AOS PROTOCOL AND PROCEDURE: MICROBES IN LAKES AND NON-WADEABLE STREAMS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	02/10/2014	ECO-01178	Initial release
B	08/29/2014	ECO-02210	Minor updates based on feedback from the field
C	01/09/2015	ECO-02621	Migration to new protocol template
D	06/01/2015	ECO-02697	Minor updates to equipment, removed lake sampling depth information (refer to lake water chemistry), updates to shipping and labeling, flash-freezing filters in the field, and removal of RNAlater preservation.

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1 OVERVIEW

1.1 Background

This document describes the required protocols for conducting field sampling of microbes in lakes and non-wadeable streams. 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 and so concurrent sampling ensures comparison between drivers and effects. Microbes also form biofilms in stream beds which are important to the productivity of the system. Collecting basic measures of biomass, enzymatic activity, and DNA will enable researchers and managers to monitor 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 into and between lakes, and large storm events can greatly 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 Sampling Strategy (RD[08]). It is therefore very 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 technique 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

This protocol is based on collection techniques used by the Arctic and Antarctic LTER programs and Dr. Byron Crump, University of Maryland Center for Environmental Studies Horn Point Laboratory. Collection techniques have been standardized to be consistent with the NEON lake water chemistry protocol.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

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

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.002194	Datasheets for AOS Protocol and Procedure: Microbes in Lakes and Non-Wadeable Streams
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling
RD[08]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[09]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[10]	NEON.DOC.001190	AOS Protocol and Procedure: Water Chemistry Sampling in Lakes and Non-Wadeable Streams
RD[11]	NEON.DOC.001196	AOS Protocol and Procedure: Stream Riparian Mapping
RD[12]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[13]	NEON.DOC.014048	TOS Protocol and Procedure: Soil Physical, Chemical, and Microbial Measurements
RD[14]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

2.3 Acronyms

Acronym	Definition
^{o}C	Degrees Celsius
CTD	Conductivity, temperature, and depth sensor
DI	De-ionized water
DNA	Deoxyribonucleic acid
HDPE	High Density Polyethylene
L	Liter
LTER	Long Term Ecological Research
m	Meter
mL	Milliliter
µm	Micrometer
mm	Millimeter
mRNA	Messenger RNA
PFD	Personal flotation device
qt	Quart
RNA	Ribonucleic acid
s	Second

2.4 Definitions

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

Euphotic zone: The depth to which sufficient light for photosynthesis penetrates (0.5-1% of light).

Hypolimnion: The dense bottom layer of a stratified lake that sits below the thermocline. This layer is denoted by cooler summer temperatures and slightly warmer winter temperatures relative to the epilimnion.

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

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

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

Stratified: Indicating the presence of a thermocline.

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 defined by the hypolimnion. The warmer upper layer is termed the epilimnion.

Thalweg: The line of least resistance to water flow in a stream or river, often the line of maximum water velocity.

3 METHOD

The field protocol used by NEON for collecting aquatic surface water microbe samples in lakes and non-wadeable streams follows the Arctic and Antarctic LTER standard operating procedures. Samples are taken at the same time and location as water chemistry samples, which are determined by the size and depth of the lakes and non-wadeable streams. Sampling is co-located with Lake Water Chemistry (RD[10]). Aquatic microbe sampling in lakes and non-wadeable streams occurs 6 times per year on the same day each month, where possible, at each NEON location based on statistical analysis of environmental parameters. Details on sampling locations are provided in the Lake Water Chemistry (RD[10]) protocol. Microbial samples are collected both as whole sample (water or substratum) and on filters for archival and analysis.

