
B	02/08/2017	ECO-04359	Update NEON template; Update sample ID template; Update field and lab sterilization SOP; Lengthen holding time on cell count samples
C	02/13/2018	ECO-05326	Add barcode labels, change formaldehyde concentration, update shipping hold times, add benthic large substrate sampler, metagenomics labeling for mid-summer samples, move datasheets to appendix, update D14 bout dates, cell count packaging

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1 OVERVIEW

1.1 Background

This document describes the required protocols for conducting field sampling of microbes in aquatic habitats. Microbes mediate nutrient cycling in all habitats. Linking activity and community composition to chemistry measures will enable a mechanistic understanding of ecosystem function. Temperature, nutrient and carbon availability, physical dispersal in water flow, and competition control microbial community composition and activity so concurrent sampling ensures comparison between drivers and effects. Microbes also form biofilms in the benthos which are important to the productivity of the system. Collecting basic measures of biomass, enzymatic activity, and DNA will enable researchers and managers to assess changes in this key ecosystem group of organisms.

Aquatic microbes are different from those in terrestrial systems mainly due to dispersal across habitats. Stream flow transports bacteria downstream as well as into and between lakes. Additionally, large storm events can increase the similarity of microbial communities between sampling sites such as the inlet and outlet of a lake. The potentially large impact of stream flow on microbial communities has been incorporated into the NEON Aquatic Sample Strategy (RD[07]). Within the benthos, there is heterogeneity in community composition, particularly where substrata and flow rates differ. It is therefore important to ensure that microbes are collected using sterile technique, so that *in situ* diversity is preserved and cross contamination is minimized.

Concurrent sampling with environmental drivers as well as using sterile sampling techniques will enable comparison within a habitat and also across regions to determine patterns in biogeography as well as relationships with temperature changes or other habitat characteristics.

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

Collection techniques have been standardized to be consistent with the NEON water chemistry protocol and stream algal protocol. Collection techniques are based closely on the protocols of the USGS National Water Quality Assessment (Moulton et al. 2002), the EPA Rapid Bioassessment Program (Stevenson and Bahls 1999), Antarctic LTER programs (Lisle and Priscu 2004), Arctic Streams LTER program (Slavik et al. 2004), Methods in Stream Ecology (Lowe and LaLiberte 2006), and the University of Maryland Center for Environmental Studies Horn Point Laboratory, with the addition of the protocols of LaRouche et al. 2012. Lydia Zeglin (Kansas State University) and Lee Stanish (NEON FSU) also contributed to the methods provided in the following SOPs.

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

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2, Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.003041	Datasheets for AOS Protocol and Procedure: Aquatic Microbial Sampling
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.004257	NEON Standard Operating Procedure (SOP): Decontamination of sensors, field equipment and field vehicles
RD[09]	NEON.DOC.003045	AOS Protocol and Procedure: Periphyton and Phytoplankton Sampling
RD[10]	NEON.DOC.002905	AOS Protocol and Procedure: Water Chemistry Sampling in Surface Waters and Groundwater
RD[11]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology

RD[12]	NEON.DOC.014048	TOS Protocol and Procedure: Soil Physical, Chemical, and Microbial Measurements
RD[13]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[14]	NEON.DOC.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD[15]	NEON.DOC.003039	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling
RD[16]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[17]	NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling
RD[18]	NEON.DOC.002792	AOS Protocol and Procedure: Secchi Disk and Depth Profile Sampling in Lakes and Non-wadeable Streams

2.3 Acronyms

Acronym	Definition
°C	Degrees Celsius
DI	De-ionized water
DNA	Deoxyribonucleic acid
ETOH	Ethanol
HDPE	High-density polyethylene
L	Liter
LTER	Long Term Ecological Research
m	Meter
mL	Milliliter
µm	Micrometer
mm	Millimeter
PFD	Personal flotation device
qPCR	Quantitative polymerase chain reaction
qt	Quart
s	Second
USEPA	US Environmental Protection Agency
USGS	US Geological Survey

2.4 Definitions

Bryophyte: Aquatic moss, liverworts, or hornworts lacking true vascular tissues.

Cobble: Medium-sized rocks in the bottom, geologically defined as 64 to 256 mm diameter. Cobbles are larger than pebbles (4-64 mm), and smaller than boulders (>256 mm).

Epilimnion: Top layer of water of a stratified lake, denoted by highest temperatures and least dense water. Typically occurs in the summer.

Epilithon: Periphyton colonizing rock substrata.

Epipelon: Periphyton colonizing silt substrata.

Epiphyton: Periphyton colonizing surface of aquatic plants.

Epipsammon: Periphyton colonizing sand substrata.

Epixylon: Periphyton colonizing woody substrata.

Euphotic zone (or "Photic zone"): The upper layer of lake water where sunlight penetrates and photosynthesis can occur. Specifically, the depth to which 1% of surface light penetrates.

Hypolimnion: The dense bottom layer of a stratified lake that sits below the thermocline. This layer is cooler than the surface water and has less circulation.

Hydrograph: A diagram depicting the change in discharge (m^3) over a given time (s).

Integrated: A sample that is composed of multiple samples in the water column.

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

Macrophyte: Aquatic plant with vascular tissues.

Metalimnion: The layer of water in a stratified lake that sits between the hypolimnion and the epilimnion. Often equated with the thermocline.

Pelagic: The part of the lake that is not near shore or close to the bottom.

Periphyton: Mixture of algae, cyanobacteria, microbes, and detritus that coats submerged surfaces in most bodies of water

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

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

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

S1 and S2: Locations of NEON aquatic sensors.

Sand: Small sediment particles, 0.25-4 mm diameter.

Secchi depth: Depth that visible light penetrates, usually approximately 10-15% of light transmission.

Seston: Organic and inorganic particles suspended in the water column.

Silt: Very small sediment particles, 3.9-62.5 µm diameter.

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

Stratified: Layers within the system (e.g., warm and cold water layers indicate thermal stratification in a lake).

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

Thermocline: A distinct layer in a body of water where the change in temperature is more rapid than increasing depth - usually a change of more than 1 ^{o}C per meter. The denser and cooler layer below the thermocline is the hypolimnion, warmer upper layer is termed the epilimnion.

3 METHOD

The goal of the Aquatic Microbial Sampling Protocol is to determine structure and function of benthic and surface water microbial communities, and to provide data that can be linked to other AOS datasets.

Surface water microbes

Surface water microbes are collected 12 times per year in **wadeable streams** and 6 times per year in **lakes and non-wadeable streams (rivers)**, at the same time and location as standard recurrent water chemistry samples (RD[10]). Sample timing is based on statistical analysis of environmental parameters. Details on sampling locations and timing are provided in the Surface Water Chemistry Sampling in Aquatic Habitats (RD [10]). Surface water samples are collected as 1) a preserved water sample for cell counts and 2) on filters for archive and analysis.

Benthic microbes

Benthic microbes are collected 3 times per year in **wadeable streams** at the same time and location as algal periphyton samples (RD[09], Appendix D). Benthic microbes are not collected in lakes and non-wadeable streams. Benthic biofilms are collected in wadeable streams during periods of stable stream flow (e.g., when the stream is not flooding, defined as >3x median discharge for the preceding year) (Clausen and Biggs 1997, Stevenson and Bahls 1999) using a series of scrubbing procedures, depending on habitat and substratum type (Moulton et al. 2002). Additional details may be found in Periphyton and Phytoplankton Sampling (RD [09]). Microbial samples are collected both as whole sample (plant or sediment grab) and on filters for archive and analysis.

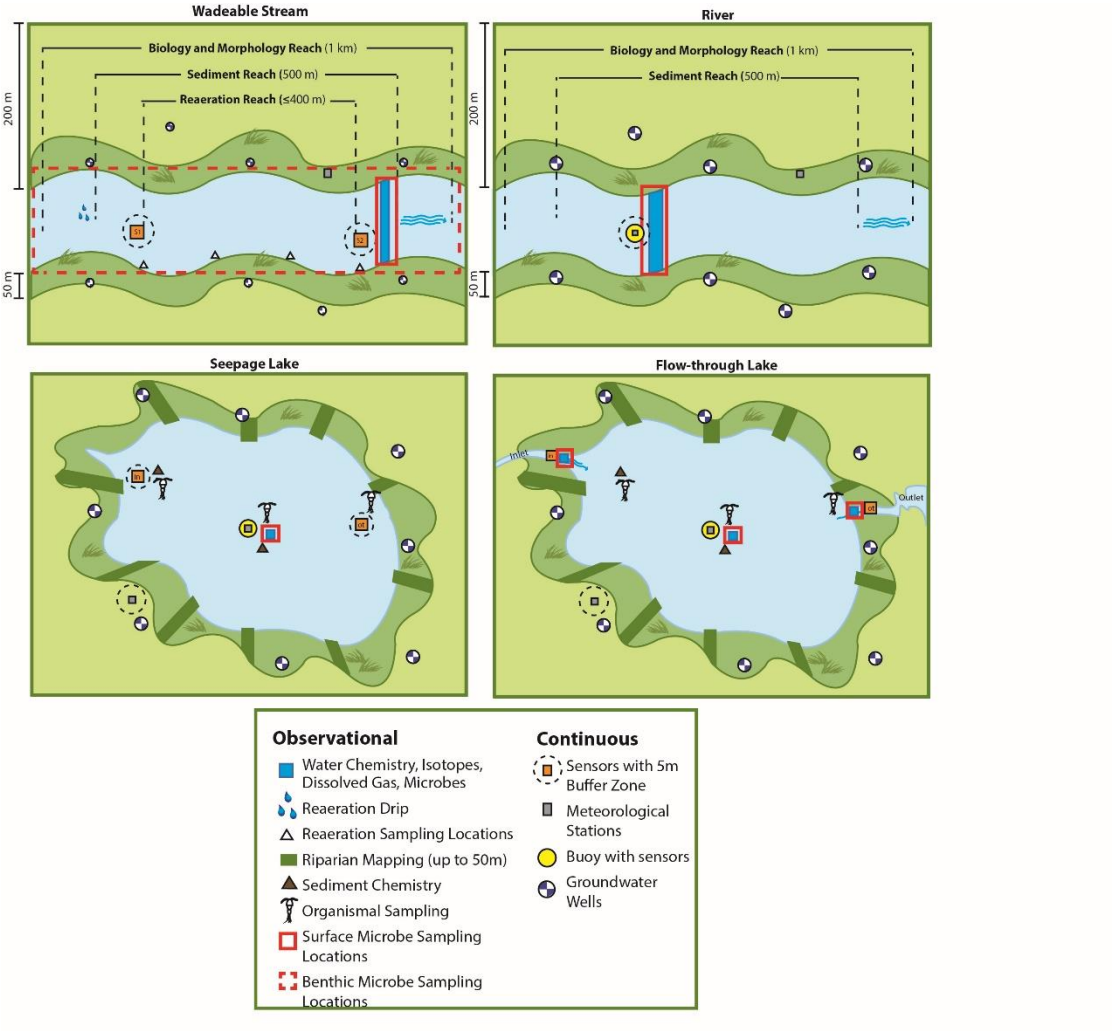

Figure 1. Generic site layouts with microbe sampling locations in red. Seepage lakes have no true inlet or outlet stream. In flow-through streams, inlet and outlet infrastructure are located in the inlet or outlet stream channel.

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

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

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Surface water microbes

Surface water microbes will be collected 12 times per year in **wadeable streams** and 6 times per year in **lakes and rivers**. Sampling will occur at the same time, location and sampling depth as standard recurrent water chemistry samples (RD[10]). Microbial samples may be collected either just before or just after chemistry sample collection as long as the water column remains undisturbed.

Benthic microbes

Benthic microbial sampling in **wadeable streams** occurs three times per year at each site, roughly spring, summer, and autumn. Benthic microbial samples are collected at the same time and location as periphyton samples (RD[09]) and may be collected before, after, or during periphyton sampling as long as they are collected from undisturbed substrate. Sampling must be scheduled within the first 21 days of the 1 month window specified in Appendix D with a minimum of two weeks between sampling dates. Accommodations for local weather conditions (e.g., late ice-off) may be made that cause the sample date to fall outside of the pre-determined window. Use NEON's problem reporting system to report sampling efforts that take place outside of the defined sampling window.

