AOS PROTOCOL AND PROCEDURE: WATER CHEMISTRY SAMPLING IN SURFACE WATERS AND GROUNDWATER

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	07/21/2015	ECO-03068	Initial release of merged protocols (Supersedes NEON.DOC.000694, NEON.DOC.001190 and NEON.DOC.001219 which are now OBSOLETE)

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1 OVERVIEW

1.1 Background

This document describes the required protocols for conducting field sampling of water chemistry in lakes, non-wadeable rivers, wadeable streams, and groundwater. Water chemistry involves both the physical properties of water, as well as the substances within the water. It is influenced by a multitude of factors such as the local geology, rainwater chemistry, and other atmospheric and terrestrial inputs such as dust and allochthonous compounds. Water is a unique compound due to its physical properties such as bonding, electronic structure and chemistry. Its structure imparts a fundamental ability to hold chemical constituents. Further, the character of water can change as a function of physical and biological processes, namely retention, redox reactions, evaporation and adsorption and metabolism. This can impart a large influence on the biotic assemblage and its tolerance to shifts in chemistry. Thus, water chemistry varies spatially and temporally, depending on the watershed characteristics, primary surface and sub-surface hydrologic flowpaths and the turnover time of the water. The character of the water chemistry allows one to determine the quality of a water body and helps understand ecosystem function and health.

Lake, stream, and ground water chemical parameters include concentration, load, and yield. Concentration is the amount of a constituent in a volume of water (e.g., mg/L). Load is the total amount of a constituent transported per unit time:

$$\mathbf{L = CQ}$$

Where: L = Load (mg/s)

C = Concentration (mg/L)

Q = Discharge (L/s)

Loads are typically calculated on an annual basis (e.g., Kg/year). Constituent yield is the transported load per unit of drainage area (e.g., Kg/Ha/year), and is useful in comparing loads from watersheds of differing sizes.

Water chemistry provides valuable information to help inform scientists, managers and decision makers regarding the response of the aquatic ecosystem to natural and anthropogenic changes. Therefore, characterizing lake and stream water chemistry can provide an early warning sign of ecosystem degradation resulting from contaminant inputs, nutrient additions, sediment runoff, and overuse of the resource (Nevers & Whitman, 2007). Sources of such impacts may be far-ranging and include atmospheric deposition, contamination from the watershed, industrial or residential development, waste disposal, water level control, mining, herbicide use, timber production, building of dams and levees, and non-native species invasions (Nevers & Whitman, 2007). Aquatic biota are tolerant of small changes in chemistry; however, large shifts in chemistry can have dramatic effect on the biotic community structure and function through processes such as nutrient uptake and retention. Long-term observations provide an effective means of keeping track of possible impacts and ecological status (water quality).

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

The surface water component of this protocol is derived from the United States Geological Survey, National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water Resources Investigations, Book 9, Chapter A4, Version 2.0, 9/2006.

The groundwater component of this protocol is derived from the Environmental Protection Agency Report: Low-Flow (Minimal Drawdown) Ground-Water Sampling Procedures (Puls, R.W., and Barcelona, M.J., 1996, Report EPA/540/S-95/504) and the United States Geological Survey, National Field Manual for the Collection of Water-Quality Data (U.S. Geological Survey TWRI Book 9, Chapter A4, Version 2.0, 9/2006).

The laboratory component of this protocol is derived from the U.S. Geological Survey Techniques of Water Resources Investigations, Book 9, Chapter A6., sec 6.6, Version 2.0, 9/2006 Chapter A6.6, Version 4.0, 9/2012.

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.000824	Data and Data Product Quality Assurance and Control Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.014051	Field Audit 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.002906	Datasheets for AOS Protocol and Procedure: Water Chemistry Sampling in Surface Waters and Groundwater
RD[06]	NEON.DOC.002792	AOS Protocol and Procedure: Secchi Disk and Depth Profile Sampling in Lakes and Non-wadeable Streams
RD[07]	NEON.DOC.001646	General AQU Field Metadata Sheet
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.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[11]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

2.3 Acronyms

Acronym	Definition
A/R	Acid-rinsed
ALK	alkalinity
ANC	Acid Neutralizing Capacity
ASR	Analytical Services Request
C/B	Cleaned and burned
^oC	Degrees Celsius
DI	Deionized
FIL	Filtered Chilled
GF/F	Grade F Glass Fiber Filter
GW	Groundwater
H_2SO_4	Sulfuric acid
ha	Hectare
HDPE	High-density polyethylene
kg	Kilogram
L	Liter
lb/in	Pounds per inch
m	Meter
M	Molar
m^3	Cubic meter
mg	Milligram
mg/L	Milligrams per liter
meq/L	Milliequivalents per liter
mL	Milliliter
µS/cm	Microsiemens per centimeter
N	Normal
OW	Observation Well
P&P	Procedure and Protocol
PCN	Total Particulate Carbon and Nitrogen
PSI	Pounds per square inch
PPE	Personal Protective Equipment
RAW	Raw Untreated
s	Second
SOP	Standard Operating Procedure
SPC	Specific Conductance
µS/cm	Microsiemens per centimeter
USGS	United States Geological Survey

2.4 Definitions

Alkalinity: The buffering capacity of a water body, or the ability of solution to neutralize acids to maintain a fairly stable pH, which is important for agriculture, wastewater, contamination determination, ecosystem health etc. Good water buffers include compounds such as bicarbonates, carbonates and hydroxides, which combine with H^+ ions in the water and increase the pH to prevent acids from building up in a solution.

Acid Neutralizing Capacity (ANC): Measure of the overall (total) buffering capacity of water or the ability to neutralize acid and maintain a constant pH. Acid neutralizing capacity is similar to Alkalinity, but is measured on an unfiltered water sample, rather than a filtered one.

Conductivity: A measurement of the electrical conductance per unit distance in an aqueous solution.

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

Headspace: A gaseous space above a closed liquid sample

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

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.

pH: A measure of the acidity or basicity of an aqueous solution.

Thalweg: The deepest part of a stream channel.

Thermocline: The vertical section of the lake where the rate of decrease in temperature with increasing depth is greatest. The denser and cooler layer below the thermocline is defined by the hypolimnion. The warmer upper layer is termed the epilimnion.

3 METHOD

The field protocol used by NEON for collecting **surface water** chemistry samples follows the general requirements set forth by the 2011 USGS National Water-Quality Assessment (NAWQA) Program and the Arctic LTER standard operating procedures (SOP). The field protocol used by NEON for collecting **groundwater chemistry** samples in small (2-in diameter) shallow (<100 ft depth) groundwater observation wells follows the general procedure for minimal drawdown sampling detailed by EPA report Low-Flow (Minimal Drawdown) Ground-Water Sampling Procedures (Puls and Barcelona, 1996). Sample handling and preparation portions of this protocol follow the general requirements set forth by the USGS National Water-Quality Assessment (NAWQA) Program (USGS 2006). This protocol describes the collection, field processing, preservation (if applicable) and shipping of total, dissolved, and particulate nutrients samples, as well as anions, cations and general chemistry (i.e., conductivity and pH). Additionally, samples are collected for alkalinity and acid neutralizing capacity (ANC) and are measured at the Domain Support Facility to reduce the error associated with changes in the chemical composition of a sample due to chemical dissolution or precipitation as well as the loss of CO_2.

The sampling strategy for surface water is specific to the type of waterbody. The strategies for sampling wadeable streams, non-wadeable streams, lakes, and groundwater are outlined below.

The majority of the NEON **wadeable stream** sites are shallow and narrow, rendering the use of isokinetic (i.e., sampling at same velocity as the main stream) samplers for depth-integrative sampling impractical. Thus, the following protocol outlines the use of a dip sampling method in the main section of streamflow (i.e., thalweg). This method assumes the stream channel is completely mixed. The water chemistry sampling location should be located, when possible, within 1 meter downstream of the main stream sensor set (sensor set 2) so that the sensor measurements can be validated with stream water chemistry samples (Figure 1). The sampling location should be typical of the entire reach. The sampling location should be located away from, or upstream of, any major local disturbances and other areas where NEON sampling activities commonly occur. In streams with a shallow water column, field personnel must be cautious not to disturb the benthic sediments when sampling. Disruption of the sediments by walking or by sampling too close to the stream bottom can contaminate samples. Thus, always sample upstream from wading activity and minimize suspension of sediments when sampling. If sediments are disrupted, wait until the area has cleared before sampling.

In **non-wadeable streams**, NEON will collect one sample at 0.5 m depth just downstream of the sensor set (Figure 2) in the area representing the steam thalweg (the deepest part of the stream, where access is possible). If the non-wadeable stream is stratified, an epilimnion sample at 0.5 m will be collected to align with dissolved gas sampling and an integrated sample of the hypolimnion is taken, as in stratified lakes (Figure 8). 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 2-3 times as long as the depth of the lake or stream. If sediments are disrupted, wait until the area has cleared before sampling.

Three locations per **lake** will be sampled, notably the deepest part of the lake (at the buoy), a location near the most prominent inlet (5-10 m from the inlet mouth and in at least 1 m of water depth), and one near the outlet (5-10 m into the lake from the outlet and in at least 1 meter of water depth). These locations are collocated with the lake buoy, inlet, and outlet infrastructure (Figure 2), respectively. Water chemistry samples should be collected within 1 m of the infrastructure to ensure comparability between grab samples and continuously monitoring sensors. NEON will collect one sample each at the inlet, outlet, and buoy locations in unstratified lakes at 0.5 m depth to maintain comparability with dissolved gas measurements (Figure 8). If the system is stratified, NEON will collect samples at 0.5 m depth at the inlet, outlet, and buoy locations to align with dissolved gas sampling, as well as an integrated sample of the hypolimnion at the buoy (Figure 8). Note that at these inlet and outlet locations the samples are always taken at the surface (0.5 m depth).

For **groundwater** sampling, samples are budgeted on a basis of an average of one sample per well per year at each site, or a total of 8 samples per year per site (Figure 3). Due to the limited number of samples available it is anticipated that a subset of wells will be sampled at multiple times per year (likely 4 wells at 2 times per year). This will allow for evaluation of seasonal responses in groundwater constituent concentrations.

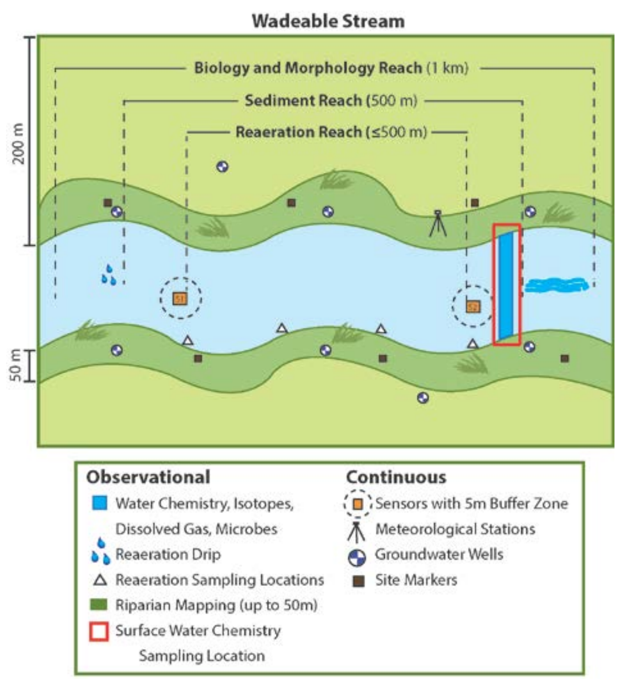


Figure 1. A generic wadeable stream layout with water chemistry sampling locations

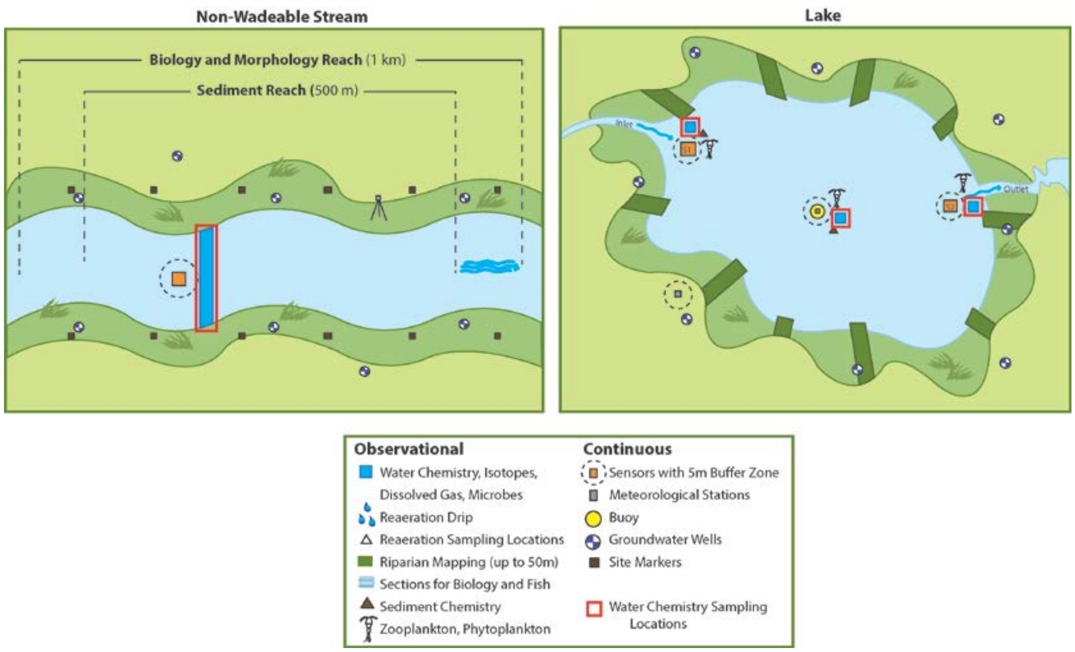


Figure 2. Generic site layouts for lakes and non-wadeable streams with water chemistry sampling locations