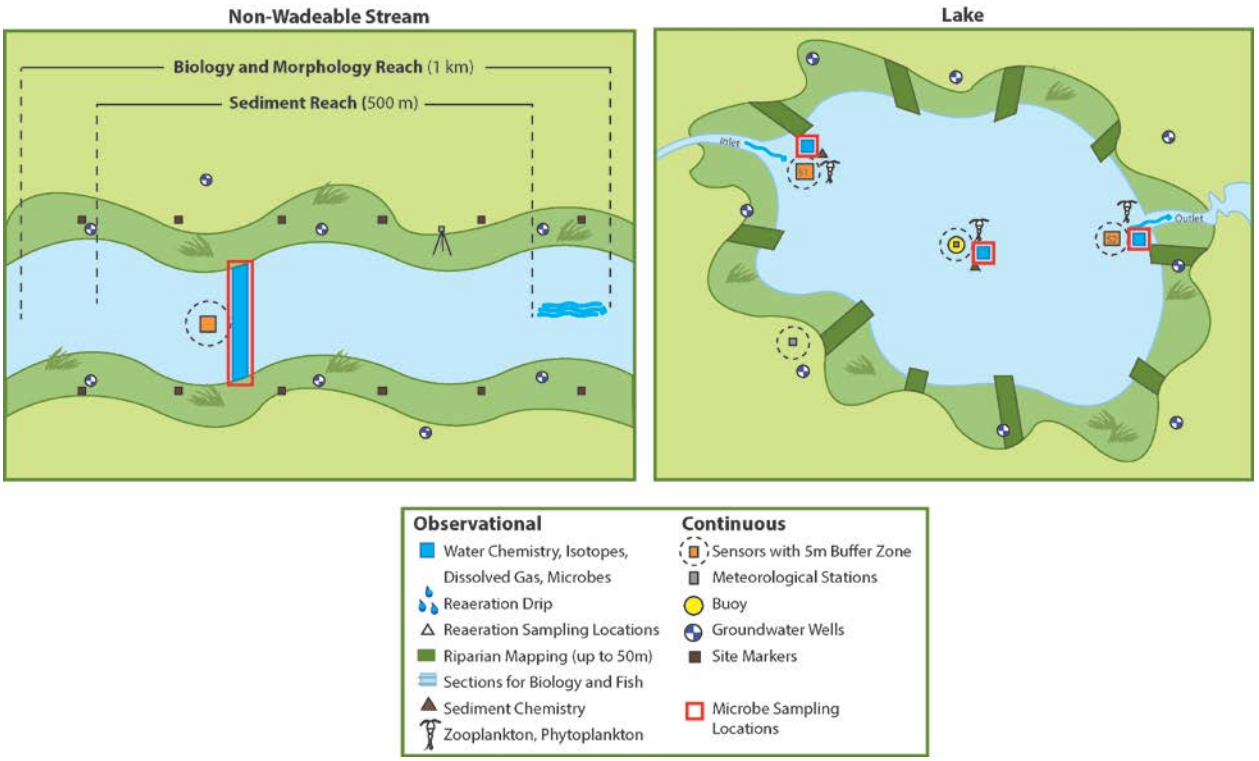


Figure 1. Generic site layouts for lakes and non-wadeable streams with microbe sampling locations

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

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

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

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Surface water microbes in lakes and non-wadeable streams will be collected up to 6 times each year. Surface microbe samples are collected at the same time and location as monthly water chemistry samples on a bi-monthly basis (RD[10]). Microbial samples may be collected either before or after chemistry sample collection as long as the water column remains undisturbed.

4.2 Criteria for Determining Onset and Cessation of Sampling

Sampling will occur every other month along with monthly water chemistry sampling, and will be collected under the ice at north sites.

4.3 Timing for Laboratory Processing and Analysis

Filtered samples must be flash-frozen in the field and may be held at the domain lab at -80 ^{o}C for up to 30 days before shipping. Cell count samples must be preserved in formalin in the field and may be held at the domain lab at 4 ^{o}C for up to 7 days before shipping. For additional storage and shipping timelines see SOP E.

4.4 Sampling Timing Contingencies

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

Table 1. Contingent decisions

Delay/Situation	Action	Outcome for Data Products
Hours	If circumstances occur that impede sampling (e.g., wildlife, weather), discard samples and start over the next day that conditions permit.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.
	If circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes

	the weather requirements below, continue to collect samples.	sampling to occur outside of the sampling window, data must be flagged.
	If weather conditions deteriorate and the lake/non-wadeable stream becomes too windy (>9 km hr^{-1}) to hold the boat stationary over a sampling point, return to shore and wait in a safe location for 30 minutes. If wind subsides, resume sampling, if not, return to the Domain Support Facility and sample at another time.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data must be flagged.

4.5 Sampling Specific Concerns

1. Sampling too soon after a disturbance event (e.g., flooding) can dramatically decrease biomass and diversity.
2. Should it begin to rain during microbial sampling, collect water samples in a 4-L jug and return the container on ice to the lab or base-camp where samples can be filtered within 3-4 hours.
   a. Water jugs must be shaken before sub-sampling or filtration to re-suspend particulates and homogenize water. If at any point you believe contamination has occurred, discard samples and resample.
3. Care must be taken to avoid contaminating the sample with re-suspended bed sediment. Such contamination may be minimized by anchoring the boat upwind (or upstream) of the sampling site, and using an anchor line 3 times as long as the depth of the lake or stream.
4. Equipment must be sterilized in the lab, and any equipment used for multiple samples in the field must be field-sterilized during sampling. Gloves are required to maintain sterility of the sampling equipment and to decrease contamination of microbial samples by human or cross-site microbes while in the field.
5. Take care to keeping track of the volume of water used for filtering, these data are very important for conversion to higher data products.
6. 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 SAFETY

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

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

field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

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

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

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

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 2. Equipment list – General equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Site-specific Bathymetry Map (RD[12])	Navigating to sampling locations	1	N
	R	Clipboard	Recording data	1	N
	R	Secchi and depth profile field data sheets (all-weather paper; RD[07])	Determining the depth of the euphotic zone	1	N
	R	Cooler (9 qt)	Keeping samples cool	1	N
	R	Cryogenic gloves	Handling dry ice	1 pair	N
	S	Ice auger	Sampling ice-covered lakes in winter	1	N
Consumable items					
	R	Aquatic field metadata sheet (RD[06])	Recording metadata	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Field data sheets (all-weather paper; RD[05])	Recording data	1	N
	R	Pencils	Recording data	2	N
	R	Permanent markers	Labeling samples	2	N
	R	Nitrile gloves, powderless	Keeping collection method sterile	10	N
	R	Resealable bags, gallon	Keeping collection method sterile	20	N
	R	Pre-printed adhesive labels (all-weather), 1”x2”	Labeling samples	1 sheet	N
	R	Ice or chemical ice packs	Keeping samples cool	2	N
	R	Dry ice	Flash-freezing filters in the field	0.5-1 kg	Y