4.2 Criteria for Determining Onset and Cessation of Sampling

Surface water microbes

At **lake and river sites**, sampling will occur with monthly or bi-monthly recurrent water chemistry sampling, including dates that require sampling under the ice at northern sites. At northern lakes, sample microbes with every other chemistry bout as the bi-monthly schedule changes due to ice-on and ice-off.

Benthic microbes

For **wadeable stream** benthic samples, a range of dates for each site were determined *a priori*, based on historical data including ice on/ice off, streamflow, the accumulation of degree days, weather, and riparian phenology (Appendix D). Benthic microbes will be collected together during periods of stable stream flow (Stevenson and Bahls 1999).

4.3 Timing for Laboratory Processing and Analysis

Filtered samples and grab samples (sediment and plant material) must be flash-frozen in the field and may be held at the domain lab at -80 °C until shipping. Cell count samples must be preserved in formaldehyde in the field and may be held at the domain lab at 4 °C for up to 60 days before shipping. For additional storage and shipping timelines see SOP F.

4.4 Sample Timing Contingencies

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

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and conditions become unsafe (e.g. approaching thunderstorm, rapid increase of water level in the **wadeable stream**), or the **lake/river** becomes too windy (>32 km hr^{-1}) and has unsafe wave heights (>1 m) so that the boat cannot be held stationary over a sampling point while at anchor, return to shore and wait in a safe location for 30 minutes. If conditions improve, resume 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 circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the streamflow or weather requirements, continue to collect samples after the delay. If conditions do not improve, discard any previously collected samples at the site or at the Domain Support Facility and start over on the next appropriate sampling day.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
14 Days	Benthic samples: If flooding occurs on or <14 days prior to the targeted sampling date in a **wadeable stream** (>3x median discharge for the preceding year; Clausen and Biggs 1997) or unsafe wading conditions occur (Lane and Fay 1997), wait a minimum of 14 days after	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.

	the water level drops below 3x median discharge and is safely wadeable and biofilm can recolonize.	
	Surface samples: Preserved cell count samples may be held for a maximum of 60 days at 4 °C in the domain lab if circumstances do not allow shipping to the external lab.	Sample degradation is likely during the 60 day window, however preliminary studies at the external lab show that samples are relatively stable until 60 days. Data shipped within this window are not flagged.
6 months	Frozen samples (filters, sediment, and plant material) may be held for up to 6 months at -80 °C in the domain lab if circumstances do not allow shipping to the external lab.	Holding samples longer than expected affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected and samples do not need to be flagged if held for ≤6 months.

4.5 Criteria for Permanent Reallocation of Sampling within a Site

Surface water microbe sampling will occur on the schedule described above at 1 location per stream or river site or 3 locations per lake site. Benthic microbes will occur on the schedule described above at 8 locations per stream site in two different habitat types. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (relocatable sites). However, circumstances may arise requiring that sampling within a site be shifted from one particular location to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given location becomes compromised, a problem ticket should be submitted by Field Operations to Science.

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

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

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

Situation	Action	Outcome for Data Products	Considerations
Wadeable stream site with <500 m aboveground stream length due to stream size or permitting restrictions	Habitat available may be insufficient to accommodate all 8 samples without causing harm to the stream. Reduce sampling by collecting samples only in the dominant habitat type (5 samples total).	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[09], RD[14], RD[15]).
Wadeable stream site with seasonal drying	If the stream experiences seasonal drying such that the chosen habitat types have disappeared, select the next dominant habitat type in C.1, or use the sub-dominant habitat if still present. Reduce sampling by collecting samples only in the dominant habitat type (5 samples total). Enter "sampling impractical" for the missing habitat type.	Less standardization of the dataset.	Habitat types sampled should be present throughout the year. The decision to switch habitat types should only occur during periods of extreme drying such that typical habitat types are no longer present at the site.

4.6 Sampling Specific Concerns

1. Care must be taken to avoid contaminating samples with re-suspended bed sediment.
   a. In **wadeable streams**, minimize contamination by entering the stream downstream of the sampling location.
      i. The sampling location should be located in a flowing section of the stream that is deep enough to sample without disturbing the stream sediments.
      ii. If sediments are disrupted, wait until the area has visually cleared before sampling.
   b. In **lakes and rivers**, contamination may be minimized by anchoring the boat upwind (or upstream) of the sampling site, and using an anchor line 2 times as long as the depth of the lake or stream.
2. All reusable equipment must be cleaned and sterilized in the lab, and any equipment used for multiple samples in the field must be field-sterilized during sampling. Gloves, cleaned with an ethanol wipe, are required to maintain the cleanliness of the sampling equipment and to decrease contamination of microbial samples by human or cross-site microbes while in the field.
3. Take care to keep track of the volume of water used to scrub the sample and the volume of water used for filtering, these data are very important for conversion to higher data products.
4. Failure to completely mix sample before filtering can result in skewed results. All subsamples are meant to be representative of one-another, so careful mixing is a necessity.
5. Should it begin to rain during **surface microbe** sampling, collect surface water samples in 4-L containers and return the containers on ice to the lab or base-camp where samples can be subsampled or filtered within 3-4 hours of sample collection.
   a. Water jugs must be shaken for 15 seconds before sub-sampling or filtration to re-suspend particulates and homogenize water. If at any point you believe contamination has occurred during subsampling, discard samples and resample.
6. For **benthic microbe** sampling in **wadeable streams**, sampling too soon after a disturbance event (e.g., flooding, drying, or wildlife crossing a stream) can dramatically decrease biomass and diversity, be sure to wait at least 14 days for recolonization to occur.

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 EHSS Policy, Program and Management Plan (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Ecologist have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

Field staff must wear protective nitrile gloves when handling microbial samples to prevent cross-contamination and to protect from chemicals used in preservation. Safety Data Sheets (SDS) shall be made available for all chemicals used in this work (glutaraldehyde). Whenever chemicals are used, follow requirements of the site-specific Chemical Hygiene and Biosafety Plan (AD[03]) for laboratory safety and NEON EHSS Policy, Program and Management Plan (AD[01]).

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

1. Activities in **wadeable streams** should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 10 ft^2/s (0.93 m^2/s; Lane and Fay 1997).
2. In **lakes and rivers**, site-specific hazards may be encountered that necessitate sampling from the boat, without dismounting from the vessel. In addition, use extra caution in waters where alligators are present and maintain a safe distance from hazards.
3. When working around ice, refer to (AD[02], Section 10.3 Winter Water Safety. Do not attempt to walk on frozen lake if depth of ice is less than 6” (+15cm) or operate UTV or snowmobile on frozen lake if depth of ice is less than 8” (20cm). Use caution and good judgment to carefully evaluate site conditions including ice strength. Local guidelines from natural resource officials, property owners or hosts, and domain managers should be consulted regarding work on ice, prior to deploying employees and equipment. Do not continue if the risk is too great.
4. All personnel must be wearing a personal flotation device (PFD) prior to entering a boat.
5. All personnel shall have access to a form of communication with other team members such as a two-way radio.
6. Be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, water release from dams, etc.).

6 PERSONNEL 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 3. Equipment list – General equipment

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
RD[11] or RD[12]			R	Site-specific Stream Morphology, Rapid Habitat Assessment, or Bathymetry Map	Determining sampling locations	1	N
MX110075 MX102739	Forestry Suppliers, Inc. Cabela's Inc. Recreational Equipment Inc.	39481 IK-270217 895022	R	Handheld GPS unit (with batteries, ±4 m accuracy) or Humminbird™	Navigating to sampling locations	1	N
MX102941 MX102942	Forestry Suppliers, Inc. Grainger	53190 1GEJ1	R	Clipboard	Recording data	1	N
MX106668 (M) MX106680 (L) MX107549 (XL)	Fisher Scientific Company, LLC	11394305 11394200 11394328	R	Cryogenic gloves	Handling dry ice	1 pair	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
			R	Cooler (9-28 qt)	Field sample storage; use size appropriate for number of samples being collected	2	N
MX100491 MX100494 MX107505	Ben Meadows Co., Inc. Grainger, W.W. Forestry Suppliers, Inc. Cabela's		R	Waders (hip or chest) or knee boots	Boating or wading	1 pair per person	N
Consumable items							
RD[06]			R	Aquatic Field Metadata Sheet	Recording metadata in case tablet fails	1	N
RD[05] MX103942	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	010510-1 49247	R	Field datasheets (all-weather paper)	Recording data in case tablet fails	1	N
MX111388	CDW-G	4452963	R	Mobile data entry tablet	Field data entry	1	N
MX106268	Amazon Capital Services Inc.	7530-01-498-9209	R	Pre-printed adhesive labels (all-weather, 1”x 2-5/8" (e.g., Avery 5661))	Labeling samples	20	N
			S	Adhesive barcode labels	Labeling sample bottles with barcode-readable	1 sheet	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
			R	Pencils	Recording data	2	N
MX102002	Grainger, W.W.	1JU51	R	Permanent markers	Labeling samples	2	N
			R	Ice or chemical ice packs	Keeping cell count samples cool	2	N
MX100212			R	Dry ice	Flash-freezing samples in the field	0.5-1 kg	Y
			R	Nitrile gloves, powderless	Sterile collection methods	10	N
MX104844	Grainger, W.W.	5LH30	R	Resealable bags, gallon	Containing sterilized equipment	20	N

R/S=Required/Suggested

Table 4. Equipment list – Lab sterilization

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
			R	Wash basin	Container for sterilization solutions	1	N
MX100373	Cole-Parmer	HV0751802	R	Peristaltic pump head	Filtering DI water	1	N
MX100383	Grainger, W.W.	3HFV9	R	18V drill pump	Filtering DI water	1	N
MX100364	Thomas Scientific, Inc.	1207W05	R	Peristaltic pump tubing	Filtering DI water with peristaltic pump	1	N
MX100662	Fisher Scientific Company, LLC	141697G	R	Flexible tubing, 3/8” inner diameter	Filtering water with peristaltic pump	2	N
MX100376	Fisher Scientific Company, LLC	1531528C	R	Tubing connector	Attaching C-flex to peristaltic tubing	1	N
MX100665 MX105571	Thomas Scientific, Inc.	1709C04 (clear) 1212W94 (clear jug)	R	1 L HDPE bottle or 4 L HDPE jug with lid, sterilized	Container for filtered DI	1	N
Consumable items							

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
			S	Lab drying rack	Drying equipment	1	N
MX100213 MX100202	Fisher Scientific Company Thomas Scientific, Inc.	4355601 C954K61	S	ETOH, 95%	Sterilizing lab bench	1 L	Y
MX104102	Thomas Scientific, Inc.	2902G05	R	Mild detergent (Alconox or Liquinox)	Sterilizing equipment	<1 gallon	N
MX107386	Thomas Scientific, Inc. Fisher Scientific Company, LLC	1208R78 SVGPL10RC	R	0.22 µm Sterivex SVGP® L10RC capsule filter with luer lock outlet	Filtering DI	2	N
			R	Deionized water	Rinsing equipment, preparing filtered DI	4 gallons	N
MX104844	Grainger, W.W.	5LH30	R	New gallon zip top bags	Storing sterilized equipment	5	N