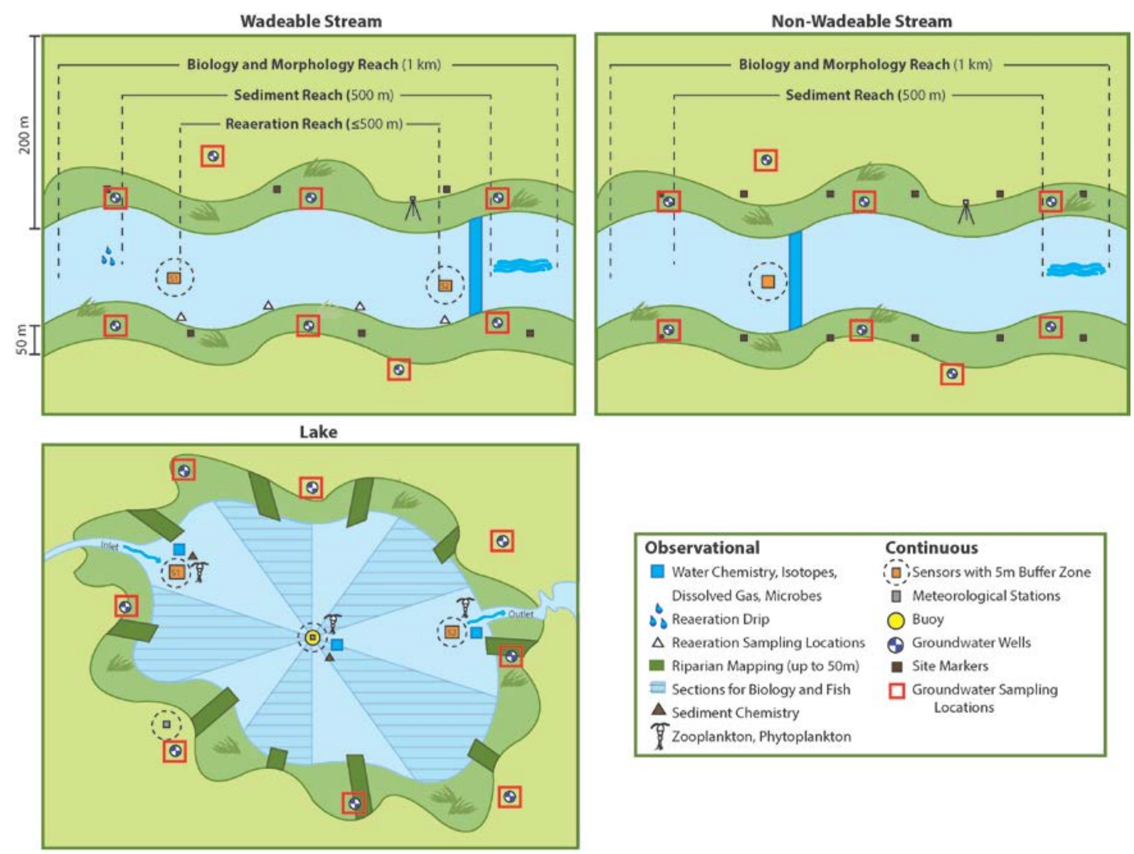


Figure 3. Generic layout of groundwater sampling locations in wadeable stream, non-wadeable streams, and lake sites

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[06]). 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[04]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Wadeable and Non-wadeable stream water chemistry sampling occurs up to 26 times per year (approximately every other week) at each NEON location. When applicable, chemistry samples should be collected on Tuesday to coincide with NEON atmospheric wet chemistry sampling, as well as the National chemistry sampling efforts. It is advised not to collect samples on Friday. A range of dates for each site will be determined *a priori*, based on historical stream discharge data. These criteria will be detailed in the NEON Aquatic Sample Strategy Document (RD[08]).

Lake water chemistry will be collected 12 times per year (approximately monthly and during shoulder seasons to capture ice-on and ice-off events). Other than event based sampling, chemistry samples are preferably collected on Tuesday to coincide with other National chemistry sampling efforts. Sampling timing is provided annually by Science Operations and shall be outlined in the NEON Aquatic Sampling Strategy Document (RD[08]).

Groundwater chemistry samples are collected twice per year from a subset of wells, selected on a site-by-site basis, during each sampling event. Periodic changes to the selected subset of wells may occur during the life of the Observatory and are guided by various parameters. For example, changes in hydrologic conditions (dry wells, changes in hydrologic flow paths) or infrastructure (damaged wells). Groundwater chemistry samples shall be collected within +/- 1 day of the stream water chemistry sampling event, when possible.

4.2 Criteria for Determining Onset and Cessation of Sampling

The timing of sampling allows researchers to assess aquatic biogeochemistry cycles, and therefore timing depends on the dominant driver(s) of nutrient flux within each system. Timing of sampling is site-specific and determined by rules developed using historical meteorological, physical and environmental data ice on- ice- off dates, including lake levels, stratification, discharge and riparian greenness (see RD[08]).

For example, **wadeable streams** with little or no flow during the summer dry-season or completely frozen streams during the winter are sampled more intensively during wet periods. Systems that have a snowmelt-dominated or storm-dominated flow regime are sampled more intensively during time periods of high flow, when the majority of the nutrients are moving through the system and sampled sporadically during times of baseflow. Stream systems that are heavily influenced by autumn leaf fall and winter rains are more heavily sampled in autumn and winter.

Non-wadeable streams are sampled approximately twice monthly with more intensive sampling occurring during high flow periods.

Lakes that stratify are sampled just before and just following turnover in both the spring and autumn season. Other higher intensity sampling may occur following a major storm event. Lakes that do not stratify and the remaining samples of stratified systems are taken approximately every month throughout the year when conditions allow. When sampling during the winter, the last winter sample shall be collected 1 month prior to the annual average ice-off date. The first spring/summer sample shall be collected within 1 week after ice-off, assuming safe access conditions to the lake. Ice-off in lakes is defined by the permanent loss of ice in spring from the center of the lake. Ice-on in lakes is defined by the permanent ice coverage in the fall of the central part of the lake.

Groundwater sampling dates are guided by the hydrologic cycle of the system that the site is located in. Samples are targeted for the early spring when precipitation events begin to increase the groundwater flow rate towards the streams; and for late fall when the production of surface constituents have had sufficient time to infiltrate into the groundwater. The timing of these seasons is site dependent, but will occur based on the streams cumulative discharge with the first sample occurring when the stream is between 20-30% and the second sample when the stream is between 70-80% of the predicted annual cumulative discharge based on historic discharge data. The timeframe for collecting samples will range from a 2-week to a 2-month window of time with which to align a groundwater chemistry sampling event with a stream sampling event. This range is dependent on the discharge characteristics unique to each stream. Lake sites are sampled following a similar approach for timing, and are sampled in the spring and fall with groundwater sampling dates linked to surface water chemistry sampling events. Date ranges for sampling are provided in the site-specific sample strategy document.

4.3 Timing for Laboratory Processing and Analysis

For external laboratory analysis, samples should be processed (i.e. filtered) as soon as possible, preferably within 3 hours, and shipped to the water chemistry lab within 24 hours, when possible, to ensure sample integrity. Samples must be kept cold (~4 ± 2°C) to reduce nutrient transformation. Water jugs must be shaken before filtration to re-suspend particulates and homogenize water. For further storage and shipping information see SOP 7: Standard Operating Procedures.

For internal laboratory analysis, samples for alkalinity and ANC should be kept on ice or refrigerated at 4°C ± 2°C. Laboratory analysis should be completed as soon as possible after returning from the field. Alkalinity and ANC samples should be processed within 24 hours, when possible. Samples analyzed after the 24 hours window will be flagged. The maximum allowable time period between sample collection and analysis is 72 hours. It is advised to not collect field samples on Friday, unless alkalinity and ANC can be measured in the Domain Support Facility on Friday afternoon. For **groundwater**, alkalinity and ANC samples should be processed within 24 hours, when possible. However, given that groundwater chemistry sampling logistics may make titrations within 24 hours impossible, the maximum allowable time period between sample collection and analysis is 72 hours.

It is advised to not collect field samples on Friday given shipping and domain laboratory processing requirements.

4.4 Sampling Timing Contingencies

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

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/non-wadeable stream** becomes too windy (>9 km hr^{-1}) and has unsafe wave heights (<1 m) to hold the boat stationary over a sampling point) 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.	No adverse outcome.
	If stream is ice-covered but still flowing, the ice should be broken in order to sample the stream. Be sure to bring a shovel or other long-handled tool if the surface ice is expected to be hard to break.	No adverse outcome.
	If sampling stirred up sediments or added chemical constituents to the water within the past hour, allow the water to clear and disturbance to pass or sample in a different location upstream/upwind of the disturbance.	No adverse outcome.
	If water samples cannot be processed in situ (due to field conditions, time limits, etc.), collect water samples in two 4 L jugs and return the samples on ice to the designated sample processing location to filter. The filtration should be completed in 3 hours and must be completed within 6 hours of sample collection. Samples must be kept cold (~4°C) to reduce nutrient transformation. Water jugs must be shaken before filtration to re-suspend particulates and homogenize water.	No adverse outcome.
	If the water chemistry sampling location is too shallow to obtain a sample, sample in a nearby location where water is deep enough to obtain a clean, sediment free, sample, and note this change during data collection by noting the new GPS position on the field sheet.	No adverse outcome.
	If low discharge or lake levels render some habitat dry or the flow is so low that the stream appears to be a series of pools not connected by surface water, continue sampling in the water chemistry sampling locations provided the sample bottle could be filled without disturbing sediments. Be sure to note this in the comments section of the field sheet during data collection.	No adverse outcome.
	If the **lake** is partially frozen, sample only when 0.5 m of water are available below the ice surface. If 0.5 m is not available, move to	No adverse outcome

	another location within 10 m of the original GPS coordinate, where the water column below the ice has between 0.5 and 1.5 m depth. For littoral sampling locations, do not extend this zone beyond 1.5 m depth and/or 20 m from the shoreline. Note this change during data collection by noting the new GPS position on the field sheet.	
	When sampling a **groundwater** well following the low flow method, if the well goes dry, turn off the pump and wait for groundwater to return to the well, then restart the pumping and collect groundwater for sampling.	No adverse outcome.
	If temperatures are below freezing and filtration equipment is not functional in situ, collect sample and filter in a sheltered area, such as the field vehicle or return to the Domain Support Facility for filtration.	No adverse outcome.
Days – Months	If the water body is entirely dry or frozen to the ground, note during data collection.	No adverse outcome.
	If site conditions dictate that **stream** sampling is not possible due to the stream being dry, then postpone the **groundwater-sampling** event until flow returns in the stream.	No adverse outcome
	If temperatures are below freezing and water in the **groundwater** pump discharge line is freezing, stop sampling and reinitialize the sampling effort when ambient temperatures are above freezing. Since GW sampling occurs at most twice a year, the events should be timed with above freezing weather conditions.	No adverse outcome.
	In some locations the **groundwater** level will drop below the bottom of the well either seasonally (e.g. Taiga and Tundra sites) or in periods of drought, which are likely to occur at some point during the life of the Observatory. Under these conditions sampling of groundwater is not possible.	No adverse outcome
	Though **groundwater** wells are generally sited for slightly elevated locations, times will occur when standing water surrounds the base of the well. In this condition postpone sampling until the ground near the base of the well is free of standing water.	No adverse outcome.
	For sites that have the "generation" **of groundwater** resulting from seasonal thawing of permafrost, sampling is targeted for times when the permafrost is sufficiently thawed to allow for collection of groundwater samples.	No adverse outcome

4.5 Sampling Specific Concerns

Samples should be processed (filtered and chilled at 4 ± 2°C) as soon as possible to reduce nutrient transformation. If necessary, sample water may be collected in a large container (may require up to 2 4 L jugs in clear systems), kept on ice or ice packs, and filtered within 3 hours (maximum of 6 hours) at a base camp or Domain Lab (i.e., if weather dictates the need to get out of the field immediately and stream discharge is increasing). Water jugs must be gently shaken before filtration to re-suspend particulates and homogenize water.

Always make note of any weather or stream conditions that could influence chemistry, including but not limited to wind, activities in the surrounding watershed, prior flood or rain events, ice, and changes in sampling locations (RD[05]). Sample collection time, processing station and processing time must be recorded on the Water Chemistry Data Sheet (RD[05]).

Surface water chemistry samples should be collected up-wind or upstream of any fieldwork disrupting the stream or lake bottom (i.e., morphology mapping, invertebrate collection, macrophyte collection, etc.).

Groundwater sampling should be timed to occur on the same day, or if necessary one day before or after a stream water chemistry sampling event. GW sampling can be time consuming, as such, conducting the groundwater chemistry sampling event over two days is also acceptable, though efforts should be taken to complete the sampling in one day.

Table 2. Groundwater sampling specific contingent decisions

Delay/ Situation	Action	Outcome for Data Products
If a groundwater well is damaged (i.e. casing is broken internally) or bent	Do not try to sample this well. It is likely that the pump may get stuck inside the well due to the limited tolerance between the pump and well casing. If this condition is observed submit a trouble ticket for a new well to be selected for sampling.	No adverse outcome.