R/S=Required/Suggested

Table 3. Equipment list – Sterilization equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Wash basin	Container for sterilization solutions	1	N
	R	Clean bench paper or paper towels	Storing equipment	1 roll	N
MX100373	R	Peristaltic pump head	Filtering DI water	1	N
MX100383	R	18V drill pump	Filtering DI water	1	N
MX100364	R	Peristaltic pump tubing	Filtering DI water with peristaltic pump	1	N
	R	Pieces of C-flex tubing, 4 feet and 2 feet in length	Filtering water with peristaltic pump	2	N
	R	Hose connector	Attaching C-flex to peristaltic tubing	1	N
	R	1 L HDPE bottle or 4 L HDPE jug with lid, sterilized	Container for filtered DI	1	N
Consumable items					
	R	ETOH, 70%	Sterilizing equipment	1 L	Y
	R	Phosphate-free detergent (Alconox or Liquinox)	Sterilizing equipment	<1 gallon	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX107386	R	0.2 µm Sterivex SVGP® 010 50 capsule filter	Filtering DI	2	N
	R	Deionized water	Rinsing equipment	4 gallons	N
	R	Gallon zip top bags	Storing equipment	5	N

R/S=Required/Suggested

Table 4. Equipment list – Sampling equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX100447	R	Secchi disk and weight	Determining the depth of the euphotic zone	1	N
	R	Braided polyester line, calibrated	Determining the depth of the euphotic zone	1	N
MX100393	R	Kemmerer sampler with rope and messenger	Collecting water	1	N
	R	Handheld GPS unit (with batteries, ± 1 m accuracy)	Navigating to sampling locations	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Handheld meter	Measuring water temperature for temperature profile	1	N
	R	Sterile 4 L HDPE jug	Integrating samples	2	N
Consumable items					
	R	Filtered DI (0.2 µm filter)	Field-sterilization and rinsing	1-4 L	N
	R	Alcohol wipes, individually-wrapped	Field-sterilization	20	N

R/S=Required/Suggested

Table 5. Equipment list – Filtering equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Pieces of C-flex tubing, 4 feet and 2 feet in length	Filtering water with peristaltic pump	2	N
	R	Hose connector	Attaching C-flex to peristaltic tubing	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	S	140 mL syringes	Alternate to peristaltic pump	3	N
	R	Filter adapters for tubing (cut-off 3 mL syringe)	Connecting peristaltic tubing and C-flex tubing	2	N
MX100373	R	Peristaltic pump head	Filtering water	1	N
MX100383	R	18V drill pump	Filtering water	1	N
MX100364	R	Peristaltic pump tubing	Filtering water with peristaltic pump	1	N
	R	500 mL plastic graduated cylinder	Measuring filtered water volume	1	N
	R	10 mL adjustable pipette	Pipetting cell count samples into vials	1	N
Consumable items					
	R	Zip tie, small or small hose clamp	Attaching C-flex tubing to hose connector	1	N
MX106249	R	20 mL scintillation vials (glass) with caps	Cell count sample container	3	N
MX106241	R	0.2 µm Sterivex SVGP® 010 50 capsule filter	DNA and RNA sample filters	6	N
	R	Luer lock male closures	Capping the Sterivex® filters	6	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Luer lock female closures	Capping the Sterivex® filters	6	N
	R	10 mL pipette tips	Pipetting cell count samples into vials	20	N

R/S=Required/Suggested

Table 6. Equipment list – Sample processing and preservation

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	3 mL HDPE syringe, luer lock end	Filtering formaldehyde	1	N
	R	Freezer (-80 °C)	Sample storage	1	N
Consumable items					
MX106257	R	10% formaldehyde, buffered (sodium borate or phosphate)	Preserving cell count samples	1 L	Y
MX106239	R	0.2 µm Acrodisc® filter	Filtering formaldehyde	2	N

R/S=Required/Suggested

Table 7. Equipment list – General boating equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Boat		1	Y
	R	Anchor with rope		2	N
	R	Oars		2	N
	R	Trolling Electric Motor		1	Y
	R	Battery (12 volt)		1	Y
	R	Safety kit for boat (e.g., flares, bailer, float with rope)		1	Y
	R	First Aid Kit		1	N
	R	Personal Flotation Devices (PFDs)		1 per person	N
Consumable items					
		(none)			

R/S=Required/Suggested

Table 8. Equipment list – Shipping materials

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Dry ice shipping container	Shipping filters	1	N
	R	Cooler, 9 qt.	Shipping cell counts	1	N
Consumable items					
	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	As needed	N
	R	Trash bag to line cooler (~13 gallon size)	Protecting against leaks	1	N
	R	Resealable plastic bags (gallon and quart size)	Protecting against leaks	6	N
	R	Dry Ice	Shipping filters	As needed	Y
	R	Ice or chemical ice packs	Shipping cell counts	As needed	N
	R	Shipping inventory (RD[14])	Provides sample information to external lab	1	N

R/S=Required/Suggested

6.2 Training Requirements

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

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

Personnel will be trained in the field protocols associated with this document, and trained in safe working practices for lake- and river-based field work. Technicians must also be trained in safe handling of formalin (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 plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

Field sampling requires two technicians for three hours in in addition to chemistry 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