R/S=Required/Suggested

Table 5. Equipment list – Surface water microbe sampling equipment

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
MX100393	Fisher Scientific Company, LLC	EW0548610	R	Kemmerer sampler with rope and messenger	**Lakes and rivers**, collecting water	1	N
MX108938			S	Ice auger	**Lake** sampling under ice	1	N
MX105571	Thomas Scientific, Inc.	1212W94	R	Sterile 4 L HDPE jug	Collecting or integrating samples	2-6	N
MX100662	Fisher Scientific Company, LLC	141697G	R	Flexible tubing, 3/8” inner diameter	Filtering water with peristaltic pump	2	N
MX100376	Fisher Scientific Company, LLC	1531528C	R	Tubing connector	Attaching C-flex to peristaltic tubing	1	N
MX106253	Thomas Scientific, Inc.	1236A26	R	Filter adapters for tubing (3 mL syringe)	Connecting peristaltic tubing and C-flex tubing	2	N
MX100373	Cole-Parmer	HV0751802	R	Peristaltic pump head	Filtering water	1	N
MX100383			R	18V drill pump	Filtering water	1	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX100364	Thomas Scientific, Inc.	1207W05	R	Peristaltic pump tubing	Filtering water with peristaltic pump	1	N
MX106250	Fisher Scientific Company, LLC	300743	R	500 mL plastic graduated cylinder	Measuring filtered water volume	1	N
MX107138	Fisher Scientific Company, LLC	FBE10000	R	10 mL adjustable pipette	Pipetting cell count samples into vials	1	N
MX106245	Fisher Scientific Company, LLC	22257152	S	140 mL syringes	Alternative to peristaltic pump	2	N
Consumable items							
			R	Small zip tie or small hose clamp	Attaching flexible tubing to hose connector	1	N
MX107140	Fisher Scientific Company, LLC	2681412	R	10 mL pipette tips, sterile	Pipetting cell count samples into vials	1 per station	N
MX106249	Fisher Scientific Company, LLC	33377	R	20 mL glass scintillation vials (cap not included)	Cell count sample container	1 per station	N
MX107204	Thomas Scientific, Inc.	9718G80	R	20 mL scintillation vial caps, polyethylene with cone-shaped liner	Cell count samples container lid	1 per station	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX107386	Thomas Scientific, Inc. Fisher Scientific Company, LLC	1208R78 SVGPL10RC	R	0.22 µm Sterivex SVGP® L10RC capsule filter with luer lock outlet	Microbe sample filters	2 per station	N
MX109186	Cole-Parmer	EW4550556	R	Luer lock male closures, sterile	Capping the Sterivex® filters	4 per station	N
MX109187	Cole-Parmer	EW4550228	R	Luer lock female closures, sterile	Capping the Sterivex® filters	4 per station	N
			S	Filtered DI (0.2 µm filter)	Field-sterilization and rinsing for multiple stations	1-4 L	N
RD[12] MX110716	VWR	TWTX3044P	S	Sterile 70% ethanol wipes (e.g., www.soscleanroom.com item TX3044P pre-wetted wipe OR TX3215 dry wipe)	Field-sterilization for multiple stations and gloves	20	N

R/S=Required/Suggested

Table 6. Equipment list – Benthic microbe sampling equipment (**wadeable streams** only)

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items								
MX103144	B&H	GESM7001	R	Template (35 mm plastic slide cassette)	Sampling area for epilithon and epixylon scrubbing	Epilithon, epixylon	1	N
			R	PVC large substrate sampler: • 2" inner diameter PVC • Rubber seal or O-ring	Sampling area for large substrate rock and wood scrubs	Epilithon, epixylon large substrate	1	N
			S	Turkey baster	Sample removal for large substrate rock and wood scrubs	Epilithon, epixylon large substrate	1	N
MX100338	BioQuip Products Inc. Fisher Scientific Company, LLC	1426B NC0095946	R	Larval insect tray, plastic	Scrubbing container in which sample is collected	Epilithon, epixylon	1	N
MX100346	Thomas Scientific, Inc. Fisher Scientific Company, LLC	1207M51 0340910C	R	Wash bottle, unitary, 125 mL	Rinsing substrate and larval tray into syringe	Epilithon, epixylon	1	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX106245	Fisher Scientific Company, LLC	22257152	R	140 mL HDPE syringe	Filtering samples	All	2	N
MX100341	Grainger, W.W.	5C936	R	Spatula (metal, flat)	Epipsammon and epipelon collection	Epipsammon, epipelon	1	N
			R	Sterilized scissors	Epiphyton collection	Epiphyton	1	N
			S	ETOH rinse or spray bottle	Field sterilization	All	1	N
Consumable items								
			R	Filtered DI (0.2 µm filter)	Field sterilization and rinsing	All	1-4 L	N
RD[12] MX110716	VWR	TWTX3044P	R	Sterile 70% ethanol wipes (e.g., www.soscleanroom.com/content/texwipe_pdf/3044p.pdf)	Field sterilization	All	20	N
MX100213 MX100202	Fisher Scientific Company Thomas Scientific, Inc.	4355601 C954K61	S	ETOH, 95%	Field sterilization, refill ETOH	All	500 mL	Y

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
					wash/spray bottle above			
MX103142	Fisher Scientific Company, LLC	19027438	R	Nylon toothbrushes for each habitat type (new toothbrush for each sample)	Epilithon and epixylon scrubbing	Epilithon, epixylon	≤8	N
MX103145	Amazon Capital Services Inc.	B005IQTSE0	S	Disposable pipets	Sample removal for large substrate rock and wood scrubs	Epilithon, epixylon large substrate	5	N
MX106246	Fisher Scientific Company	507532951	R	15 mL plastic centrifuge tubes with lids, sterile	Epipsammon and epipelon sample container	Epipsammon, epipelon	≤8	N
MX104787	Thomas Scientific, Inc.	1303N48	R	Whirl-paks®, 24 oz.	Epiphyton sample container	Epiphyton	≤8	N
MX107386	Thomas Scientific, Inc. Fisher Scientific Company	1208R78 SVGPL10RC	R	0.22 µm Sterivex SVGP® L10RC capsule filter with luer lock outlet	Microbe sample filters	Epilithon, epixylon	≤16	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX109186	Cole-Parmer	EW4550556	R	Luer lock male closures, sterile	Capping the Sterivex® filters	Epilithon, epixylon	≤16	N
MX109187	Cole-Parmer	EW4550228	R	Luer lock female closures, sterile	Capping the Sterivex® filters	Epilithon, epixylon	≤16	N

R/S=Required/Suggested

Table 7. Equipment list – Sample processing and preservation

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items								
MX106253	Thomas Scientific, Inc.	1236A26	R	3 mL HDPE syringe, luer lock end	Filtering formaldehyde	Surface water, epilithon, and epixylon	1	N
MX100238	Panasonic Healthcare Corp	MDFU56VCPA	R	Freezer (-80 °C)	Sample storage	Sterivex® filters, whole sample grabs	1	N
Consumable items								
MX106257	Thomas Scientific, Inc.	C998K62	R	10% formaldehyde, buffered (sodium borate or phosphate)	Preserving samples	Surface water, epilithon, and epixylon	1 L	Y
MX100691	Fisher Scientific Company	1337412	R	Parafilm	Sealing cell count vial lids prior to shipping	Surface water	1 roll	N
MX106239	Fisher Scientific Company	NC9192975	R	0.2 µm Acrodisc® filter	Filtering formaldehyde	Surface water, epilithon, and epixylon	2	N

R/S=Required/Suggested

Table 8. Equipment list – Shipping materials

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
			R	Dry ice shipping container	Shipping filters	1	N
MX100555	Amazon Capital Services Inc.	202021583	R	Cooler, 9 qt. or Styrofoam-lined box	Shipping cell count samples	1	N
Consumable items							
MX109205	Grainger, W.W.	30RD13	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	As needed	N
MX102717	Uline	S-5106	R	Trash bag to line cooler (~13 gallon size)	Protecting against leaks	1	N
MX104844	Grainger, W.W.	5LH30	R	Resealable plastic bags (gallon and quart size)	Protecting against leaks	6	N
MX100212			R	Dry Ice	Shipping filters	As needed	Y
			R	Ice or chemical ice packs	Shipping cell counts	As needed	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
RD[13]			R	Shipping inventory	Provides sample information to external lab	1	N

R/S=Required/Suggest

6.2 Training Requirements

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

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

Personnel will be trained in field protocols associated with this document, and trained in safe working practices for aquatic field work. Personnel must also be trained in safe handling of formaldehyde as documented in the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

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 sampling locations. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Field sampling requires two field ecologists for three hours per site in addition to other chemistry and biological sampling occurring concurrently, plus travel to and from the site. There is no lab processing associated with this protocol.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged and synced at the beginning of each field day, whenever possible.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

A.2 Microbe Sampling Preparation

1. Make sure all supplies are packed and peristaltic pump batteries are charged and the pump is in good working condition.
2. Create a filter adapter for the peristaltic pump.
   a. Remove the plunger of a 3 mL syringe (Figure 2).
   b. Connect 3/8" inner diameter C-flex tubing to the top of 3 mL syringe.

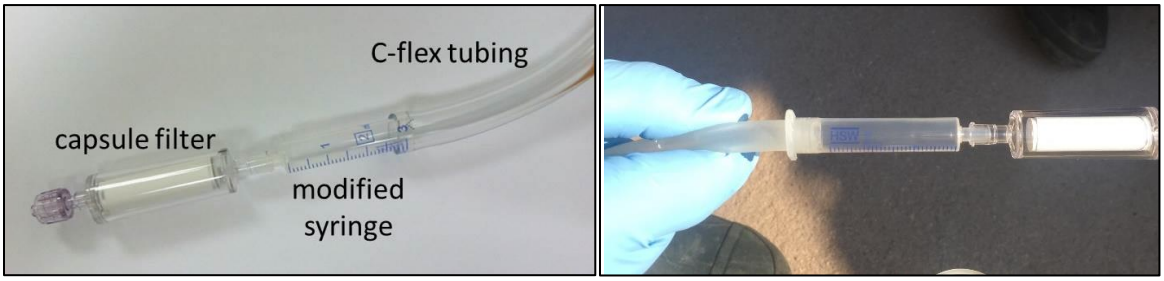

Figure 2. Filter adapter for peristaltic pump, you may put the C-flex tubing inside or outside (cut off top) of the 3 mL syringe.

3. Prepare filtered DI for rinsing.
   a. Filter DI using the peristaltic pump setup (with sterilized tubing) and a 0.2 µm Sterivex® filter.
   b. Store in sterilized 1 L HDPE bottle or 4 L jug for up to 2 weeks.
4. Sterilize any equipment that is reused between sampling bouts.
   a. Equipment to be sterilized includes:
      i. **Surface microbes**: 4 L jug, 140 mL syringe, graduated cylinder (if using prior to filtering), modified 3 mL syringe (Figure 2), pump tubing, Kemmerer (**lakes and rivers** only).
         1) Having an additional piece of peristaltic pump tubing, hose connector, and filter adapter that can be dedicated to microbe sampling (i.e., not used for water chemistry) may be easier for logistics.
      ii. **Benthic microbes (wadeable streams)**: 125 mL wash bottle, graduated cylinder, brushes, slide template, larval tray, spatula, scissors.

b. Wash in mild detergent solution.
   i. Prepare a 0.2% (v.v) solution of detergent and DI water in a wash basin.
   ii. Wearing ETOH-cleaned nitrile gloves, submerge equipment thoroughly in detergent solution. Use bottle brush and/or hands to clean equipment.
c. Triple rinse with DI water.
d. Follow last DI rinse with 0.2 μm filtered ("sterile") DI water.
e. Use drying racks to completely dry all equipment. Wipe down drying racks with 95% ethanol prior to use.
   i. If drying racks are not available, clean the benchtop with 95% ethanol and place items directly on the sterilized benchtop to dry. The drying rack is strongly preferred.
f. When dry, cap bottles and place all other equipment in clean gallon Ziploc bags to store or transport to the field.