5 SAFETY

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

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

Activities in **streams** should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 10 ft^2/s (0.93 m^2/s).

Acid must be stored in acid-safe containment cabinets in compliance with the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]). Wear nitrile gloves and eye protection when dispensing acid.

In addition to standard safety training provided by NEON, the following safety requirements are sought:

1. Due to site-specific hazards that may be encountered, technicians may perform GPS positioning around the lake, and measurements for inflow and outflow, without dismounting from the vessel. In addition, technicians are required not to put hands and ft 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. The first two lists are specific to sampling of lakes/non-wadeable streams and groundwater (no additional, specific equipment is needed to sample wadeable streams). All remaining lists are organized by task and apply to all waterbodies and groundwater. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

This protocol suggests the use of a GPS unit with WAAS, specifically a Hummingbird 1198c, used for navigating to lake sampling locations. Any GPS unit can be used as long as the navigation accuracy is <4 m for lake navigation.

Table 3. Equipment specific to sampling lakes

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Boat		1	Y
	R	Anchor with rope		1	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,		1	Y

Item No.	R/S	Description	Purpose	Quantity	Special Handling
		float with rope)			
	R	First Aid Kit		1	N
	R	Personal Flotation Devices (PFDs)		1 per person	N
MX100393	R	Kemmerer sampler	Collecting samples	1	
	S	Battery charger for 12 V batteries	Powering the Sounder Unit (Humminbird)	1	
MX100453	S	GPS unit with WAAS (Humminbird 1198c) • Echosounder transducer (83/200kHz)	Navigating to sampling locations	1	
MX100447	R	Secchi Disk	Determining the depth of the euphotic zone	1	
Consumable items					
		(none)			

R/S=Required/Suggested

Table 4. Equipment specific to sampling groundwater

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Clean 5 Gallon Bucket	Storing the groundwater well sensor and cable during sampling	1	N
MX100514	R	Hand-held conductivity meter	Measuring dissolved oxygen, temperature, and conductivity	1	N
0318830003	R	QED Sample Pro Pump	Pumping groundwater from the well	1	N
0318830001	R	QED MP-50 Compressor / Controller	Pumping groundwater from the well	1	N
0338280000	R	Battery (12V, minimum of 3.6 Ah)	Pumping groundwater from the well	8	N
0318830002	R	Bucket of ¼" x ¼" dual bonded tubing (250 ft of tubing in each bucket). Tubing is dedicated per each well for the duration of the sampling events.	Pumping groundwater from the well	1 per site, required for 1st sampling event.	Y, Must Remain Clean
Cut tubing from above line	R	Dedicated tubing for wells (in large plastic bags). Make sure to get the tubing identified for the well that is sampled. The sealable bags shall be labeled with the Well ID.	Pumping groundwater from the well. One piece of tubing dedicated to each well.	1 dedicated piece of tubing per well sampled (after 1st sampling event)	Must Remain Clean
MX110049	R	Water level tape (metric)	Measuring water height	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Consumable items					
		(none)			

Table 5. Equipment list – Sampling equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	4 L jug	Collecting water	4 (GW), 2 (Surface Water)	N
	R	Pieces of C-Flex® tubing, 1/4 in I.D. and 3/8 in outer O.D., suggested 4 ft and 2 ft in length	Pumping water into sample containers	2	N
GB07270000	R	Pump Assembly - Easy-Load Peristaltic pump head (e.g., Masterflex® L/S® Easy-Load® pump head) with Peristaltic pump tubing (e.g., L/S® 15 or L/S® 24) - 18-V Drill Pump (Power source for pump head)	Pumping stream water into sample containers	1 1 1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
		- Tubing connectors		2	
	R	18-V Drill battery charger	Pumping water into sample containers	1	N
	S	Velcro strap or c-clamp	Keeping the drill in the "on" position to pump stream water continuously	1	N
	R	Squirt bottle (125 mL)	Rinsing tubing before placing in 4 L jug	1	N
	S	Non-porous flat surface	Filtering and processing water samples	1	N
	R	Hand-held conductivity meter	Measuring conductivity	1	N
Consumable items					
MX108215	R	Pall Supor capsule filter (0.45 µm)	Collecting stream water for filtered samples	1	N
	R	Sample labels (2*4 in waterproof)	Labeling samples	10	N
	R	Permanent marker	Labeling samples	2	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	1 L jug of DI	Rinsing tubing before placing in 4 L jug	1	N
	R	Conductivity calibration solutions	Calibrating hand-held conductivity meter	1	N

R/S=Required/Suggested

Table 6. Equipment list – Water chemistry bottles for dissolved and totals (see also Figure 4)

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	250 mL HDPE [a] - Alkalinity - Acid Neutralizing Capacity (ANC)	ALK and ANC sample containers, per site	2	N
Consumable items					
	R	1 L amber bottle – A/R and C/B	Filtered water sample (FIL) container	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	250 mL amber bottle – A/R and C/B	Unfiltered water sample (RAW) container	1	N
	R	25 mm ashed GF/F filter	Filtering stream water for particulate samples (PCN)	1	N

R/S=Required/Suggested

[a] indicates sample bottles that will remain at NEON Domain Support Facilities to be analyzed. These bottles are labeled with different labels (Figure 6) and bottles can be re-used.

Table 7. Equipment list – Total Particulate Carbon and Nitrogen (PCN)

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	4 L jug	Collecting samples	1	N
	R	Vacuum pump manifold or hand pump and vacuum tubing	Filtering samples	1	N
	R	Filter Unit and Funnel	Filtering samples	1	N
	R	Graduated Cylinder, plastic, 250 mL	Measuring and adding the volume of sample into the filter funnel	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	#8 rubber stopper for filter manifold	Filtering samples	1	N
	R	1 L Polypropylene vacuum flask	Filtering samples	1	N
	R	Filter Forceps – forceps with flat ends to not poke holes in filter	Handling filter	2	N
	R	Squirt bottle (125 mL)	Rinsing the sides of the filter funnel	1	N
Consumable items					
	R	25 mm pre-ashed GFF (0.7 µm) filters	Filters for particulate sample (PCN)	20*	N
	R	Aluminum foil, pieces (~4X4 in)	Wrapping GFF filters	20*	N
	R	DI water	Rinsing the sides of the filter funnel	As needed	N

R/S=Required/Suggested
*Take extras in field

Table 8. Equipment list – Sample field storage and shipping

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Shipping cooler	Shipping samples	1	N
Consumable items					
	R	Packing material	Filling up extra space and adding absorbent material	As needed	N
	R	Resealable plastic bags (gallon and quart size)	Separately enclosing the shipping labels, ice packs and samples	As needed	N
MX102295	R	Ice or ice packs	Keeping the samples cool (water ice is preferable if logistically feasible).	As needed	N
	R	Clear packing tape, roll	Labeling shipment	1	N
	R	FedEx shipping labels	Labeling shipment and cooler return	2	N

Table 9. Equipment list – Laboratory processing: Materials and supplies for the alkalinity and ANC laboratory measurement procedure

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX100556	R	pH meter, with automatic temperature compensator - pH electrode, calibrated - Thermometer, calibrated	Reading pH of the samples	1	N
	R	Magnetic stirrer	Mixing the sample with the titrant solution	1	N
	R	Stir bars, Teflon® coated, smallest size	Mixing the sample with the titrant solution	2	N
	R	Volumetric pipets, Class A "TD" [a] – 25 mL – 50 mL – 100 mL	Measuring volume and transferring sample to glass beaker	1 1 1	N
	R	Graduated cylinders [b] – 25 mL – 50 mL – 100 mL	Measuring volume and transferring sample to glass beaker	1 1 1	N
	R	Pipette squeeze bulb	Used with volumetric pipet	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Glass beakers – 50 mL – 100 mL – 150 mL	Sample container for pH readings	1	N
	R	Squeeze bottle with DI water	Rinsing pH probe	1	N
MX100384	R	Digital titrator and mounting assembly	Adding titrant solution to sample	1	N
	R	Delivery tubes, 90° angle, transparent	Adding titrant solution to sample, 1 per titrant solution	2	N
	R	Safety – gloves, glasses, acid spill kit, lab coat	Safety	1	N
	R	Acid waste container		1	N
Consumable items					
	R	DI water (max conductivity of 1 µs/cm)	Rinsing pH probe	1	N
	R	Titrant solution - Sulfuric acid (H_2SO_4) 0.16*N* - Sulfuric acid (H_2SO_4) 1.6*N*	Added to samples in order to measure ANC and ALK	1	Y

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Sodium Bicarbonate, 1lb	Acid disposal	1	N

R/S=Required/Suggested

[a] indicates equipment specific to alkalinity measurements.

[b] indicates equipment specific to ANC measurements.

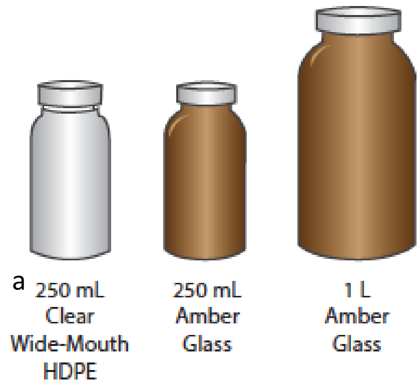


Figure 4. Water chemistry bottle types. [a] indicates sample bottles that will remain at NEON Domain Support Facilities to be analyzed. These bottles are labeled with different labels (Figure 6) and bottles can be re-used. 125 mL bottles may be used if less volume is needed – site specific.

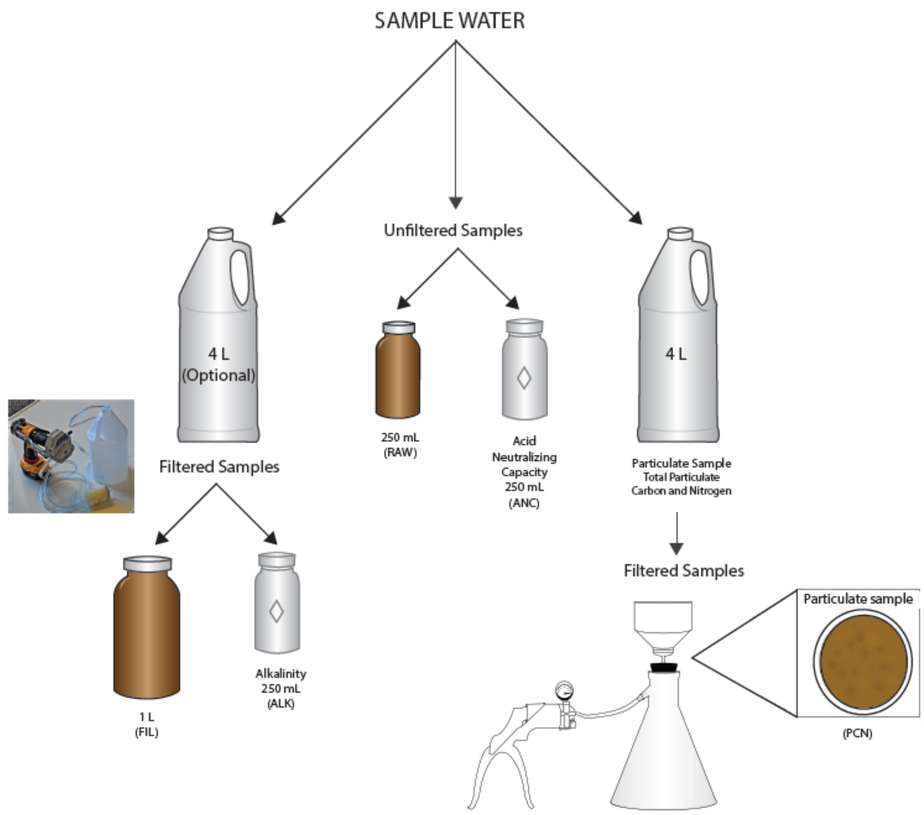


Figure 5. Flowchart of Water Chemistry Sample Collection and Filtration. ◊ Indicates 250 mL, wide-mouth sample bottles that remain at the Domain Support Facility for analysis. Letters in parenthesis indicate the codes that correspond to the chemistry labels (see Figure 6).

6.2 Training Requirements

Technicians must complete protocol-specific training for safety and implementation of this protocol. Personnel shall be trained in making water chemistry measurements and associated safety procedures.

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

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.

We estimate that surface water sampling requires 2 technicians for 1-2 hours in **wadeable and non-wadeable streams** or 1-2 hours per station in **lakes** (~ 3-5 hours per lake) each sampling day plus travel to and from the site. An additional 1-2 hours per station (l**akes, wadeable streams, and non-wadeable streams**) of laboratory work at the Domain Support Facility is expected.