1. Make sure all supplies are packed and ensure peristaltic pump and GPS batteries are charged and that the pump is in good working condition.
2. Sterilize any equipment that is reused between sampling bouts.
    a. Equipment to be sterilized includes: 4 L jug, 140 mL syringe, 125 mL wash bottle, graduated cylinder, 3/8" pump tubing adapter, brushes, slide template, larval tray, spatula, scissors, Kemmerer.
        1) Having an additional piece of peristaltic pump tubing, hose connector, and C-flex tubing that can be dedicated to microbe sampling (i.e., not used for water chemistry) may be easier for logistics.
    b. Wash in detergent solution.
        1) Prepare a 0.2% (v.v) solution of detergent and DI water in a wash basin.
        2) Wearing nitrile gloves, submerge equipment thoroughly in detergent solution. Use bottle brush and/or hands to clean equipment.
    c. Rinse with DI water.
        1) Remove equipment from detergent solution and rinse thoroughly in DI. Place on clean bench top paper.
    d. Rinse with 70% ETOH.
        1) Dilute 95% ETOH to 70%. Rinse all equipment in ETOH and allow to thoroughly dry (minimum drying time 5-10 minutes).
    e. When dry, cap bottles and place all other equipment in clean gallon Ziploc bags to transport to the field.
3. Prepare filtered DI for rinsing in the field.
    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. Attach pre-printed labels (Figure 3, RD[05]) to bottles and capsule filters (Figure 2), use permanent marker to fill out labels before going into the field.
    a. Site = 4-letter site code
    b. Date = YYYYMMDD
    c. Module = 'amc'
    d. Sample type = 'plankton'
    e. Replicate = '[1-3]'
    f. Sample ID = SITE.DATE.'amc'.sample type.replicate
5. Using the Acrodisc® 0.2 µm filter attached to a 3 mL syringe, add buffered formalin preservative to 20 mL vials for cell counts (0.9 mL per 18 mL sample) making sure to wear gloves.
6. Prepare coolers, frozen ice packs or water ice, and dry ice.

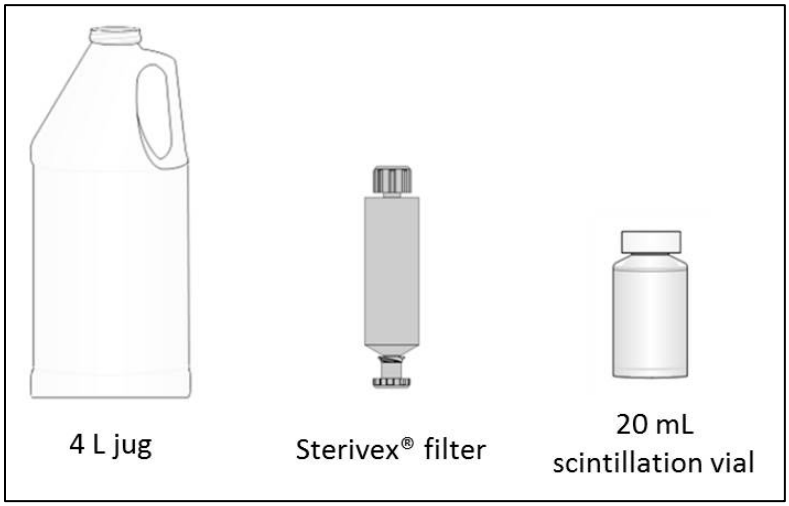


Figure 2. Sample bottles and capsule filter.

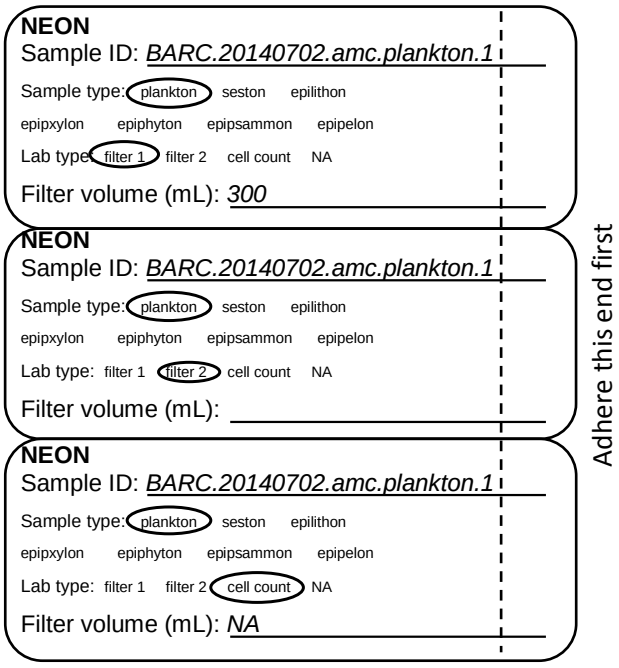


Figure 3. Example NEON microbes label. Adhere right side of label to vial first, label will overlap.

SOP B Field Sampling

Lakes will be sampled at three locations in conjunction with water chemistry samples: the central/deepest part of the lake ('C0', 'C1', 'C2', or 'C3'), the inlet ('IN'), and the outlet ('OT'). Non-wadeable streams will be sampled at one location in conjunction with water chemistry in the thalweg.

Fill out general aquatic field metadata sheet (RD[06]) and Secchi Depth and Depth Profile Sampling Datasheet (RD[07]) upon every field sampling visit.