5. Prepare labels (Figure 3, RD[05]) for bottles and capsule filters (Figure 4), use permanent marker to fill out labels as much as possible before going into the field. Leave filter volume blank until filtering has been completed. Do not adhere labels to filters until you are in the field as this compromises the sterility of the filter.
   a. Surface water samples (Figure 4)
      i. **Wadeable streams** surface water: 1 sample
      ii. **Seepage Lakes** (no inlet or outlet stream) surface water: 1-2 samples, depending on stratification
      iii. **Flow-through Lakes** (have true inlet and outlet stream) surface water: 3-4 samples, depending on stratification
      iv. **Rivers** surface water: 1-2 samples, depending on stratification
   b. Benthic samples (Figure 4)
      i. **Wadeable streams** benthic, dominant habitat: 5 samples
      ii. **Wadeable streams** benthic, secondary habitat: 3 samples

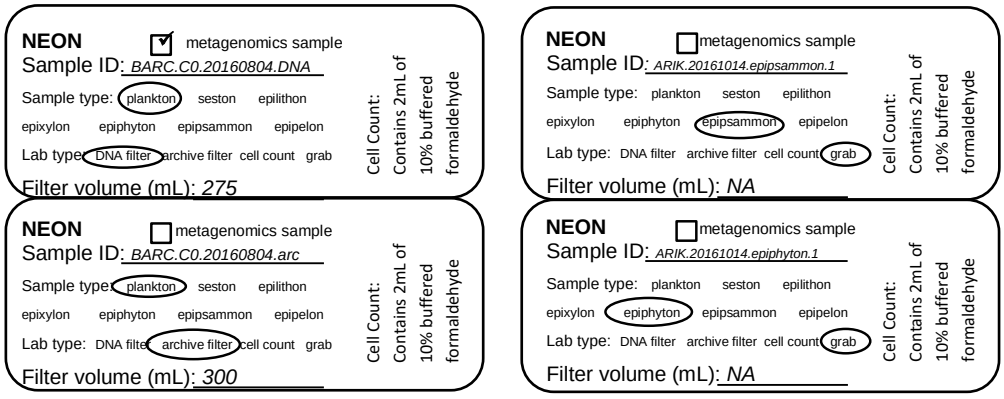

Figure 3. Example NEON microbes label. Adhere right side of label to filter or vial first, label will overlap on filters. Note that surface water samples include the location code in the sample ID (Table 9). Metagenomics samples

collected in midsummer (July or August surface water sample or Bout 2 benthic sample) will be denoted by checking the metagenomics checkbox to help the external lab.

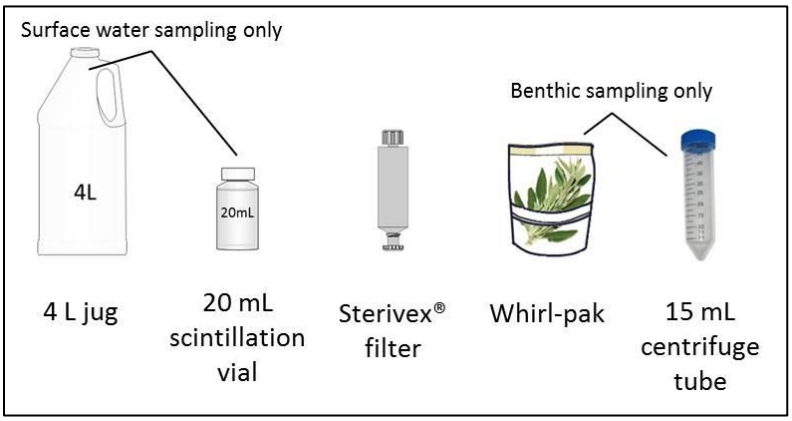

Figure 4. Sample containers.

6. When the system is available, adhesive barcode labels will be added to the sample containers and scanned by the mobile app (Figure 5). Add adhesive labels to HDPE bottles prior to going in the field and getting the bottle wet.
    a. Keep a human-readable label on each bottle with a minimum of the sample ID printed to assist with organization and shipping.

Figure 5. Example of adhesive barcode labels.

7. Human-readable sample IDs will be generated by the mobile app as follows (Table 9, Table 10). Sample IDs written on the physical sample label must match the sample if generated by the mobile app.

Table 9. Examples of surface water microbe sample IDs generated by the mobile app.

Sample type	Site type	Subsample	Example Sample ID
seston, plankton	Stream, Lake, River	cell count ("cc")	REDB.ss.20161123.cc
seston, plankton	Stream, Lake, River	DNA filter ("DNA)	REDB.ss.20161123.DNA
seston, plankton	Stream, Lake, River	archive filter ("arc")	REDB.ss.20161123.arc

Table 10. Examples of benthic microbe sample IDs generated by the mobile app.

Sample type [benthic area]	Site type	Field app populates as:	Example Sample ID
epilithon [0.002406]	Stream, Lake, River	epilithon	REDB.20161123.amc.epilithon.1
epixylon [0.002406]	Stream, Lake, River	epixylon	REDB.20161123.amc.epixylon.1
epipsammon [0.005938]	Stream, Lake, River	epipsammon	REDB.20161123.amc.epipsammon.1
epipelon [0.005938]	Stream, Lake, River	epipelon	REDB.20161123.amc.epipelon.1
epiphyton [0.01]	Stream, Lake, River	epiphyton	REDB.20161123.amc.epiphyton.1
epilithon_largeSubstrate [0.006080]	Stream, Lake, River	epilithon_largeSubstrate	REDB.20161123.amc.epilithon_largeSubstrate.1
epixylon_largeSubstrate [0.006080]	Stream, Lake, River	epixylon_largeSubstrate	REDB.20161123.amc.epixylon_largeSubstrate.1

8. Check the volume of the 125 mL wash bottle using a graduated cylinder. If it is within 5 mL, use the "fill line". If the difference is >5 mL, create a new "fill line".
9. Use the Acrodisc® 0.2 μm filter attached to a sterile 3 mL syringe, add filtered buffered formaldehyde preservative to sterile 20 mL glass scintillation vials for cell counts (2 mL per 18 mL sample) making sure to wear gloves.
10. Prepare coolers with frozen ice packs or water ice (cell counts) and/or dry ice (filters and grab samples).
11. Fill out the General Aquatic Field Metadata mobile app once per day upon every field sampling visit, and Secchi and Depth Profile mobile app in **lakes and rivers**. If other protocols are done in the same day, one record for field metadata and one record for Secchi and depth profile are sufficient.
12. **Always wear nitrile gloves.** Field sampling methods should be as sterile as possible. Have a clean/sterile place to set equipment on the boat or stream bank, and use new zip-top bags to contain cleaned items. Plastic containers or organizers work well.

SOP B Field Sampling: Surface Water Microbes

B.1 Wadeable Stream Collection

1. Samples will be collected at one location near the **wadeable stream** S2 sensor set (ss) along with surface water chemistry sampling.
   a. Collect surface water samples prior to or upstream of any benthic sampling. Confirm that no one is wading upstream during sampling.
2. **Wearing ETOH cleaned nitrile gloves**, prime (rinse) both 4 L HDPE bottles and caps 3 times with stream water, shake vigorously. Discard rinse water into stream. Do not set bottle cap down as this increases the chance of contamination.

a. If the sampling location is too shallow to use 4 L jugs, use either the peristaltic pump and tubing, or 140 mL syringe to pump water directly out of the stream.
   i. If using the pump and tubing, prime the pump tubing by pumping at least 100 mL through the tubing. Allow water to rinse the endo of the tubing as well.
b. If using the 140 mL syringe, prime (rinse) 3 times with stream water and discard downstream.

3. Standing in the thalweg, hold 4 L bottles several centimeters below the water surface with the opening pointed upstream. Tilt bottle slightly underwater to allow stream water to fill bottle. Fill one to two 4 L jugs to ensure sufficient water collection, any extra may be discarded.
   a. If the sampling location is too shallow, and you are using the pump and tubing or the 140 mL syringe, you may pump directly into the syringe or into a primed 4 L jug. See SOP B.3 for Surface Water Processing.
4. Return to the stream bank and proceed to SOP B.3 for Surface Water Processing.

B.2 Lakes and River Collection

SELECTING SAMPLING LOCATIONS

1. **Lakes** will be sampled at up to three locations in at the same time as water chemistry sampling: the central/deepest part of the lake ('c0' - non-stratified, or 'c1', 'c2', 'c3' – stratified), the inlet ('in'), and the outlet ('ot'). River**s** will be sampled at the sensor ('c0') in conjunction with water chemistry sampling.
   a. Seepage lakes will only be sampled at the buoy (c0, c1, c2, c3)
   b. Flow-through lakes will be sampled at the inlet (in), outlet (ot), and buoy (c0, c1, c2, c3)
2. Refer to Section B.2 in Water Chemistry Sampling in Surface Waters and Groundwater (RD[10]).
3. Determine the stratification conditions at the buoy from the Secchi Disk and Depth Profile Sampling in Lakes and Rivers (RD[18]), Section 7, SOP C.
4. Sample in the same locations and depths where water chemistry samples are collected RD[10]).
   a. Center (buoy)
      i. Unstratified ('c0'): 0.5 m (±5 cm)
      ii. Stratified (two samples):
         1) c1: 0.5 m (±5 cm)
         2) c2: If hypolimnion section depth (i.e. hypolimnion thickness) is <2 m, do not take any more samples (see RD[10] for details)
            (1) If hypolimnion depth/thickness ≥2 m but ≤4 m, then collect a sample from the mid-point of the hypolimnion
            (2) If hypolimnion depth/thickness >4 m, collect an integrated sample (see RD[10])
   b. Inlet ('in'): 0.5 m (±5 cm) – flow-through lake only
   c. Outlet ('ot'): 0.5 m (±5 cm) – flow-through lake only
5. If sampling under ice, core through ice and sample as above as long as there is at least 0.5 m of water depth below the ice.

a. If the water depth is <0.5 m, move to a location within 10 m of the original sampling location and note the new GPS location.
b. In lakes, do not sample at the inlet or outlet under ice, following guidelines for lake water chemistry sampling in Water Chemistry Sampling in Surface and Groundwater (RD[10]).

SAMPLE COLLECTION

1. Navigate to the sampling location. Gently lower anchors at the bow and allow boat to float back with wind or current to sampling location. Drop a second anchor at the stern to hold boat in place. Two anchors should always be used when collecting samples from the water column.
   a. Allow ~5 minutes for sediments to settle after lowering the anchor; you can use this time to prepare the sampling equipment.
   b. Use a bow anchor line 2 times the depth to prevent contamination from sediments.
   c. The boat must be anchored at the bow and stern in order to collect representative water column samples and prevent the boat from spinning.
2. Always sample near the bow of the boat to minimize the effects of the motor on the water column. When anchored, the bow of the boat tends to orient itself with the bow into the wind or current.
3. Rinse sample bottles (4 L jug) 3 times with source water at each location.
   a. Hold the bottle cap in a gloved hand, setting the cap down increases the risk of contamination.
   b. Discard rinsewater away from the location where you are sampling.
4. **Wearing ETOH-cleaned gloves**, prepare the Kemmerer sampler (Figure 6) for sampling. Pull the stoppers from the collection cylinder by holding the top and bottom stopper and giving a short, hard pull to the bottom stopper.
   a. A short, hard pull is important to keep the stoppers open. If the stoppers don't stay open, pull harder.
5. Tie the free end of the Kemmerer line to a cleat on the boat to prevent losing the sampler.
6. Carefully lower the Kemmerer sampler over the side of the boat into the water. Hold the messenger in one hand. Ensure it is secured properly to the line.
7. Prime (rinse) the Kemmerer with surface water 3 times. Allow rinse water to drain through the spigot to prime the spigot too.
8. Continue to lower the Kemmerer sampler until it reaches the desired depth by using the depth markings on the line attached to the sampler.
9. When you reached the desired depth, gently move the sampler up and down to ensure water from the correct depth is in the sampler. Drop the messenger to release the clamps and seal the sampler.
10. Pull the sampler back into the boat and dispense sample water into the collection bottles through the spout (Figure 6a).

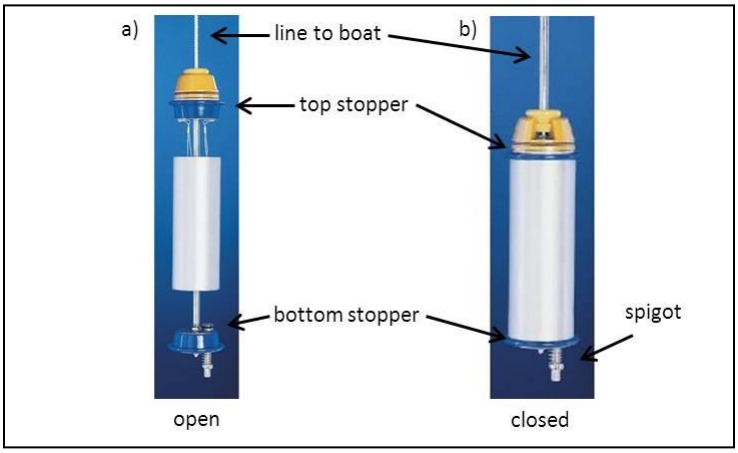

Figure 6. Vertical Kemmerer water sampler in the a) open or cocked position and b) closed (after dropping the messenger) position.