Groundwater sampling is estimated to require 1 technician up to 1-2 hours for each well that is sampled plus travel to and from the site. Sampling 4 observation wells should be able to be completed in 6-8 hours as an average. Shallow wells will require significantly less time than deeper wells to sample. An additional 1-2 hours (per well) for laboratory work at the Domain Support Facility is expected.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

1. Check the water chemistry field sampling kit to make sure all supplies are packed and ensure batteries for the peristaltic pump are charged.
2. Check the hand-held conductivity calibration and recalibrate if necessary. See Conductivity User's Manual. Be sure when calibrating and using the conductivity meter that the holes at the top of the senor are completely covered.
3. Prepare the appropriate bottles and collection devices based on type of water samples being collected (Figure 4, Figure 5) *Note: prepare 2 sets of bottles; the second set will be used as a backup.
4. **<u>Groundwater sampling only:</u>** Pack tubing individually into large sealable bags labeled for the corresponding well. If tubing is either missing or damaged, take new spare tubing from the bucket of tubing. In the first round of sampling, the tubing will need to be cut for each well (detailed below).
5. Pre-ash GF/F filters:
    a. Place layers of 25 mm GF/F filters on aluminum foil. Use multiple layers of foil if needed, filters can be touching and placed on top of one another but should not be stacked more than 3 filters deep.
    b. Place in muffle furnace ($500^{o}C$) for 6 hours. WARNING: Use designated safety equipment when working near or in the muffle furnace. DO NOT touch hot surfaces.
    a. After 6 hours, remove from furnace; stack filters using filter forceps, and place in original box. CAUTION: Ashed filters may be hot. Use designated safety equipment at all times.
    b. Label box with permanent marker to read "ASHED, Your Name, Date".
    c. Place box in in sealed zip-top bag.
    d. Ashed filter may be stored indefinitely, as long as they remain in the box and stay dry.
6. Attach pre-printed labels (Figure 6 a and b) to bottles (Figure 5).
7. Use a permanent marker to fill out bottle labels (Figure 6) before going into the field. Note that there are two different labels depending on whether the samples will be shipped to the external analytical chemistry laboratory (Figure 6a) or will be analyzed at the Domain (Figure 6b). **Labels are waterproof but should be filled out before getting wet to ensure ink is dry. SampleID** should be **siteID.stationID.date.sampleType.** StationID is the 2-digit station code where sample was taken (i.e. Station ID for streams ='ss', non-wadeable streams/rivers = 'rs' (or 'rs' and 'r2' if river is stratified, with 'rs' being the top-most layer)), in Lakes 'in', 'ot', 'c0', if center is stratified: 'c1', 'c2', 'c3', with 'c1' being the top-most layer. 'w1'-'w8' for groundwater wells) or use basketID when STREON baskets are sampled. SampleType for external lab is 'FIL', 'RAW', or 'PCN', with 'FIL' indicating a filtered sample, 'RAW' indicating an unfiltered sample, 'PCN' indicating the particulate carbon and nitrogen filter sample. Domain Lab SampleType is ALK

(filtered sample) and ANC (unfiltered sample). Circle the correct bottle type code (Figure 5, Figure 6) for each bottle.

8. Organize bottles as appropriate for your study sites.

a) b)

Figure 6. Blank NEON Chemistry Labels for a) the External Analytical Laboratory and b) Internal NEON Domain Support Facility Measurements.

SOP B Field Sampling

For all water chemistry samples:

In the field, fill out the General AQU Field Metadata Sheet (RD[07]) before collecting samples. You only need to fill out one AQU Field Metadata Sheet per **SITE** per day.

1. For each station, complete the Datasheets for Water Chemistry Sampling in Surface Waters and Groundwater (RD[05]).
   a. Measure and record temperature-corrected conductivity values on the Datasheets for Water Chemistry Sampling in Surface Waters and Groundwater (RD[05]). This data will also need to be included on the sample shipping inventory remarks section of the 'per sample' tab [RD12].
      1) Conductivity should be measured as temperature-corrected conductivity at 25°C, whenever possible. Ensure conductivity measurements are on the appropriate temperature-corrected and unit setting (i.e., setting SPC, uS/cm).
      2) Conductivity sensor is located at the top of the probe tip, so ensure probe is completely submersed in the water or readings will be inaccurate.

B.1 Wadeable Stream Collection

FIELD SAMPLING –WADEABLE STREAMS

1. ALWAYS sample in the THALWEG (the deepest location in the stream cross-section) with the bottle opening pointed upstream and into the main flow of water (Figure 7) and several centimeters below the surface (to avoid sampling floating material or surface film). You may step into the stream, but disturb the stream bottom as little as possible as you walk. Take samples UPSTREAM from where you are standing. **Be cautious when sampling. Items can easily fall into stream while bending to sample.**

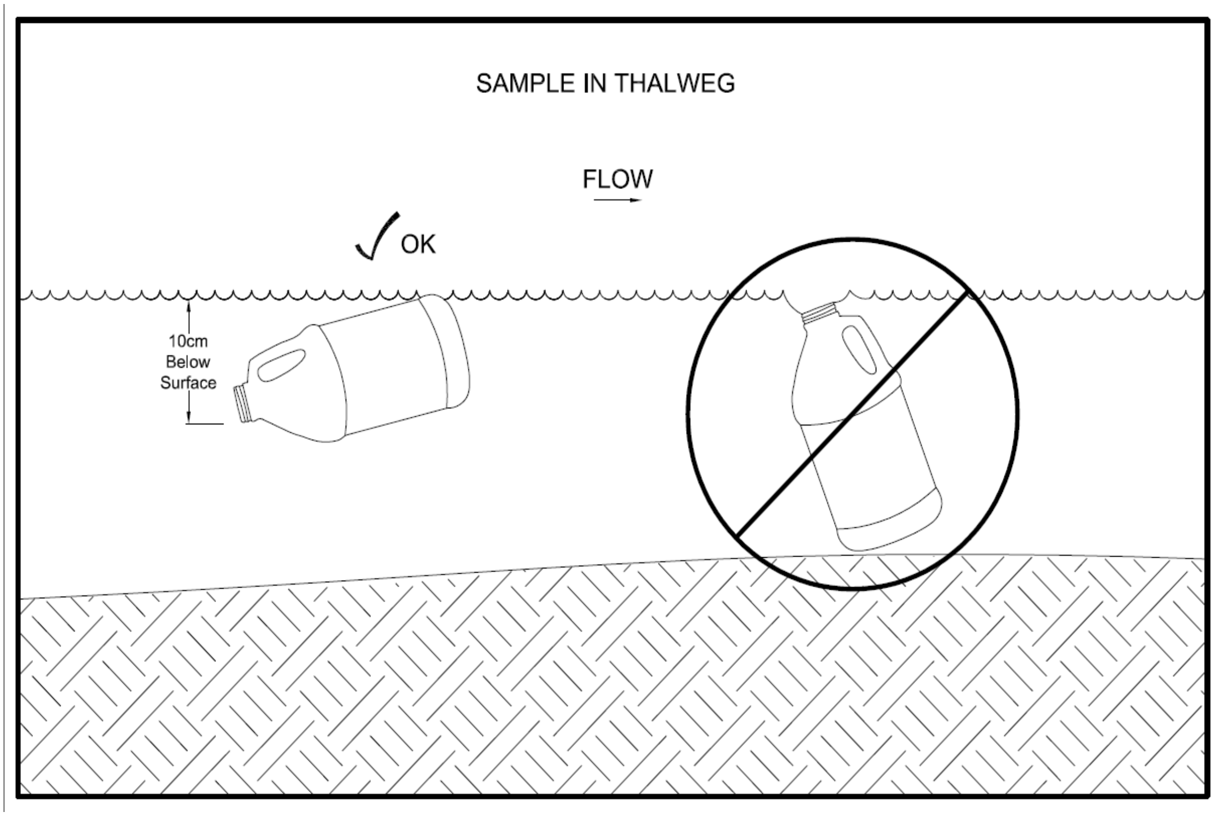


Figure 7. Diagram of proper and poor placement of a water sampling bottle

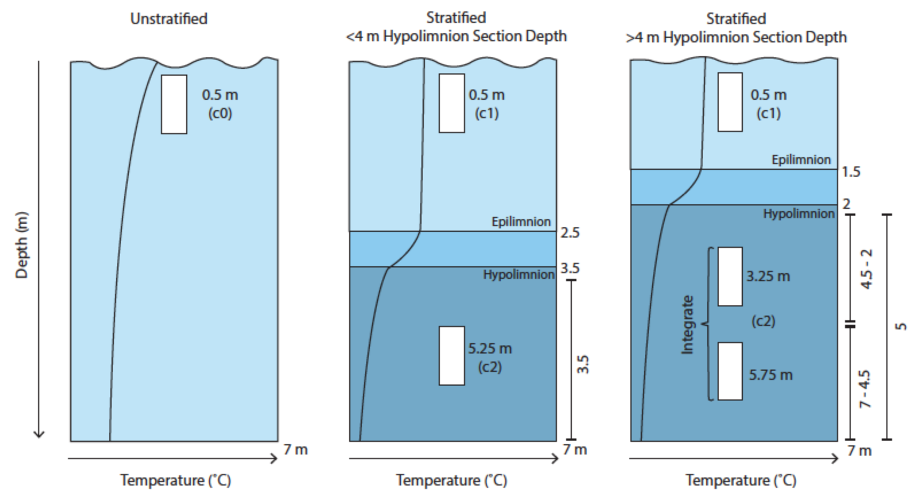
2. Rinse the collection bottles and caps with the appropriate sample water (i.e., use filtered water to rinse filtered samples):
   a. Bottles to be rinsed with stream water:
      1) 4 L jug (can be used for filtered samples and/or PCN, see below)
      2) 250 mL burned amber glass bottle for external lab
      3) ANC - 250 mL wide-mouth, HDPE – ***to be analyzed at the Domain Support Facility**.
   b. To rinse: Hold the cap in your hand (setting the cap down increases risk of contamination). Lower the collection bottle under the water surface (approximately 10 cm below the surface) so that the opening of the bottle faces upstream. Allow stream water to fill approximately $^{1}/_{5}$ of the collection bottle. Remove bottle from stream, cap and shake. Discard water downstream. Repeat 2 more times.
      1) NOTE: You may also use the field pump to pump water out of the stream (from just below the surface) and into your bottles for rinsing.
3. Proceed to SOP C.

B.2 Lake and Non-Wadeable Stream Collection

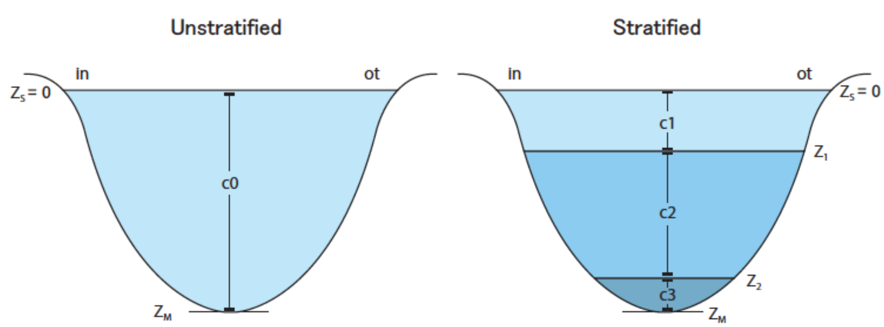
DETERMINE SAMPLING DEPTH BASED ON STRATIFIED OR NON-STRATIFIED WATERBODY

1) Determine the stratification conditions from the Secchi Disk and Depth Profile Sampling in Lakes and Non-Wadeable Streams (RD[06]), Section 7, SOP C
2) Take one sample at a 0.5 meter depth at the inlet, outlet, and buoy (the bottom of the Kemmerer should be located at 0.65 m)
3) Is the lake thermally stratified?
   a) If NO, do not take any more samples.
   b) If YES, calculate the depth of the **hypolimnion** section at the buoy. Ensure you are calculating the hypolimnion section depth, NOT the maximum lake depth (Figure 8a).
      i) If < or = 4 m, then collect a sample from the midsection of the hypolimnion depth.
      ii) If >4 m, then divide the hypolimnion depth by 2 and collect a sample in the midsection of both those layers. Integrate the samples from the hypolimnion into 1 sample.
4) Note the maximum depth of the lake or non-wadeable stream on the datasheet. If the lake or non-wadeable stream is stratified, also note the upper and lower depth of each section for each integrated sample (Z_{upper}, Z_{lower}) to enable the total depth per section to be known (Figure 8).
5) During winter sampling:
   a) Core through the ice.
   b) Determine the total depth of water below the ice.
   c) Take samples from below the ice as per an unstratified lake or non-wadeable stream.
      i) A minimum of 0.5 m of water below the ice is required to sample.
      ii) If < 0.5 m of water depth is available in the central (buoy) location, then move to a location that is within 10 m of the original location and note the new GPS location.
      iii) If < 0.5 m of water depth is available in the littoral (inlet and outlet) locations, then move to a location that is within 10 m of the original location and note the new GPS location. Do not move farther than 20 m from the shoreline or below a depth of 1.5 m.

a)



b)



		Upper	Lower	Max Depth
c0	⟶	Z_S	Z_M	Z_M
c1	⟶	Z_S	Z_1	Z_M
c2	⟶	Z_1	Z_2	Z_M
c3	⟶	Z_2	Z_3	Z_M

Figure 8. Example of an unstratified and stratified lake water column a) sampling depths with placement of thermocline and b) identification of upper and lower section depths. In deep lakes, 2 thermal stratifications may occur, creating 3 sections.

FIELD SAMPLING –LAKES AND NON-WADEABLE STREAMS: USING A KEMMERER SAMPLER

1. Take your water sample from the windward (the upwind) side of the boat to lessen any contamination from the boat.
2. Record the Date and the time of day (use local, military time; ex. 13:46) that samples were collected in the Surface Water Chemistry Field Sampling Datasheet (RD[05])
3. Prepare Kemmerer sampler for sampling and check the knot at the bottom of the sampler for tightness and size. The knot should be sufficiently large so that it will not pull through the central tube of the sampler.
4. Cock the sampler by pulling the trip head into the trip plate by holding the top and bottom stoppers and giving a short, hard pull to the bottom stopper.
5. Holding the rope securely in one hand, lower the Kemmerer sampler gently, allowing it to fall to the desired depth with the other hand. Be sure you hold the messenger securely on the rope (Figure 9b). Depth markings are found on the rope.
6. When the desired depth is attained, drop the messenger to release the clamps and seal the sampler.
7. Retrieve the sampler from the water column. Water is dispensed into the appropriate containers/sample bottles through the spout (Figure 9a).
8. Repeat steps 1 through 7 for each sample.