B.1 Determining Thermocline and Euphotic Depth

1. Locate the deepest part of the lake or thalweg in non-wadeable streams using GPS coordinates and the bathymetry site map (RD[11], RD[12]). Thermocline and euphotic depth are only measured at the center location of the lake.
2. Position and anchor the boat. Carefully lower the anchor so as not to suspend the sediments. Such contamination may be minimized by anchoring the boat upwind (or upstream) of the sampling site, and using an anchor line 3 times as long as the depth of the lake or stream. If sediments are disrupted, wait until the area has cleared before sampling.
3. Determine if and where thermal stratification is occurring. Stratification occurs where the rate of decrease in temperature with increasing depth is greatest (usually >1 ^{o}C per 0.5 m depth change)
4. Put the calibrated handheld meter into the water and slowly lower through the water column at 0.5 m intervals to note depth and water temperature (for probe calibration instructions, refer to http://www.ysi.com/media/pdfs/038503-YSI-Model-85-Operations-Manual-RevE.pdf).
    a. Note the depth and water temperature every 0.5 m on the field data sheet(RD[07]).
    b. Thermal stratification occurs where the rate of decrease in temperature with increasing depth is greatest (usually >1 ^{o}C per 0.5 m depth change).
5. Determine the depth of the euphotic zone (depth to which 1% of light penetrates) using the Secchi disk.
    a. Lower the Secchi disk slowly into the water on the shady side of the boat (or cut the glare from the sun using your hand or other object) until the white quadrants disappear from view. NOTE: Do not wear sunglasses as this will interfere with the readings.
    b. Record depth read from the lines on the Secchi rope to the nearest 0.1 m on the Secchi depth field data sheet.
    c. Lower the Secchi disk approximately 0.5 m deeper than the first reading.
    d. Slowly pull the disk up until the white quadrants reappear, record depth to nearest 0.1 m on field data sheet as "Secchi 2".
    e. Take the mean of the two depths and record on the field data sheet (RD[07]).

Figure 4. Illustration of Secchi disk lowered into water with shading

f. Take microbe samples from the upwind side of the boat to lessen any contamination from the boat.

B.2 Selecting Sampling Depths

Samples should be taken at the same time and location as water chemistry samples (RD[10]). Refer to SOP A in AOS Protocol and Procedure: Water Chemistry Sampling in Lakes and Non-wadeable streams for specific sampling depth instructions (RD[10]).

1. Take one surface sample at 1 meter depth at each sampling location (center, inlet, and outlet).
2. At the center location: Is the lake thermally stratified?
   a. If NO, take an integrated sample from the epilimnion (location = "C0").
   b. If YES, take one integrated sample from the epilimnion (location = "C1" and one integrated sample from the hypolimnion (location = "C2").

B.3 Sampling Using the Kemmerer Sampler

1. Prepare Kemmerer sampler (Figure 5) for sampling by pulling 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.
2. Tie the free end of the Kemmerer line to a cleat on the boat to prevent losing the sampler.
3. 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.

4. 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.
5. Continue to lower the sample until it reaches the desired depth by using the depth markings on the line attached to the sampler.
6. 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.
7. Retrieve the sampler from the water column. Water is dispensed into the collection bottles through the spout (Figure 5a).
8. Repeat for each sample.

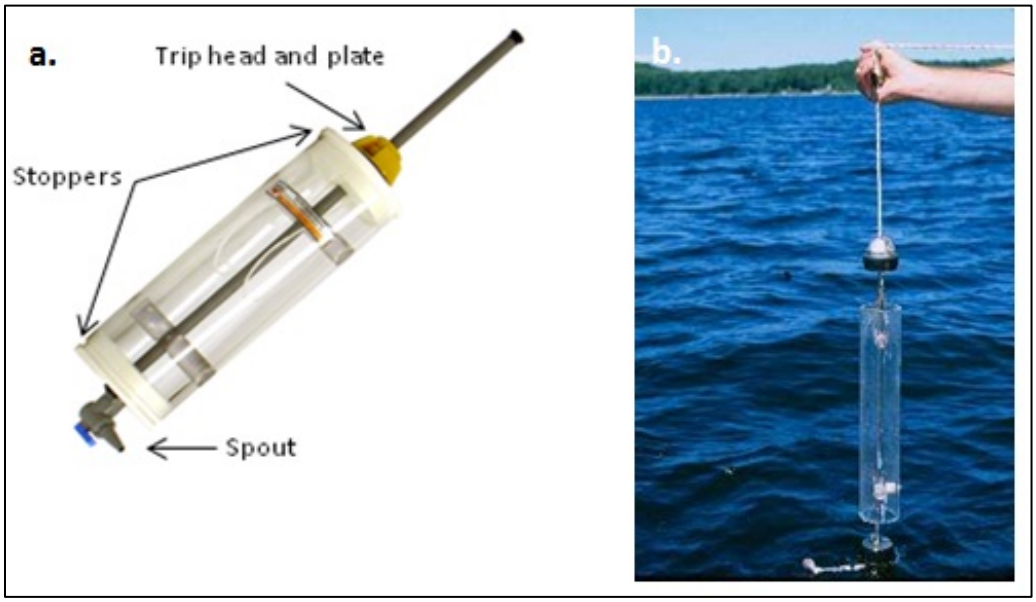


Figure 5. Illustration of Kemmerer sampler for water sampling (a). Illustration of how the Kemmerer is lowered into the water is held by the rope and messenger (b).

9. **Wearing clean nitrile gloves**, rinse 4 L jugs bottles with water from the Kemmerer sampler 3 times, shake vigorously.
   a. Hold the cap in your hand (setting the cap down increases risk of contamination).
   b. Discard water away from the area you are sampling (other side of the boat or downstream of any current).
10. While still wearing nitrile gloves, collect water samples using the Kemmerer sampler.
    a. Collect Kemmerer samples to be integrated in a 4 L container (Figure 3) in the boat. Fill 4 L container completely.
    b. Process samples from 4 L jug
       1) **Cell counts**: Use pipette with new sterile tips (if tips are not sterile, transport clean tips from the lab into the field in clean Ziploc bags) to add 18 mL of sample to 20 mL glass

scintillation vial containing preservative. Recap bottle and invert 5 times to mix. Immediately preserve samples with formalin in the field.

2) Place cell count vials in cooler with ice packs. DO NOT FREEZE.