11. Allow at least 100 mL to drain through the spigot to rinse and residual water, discarding away from where you are sampling. Wipe the end of the spigot with an ETOH wipe.
12. Collect samples to be integrated in 4 L jug.
13. Repeat for each sample, priming the Kemmerer with source water at each new location.
14. Proceed to Section B.3 for Surface Water Processing.

B.3 Surface Water Processing

Processing may be conducted at the domain support facility in case of inclement weather within 4 hours of collection provided 4 L jugs are appropriately labeled. Field processing and preservation is preferred. Two Sterivex® filters and 1 cell count vial are produced per surface water sample (Figure 7).

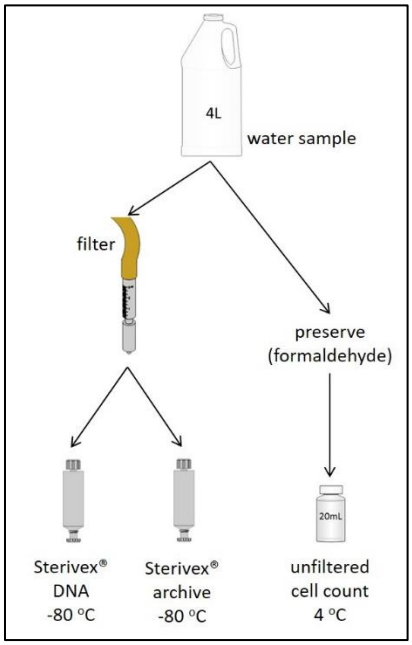

Figure 7. Schematic for surface water sample partitioning

CELL COUNTS

1. Shake 4 L jug to mix sample.
2. Use adjustable pipette with new sterile tips to transfer 18 mL of sample from 4 L jug to 20 mL glass scintillation vial containing 2 mL buffered, filtered formaldehyde preservative. Samples must be immediately preserved with formaldehyde.
3. Recap scintillation vial and invert at least 3 times to mix.
4. Wrap parafilm around the lid to prevent leaks.
5. Place cell count vial in cooler with ice packs, do not freeze.
6. Enter data in the mobile app. If not available, fill out field data sheet in pencil (Appendix F).
   a. If available, scan the barcode label with the tablet (Figure 8).
   b. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.

Figure 8. Barcode label scanning.

FILTERS

1. **WEAR ETOH-CLEANED GLOVES**, the same gloves can be worn while collecting the sample.
2. Set-up filter apparatus on stream bank, boat, or other relatively level surface:
   a. Set peristaltic pump speed below manufacturer specifications (45 psi for Millipore Sterivex SVGP® filter). If pump speed is set too high, the filter can rupture.
   b. Check that the 3 mL syringe adapter is in place to connect pump tubing to the capsule filter (Figure 2).
   c. Attach 3/8" inner diameter C-flex tubing to the peristaltic pump. Use a zip tie or small hose clamp to firmly attach the tubing to the hose connector, if needed.
3. Rinse tubing by pumping ~100 mL of sample water completely through the tube.
4. After tubing is flushed with sample water, open Sterivex® filter and attach to the luer lock end of 3 mL syringe/filter adapter.
5. Begin pumping water through the filter using peristaltic pump. Make sure the tube is filled with water to reduce air and reduce the potential to blow a hole in the filter.
   a. A clean, sterilized 140 mL syringe maybe also be used, connected directly to the capsule filter, if peristaltic pump is unavailable.
6. Filter >500 mL (≥1 L if possible, 2 L maximum) of surface water through the filter, discarding the filtrate in a 500 mL graduated cylinder to keep track of the filter volume. Stop filtering when filter clogs with sample or filtering becomes very slow.
   a. You may either pump filtered water into the graduated cylinder to measure the volume filtered (recommended, Figure 9), or pump water out of the graduated cylinder into the filter (this requires sterilization of the graduated cylinder).
   b. Filter <500 mL if filter becomes clogged and record filter volume.

Figure 9. Pump sample water out of the 4 gallon HDPE jug, through the peristaltic pump, the through the Sterivex® filter, and into a graduated cylinder to measure the volume filtered.

7. Label capsule filter with an adhesive label marked in permanent marker (Figure 3). Use a barcode label if available.
8. Filter two capsule filters per site for each parent sample (Figure 7). Filters should remain in original packaging until filtration.
9. When finished, remove filter from set up. Using the 140 mL syringe, draw air into the syringe and push air gently through the filter with 140 mL syringe to remove excess water from filter (about 1-2 syringes of air should be enough). If measuring water volume after it has been filtered, include this water in the sample volume as it has passed through the filter.
10. Cap ends of filter with luer lock male and female end caps (Figure 10).

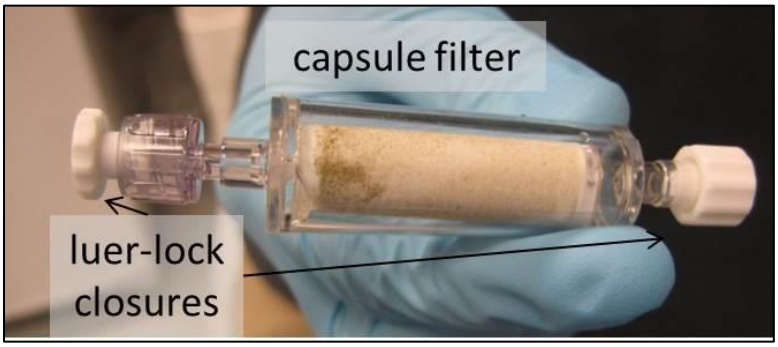

Figure 10. Capsule (Sterivex®) filter and luer lock closures

11. Check the "metagenomics" box on DNA filters collected during midsummer. Every site should have 1 set of surface water DNA sample and 1 set of benthic samples designated for metagenomics annually.
    a. Surface water DNA filters
        i. Check the box if the sample was collected in July
        ii. If working at a lake/river and no sample was collected in July, use an August sample
    b. Benthic sample
        i. Check the box for all DNA or grab samples collected during Bout 2
12. Record filter volume in the mobile app and on the adhesive label (Figure 9, RD[05]) for each sample.
    a. If available, scan the barcode label with the tablet.
    b. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
13. Separate labeled filters by DNA or archive in zippered plastic bags or Whirl-paks® and flash-freeze on dry ice in the field. Ensure that samples remain frozen until returning to the domain lab.
14. Proceed to Sample Preservation (SOP C.7).

SOP C Field Sampling: Benthic Microbes (wadeable streams only)

Benthic biofilm microbial samples are collected in wadeable streams at the same time and location as algal periphyton samples (see RD[09]), but collected independently, i.e., from different cobbles within the same habitats. Benthic microbes do not need to be collected on the same day as surface water microbes.

It is recommended, but not required, that three people work in the field together on the benthic microbes and periphyton protocols:

- 1 person collecting periphyton samples
- 1 person wearing sterile gloves collecting benthic microbe samples
- 1 person wearing sterile gloves to assist the microbe sampler with items on the bank

C.1 All Benthic Substrata

1. Sample the sample habitat/substratum type as for the periphyton protocol (RD[09]).
   a. Determine the dominant habitat and second-most dominant habitat based on the Rapid Habitat Assessment (RD[10]) for the site. Habitat suggestions for NEON sites are provided in Appendix E.
   b. The habitat type chosen should be present during all sampling bouts.
   c. All 5 (or 3) samples must be taken from the same habitat type on each sampling bout, unless a major event (i.e., a flood or dewatering of the stream) causes significant changes to the stream channel and the habitat type is no longer present.
   d. If there is only one clear habitat at the site, sample two different types of substrata using the sampling methods below (e.g., in a slow-moving run, collect 5 epilithon samples and 3 epipsammon samples).
2. Target substratum types in order of sampling preference (see Definitions, 2.4). If the preferred substratum is present in high enough density, that takes precedence over a less preferred, more dominant substratum type (e.g., if silt is the dominant substratum type but cobbles are present in high enough density to sample consistently, cobble sampling takes priority; Porter et al. 1993).
   a. Cobble (epilithon) → follow sampling procedure SOP C.3
   b. Woody snag (epixylon) → follow sampling procedure SOP C.3
   c. Boulder/bedrock (epilithon large substrate) → SOP C.4
   d. Large woody debris (epixylon large substrate) → SOP C.4C.4
   e. Plant surface (epiphyton) → follow sampling procedure SOP C.5
   f. Sand (epipsammon) → follow sampling procedure SOP C.6
   g. Silt (epipelon) → follow sampling procedure SOP C.6
3. Choose sampling locations with shallow, flowing water that appear to be historically wetted (i.e., usually underwater). Avoid areas that have been recently dried. Signs of recent drying include: extremely shallow areas, rocks that have nothing growing on them, and rocks that are not slippery to the touch.
4. Choose sampling locations that are representative of the periphyton/biofilm cover of the reach (i.e., not extremely dense or extremely sparse cover relative to nearby substrata) and representative of the light regime within the reach (i.e. do not sample in the one sunny patch of a heavily canopied stream).
5. Do not collect samples within a 5 m radius of the aquatic instrumentation.

6. Do not sample substrata where you or other field people/animals have walked or locations that appear recently disturbed (e.g., overturned rocks, footprints, dislodged plants, other evidence of wildlife, cattle, humans, etc.).
7. Avoid substrata that are close to the stream/river bank or lakeshore and may be exposed to frequent drying.
8. Choose sampling locations that are exposed to ambient light (e.g., not under a log or cut bank).
9. Unless sampling epiphytes, avoid substrata that are heavily colonized with aquatic plants, bryophytes, invertebrates, or have leaf litter clinging to the surface. You may brush some invertebrates off the surface, but the presence of aquatic plants and bryophytes may skew the results.
10. Avoid substrata that have noticeably tumbled or been recently disturbed.
11. Do not collect all samples from the same location.
    a. In **wadeable streams**, collect each sample from a different habitat unit (i.e., 5 riffles samples should be collected from 5 separate riffles).
12. 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.

C.2 Field Sterilization

1. Any equipment that is not sterile from the manufacturer upon use, or is reused from sample to sample or bout to bout, must be field-sterilized. Any equipment used from bout to bout must also be cleaned appropriately in the domain support facility prior to sampling, see SOP A.
2. At each location prior to collecting a sample, prime sterile equipment and gloves with native water by rinsing ≥3 times.
3. After collection at a location, wipe equipment and gloves with an ethanol wipe. You may spray with an ethanol sprayer, however the surface of the equipment still need to be wiped off with an ethanol wipe. If using both the sprayer and the wipe, you may prolong the life the wipe by folding over to a new side, and storing in a zip-top bag until the next sampling location.
    a. For equipment that is not easily cleaned with an ethanol wipe such as the Kemmerer or tubing, prime well (triple rinse) with source water at each sampling location within the same site.
4. Store smaller ethanol-cleaned equipment in a secondary containment zip-top bag for transport to the next sampling location. Keep domain lab-cleaned equipment in a separate bag. Larger equipment such as the Kemmerer sampler at lakes should be cleaned as well as possible with ETOH wipes, rinsed with sterile DI, then closed until reaching the sampling location.