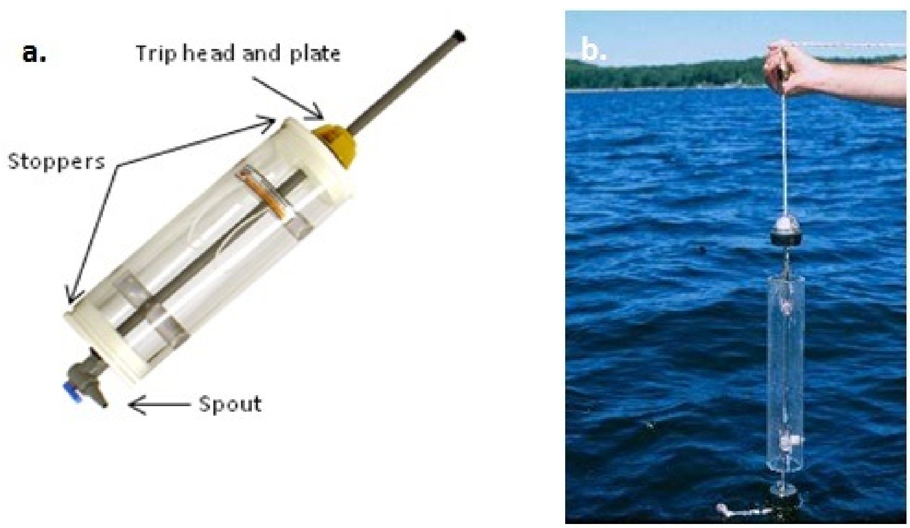


Figure 9. Illustration of Kemmerer sampler for water sampling (a). Illustration of how the Kemmerer is lowered into the water by holding the rope and messenger (b)

9. Rinse the collection bottles and caps with the appropriate sample water (i.e., use raw sample water for unfiltered samples and use filtered water to rinse filtered samples) (NOTE: you may just fill two 4 L jugs to be used for all raw and filtered water in SOP C):
   a. Bottles that can be rinsed with raw sample water:
      1) 4 L jug(s) (can be used for filtered samples and/or PCN, see below)

2) 250 mL burned amber glass bottle for external lab (code RAW)
3) ANC - 250 mL wide-mouth, HDPE – ***to be analyzed at the Domain Support Facility**.

To rinse: Hold the cap in your hand (setting the cap down increases risk of contamination). Empty part of the Kemmerer sampler into the collection bottle. Fill approximately $^{1}/_{5}$ of the collection bottle with water. Cap bottle and shake. Discard water away from the area you are sampling (other side of the boat or downstream of any current). Repeat 2 more times.

B.3 Groundwater Collection

To collect groundwater samples from the wells several steps need to be completed. In summary, locate and unlock the well (combo for lock is same for all NEON locks on site); remove the sensor and cable from the well; measure the water depth:

- If sufficient water depth: set-up the pump, attach tubing, and position pump in well; pump water from well until stable water quality parameters are obtained; collect two 4 L jug samples of water for filtering and processing.
- If insufficient water depth: use the bailer method.
- If the water level is below the minimum water depth: Do not obtain water samples by either method.

The following sections provide specific details for each step summarized above. Figure 10 provides a schematic showing the logic process for determining minimum water depth for obtaining samples, which method to select, and the length of tubing required for each well.

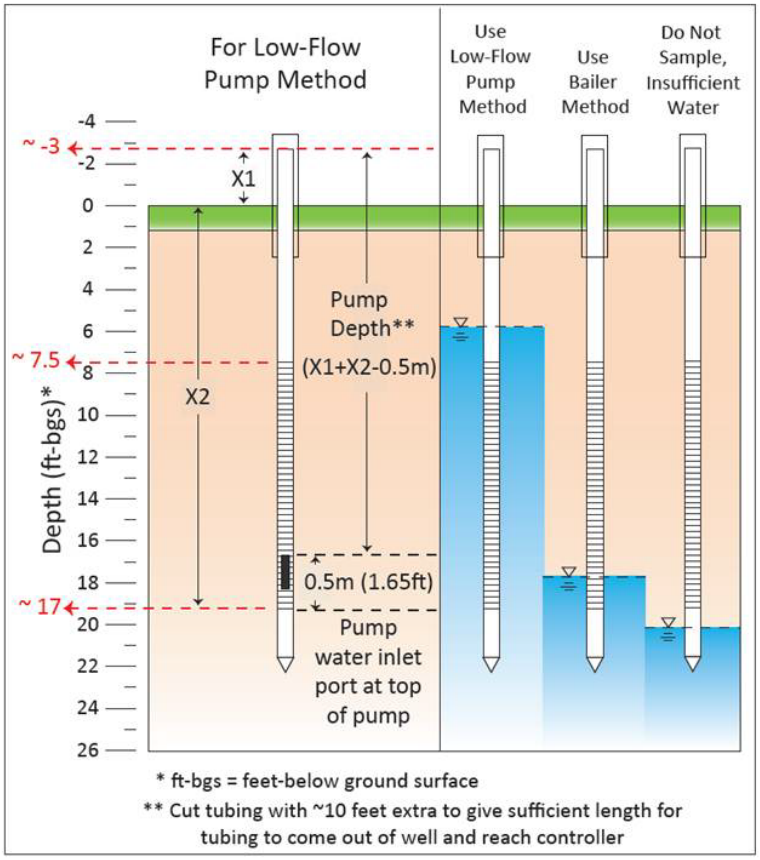


Figure 10. Procedure to determine which sampling method to select and the length of tubing for the pump in the well.

LOCATE WELL AND MEASURE DEPTH TO WATER

The NEON groundwater observation wells (OW) will look different from Site to Site and may be camouflaged at sites within National Parks. Figure 11a) below shows what a typical OW looks like. Wells can be difficult to locate the first time. A well map with GPS coordinates should be taken to the field the first time.

1. Measure the depth to water in the well

    a. Attach the water level tape to the outer steel casing of the well if this is present (may not be in some sites) (Figure 11b)
    b. Note the depth to water and time on the datasheet (RD[05]). The inner PVC well casing should have a black mark on it to indicate the spot on the well casing to take the reading. The water level tape is read like a standard ruler or survey tape as shown in Figure 11c.
    c. Turn the water level tape on by turning the dial on the side of the reel, and slowly lower the tape down into the well. The water level tape will give an audible signal when it

reaches the water in the well (the knob used to turn the unit on is also the volume control).

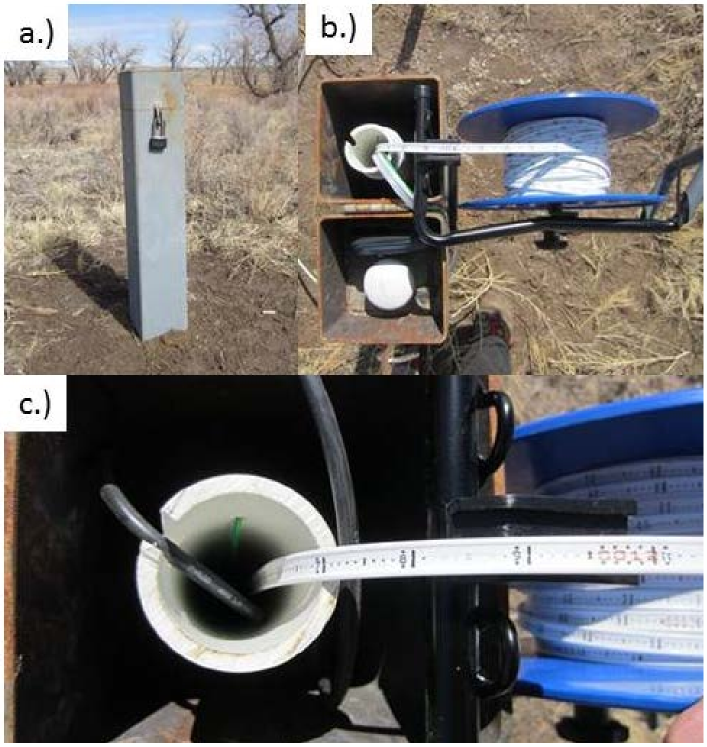


Figure 11. (a.) Standard groundwater well at a NEON site (b.) Water-level tape attached to outer well casing. (c.) Reading the depth to water from the water-level tape. The tapes are marked in “decimal feet” with each foot increment marked in red. Readings are taken at the top of the inner PVC casing.

2. Remove Groundwater Well Sensor from Well
   a. Pull the sensor cable and mounting cable out of the well gently so as to not damage the sensor (they are sensitive to shock).
   b. Place the sensor and coiled sensor cable in a clean 5 gallon bucket. The end of the sensor cable will either be attached directly to a telemetry unit or to a blue desiccant canister. If attached to a telemetry unit, then do not disconnect. If attached to a desiccant canister then when putting in the bucket make sure to have the canister positioned so it is not in water in the bucket.

EXTRACT GROUNDWATER FROM WELL: LOW-FLOW PUMP METHOD

This method is used when the elevation of the water in the well is more than 1.65ft (0.5m) greater than the bottom of the screened interval of the well as illustrated in Figure 10 above.

1. Cut Tubing to Correct Length. The tubing used to sample the groundwater wells is dual bonded tubing with one line for air delivery to the pump and one line for water discharge from the pump. Tubing used to sample a well is dedicated to that specific well and should not be used to sample other wells. The first time a well is sampled the tubing will need to be cut to the correct length. The top of the pump is positioned in the well at 0.5m above the bottom of the screened interval of the well; see Figure 10 above for details. Well construction logs should be used to determine where the screened interval of the well is relative to the ground surface; the set of well construction logs are at each Domain Office for sites in that Domain.
    a. Use Figure 10 above to determine the length of tubing to hang the pump in the well at the specified depth. Make sure to add an extra 7-10 ft to the length of the tubing so that the tubing can extend from the top of the well to the ground surface at a location suitable for positioning the pump controller. From the example in Figure 10 above, the length of tubing required for the well is (17 + 3 – 1.65) ft + an extra 7-10 ft to get the tubing to a sufficient location on the ground surface. The total length required then for this particular well is about 25-28 ft.
2. Set-up the Sample Pump. There are a few components to the sample pump: The sample pump, controller/air compressor, air lines, a battery, and a flow-thru cell.
    a. Assemble Pump – The pump has push-in style fittings for connecting the air and water lines to the pump. The push-in fitting plate (a thin metal disk) needs to be changed out between sampling events as the tubing doesn't come out of the plate once installed in it. To change the fitting plate, unscrew the 3-in tall cylinder at the top of the pump, remove the disk with the "A" and "W" on it, and then place the fitting plate on the top of the pump with the holes lining up, then reassemble the pump. Note: make sure that the small metal plate has the "TOP" facing upwards. Figure 12 (a-d) illustrates this for each step.

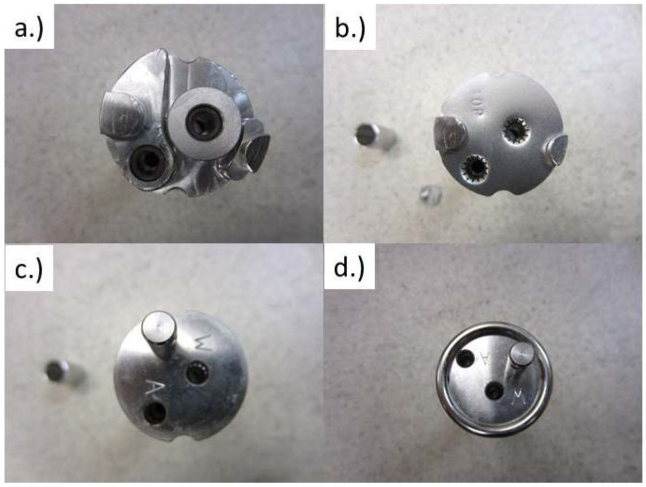


Figure 12. Assembly of the fitting plate at the top of the pump for holding the air and water lines. (a) Bare top of pump. (b) Fitting plate added ("TOP" must face upwards). (c) Top plate added. (d) Collar added to lock parts (a-c) together.

b. Change bladder (if needed) – The pump uses a bladder to hold water drawn in from the well and compressed air that surrounds the bladder to discharge the water from the pump and out of the discharge lines to the surface. Periodically this bladder will need to be replaced as it will develop holes from use or will be contaminated with sediment. To do so, open the lower portion of the pump to expose the bladder and then cut the old bladder off and reinstall a new one by sliding the new bladder over the bottom port on the top of the pump. Figure 13 shows the components of the pump including the bladder.