11. Filtered water samples (Note: filtering can be conducted in the domain lab in cases of inclement weather within 3-4 hours of collection provided 4L jugs are appropriately labeled):
    a. Set-up filter apparatus on relatively level surface:
        1) **WEAR GLOVES**, the same gloves can be worn while collecting the sample.
        2) Set peristaltic pump speed below manufacturer specifications (45 psi for Millipore Sterivex SVGP® filter). If pump speed is set too high, the filter can blow out.
    b. Check that the 3 mL syringe adapter is in place to connect pump tubing to the capsule filter (Figure 6).

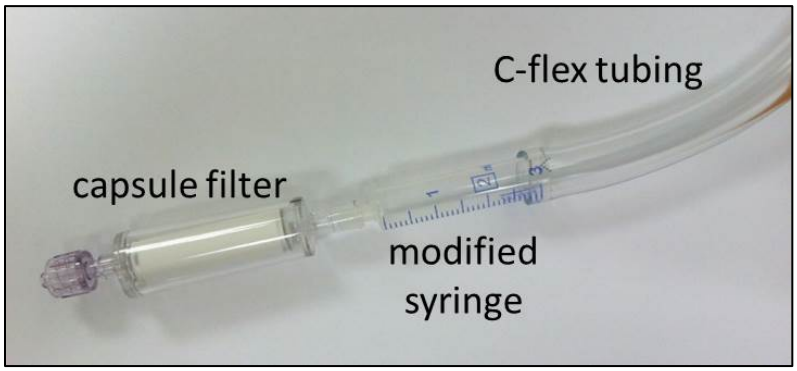


Figure 6. Water filtration setup.

    c. Label capsule filter with an adhesive label marked in permanent marker.
    d. 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.
    e. Rinse tubing by pumping 100 mL of sample water completely through the tube.
    f. When tubing is flushed with sample water after rinsing, attach filter and 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.
        1) A clean (sterilized and rinsed three times in filtered DI) 140 mL syringe maybe be used, connected directly to the capsule filter, if peristaltic pump is unavailable.
    g. Filter >500 mL (1 L or more if possible) of lake (or non-wadeable stream) water through the filter, discarding the filtrate. Keep track of volume filtered with the 500 mL plastic graduated cylinder. You may either pump filtered water into the graduate cylinder to measure (recommended), or pump water out of the graduated cylinder to measure (Figure 7). Stop filtering when filter clogs with sample. Only filter a maximum of 2 L each for the Millipore Sterivex SVGP® capsule filter. Note that filtering the second 4 L bottle may be required for very clear water to collect all samples.

Figure 7. A field technician demonstrates pumping sample water out of the 4 gallon HDPE jug, through the peristaltic pump, the trough the Sterivex® filter, and into a graduated cylinder to measure the volume filtered.

1) Filter 2 capsule filters per site for each parent sample (Figure 8). Filters should remain in original packaging until filtration.

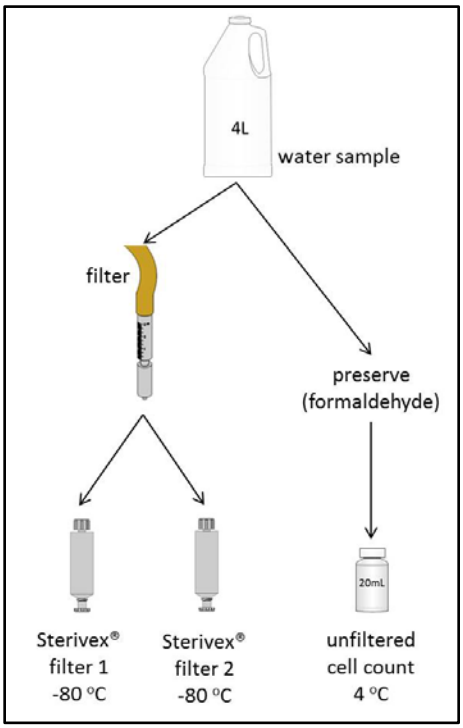


Figure 8. Schematic for microbe sample partitioning

2) When finished, remove filter from set up and push air gently through the filter with 140 mL syringe until dry.
3) Cap ends of filter with luer lock male and female end caps (Figure 9).

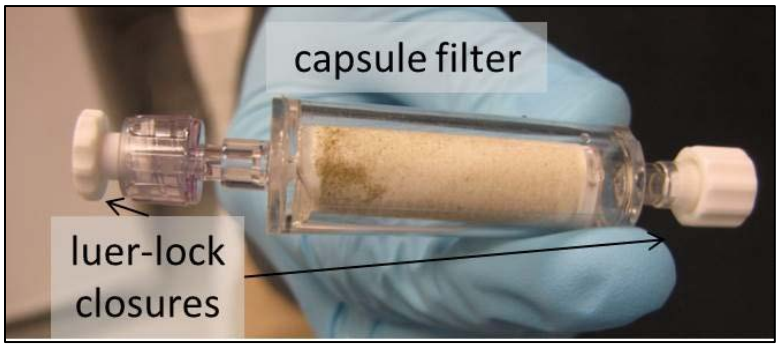


Figure 9. Capsule filters with closures.

h. Record volume of filtration on field data sheet (RD[05]; Figure 10;) for each sample.