C.3 Epilithon (Rock Scrubs) and Epixylon (Wood Scrubs) Microbes

1. Prepare 1”x2” adhesive labels (RD[05]), plan for two capsule filters for each parent sample (Figure 12). Use barcode labels if available.

a. **Wadeable streams**, dominant habitat: 10 filters/labels
b. **Wadeable streams**, secondary habitat: 6 filters/labels

2. **Wear gloves.** Replace gloves between habitat types.
   a. Field-sterilize gloves with alcohol wipes after sampling at a location and before moving to the next location in the same habitat type.
   b. Field-sterilized materials can be contained in a specified zip-top bag for field-cleaned items, and not mixed with lab-cleaned or sterile items.
3. A clean, sterile tray or tote is recommended for setting equipment on the stream bank (Figure 11).
4. Prime all field equipment with stream water and set in a clean location (e.g., tray, tote, or new plastic bag).
5. Select five cobbles and/or pieces of woody debris from the dominant habitat or three from the secondary habitat. Select cobbles and/or pieces of woody debris that meet both the requirements in SOP C.1 AND the following:
   a. Larger than the scrubbing template (i.e., > 2 inches in diameter).
   b. **These are NOT composite samples.** Only one cobble/piece of woody debris is required per sample.
6. Note the dominant substratum size class at the sampling location in the mobile app.
7. Place cobble/woody debris top-side up in the white larval tray. Take care to keep the cobble top-side up. This is the surface that has been exposed to the sunlight, and will be the portion of the cobble that you sample.
   a. You may clean the bottom of the cobbles with your gloved hands to remove excess material so it doesn't get in the tray and contaminate the sample.
8. Be sure to keep cobbles/woody debris moist with native water until scrubbing.
9. Proceed to a location on the stream bank or boat to process the sample. This location should be out of direct sunlight if possible.

Figure 11. Template placement for epilithic microbe sampling protocol. Note sterile organizer used to hold equipment on the stream bank.

10. Fill the primed 125 mL wash bottle with 0.2 μm filtered DI to the "fill line" (as marked on bottle).
    a. Make sure that bottom of the meniscus lines up with the "fill line".
    b. **Since this is a volume-based sampling technique, it is important to keep the sample volume consistent and record the volume of water used.**
11. Holding cobble/woody debris underwater, briefly rinse any leaves and/or large invertebrates from surface using stream water (you can use your hand to gently sweep insects off the cobble/woody debris surface, but take care not to scrub hard and dislodge biofilm).
    a. Recheck cobble, if there are more than 10 invertebrates attached to the substratum within your template, discard and choose a new cobble/piece of woody debris.
    b. If there is growth of aquatic plants or bryophytes that falls within your template, discard and choose a new cobble.
12. Place cobble/woody debris right-side up (the side exposed to the sun) in white tray and pour any excess water out of the tray.
13. Place white slide template on top of cobble/woody debris (surface that was exposed to light at the stream bottom; Figure 11). Check cobble/woody debris again for colonization of invertebrates, bryophytes, or plants.
14. Holding the template firmly in place on the cobble/woody debris, begin scrubbing inside the template (scrub gently if woody debris).

a. Both cobbles and woody debris are scrubbed using a **toothbrush** for this protocol. Prime toothbrush with stream water prior to sampling by working stream water into the bristles using your fingers.
b. Use a new toothbrush for each sample, discard after use.
c. Be sure to hold the template in place, as slipping would change the area you are sampling (Figure 11).
d. Scrubbing should be similar to brushing your teeth.

15. Periodically rinse the inside area of the template using the 0.2 µm filtered DI in the 125 mL wash bottle **while holding the template in place**. Allow water to run into the white tray – **DO NOT DISCARD** rinse-water.
16. Continue scrubbing until the inside of the template is clean.
17. Remove template. There should be a clean rectangle left from your scrubbing. This is harder to see on woody substrates.
18. Using the wash bottle, rinse the template (front and back), the cobble/woody debris, scrub brush, and gloved fingers into the tray. If you need more water for rinsing, refill the 125 mL wash bottle with 0.2 µm filtered DI water and keep track of the volume used with a graduated cylinder.
    a. Keep total volume <140 mL so the sample will fit in the syringe.
19. If there is any remaining water in the 125 mL wash bottle, empty this into the tray. All rinse-water should now be in the white tray and should total 125 mL (or more if additional water was needed for rinse). **DO NOT DISCARD.**
20. Discard cobble/woody debris in the stream where it will not be sampled again during this bout, or on the stream bank. There is no composite sampling for this protocol.
21. Carefully swirl contents of tray (scrubbed material + rinse-water) to re-suspend the sample.
22. Carefully pour sample water into primed 140 mL syringe with a luer lock end cap attached. There may be some sand left in the bottom of the tray, this is ok. If you feel that the sample wasn't properly mixed, you may pour the sample back in the tray and swirl again.
23. Attach Sterivex® filter to end of 140 mL syringe. Filter ~50 mL of sample, or until filter becomes clogged.
    a. You do not need to filter all of the material in the syringe.
    b. Keep track of the volume filtered.
24. After filtering the sample, remove the filter from the syringe, pull in air, replace the filter and push air through the filter to dry prior to freezing. This may require 1-2 passes of air from the syringe.
25. Cap both ends of the capsule filter tightly with luer lock caps, place in a Whirl-pak® bag and flash-freeze on dry ice in the field.
26. Repeat Steps 23-25 for second Sterivex® filter (Figure 11).

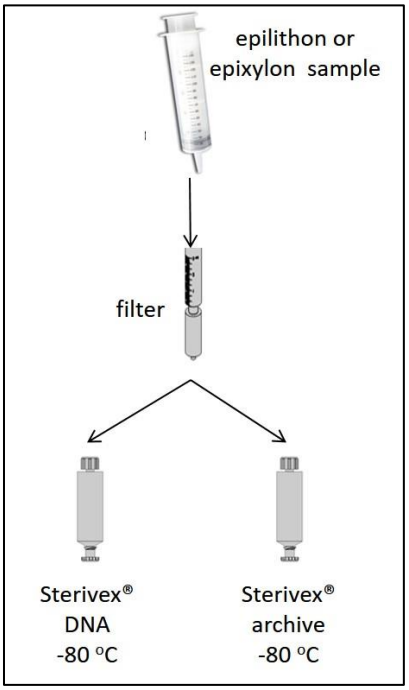

Figure 12. Schematic for benthic biofilm sampling via scrub slurry (SOP C).

27. Enter data in the mobile app. If not available, fill out field data sheet in pencil (Appendix F, RD[05]).
    a. If available, scan the barcode label with the tablet.
    b. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
28. Rinse tray, template, and syringe with stream water and field sterilize with ethanol and quickly shake to air dry before moving to the next sampling location.
29. Process remaining cobbles/woody debris in the reach one at a time.
30. Discard sampled cobble/woody debris in stream or on the bank before you leave the site.
31. Recheck labels and place all sample filters on dry ice. Use resealable bags or Whirl-paks® to separate filters by DNA or archive from each sample.

C.4 Epilithon (Rock Scrubs) and Epixylon (Wood Scrubs) – Large Substrate

This section is for epilithon and epixylon on substrates that you are not able to pick up to sample, e.g., boulder, bedrock, and large woody debris. Note that this sample is NOT a composite.

1. Fill out labels in pencil (all-weather adhesive labels, Figure 3, RD[05]), used barcode labels if available
    a. **Wadeable streams**, dominant habitat: 5 labels
    b. **Wadeable streams**, secondary habitat: 3 labels
2. **Wear gloves.** Replace gloves between habitat types.

a. Field-sterilize gloves with alcohol wipes after sampling at a location and before moving to the next location in the same habitat type.
b. Field-sterilized materials can be contained in a specified zip-top bag for field-cleaned items, and not mixed with lab-cleaned or sterile items.

3. A clean, sterile tray or tote is recommended for setting equipment on the stream bank (Figure 11).
4. Use the large substrate sampler and modified toothbrush for sampling. Wipe the inside of the sampler with an ethanol wipe prior to sampling. Use a new toothbrush for each sample.
5. Prime all field equipment, including the turkey baster, with stream water and set in a clean location (e.g., tray, tote, or new plastic bag).
6. Rinse the inside of the amber wide-mouth HDPE sample bottle with native water. Fill bottle ~1/4 full, cap, and shake vigorously. Discard rinse water into stream or onto the bank. Rinse each bottle 3 times. Recap bottle and set aside.
7. Select a sampling location that is shallower than the PVC tube on the sampler. Choose substratum that is free of plants/bryophytes and minimal macroinvertebrate colonization.
8. Fill the 125 mL wash bottle to the fill line with stream water.
   a. You may use as much rinsewater as you need, as long as you keep track of the volume used and record in the mobile application.
9. Place the PVC periphyton sampler tightly on the substratum to be sampled. Hold the sampler as allowing it to slip will affect the benthic area sampled.
10. Using a modified toothbrush (Figure 13 A), scrub the substratum within the PVC tube.

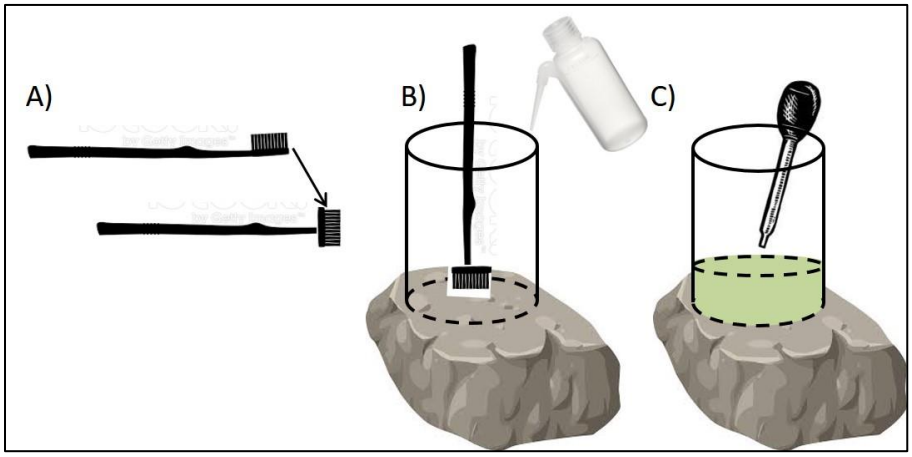

Figure 13. Schematic of PVC periphyton sampler. A) Cut off the head of a toothbrush, and re-adhere it horizontally. B) Set the PVC sampler on the substrate, and scrub the substrate surface using the modified toothbrush. Rinse rock surface. C) Remove water + algae sample with a turkey baster or disposable pipet.

11. When finished scrubbing, use the turkey baster to pipet the sample out of the PVC tube. Place the sample directly into the sample bottle.
12. Pipet all of the scrubbed sample out of the PVC tube. Before moving the tube, rinse the sides of the tube and the substratum at least once with the wash bottle, and pipet into the sample bottle.

Continue to rinse and pipet until it appears that the water is relatively clear and free of scrubbed algae.

13. When finished removing the sample from the PVC tube, remove the tube from the stream.
14. Ensure that the amount of water in the sample bottle is known. You may add the remainder of the wash bottle directly to the sample bottle if necessary.
15. Carefully pour sample water into primed 140 mL syringe with a luer lock end cap attached.
16. Attach Sterivex® filter to end of 140 mL syringe. Filter ~50 mL of sample, or until filter becomes clogged.
    a. You do not need to filter all of the material in the syringe.
    b. Keep track of the volume filtered.
17. After filtering the sample, remove the filter from the syringe, pull in air, replace the filter and push air through the filter to dry prior to freezing. This may require 1-2 passes of air from the syringe.
18. Cap both ends of the capsule filter tightly with luer lock caps, place in a Whirl-pak® bag and flash-freeze on dry ice in the field.
19. Repeat steps above for second Sterivex® filter (Figure 11).
20. Enter data in the mobile app. If not available, fill out field data sheet in pencil (Appendix F, RD[05]).
21. If available, scan the barcode label with the tablet.
22. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
23. Rinse all equipment well with stream water prior to moving to the next sampling location.
    a. Repeat above steps until all locations have been sampled.
    b. **Wadeable streams**, dominant habitat: 5 composite samples
    c. **Wadeable streams**, secondary habitat: 3 composite samples

C.5 Epiphytic (Aquatic Plant Surfaces) Microbes

1. Using 1”x2” adhesive labels (RD[05]), label one Whirl-pak® per sample (Figure 3). Use barcode labels if available.
    a. **Wadeable streams**, dominant habitat: 5 Whirl-paks®
    b. **Wadeable streams**, secondary habitat: 3 Whirl-paks®

2. **Wear gloves.** Replace gloves between habitat types.
    a. Field-sterilize gloves with alcohol wipes after sampling at a location and before moving to the next location in in the same habitat type.
    b. Field-sterilized materials can be contained in a specified zip-top bag for field-cleaned items, and not mixed with lab-cleaned or sterile items.
3. Select five plants from the dominant habitat or three from the secondary habitat. Select plants for sampling that are well-colonized with epiphytes (Figure 14) and that meet both the requirements in SOP C.1 AND the following:
    a. Sample from only 1 plant species to standardize sampling.
    b. The plant species should be common (i.e., accounts for >50% of the aquatic plants) in the reach throughout the year.

c. Plants should not be covered by sediments.