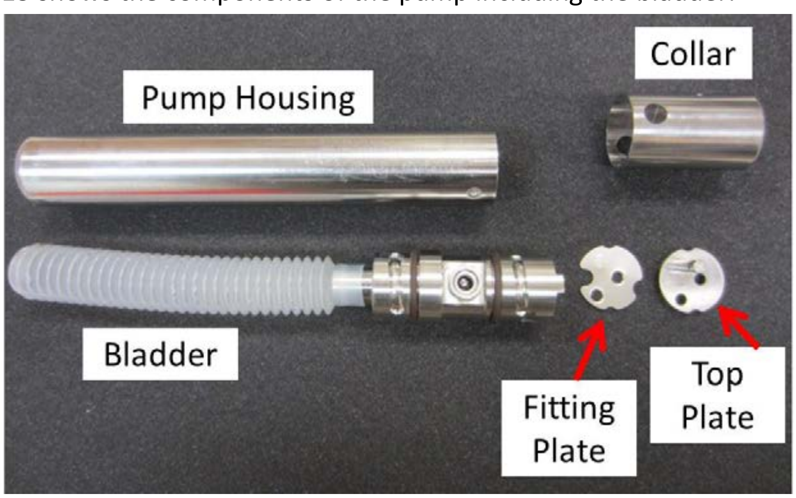


Figure 13. Components of the sampling pump

c. Attach Tubing Lines and Cable
   1) To connect the tubing to the pump, separate the two lines for about 5" of length and then push each tubing line through the holes in the top of the pump. The top

plate is denoted with an "A" for the GREY air-line and a "W" for the clear water-line. The lines should push into the pump top by about 0.5-in. A little water dabbed on the ends of the tubing help facilitate inserting the tubing in the pump.

2) The plastic cable used to hold the pump is the same cable used to hold the sensor in the well, and is the correct length for hanging the pump in the well. Disconnect the cable from near the sensor on the sensor cable by opening the quick-link and attaching the quick-link to the top of the pump. The other end of the cable will already be connected well dock (metal ring) at the top of the well with another quick-link. Do not disconnect this side of the cable. If the cable is not connected to the pump then there is potential to lose the pump down the well.

3. Place Pump in Well: Once all tubing is connected between the pump and controller, gently lower the pump into the well holding onto the air and water discharge lines and plastic coated cable until the pump rests on the cable.

4. Set-up the Controller / Compressor / Flow-Thru Cell

   a. Connect the blue air-line tubing contained in the controller kit to the AIR OUT port on the controller compressor, and then the GREY air-line coming from the pump to the other push-in fitting on the opposite end of the blue air-line. To remove the GREY air-line from the blue air-line pull the thin black collar back toward the brass fitting and pull the tubing out of the push-in fitting.
   b. Connect the water discharge line to the flow-thru cell by connecting the WHITE water-line to the bottom push-in style port. Place the hand-held water quality probe (YSI PRO2030) into the top of the flow-thru cell through the black rubber bushing. A short (2 foot) water outlet line should already be connected to the top port of the cell. If one is not present then cut a piece of tubing from the tubing bucket and install it.
   c. Operation of the Controller:
      1) Once the pump is placed in the well and all the air and water lines are connected then turn the pump on by first checking to make sure the "Throttle" (regulator dial) is turned counter-clockwise to till it stops.
      2) Connect the controller / compressor to the battery, this will turn the compressor on, but no air should come out of the controller yet.
      3) Slowly turn the throttle clockwise to being adding air pressure to the air-line to the pump. As a rule of thumb 1 PSI of air pressure is required to lift water in the pump line 2 ft. So if the depth of the pump is 17 ft then the controller should be set to a minimum of 9 PSI and a maximum of 15PSI. The max PSI should not be more than 10 PSI over the minimum pressure required to lift the water. The pump should begin to discharge water in pulses.

The function of the controller is to control the length of time that water is allowed to enter into the pump, the length of time that air is sent to the pump to discharge the water in the pump, and the air pressure used to discharge the pump (i.e., compress the bladder in the pump). The controller has two main modes of operation for this, a manual "MN" mode where the user specifies the length of time for each step, and a preset "ID" mode where there are predetermined settings. Pushing the "MODE" button on the controller toggles between the different modes of operation and the "UP" and "DOWN" arrows show the settings within each mode. Either mode is acceptable to use but ID mode is easier.

When selecting the pump air-inlet and water discharge times, the main aim is to achieve a relatively continuous flow into and out of the flow-thru cell ranging between 100 and 500 mL/min, which is measured by putting the discharge line from the flow-thru cell in to a graduated cylinder and measuring the flow over 30sec or 1min intervals periodically through-out the sampling event. Ideally once the flow rate is set it will be maintained at this rate for the duration of the sampling event for the well.

5. Monitor water level: Water-level within the well should be monitored every few minutes using the water-level tape in the procedure detailed earlier. The aim is to select a pumping rate from the well that does not cause the static water level within the well to decline by more than 10% of the value initially noted before pumping started. As an example, if the static water level in the well was measured at 10.24 ft from the top of the casing prior to turning the pump on then the decline in the well should be limited to about 1.25 ft (i.e., measured water depth should not be less than 11.50 ft from the top of the casing). If the water depth declines more than the 10% threshold, then select a decreased pumping rate by either choosing a shorter discharge time on the controller or turning the throttle counter-clockwise to decrease the air pressure delivered to the pump.

6. Monitor water quality: While the pump is discharging water from the well, monitor the water quality parameters Dissolved Oxygen (mg/L), Specific Conductance (uS/cm), and Water Temperature (°C) to provide a metric to determine when the water is ready to be collected for sampling.

    a. Specific conductance should be measured as temperature-corrected conductivity at 25°C, whenever possible. Ensure conductivity measurements are on the appropriate temperature-corrected and unit setting (i.e., setting SPC, uS/cm).
    b. Take readings from the hand-held meter approximately every 3-5 minutes during the pumping event and noted on the sampling sheet in addition to the time.

Once readings stabilize, by varying less than 10% over 3 consecutive readings spaced a minimum of 3 minutes between readings, then the water being discharged from the well is ready to be collected for sampling. Once the well water is ready to be collected for sampling, it should be collected directly from the water line coming from the pump.

c. Compress the push-in fitting on the flow-thru cell and disconnect the WHITE water-line and put the line into the 4 L jug for this well.
d. The following sections detail the actual water samples to be collected and the procedure to do so. One to two 4 L jugs of water will need to be collected from each well and should be collected all at one time.
e. **Record** water quality parameters (SPC, DO, and Temp) at the time of sampling using the hand-held meter on the field datasheet (RD[05]).
f. **Record** the sampling method and approximate volume of water discharged/removed from the well prior to collecting water for samples (Multiply the discharge rate by the length of time of discharge).

Bailer Tube Method

The bailer tube method is a second, less robust, method for obtaining samples from wells. This method is applied for wells where the elevation of the water level in the well is less than 0.5m above the bottom of the screened interval of the well as shown in Figure 10.

1. This method is much simpler than the Low-Flow method and is performed by evacuating the water in the well using a "bailer tube" which is a small diameter hollow tube that has check ball in the bottom of it.
2. Lower the tube into the well on the end of a string below the static water level and allowed to fill-up with water.
3. Pull the tube out of the well and dump contents into a bucket. This process repeats until **three** "well-volumes" are removed from the well.

A well-volume is defined as the volume of water contained within the well and is calculated as:

$$Well\ Volume = (Total\ Well\ Depth - Depth\ to\ Water)\ x\ \pi\ r^2$$

Where the "Total Well Depth" and "Depth to Water" are measured from the top of the PVC well casing and "r" is the radius of the well (2" diameter, 1" radius, r^2 = 0.00694 ft^2). As an example Figure 10 above for the bailer tube method shows a water level in the well of ~ 17.75 ft and a total depth of ~22.00 ft, with an assumed well diameter of 2-in, this yields a well volume of (22.00 – 17.75)ft x π x (0.00694)ft^2 = 0.0926 cubic ft of water, or about 0.70 gallons of water, and three well-volumes equal to 2.1 gallons of water. **Note:** 1 cubic foot = 7.48 gallons.

1. After three well volumes are removed from the well, collect sample water by again using the bailer tubes to pull water from the well and pouring the water directly into the clean 4 L jugs.

2. **Record** water quality parameters (SPC, DO, and Temp) at the end of the three well volumes by filling a small bucket or bottle with well water and using the hand-held meter to measure the parameters on the field datasheet (RD[05]).
3. **Record** the sampling method and approximate volume of water discharged/removed from the well prior to collecting water for samples.

FIELD SAMPLING - GROUNDWATER

After recording water quality parameters, sampling method, and approximate volume of water discharged:

1. Rinse a 4 L collection bottle and cap with the appropriate sample water (i.e., use filtered water to rinse filtered samples)
   a. To rinse: Hold the cap in your hand (setting the cap down increases risk of contamination). Fill water from pump into jug until about 1/5 full, shake to rinse the bottle and discard water away from the well. Repeat 2 more times.
2. Collect an unfiltered water sample: Fill the 4 L jug (or more if necessary).
   Collect water in a 4 L jug to be divided and filtered into appropriate containers (Figure 5). Cap the jug, set aside for SOP C. Set the jug away from the sun and heat.

SOP C Sample Processing

In **wadeable streams**, fill the collection bottle by placing the bottle 10 cm below the water surface with the opening pointed upstream (Figure 7) or pump water directly out of the stream from 10 cm below the water surface. If you are not filtering directly out of the stream, collect water in a 4 L jug to be filtered into appropriate containers (Figure 5). In **lakes and non-wadeable streams**, use the Kemmerer to collect unfiltered water in one - two 4 L jugs from the desired depth. Cap the jug, set aside for Section C.2 (Collect Filtered Water). For **groundwater** sampling, collect water from the white/clear tubing coming out of the well while the pump is running (after values have stabilized, as described above).

C.1 Collect Unfiltered Water (RAW)

1. Collect unfiltered water samples using the appropriate sampling method, described above.
2. If you collected the sample in a 4 L jug, use sample water in the jug (shaken to re-suspend particles) to rinse (3 times) the sample bottles for the 2 samples that do not require filtering (Figure 5):
    a. 250 mL burned amber glass bottle for external lab (Code RAW)
    b. Acid Neutralizing Capacity: 250 mL wide mouth HDPE – **FILLED** (Code ANC) ***to be analyzed at the Domain Support Facility.**
3. Fill the collection bottles to the rim and close cap tightly.
4. Collect an additional 4 L jug to be filtered for Total Particulate Carbon and Nitrogen (code PCN) analysis (NOTE: the **filter** is your sample and will be analyzed for PCN; SOP C.5). You may need less than 4 L. This is site specific.
5. IMMEDIATELY chill samples (4°C ± 2 °C). DO NOT FREEZE.

C.2 Collect Filtered Water Samples (FIL)

In **wadeable streams** and **groundwater** sampling, you can filter directly from the pump tubing. For **lakes** and **non-wadeable streams,** you can filter directly out of the 4 L jug. You may also wish to use the 4 L jug for **wadeable stream** samples, as well.

1. Set-up of Peristaltic Pump Apparatus (Figure 14):
    a. The peristaltic pump should be fitted with peristaltic tubing connected to ¼ in Inner Diameter (I.D.) C-Flex tubing on either end (**a**).
    b. Place a **CLEAN** end of the tubing in the 4 L collection jug (**b**). Rinse tubing with DI water before placing in jug if necessary.
    c. Attach the other end of the tubing to a $^{3}/_{8}$ – ¼ in tubing connector, which is then attached to the peristaltic tubing and pump (**c**).
    d. The other end of the pump should connect to a $^{3}/_{8}$ – ¼ in tubing connector.
    e. Attach one end of ¼ in C-flex tubing (2 ft long) to the tubing adaptor (**d**).

   f. Using the drill peristaltic pump, rinse tubing with approximately 100 mL of sample water. The direction of the drill pump can be changed, if necessary.
   g. When tubing has been rinsed and is mostly filled with water (i.e., no large air pockets), attach the end of the outflow tubing to an unused filter capsule fitted with a tubing connector (**e**).
      1) **NOTE:** make sure to attach filter so that the direction of flow follows the flow arrow on the capsule filter.
      2) Filter approximately 100 mL of sample water to rinse the filter, and discard this rinse water

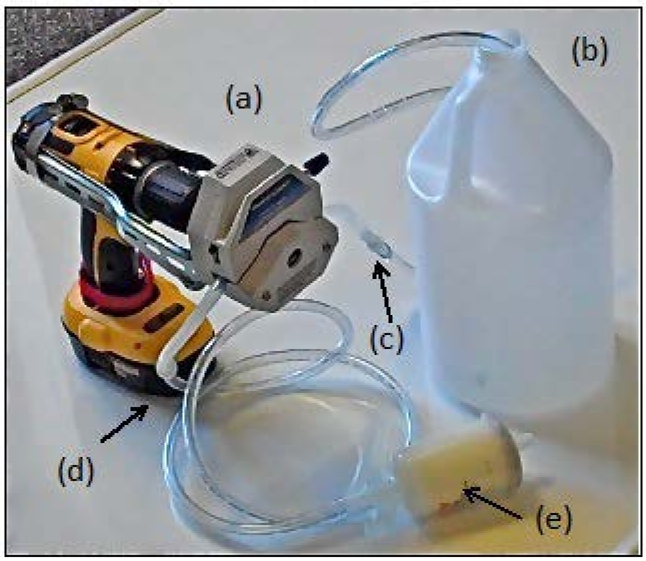


Figure 14. Pump and filter setup, including (a) a peristaltic sampling pump (modified from Woessner 2007), (b) a 4 L sample bottle, (c and d) tubing connectors to connect peristaltic and C-flex tubing, and (e) a capsule filter

2. Filter setup:
   a. Use Velcro or a c-clamp to secure drill trigger at desired speed. Do not pump too fast, or you could blow out the filter.
   b. When the tubing has been rinsed (Step 1), and is filled with water attach filter and begin pumping water through the filter. Making sure the tube is filled with water will reduce air being forced through the filter and the potential to blow a hole
3. Rinse sample bottles and caps with <u>filtered</u> water. You may wish to secure the drill trigger at desired speed, thus freeing one hand while filtering.
   a. Filter approximately 25 mL into the 1 L glass bottle (FIL) and the 250 mL HDPE bottle (ALK). Cap and shake to rinse.
   b. Repeat rinsing 2 more times

4. Ensure the tubing stays completely submerged in the water at all times.
5. Fill filtered glass sample bottle (FIL, Figure 5) to the neck. **Fill ALK bottle completely (NO HEADSPACE).**
6. Place samples in cooler with ice to keep cool (4°C ±2°C) until returned to lab. Group ALK and ANC samples together and ensure they will not be accidentally shipped to the water chemistry analytical laboratory.
7. IMMEDIATELY chill samples (4°C ± 2°C). DO NOT FREEZE.
8. Dispose of the capsule filter after all samples have been filtered. These are one time-use filters.