NEON Lake Microbe Collection Collection
Lakes and Non-wadeable Streams

Site (4-letter code): *SUGG* **Recorded by:** *sparker*
Date (YYYYMMDD): *20140702* **Collected by:** *jstewart*
Local time (HH:MM): *17:00* **Sampling protocol & Rev.:** *NEON.DOC.001200vB*

Location ID	Sample type	Replicate	Lab type	Sample ID	Depth 1 (m)	Depth 2 (m)	Depth 3 (m)	Filter volume (mL)	Notes
C0	plankton	1	filter 1	SUGG.20140702.amc.plankton.1	1.0	1.5	NA	500	
C0	plankton	1	filter 2	SUGG.20140702.amc.plankton.1	1.0	1.5	NA	500	
C0	plankton	1	cell count	SUGG.20140702.amc.plankton.1	1.0	1.5	NA	NA	
IN	plankton	2	filter 1	SUGG.20140702.amc.plankton.2	1.0	NA	NA	500	
IN	plankton	2	filter 2	SUGG.20140702.amc.plankton.2	1.0	NA	NA	500	
IN	plankton	2	cell count	SUGG.20140702.amc.plankton.2	1.0	NA	NA	NA	
OT	plankton	3	filter 1	SUGG.20140702.amc.plankton.3	1.0	NA	NA	500	
OT	plankton	3	filter 2	SUGG.20140702.amc.plankton.3	1.0	NA	NA	500	
OT	plankton	3	cell count	SUGG.20140702.amc.plankton.3	1.0	NA	NA	NA	

Figure 10. Example of field data sheet (RD[05]).

i. Place samples in a zippered plastic bag and flash-freeze on dry ice in the field. Ensure that samples remain frozen until returning to the domain lab.

12. Field-sterilize gloves and any reused equipment between sample locations
    a. Wipe surfaces of all reusable equipment (e.g., spatula) with new alcohol wipes. After cleaning, rinse with filtered DI.

B.4 Sample Preservation

Cell count samples:

1. Cell counts must be preserved immediately in the field by adding 0.9 mL of 0.2 µm (Acrodisc® filter) filtered buffered formalin to cell counts samples using a 3 mL syringe or pipet for every 18 mL of sample.
    a. Maximum time to preserve cell counts = 4 hours.
2. Recap bottle, and invert to mix ~5 times.
3. Put samples in cooler with ice packs.
4. Chill sample at 4°C upon return to lab. Samples can be held for 7 days before shipping.

Sterivex® filters:

1. Flash-freeze filters on dry ice in the field immediately after collection.
2. Freeze capped Sterivex® filters at -80 °C upon return to lab. Filters may be held for up to 30 days before shipping.

B.5 Ending the Sampling Day

1. Sterilize equipment and place in 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.
    b. Restock the sampling kit, preservation vials, nitrile gloves, filters, luer locks, resealable bags, etc. Refer to equipment list in Section 6.1.
2. Equipment maintenance, cleaning and storage
    a. Charge drill pump batteries.
    b. Wash all equipment that has come in contact with stream water and does not need to be sterilized according to the NEON Aquatic Decontamination Protocol (RD[09]).
    c. Dry all items completely before storing.

SOP C Laboratory Sampling and Analysis

If inclement weather prevents field filtering and preservation, surface water samples may be collected in bulk and processed back at the domain lab following the field standard operating procedure outlined in SOP B. Samples must be process within 4 hours of collection.

Samples should be stored as outlined in preservation, SOP B.4.

SOP D Data Entry and Verification

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

SOP E Sample Shipment

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

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

E.1 Cell counts

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., take note of lab weekend and holiday receiving schedule.
2. Place scintillation vials into one or several gallon-size resealable zip-top bags, grouped by site.
3. Line a cardboard box or 9 qt cooler with a trash bag to prevent leaks.
4. 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.
5. Include shipping inventory/manifest in additional zip-top bag.
6. Ship ground on ice or ice packs.

E.2 Filters

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 AD[03].

E.3 Handling Hazardous Material

Formalin in the concentration and volume shipped by NEON is not considered hazardous.

E.4 Supplies/Containers

See sections E.1, E.2, and Table 8 for specific shipping materials.

E.5 Timelines and Conditions

1. **Cell counts**: Formalin-preserved samples may be stored at 4 ^{o}C for up to 7 days. Do not freeze.
2. **Sterivex® filters**: Filters may be stored at -80 ^{o}C for up to 30 days.

E.6 Grouping/Splitting Samples

Group samples by site per bout.

E.7 Return of Materials or Containers

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

E.8 Shipping Inventory

Shipments are to have a hardcopy of the shipping inventory (RD[14]) 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.

E.9 Laboratory Contact Information and Shipping/Receipt Days

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

8 REFERENCES

Crump, B.C., H.E. Adams, J.E. Hobbie, and G. W. Kling. 2007. Biogeography of freshwater bacterioplankton in lakes and streams of an Arctic tundra catchment. Ecology 88:1365-1378

Hobbie, J.E., J. Daley, and S. Jasper. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. Applied Environmental Microbiology 33:1225-1228.

Lisle J.T. and J.C. Priscu. 2004. The occurrence of lysogenic bacteria and microbial aggregates in the lakes of the McMurdo Dry Valleys, Antarctica. Microbial Ecology 47(4):427-439.

Spigel, R.H. and J.C. Priscu. 1996. Evolution of temperature and salt structure of Lake Bonney, a chemically stratified Antarctic lake. Hydrobiologia 321:177-190.

Spigel, R.H. and J.C. Priscu. 1998. Physical limnology of the McMurdo Dry Valley lakes. *In* Ecosystem dynamics in a polar desert: The McMurdo Dry Valleys, Antarctica, J.C. Priscu (Ed), pp. 153-187. American Geophysical Union.