4. Using sterile scissors, cut approximately 5 cm length of heavily colonized plant (include leaves and stem if present).
   a. Sample only portions of plant that are underwater.
   b. Reuse scissors from sample to sample. Field-sterilize with alcohol pads after sampling and prime with local stream water at the next sampling location.
   c. Be gentle with the plants as epiphyton/sample material may be easily dislodged.

Figure 14. Example of epiphytes growing on bulrush in a Colorado stream.

5. Place plant material in a Whirl-pak®. Do not add water to sample.
6. Label outside of Whirl-pak®. Use a barcode label if available.
7. Close Whirl-pak® bag and flash-freeze on dry ice. Place all Whirl-paks® from the same site in one plastic bag so they are organized and not damaged by dry ice.
   a. Close the Whirl-pak® by holding the wire tabs at either side of the bag (Figure 15), then whirl the bag at least 3 complete revolutions to form leak-proof seal. Rather than whirling, you may also fold the top over as tightly as possible at least 3 times. Bend the wire ends over onto the bag to complete.

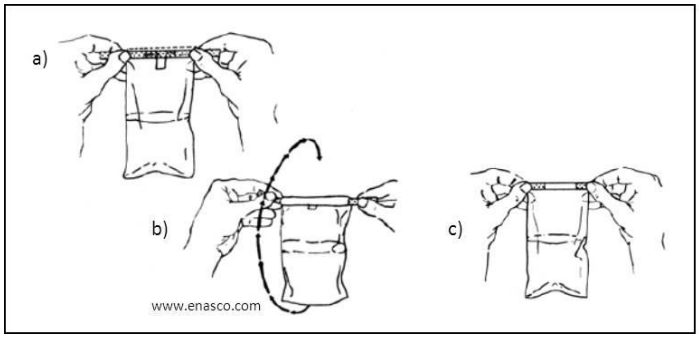

Figure 15. How to close a Whirl-pak® bag: a) hold the wire tabs; b) whirl the bag 3 complete revolutions (or fold the top over); and c) fold the wire ends over to close.

8. Enter data in the mobile app. If not available, fill out field data sheet in pencil (Appendix F, RD[05]).
   a. If available, scan the barcode label with the tablet.
   b. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
9. Repeat above steps until 5 samples (if this is the dominant habitat) or 3 (if this is the secondary habitat) have been collected.
10. No further filtering or processing occurs at the domain lab. Freeze samples at -80 °C and send to external lab for extraction (similar to TOS soil microbe samples, RD[12]).

C.6 Epipsammon (Sand) and Epipelon (Silt) Microbes

1. Using 1”x2” adhesive labels (RD[05]), label one sterile 15 mL plastic tube per sample (Figure 4). Use barcode labels if available. **NOTE:** Tubes may NOT be reused from the last sampling trip.
   a. **Wadeable streams**, dominant habitat: 5 tubes/labels
   b. **Wadeable streams**, secondary habitat: 3 tubes/labels
2. **Wear gloves.** Replace gloves between habitat types.
   a. Field-sterilize gloves with alcohol wipes after sampling at a location and before moving to the next location in in the same habitat type.
   b. Field-sterilized materials can be contained in a specified zip-top bag for field-cleaned items, and not mixed with lab-cleaned or sterile items.
3. Select five locations from the dominant habitat or three from the secondary habitat. Select locations to sample that meet the requirements in SOP C.1.
4. Note the dominant substratum size class (silt or sand) at the sampling location in the mobile app.
5. Fill the 125 mL wash bottle with 0.2 µm filtered DI if needed for rinsing.
6. Uncap sterile centrifuge tube and carefully press the tube into the upper 3 cm of substrate (like a cookie cutter). Take care not to disturb the substratum before placing tube on bottom. If substratum is disturbed, find a new sampling location.
7. Prime spatula and/or gloves with source water.
8. Slide spatula under tube to enclose the sample. Holding the tube tightly to the spatula, lift out of water. Gently rinse excess silt not enclosed by tube from spatula. It is okay if minimal sample is lost.

a. You may also use a clean, gloved hand instead of the spatula.

9. Invert tube, carefully slide spatula from top of tube and cap tightly. Take care not to spill sample. You may use your gloved fingers to help get the sample into the bottle from the spatula.
   a. No filtering occurs with these samples, these are whole samples that will be extracted at the external lab.
   b. These are not composite samples.
10. Enter data in the mobile app. If not available, fill out field data sheet in pencil (Appendix F, RD[05]).
11. Field-sterilize prior to moving to next sampling location.
12. Repeat steps 1-11 until samples have been collected from all selected locations.
13. Recheck labels and place all sample filters on dry ice. Use resealable bags to contain all samples from the same habitat.
14. No further filtering or processing occurs at the domain lab. Freeze samples at -80 °C and send to external lab for extraction (similar to TOS soil microbe samples, RD[12]).

C.7 Sample Preservation

CELL COUNT SAMPLES

1. If filtered formaldehyde was not already added to the 20 mL scintillation vials in the lab (SOP A), add 2 mL of 0.2 µm (Acrodisc® filter) filtered buffered formaldehyde to cell counts samples using a 3 mL syringe or pipet for every 18 mL of sample.
   a. Maximum time to process and preserve cell counts = 4 hours.
2. Recap bottle, and invert to mix ~3 times.
3. Put samples in cooler with ice packs. Do not freeze.
4. Chill sample at 4°C upon return to lab. Samples may be stored at 4°C for a maximum of 60 days to allow for bundling of two months-worth of samples into one shipment (streams only as lakes/river sample every other month).

STERIVEX® FILTERS

1. Flash-freeze capped Sterivex® filters on dry ice in the field immediately after collection.
2. Store filters at -80 °C upon return to lab. Filters may be held at -80 °C until shipping.

WHOLE SEDIMENT (EPIPSAMMON, EPIPELON) OR PLANT (EPIPHYTON) SAMPLES

1. Flash-freeze whole samples on dry ice in the field immediately after collection.
2. Store whole samples at -80 °C upon return to the lab. Samples may be held at -80 °C until shipping.

C.8 Ending the Sampling Day

1. Sterilize and dry equipment and place in new, clean zip-top bags for the next sampling bout.

a. Having a microbe-dedicated set of peristaltic pump tubing, connector, and c-flex tubing may be logistically helpful, or you can sterilize and reuse the tubing used for water chemistry.

2. Refreshing the sampling kit
   a. Replace Whirl-pak® bags and filters, nitrile gloves, luer locks, resealable bags, toothbrushes, etc. Refer to equipment list in Section 6.1.
   b. Discard and replace any broken templates and used toothbrushes.
   c. Restock the cell count preservation vials.
3. Equipment maintenance, cleaning and storage
   a. Charge drill pump batteries.
   b. Wash all equipment that has come in contact with site water and does not need to be sterilized according to the NEON Aquatic Decontamination Protocol (RD[08]).
   c. Dry all items completely before storing.

SOP D Laboratory Sampling and Analysis

Surface water microbes

If inclement weather prevents field filtering and preservation, surface water samples may be collected in bulk and processed back at the Domain Support Facility following the field standard operating procedure outlined in SOP C.7. Samples must be processed within 4 hours of collection.

All microbe samples

Samples should be stored as outlined in preservation, SOP C.7.

SOP E Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription error and improve data quality. If available, adhesive barcode labels should be used and scanned into the mobile application. Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL).

Given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

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

SOP 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 NEON's CLA intranet site.

Shipments are to include a hardcopy of the "per sample" tab of the shipping inventory (RD[13]) 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 Cell Count Shipping

1. Cell count samples must not freeze. Take care to avoid shipping at times when the samples may be subject to sitting for long periods in a frozen warehouse (e.g., note lab weekend and holiday receiving schedule).
2. Add parafilm around the cap of the cell count vials prior to shipping to minimize leaks.
3. Place scintillation vials into one or several gallon-size resealable zip-top bags, grouped by site.
4. Line a cardboard box or 9 qt cooler with a plastic bag to prevent leaks.
    a. Using a Styrofoam lined box or cooler may help regulate temperature.
5. Place all vials right-side up inside the liner bag. Add packing material (Vermiculite or other) to take up excess space in container and cushion samples.
    a. If using 9 qt coolers, include return shipping label for external lab to send cooler back.
6. Include shipping inventory/manifest in additional zip-top bag.
7. Mark the outside of package "Refrigerate on Arrival".
8. Ship overnight on ice or ice packs. Formaldehyde in these concentrations is not considered hazardous.

F.2 Filters, Plant, and Sediment Sample Shipping

1. All filters and whole samples must remain frozen at -80 ^{o}C. Do not ship on Fridays and ensure that the receiving lab will be open when the shipment arrives (e.g., take note of holiday schedules).
2. Place filters and samples in resealable zip-top bags grouped by site/date.
3. Place inside small dry-ice shipper.
4. Keeps filters/bags from directly touching dry ice using cardboard or additional bags.
5. Include shipping inventory/manifest in additional zip-top bag.
6. Follow instructions for shipping overnight on dry ice in AD[03].

F.3 Handling Hazardous Material

Formaldehyde in the concentration and volume shipped by NEON for this protocol is not considered hazardous.

F.4 Supplies/Containers

See sections F.1, F.2, and

Table **8** for specific shipping materials.

F.5 Timelines and Conditions

1. **Cell counts**: Formaldehyde-preserved samples may be stored at 4 °C for up to 60 days. Do not freeze.
   a. Cell count samples from 2 consecutive months of sampling should be bundled for shipping. Samples must be received by the lab by the Friday of the shipping week to ensure that they will be included in the next analytical run.
2. **Sterivex® filters**: Filters may be stored at -80 °C until shipping.
   a. Frozen samples may be held for up to 6 months if the lab is not able to receive samples (Table 1). Shipping may occur on the same schedule as TOS microbial soil shipping.
3. **Whole samples (plants, sediment):** Samples may be stored at -80 °C until shipping.
   a. Frozen samples may be held for up to 6 months if the lab is not able to receive samples (Table 1). Shipping may occur on the same schedule as TOS microbial soil shipping.

F.6 Grouping/Splitting Samples

Group samples by site per bout.

F.7 Return of Materials or Containers

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

F.8 Shipping Inventory

Shipments are to have a hardcopy of the shipping inventory (RD[13]) 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.9 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on NEON's CLA intranet site.

8 REFERENCES

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 11. Datasheets associated with this protocol

NEON Doc. #	Title	Mobile Application
NEON.DOC.003041	Datasheets for AOS Protocol and Procedure: Aquatic Microbial Sampling	(AOS) Water Chemistry [PROD] *(use for surface microbes)* (AOS) Algae – Field [PROD] *(use for benthic microbes)*
NEON.DOC.001646	General AQU Field Metadata Sheet	(AOS) Field Metadata and Gauge Height [PROD]
NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory	Shipping App [PROD]
NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling	(AOS) Secchi [PROD]

Datasheets can be found in Agile or the NEON Document Warehouse, user guides for mobile applications may be found in NEON's internal sampling support library.

APPENDIX B QUICK REFERENCES

B.1 Microbe Sampling Preparation

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

Step 2 – Makes sure equipment has been sterilized.

Step 3 – Prepare adhesive labels and barcode labels.

Step 4 – Ensure the General AQU Field Metadata app is completed per field site visit and Secchi and Depth Profile app in lakes and rivers (RD[17]).