C.3 Total Particulate Carbon and Nitrogen (PCN) Sample Collection

Use the water collected in a 4 L jug as described in step 5 of SOP C.1. Filter with vacuum pump and filter funnel to obtain a particulate sample on filter (Figure 15).

1. Rinse filter unit, filter screen and filter funnel (Figure 15) with DI water, making sure no particulates remain on the filter screen or funnel.
2. Insert the stem of the filter unit into the hole in the middle of the rubber stopper and insert the stopper into the filter flask.
3. Remove the filter funnel from the base, leaving the filter unit and screen resting on the manifold stem.
4. Use filter forceps to remove a 25-mm pre-ashed GFF filter from the box and place the filter on the screen of the filter unit.
5. Replace the filter funnel, rinsing with DI if necessary before replacing. Make sure that the filter is in the center of the filter unit. Ensure there are no gaps between the side of the filter and the filter unit, and that there are no holes in the filter itself.
6. Place the filter box back in the Ziploc bag to keep the filters from getting wet or blowing away.
7. Attach the vacuum pump tubing to the filter flask (Figure 15).
8. Rinse the filter with DI water. Use the vacuum pump to create suction in the flask and draw the DI water through the filter.
9. Shake the 4 L jug of water you collected at the lake/stream in order to resuspend and uniformly mix the particles (approximately 15 s).
10. RINSE the clean plastic 250 mL graduated cylinder with 25 mL of sample water. Turn cylinder on its side and rotate to rinse all sides of cylinder. Discard the rinse water.
11. Resuspend particulates (shake for 15 s) and immediately pour the water into the filter funnel. Be careful: the funnel only holds 200 mL of sample
12. Use vacuum pump to pull water through the filter (do not exceed more than 15 lb/in of pressure). IMPORTANT: BE CAUTIOUS OF THE VOLUME OF WATER IN THE FILTER FLASK SO YOU DO NOT SUCK WATER INTO THE VACUUM PUMP. DISCARD FILTERED WATER WHEN WATER IN FLASK REACHES THE FLASK NECK.
    a. **NOTE:** Do not exceed 15 in. Hg vacuum on the pump gauge.

13. Repeat previous 2 steps until water starts to move more slowly the filter slowly and particulates are visible on the filter. At that point, add water in smaller increments until the filter is clogged and water no longer passes through (< 1 mm of particulates material thickness). This may not be obtainable in waters with low particulate (i.e., clearwater lakes), in which case filter a minimum of 2 L of sample.
14. **IMPORTANT: Keep track of the amount of water you filter**. We will need the total volume filtered for the PARTICULATE calculation. See step 17 and 20.
15. Use a DI water squirt bottle to rinse down the particulates on the sides of the filter funnel.
16. Continue to pump until all the water is drawn through the filter.
17. Release the vacuum and record the TOTAL volume of water filtered for the PCN sample on the shipping inventory (RD[11]). Be sure to include the appropriate units (mL). **NOTE: All the water in the tower should be filtered once poured because particles will start to settle. DO NOT add more water than you can filter.**
18. Using clean filter forceps, remove filter funnel from the filter unit; **fold filter into quarters**. Folding filter helps reduce loss of particulate sample. DO NOT touch filter with your hands to reduce risk of sample contamination.
19. **Be Careful.** If filter tears or rips, begin filtration over with a new filter and sample water.
20. Place filter on 4 X 4 in piece of aluminum foil, fold foil around filter and add label. Circle lab code PCN.
21. **Record total volume of water filtered** on label and field datasheet (RD[05]).
22. Double bag foiled filter in resealable plastic bag and place in cooler.
23. Using 1 L DI water jug (or more, as necessary), rinse the filter set-up (filter unit, funnel and flask) and equipment (forceps, graduated cylinder).

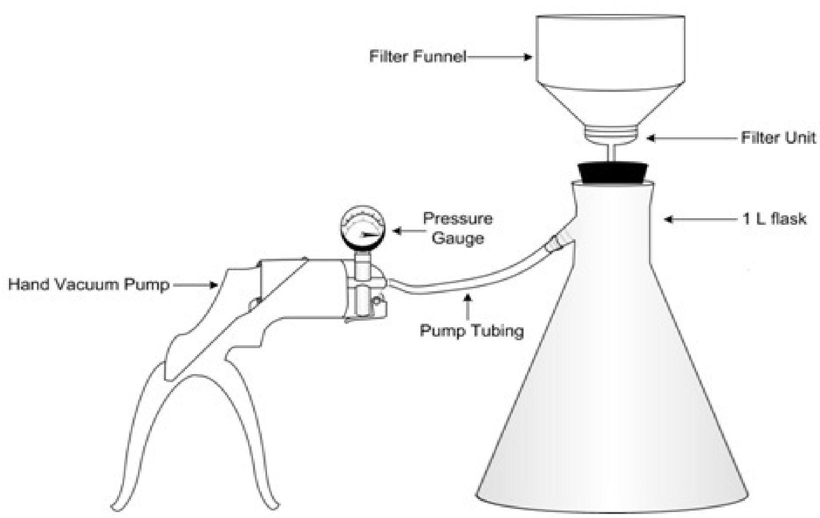


Figure 15. Filter apparatus set-up for particulates. Includes the hand vacuum pump attached to the filter flask and filter funnel/manifold

C.4 Ending the Sampling Day

Refreshing the sampling kit

1. Restock the sampling kit (shipping cooler) with new water chemistry sampling bottles with new labels attached, (alkalinity and ANC bottles can be rinsed with DI water and reused), nitrile gloves, filters, resealable plastic bags, foil, etc. Refer to section 6.1.

Equipment maintenance, cleaning and storage

1. Run clean water through the peristaltic pump to rinse tubing. Make sure to pump all water out of tubing before storage.
2. Charge drill pump batteries.
3. Triple rinse Alkalinity and ANC sample bottles with DI to be re-used.
4. Using 1 L DI water jug (or more, as necessary), rinse the filter set-up (filter unit, funnel and flask) and equipment (forceps, graduated cylinder).

SOP D Laboratory Sampling and Analysis

Alkalinity and Acid Neutralizing Capacity (ANC) are measures of the water's ability to buffer systems from changes in pH by neutralizing strong acids for filtered and non-filtered (i.e., whole-water samples), respectively. Thus, alkalinity and ANC are identical in systems without titratable particulates. Alkaline compounds include bicarbonate, carbonate and hydroxides, each of which removes H^+ ions from the water, ultimately increasing the system pH. Lakes without these alkaline compounds are often unable to buffer against changes in acidity, and therefore, any acid added to the system, such as from acid rain or wastewater effluent, may result in an immediate decrease in lake water pH. Thus, alkalinity and ANC are important measures to understand and predict how a system will respond to acidic inputs.

To determine alkalinity and ANC concentrations, a known strength of acid is added until the three main forms (bicarbonate, carbonate and hydroxide) are converted to carbonic acid. At pH 10, ~8.1, and ~5, hydroxide, if present, carbonate, and bicarbonates respectively are converted to carbonic acid.. By a pH 4.5, all bicarbonate and carbonate species should be converted to carbonic acid. The pH at which the species are converted is the equivalence point. NEON will calculate total alkalinity and ANC, thus focusing on the bicarbonate equivalence point (~pH 5). The amount of acid needed to convert the species to carbonic acid is correlated with the amount of alkalinity and ANC in the sample. NEON expresses alkalinity as meq/L.

NEON will largely follow the USGS procedures for the analysis of alkalinity and ANC using a digital titrator (Rounds 2012). Measurement will be determined at the Domain Support Facility following the Inflection Point Titration (IPT) Method for most of the NEON Aquatic sites. The IPT method is a titration method in which the sampler titrates on both sides of the expected equivalence points. The point at which the slope of the titration curve is the steepest is the inflection point. However, when alkalinity or ANC is extremely low (<0.4 meq/L or 20 mg/L) or conductivity is low (<100 µs/cm), the Gran function plot (Gran) method will be followed. This protocol focuses on the use of the IPT method, and briefly mentions the Gran method. For additional details on the IPT method or the Gran method, see the USGS protocol (Rounds 2012).

Verify the reproducibility of samples by completing a sample analysis on a replicate sample or a reference sample, at a minimum of every 10 samples. Reproducibility should be ±5%. For low conductivity (<100 µS/cm), low alkalinity (<4 mEq/L), reproducibility should be within 10%.

D.1 Sample Processing Timing

Following sample collection, alkalinity and ANC samples should be kept on ice or refrigerated at 4°C ±2°C. Laboratory analysis should be completed as soon as possible after returning from the field. Alkalinity and ANC samples should be processed within 24 hours. Samples analyzed after the 24 hours window will be flagged. The maximum allowable time period between sample collection and analysis is

72 hours. It is advised to not collect field samples on Friday, unless alkalinity and ANC can be measured in the Domain Support Facility on Friday afternoon.

D.2 Preparation

1. Turn on pH meter well in advance of sample analysis (approximately 30 minutes).
2. Allow pH buffers 4 and 7 to come to room temperature before calibration by allowing the sample bottle to sit on a lab bench until the temperature has equilibrated.
    a. Make sure buffer solution has not expired and is not reused.
        1) Discard all expired chemicals in accordance with Site Specific Chemical Hygiene Plan and Biosafety Manual or with Site Specific Chemical Disposal Procedures. Check Safety Data Sheets for more information or contact NEON Safety Department.
    b. Ensure the bottle has been capped during storage to reduce contamination.
3. Check the pH meter calibration at pH 4 and 7. DO NOT use kimwipes on pH probe tip.
4. If the pH meter is off by ≥0.1 pH units, calibrate pH meter following pH meter manual.
5. Record the meter readings on the Water Chemistry Domain Lab Data Sheet (RD[05]). If the meter is re-calibrated, record the post-calibrated pH check values.
6. Ensure sulfuric acid titrant solutions have not expired.
    a. Discard all expired chemicals in accordance with Site Specific Chemical Hygiene Plan and Biosafety Manual or with Site Specific Chemical Disposal Procedures. Check Safety Data Sheets for more information or contact NEON Safety Department.
7. Allow samples to come to room temperature by letting the sample bottle(s) sit on the lab bench until the temperature has equilibrated. You can pour out the volume of sample you will use in the titration in a labeled glass beaker, covered with plastic, to help sample come to room temperature more quickly.

D.3 Sample Processing in the Lab

1. Determine the method (Inflection Point Titration (IPT) or the Gran method) of measurement you will use by evaluating known conductivity or alkalinity measurements. Most waters will use the IPT method. However, when alkalinity or ANC is <0.4meq/L or 20mg/L or conductivity <100 µS/cm the Gran method should be followed. Record method type on the Water Chemistry Domain Laboratory Datasheet - Alkalinity/ANC Titrations (RD[05]). For additional details on the IPT or the Gran method, see the USGS protocol (Rounds 2012).

    Note for Gran Method Users: This protocol details the IPT Method, although the information in the steps is still useful to the Gran Method users. See USGS Gran method (Rounds 2012) for detailed instructions on using the Gran Method to calculate alkalinity. Contributing carbonate species will not be determined). In short, titrate to **pH 5.5** (DO NOT GO PAST 5.5 TOO FAST), and then add acid in small increments (to change pH 0.2-0.3 pH units). Titrate to pH of 3.5. Do NOT use a stir bar if conductivity is < 100 µS/cm, but swirl solution gently (do not create a vortex) between additions. Wait 15 - 30 s before recording data and adding more acid.

2. Determine the sample volume and acid normality you will use (Table 10). The majority of measurements will require a 50 mL volume with 0.16 *N* titrant. Thus, if you do not know the expected alkalinity or ANC values, start with a sample volume of 50 mL and 0.16*N* titrant, and adjust as necessary. 1.6*N* will only be used when alkalinity or ANC is greater than 4.0 meq/L, although it may not be necessary. Table 10 provides suggested sample volume and titrant normality, but should be adjusted as necessary per site. Record sample titration normality on the Datasheet (RD[05]).

Table 10. Suggested sample volume and titrant normality for alkalinity and ANC measurements based on approximate concentration ranges

Alkalinity or ANC (meq/L)	Alkalinity or ANC (mg/L as $CaCO_3$)	Sample Volume (mL)	Titrant Normality (*N*)	Minimum Beaker Size (mL)
0-1.0	0-50	100	0.1600	150
1.0-4.0*	50-200	50	0.1600	100
4.0-20	200-1000	100	1.600	150
>20	>1000	25	1.600	50

* indicates the suggested volume and titrant solution if alkalinity or ANC is unknown. ANC is acid neutralizing capacity. Table modified from USGS TWRI Book 9, Alkalinity, Version 3.0 7/2006.

3. Attach the titrant cartridge to the digital titrator body. Chemical resistant gloves and safety glasses are needed when handling the cartridge and setting up the titrator.
   a. Depress the plunger-release button and retract the plunger.
   b. Insert cartridge into the end slot of the titrator (Figure 16) and **rotate cartridge** one-quarter turn to lock into place.