Takacs C.D. and J.C. Priscu. 1998. Bacterioplankton dynamics in the McMurdo Dry Valley lakes, Antarctica: Production and Biomass loss over four seasons. Microbial Ecology 36:239-250.

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 9. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002194	Datasheets for AOS Protocol and Procedure: Microbes in Lakes and Non-Wadeable Streams
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling
NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

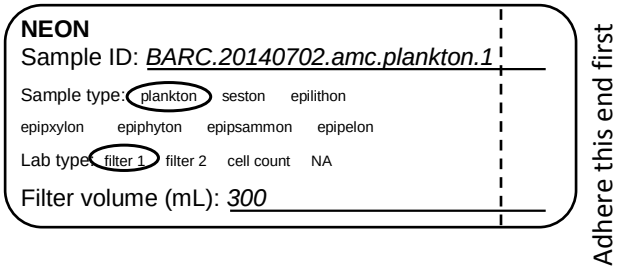
These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

B.1 Quick Steps

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

Step 2 – Prepare labels (2" * 4").



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

Step 4 – Determine the dominant habitat based on t and water chemistry sampling locations.

Step 5 – Determine the depths of the thermocline and the euphotic zone.

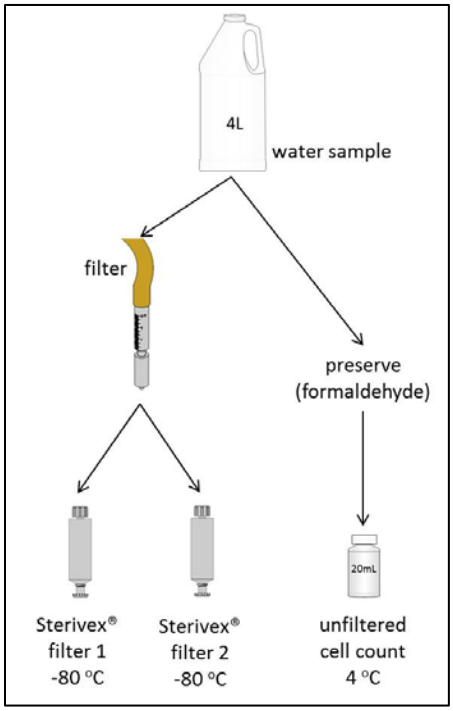
Step 6 – Determine sampling depths (same as water chemistry).

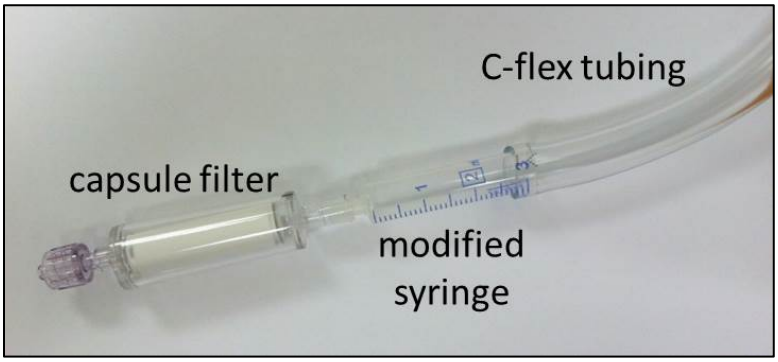
Step 7 – Collect samples using the Kemmerer and integrate the samples in a 4 L jug.

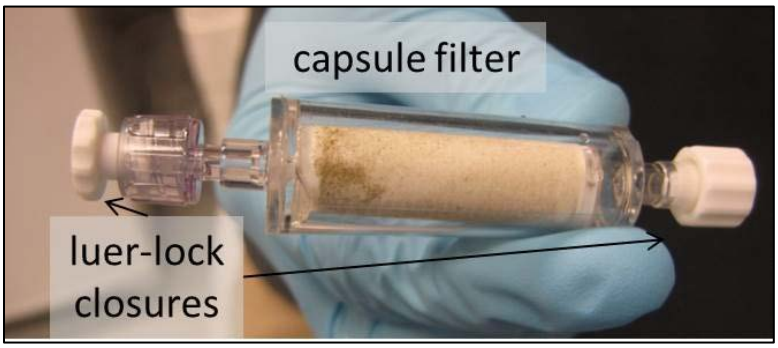
Step 8 – Process the samples from the 4 L jug

1. Cell counts:
   a. Collect in 20 mL glass vial.
   b. Add 0.9 mL of 0.2 µm (Acrodisc® filter) filtered buffered formalin.
   c. Chill sample at 4°C upon return to lab.
   d. Ship on ice overnight, but do not freeze.
2. Sterivex® filters (2 replicates):
   a. Filter >500 mL (1 L or more if possible) of lake (or non-wadeable stream) water through each capsule filter (2 filters total).
   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.2 Schematics for Surface Water Microbe Sampling







APPENDIX C REMINDERS

Before heading into the field: Make sure you…

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

Sample collection: Be sure to…

- ☑ Do not wear dark glasses or sunglasses when making the Secchi depth measurement.
- ☑ 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 formalin in the field, flash freeze Sterivex® filters in the field.

Sample processing: Be sure to…

- ☑ Filtering can be conducted in the domain lab in cases of inclement weather within 3-4 hours of collection provided 4L jugs are appropriately labeled.
- ☑ Keep track of the volume of sample filtered.
- ☑ DO NOT FREEZE cell count samples.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

See the Site Specific Sampling Strategy Document on AQU's NEON intranet site. Follow monthly water chemistry sampling dates.

APPENDIX E SITE-SPECIFIC INFORMATION

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