Step 5 – Determine habitat sampling locations and sampler type.

1. In **wadeable streams**:
   a. Sample **surface water** near the S2 sensor, same location as water chemistry.
   b. Determine **benthic** habitat from the Stream Morphology Map (RD[11]) or Rapid Habitat Assessment and periphyton sampling locations.
   c. Determine sampler type based on the habitats present and the order of preference for sampling substratum.
2. In **lakes and rivers**:
   a. Sample **surface water** at the sample locations as water chemistry.
   b. Determine the depths of the thermocline and the euphotic zone.
   c. Determine sampling depths (same as water chemistry). If the site is stratified, collect 1 sample from the epilimnion and 1 sample from the hypolimnion at the buoy location.

B.2 Steps for Surface Water Microbe Sampling

Step 1 – Collect samples:

1. Wear gloves
2. In **wadeable steams**, collect in the thalweg using 4 L jugs.
3. In **lakes and rivers**, collect samples using the Kemmerer and integrate the samples in a 4 L jug.

Step 2 – Process the samples

1. Wear gloves
2. Cell counts:
   a. Collect in 20 mL glass scintillation vial.
   b. Add 2 mL of 0.2 µm (Acrodisc® filter) filtered buffered formaldehyde.
   c. Chill sample at 4°C upon return to lab.
   d. Ship on ice overnight, but do not freeze.
3. Sterivex® filters (2 subsamples):
   a. Filter >500 mL of stream water through each capsule filter (2 filters total) until filter clogs.

b. Flash-freeze on dry ice in the field.
c. Freeze at -80 °C upon returning to the lab.
d. Ship on dry ice overnight.

B.3 Steps for Benthic Biofilm Sampling in Wadeable Streams

Step 1 – Determine habitat and sampling method.

Step 2 – Collect samples:

1. **Wear gloves**.
2. Epilithic (rock scrubs) and epixylic (wood scrubs) microbes:
   a. Scrub the cobbles or wood into a larval tray and add to 140mL syringe
   b. Sterivex® filters:
      i. Filter ~50 mL through each capsule filter (2 filters total).
      ii. Flash-freeze on dry ice in the field.
      iii. Freeze at -80 °C upon returning to the lab.
      iv. Ship on dry ice overnight.
3. Epipsammic (sand) and epipelic (silt) microbes:
   a. Collect samples in a centrifuge tube.
   b. Flash-freeze on dry ice in the field.
   c. Freeze samples at -80 °C upon returning to the lab.
   d. Ship on dry ice overnight.
4. Epiphytic (aquatic plant surfaces) microbes:
   a. Cut approximately 5 cm length of heavily colonized plant.
   b. Place in a Whirl-pak®
   c. Flash-freeze on dry-ice in the field.
   d. Freeze samples at -80 °C upon returning to the lab.
   e. Ship on dry ice overnight.

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare all equipment including labels and barcode labels.
- ☑ Pre-print adhesive labels.
- ☑ Sterilize equipment.

Sample collection: Be sure to…

Surface microbes

- ☑ Field-sterilize equipment between samples. Sterilize all equipment that is reused between sampling bouts.
- ☑ Wear clean nitrile gloves.
- ☑ Filtering a second 4 L bottle may be required for very clear water to collect all samples.
- ☑ Preserve cell count samples with formaldehyde in the field, flash freeze Sterivex® filters in the field.

Benthic microbes in wadeable streams

- ☑ Field-sterilize equipment between samples. Sterilize all equipment that is reused between sampling bouts.
- ☑ Determine the dominant habitat and second-most dominant habitat based on the Stream Morphology Map (RD[11]) or Rapid Habitat Assessment. Sample in same locations as periphyton.
- ☑ Choose the appropriate sampler.
- ☑ Start sampling at the bottom of the reach, working upstream so as not to decrease visibility and disrupt benthic biofilm communities.
- ☑ Spread samples out along the reach.
- ☑ For epilithon/epixylon samples, keep the sample volume consistent and record the volume of water used. Do not discard rinse water from the larval tray as this is your sample.
- ☑ Do not sample anywhere you or other field people/animals have walked, or locations that appear recently disturbed.

Sample processing: Be sure to…

- ☑ Filtering can be conducted in the domain lab in cases of inclement weather within 4 hours of collection provided 4L jugs are appropriately labeled.
- ☑ Keep track of the volume of sample filtered.
- ☑ DO NOT FREEZE cell count samples.
- ☑ Flash-freeze filters and grab samples on dry ice in the field.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for benthic biological sampling bouts in **wadeable streams**. Surface water samples follow monthly water chemistry sampling dates. General guidelines for developing these dates are presented in the NEON Aquatic Sample Strategy Document (RD[07]). Also see the Site Specific Sampling Strategy Document on NEON's FOPS intranet site. Benthic samples during Bout 2 will be marked for metagenomics analysis.

Domain	Site	Bout 1	Bout 2	Bout 3
D01	Hop Brook	11Apr-9May	9Jul-6Aug	3Oct-31Oct
D02	Lewis Run	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D02	Posey Creek	19Mar-16Apr	5Jul-2Aug	18Oct-15Nov
D04	Rio Guilarte	26Jan-23Feb	21Jun-19Jul	9Nov-7Dec
D04	Rio Cupeyes	24Jan-21Feb	21Jun-19Jul	10Nov-8Dec
D06	Kings Creek	23Mar-20Apr	3Jul-31Jul	3Oct-31Oct
D06	McDiffett Creek	23Mar-20Apr	3Jul-31Jul	3Oct-31Oct
D07	Leconte Creek	15Mar-12Apr	30Jun-28Jul	12Oct-9Nov
D07	Walker Branch	9Mar-6Apr	1Jul-29Jul	19Oct-16Nov
D08	Mayfield Creek	5Mar-2Apr	29Jun-27Jul	31Oct-28Nov
D10	Arikaree River	21Mar-18Apr	4Jul-1Aug	20Sep-18Oct
D11	Pringle Creek	17Feb-17Mar	29Jun-27Jul	23Oct-20Nov
D11	Blue River	7Mar-4-Apr	1Jul-29Jul	12Oct-9Nov
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	12Jan-11Feb	24Mar-23Apr	3Jun-3Jul
D15	Red Butte Creek	29Mar-26Apr	6Jul-3Aug	29Sep-27Oct
D16	McRae Creek	10Apr-8May	11Jul-8Aug	23Sep-21Oct
D16	Martha Creek	6Apr-4May	5Jul-2Aug	22Sep-20Oct
D17	Teakettle 2 Creek	14Apr-12-May	8Jul-5-Aug	23Sep-21Oct
D17	Upper Big Creek	14Apr-12-May	8Jul-5-Aug	23Sep-21Oct
D18	Oksrukuyik Creek	21May-18Jun	29Jun-27Jul	7Aug-4Sep
D19	Caribou Creek	2May-30May	26Jun-24Jul	18Aug-15Sep

APPENDIX E SITE-SPECIFIC INFORMATION: HABITAT AND SUBSTRATA RECOMMENDATIONS

See the Site Specific Sampling Strategy Document on NEON's FOPS intranet site.

Domain	Site	Benthic habitat 1 (5 reps)	Benthic habitat 2 (3 reps)	Surface water
D01	Hop Brook	Riffle (epilithon)	Pool (epipsammon)	ss (S2)
D02	Lewis Run	Run (epipelon)	Riffle (epilithon)	ss (S2)
D02	Posey Creek	Riffle (epilithon)	Pool (epipelon)	ss (S2)
D03	Flint River	NA	NA	c0
D03	Lake Barco	NA	NA	c0
D03	Lake Suggs	NA	NA	c0
D04	Rio Guilarte	Riffle (epilithon)	Pool (epilithon)	ss (S2)
D04	Rio Cupeyes	Riffle (epilithon)	Run (epilithon)	ss (S2)
D05	Crampton Lake	NA	NA	c1, c2
D05	Little Rock Lake	NA	NA	c1, c2
D06	Kings Creek	Riffle/run (epilithon)	Pool (epilithon)	ss (S2)
D06	McDiffett Creek	Riffles (epilithon)	NA	ss (S2)
D07	Leconte Creek	Riffle (epilithon)	Pools (epipsammon)	ss (S2)
D07	Walker Branch	Riffle (epilithon)	Runs (epixylon)	ss (S2)
D08	Mayfield Creek	Riffle/run(epixylon)	Run (epipsammon)	ss (S2)
D08	Black Warrior River	NA	NA	c0
D08	Tombigbee River	NA	NA	c0
D09	Prairie Lake	NA	NA	c0
D09	Prairie Pothole	NA	NA	c0
D10	Arikaree River	Run (epiphyton)	Pool/Run (epipsammon)	ss (S2)
D11	Pringle Creek	Run (epipsammon)	Riffle (epilithon)	ss (S2)
D11	Blue River	Run (epilithon)	Riffle (epilithon)	ss (S2)
D12	Blacktail Deer Creek	Riffle (epilithon)	Run (epilithon)	ss (S2)
D13	Como Creek	Riffle/Step pool (epilithon)	Run (epipsammon)	ss (S2)
D13	West St. Louis Creek	Riffle (epilithon)	Pool/Step pool (epipsammon)	ss (S2)
D14	Sycamore Creek	Riffle/Run (epilithon)	Pool (epipsammon)	ss (S2)
D15	Red Butte Creek	Step pool (epipelon)	Run (epilithon)	ss (S2)
D16	McRae Creek	Step pool/Riffle (epilithon)	Step pool/Run (epilithon)	ss (S2)
D16	Martha Creek	tbd	tbd	ss (S2)
D17	Teakettle 2 Creek	tbd	tbd	ss (S2)
D17	Upper Big Creek	tbd	tbd	ss (S2)
D18	Oksrukuyik Creek	Run (epilithon)	Riffle (epipelon)	ss (S2)
D18	Toolik Lake	NA	NA	c1, c2, in, ot
D19	Caribou Creek	Riffle (epilithon)	Pool (epipsammon)	ss (S2)

APPENDIX F PAPER DATASHEET EXAMPLES

See also RD[05] for blank paper datasheets.

NEON Microbe Collection

Site ID: *POSE*
Date: *2016-09-29*
Local time: *13:15*

Sampling protocol: *NEON.DOC.003044* **Rev:** *B*
Recorded by: *sparker@battelleecology.org*
Collected by: *jstewart@Field-ops.org*

BENTHIC

Named location	Sampling impractical	Habitat type	Substratum size class	Sample type	Sample number	Field Sample volume (mL)	Sample ID	DNA filter volume (mL)	Archive filter volume (mL)
re		*riffle*	*cobble*	*epilithon*	*1*	*125*	*POSE.20160929.epilithon.1*	*45*	*30*
re		*riffle*	*cobble*	*epilithon*	*2*	*125*	*POSE.20160929.epilithon.2*	*50*	*50*
re		*riffle*	*cobble*	*epilithon*	*3*	*250*	*POSE.20160929.epilithon.3*	*40*	*40*
re		*riffle*	*cobble*	*epilithon*	*4*	*250*	*POSE.20160929.epilithon.4*	*48*	*45*
re		*riffle*	*pebble*	*epilithon*	*5*	*250*	*POSE.20160929.epilithon.5*	*50*	*50*
re		*pool*	*silt*	*epipelon*	*1*	*NA*	*POSE.20160929.epipelon.1*	*NA*	*NA*
re		*pool*	*silt*	*epipelon*	*2*	*NA*	*POSE.20160929.epipelon.2*	*NA*	*NA*
re		*pool*	*silt*	*epipelon*	*3*	*NA*	*POSE.20160929.epipelon.3*	*NA*	*NA*

NEON Microbe Collection

SURFACE WATER

Named location	Sampling impractical	Sample type	Sample number	Sample ID	Sample depth 1 (m) [lake/river]	Sample depth 2 (m) [lake/river]	DNA filter volume (mL)	Archive filter volume (mL)	Cell count sample volume (mL)	Cell count preservative volume (mL)
ss		*seston*	*1*	*POSE.20160929.seston.1*			*500*	*425*	*18*	*1.8*