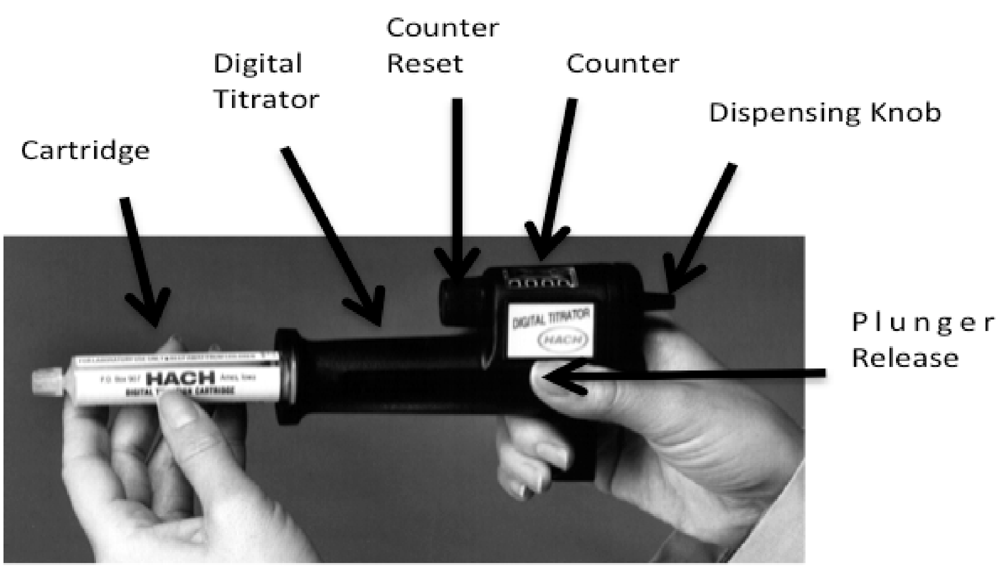


Figure 16. Inserting titrant cartridge into digital titrator. Photo from the Hach Digital titrator manual

c. Depress plunger-release button and push plunger forward until it is touching inside of cartridge. If plunger will not engage with the cartridge, ensure that the cartridge has been rotated one-quarter turn and is locked into place.
d. Attach titrator set up to titrator bracket on the mounting bracket.

4. While wearing gloves, remove cap on titration cartridge and insert a clean titration tube into the cartridge tip (Figure 16). If tube is new, label tube with correct normality. You may need to turn the titrator upright so the bubble comes to the tip. Store the cap in alkalinity test kit, so that you do not lose the cap. You will need to recap the cartridge when finished.

Figure 17. Digital titrator with titrant cartridge and titration tube attached. Photo from the Hach Digital titrator manual.

5. Turn the delivery knob to expel a few drops of titrant into a discard/acid waste container (Figure 18). This should remove air bubbles from the tube.

Figure 18. Diagram of procedure to expel acid from digital titrator set-up into a temporary acid waste container.

6. **Reset the counter to zero** and wipe the tip of the tube with a soft, lint-free tissue, such as a Kimwipe®. Once set to zero, do not turn the delivery knob.
7. Place a clean, small, magnetic stirrer into the appropriate sized beaker (Table 10). Do not use a stir bar if conductivity is less than 100 µs/cm. Using a stir bar in low conductivity water will

increase the diffusion of gases into the sample and alter the pH. If you have low conductivity water, after each titration, swirl the sample lightly by moving the beaker slowly in one circular motion. Do not swirl so fast that you create bubbles or a vortex in the sample. Allow the sample to stabilize before recording the data and continuing titrations.

8. Shake sample bottle for 30 s to homogenize.
9. Using a pipette (for alkalinity, filtered sample) or a graduated cylinder (for ANC, unfiltered sample), measure out the appropriate volume of sample and transfer to appropriate glass beaker (Table 10). Note: a pipette is a more accurate measuring device, and should be used on filtered alkalinity samples. Since particulates may get caught in the pipette tip, use graduated cylinders when measuring for non-filtered ANC samples.
   a. A small amount of sample will remain in the pipette tip when dispensing the sample from the pipette to the beaker. Touch and hold the tip of the pipette to the beaker wall and allow pipette to drain. Once flow from pipette stops, hold tip against the beaker wall for 10 more seconds to remove the majority of sample. A small volume of sample will be retained in the pipette.
10. Place the beaker on the stir plate and turn the power on. Stir should be slow and steady to avoid creating a vortex in the beaker.
    a. If sample splashes on wall of beaker, spray it down with DI water. Adding DI will not influence the titration reactions.
    b. If sample splashes out of beaker, start over.
11. Rinse pH meter and temperature sensor with deionized water and carefully blot dry with lint-free cloth. Be cautious not to rinse probes over sample.
12. Insert pH meter and temperature sensor into sample water, making sure to not touch the stir bar or the sides and bottom of the beaker.
    a. Sample solution must cover the pH sensor bulb, sensor reference electrode and temperature sensor (Figure 18). Increase volume, using pipettes or graduated cylinders, as necessary, or change beaker size, being sure to transfer the entire sample by rinsing beaker with DI into the smaller beaker. Volume of rinse DI should not be included as part of the sample volume.
13. **Record:** Start time of titration, initial sample pH and temperature (°C), sample volume, titrant normality (0.16 or 1.60 *N*), and initial titrator count (should be reset to zero) (RD[05]).
14. Insert the digital titrator tip into the sample in the beaker without touching the stir bar. Tip should be immersed in the sample (Figure 19).

Figure 19. Diagram of titration set-up with digital titrator, stir bar, pH meter and temperature probe. Ensure nothing is touching the sides and bottom of the beaker or the stir bar

15. **Add Titrant** (Table 11). After each addition of titrant, allow the stirrer to homogenize the sample for 15 – 30 s or gently swirl the sample if you have a low conductivity sample. Record pH and counter reading on the Alkalinity/ANC laboratory data sheet (RD[05]). You do not need to fill out the grey-celled columns. They will be calculated in a spreadsheet. Near equivalence points (pH ~10, 8.1 and 5), pH can change rapidly (Figure 20). **This protocol focuses on total Alkalinity and ANC, thus focusing on the bicarbonate equivalence point at pH ~5. If you add titrant too fast or in too great of increments, you will miss the inflection point completely!** Therefore, you must add titrant in smaller increments around these points, being sure to provide ample mixing time before the readings. After adding titrant, wait 15 - 30 s before recording and continuing the titration.
    a. pH ≥5.5 - Titrate with larger increments to just above a pH 5.5. Do not add in increments that are so large that you skip this region completely. **After each addition of titrant, allow the stirrer to homogenize the sample for 15 – 30 s. Record pH and counter reading on the Alkalinity/ANC laboratory data sheet (RD[05]).**
    b. pH <5.5 - <u>Bicarbonate equivalence point</u>. Cautiously and slowly add titrant in small (but not less than three counts on the digital titrator) increments from pH 5.0 to ≤4.0. If using the Gran method, add acid in small increments (to change pH 0.2-0.3 pH units) to pH ≤3.5. Titrate to pH ≤3.0 for samples with high organic acids or if sample range is unknown. **After each addition of titrant, allow the stirrer to homogenize the sample for 15 – 30 s. Record pH and counter reading on the Alkalinity/ANC laboratory data sheet (RD[05]).**

Table 11. Guidelines for sulfuric acid titration for alkalinity and ANC sample analysis

pH	Titration addition guidelines
≥5.5	Add in larger increments, but do not skip region entirely
pH <5.5	Add in small increments, no less than 3 counts

16. When possible, enter data into computer spreadsheet and graph the titration curve (change in pH divided by change in titrant volume (y-axis) by volume of titrant added (X-axis) (Figure 20).
    a. If more than one inflection point occurs in proximity, the true inflection point has been missed, and a duplicate sample should be analyzed being sure to take precaution and add titrant in smaller increments around the inflection point.

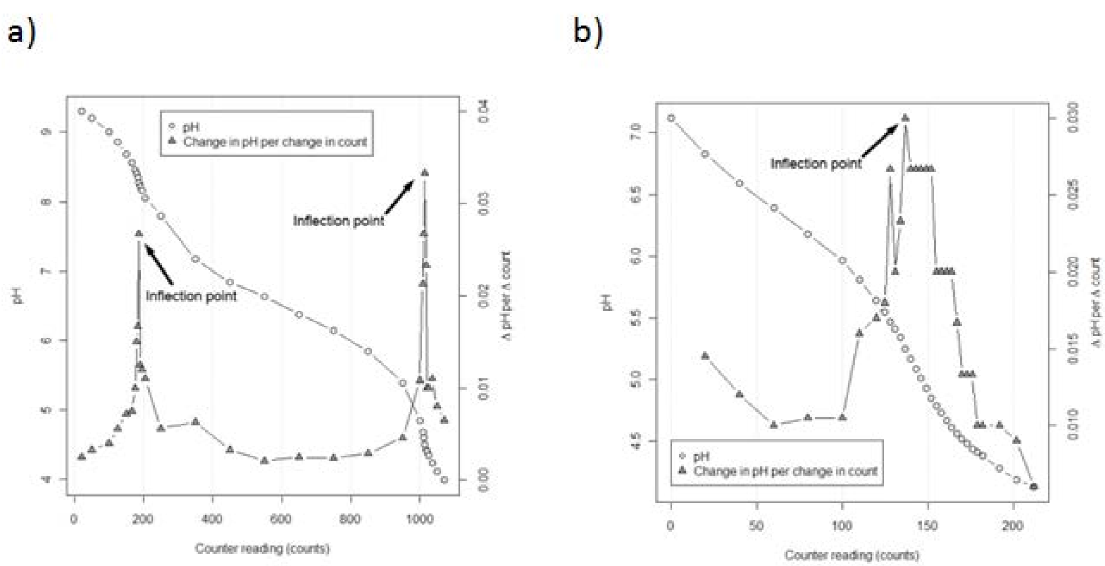


Figure 20. Example of inflection point titration using a digital titrator of a) a high alkalinity sample and b) a low alkalinity sample. Note difference in Y-axis scale. (Modified from USGS TWRI Book 9, Alkalinity, Version 3.0 7/2006).

17. When titration is finished, use soda ash or soda bicarbonate to return the sample pH to a pH 6 - 9. Use a pH meter to ensure the proper pH level.
18. Dispose of sample.
19. Repeat for the second alkalinity/ANC sample.
20. Remove digital titrator from beaker. Depress plunger release and retract plunger to remove cartridge. Remove titrator tube. Cap cartridge tip.
21. Immediately double rinse titration tube and glassware with DI water and blot dry with lint-free soft paper tissue.
22. Place titration tubes in clean, sealable bag labeled with the titration normality (0.16 or 1.6 *N*).
23. Titration tubes can be reused if rinsed well, but should be only used for the same titrant normality. When tubes begin to show wear (e.g., stretching at the end that attaches to titrant cartridge), replace with a new one.

24. Store all glassware, titrator, titrator tubes, and chemicals appropriately.
25. Rinse and re-use 250 mL alkalinity and ANC sample bottles.

D.4 Ending the Processing Day

Refreshing the laboratory supplies

1. Check expiration date of sulfuric acid titrant and pH buffer solutions. Order more if expiration has passed or will be passed within the next month.
2. Ensure you have enough equipment for the next sampling event. Refer to section 6.1.

Equipment maintenance, cleaning and storage

1. Double-rinse glassware and titrator tubes with DI water immediately after use. Glassware, titrator, titrator tubes and chemicals should be clean and dry before storage. Titrator tubes should be stored in resealable plastic bags and labeled with the titrant normality for which they were used. Store alkalinity kit parts in the blue field case. Store cartridges in a resealable plastic bag in a cool, dark place or a frost-free refrigerator.
2. Titrators do not require calibration. However, to ensure that the titrators have maintained calibration, annually perform a calibration check, using a known sample, of the accuracy and precision of the digital titrator.

SOP E Data Entry and Verification

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

SOP F Sample Shipment

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

Shipments are to have a hardcopy of the "per Sample" tab of the shipping inventory (RD[11]) 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. ShipmentID (shipment tracking #) must be included in the electronic version of the shipping inventory, but is not necessary for the hard copy. Also include the shipment tracking # in the email.

F.1 Handling Hazardous Material

N/A

F.2 Supplies/Containers

NOTE: Shipping vessels and materials vary with the number of sites and site type.

1. Pack glass bottles in packing material for protection from breaking.
2. Place samples into the cooler (suggested: 9 qt for 1 station and 12 qt for 2 stations) and add ice or ice packs (0˚C ice packs). **ALL** water chemistry samples should be surrounded by the ice packs, including the filter (wrapped in foil and placed in a resealable plastic bag). Water ice allows you to better surround each bottle and keep the samples cool.
   a. There should be at least an equal volume of ice and samples in the cooler.
   b. More ice packs will be required in the summer, since coolers may sit outside in the sun for several hours.
   c. If water ice is used, line cooler with a small trash bag and tie up to prevent water leakage if ice melts. Additionally, if ice is used, care must be taken to pack sample bottles securely, in case ice melts (e.g., wrapping bottles in bubble wrap and placing in a resealable bag, or placing ice in resealable bags, so if the ice melt the water filled bag will still provide a cushion for the bottles).
3. Surround bottles with absorbent packing material.
4. Fill remaining space with regular packing material.
5. Place 'per sample' tab of AOS shipping inventory (RD[11]) in a resealable plastic bag and tape to the inside top of cooler. Include a return shipping label (F.6)
6. Tape the cooler shut and ship to appropriate address

F.3 Timelines

1. Ship samples to the External Water Chemistry Laboratory immediately following processing, when possible. Ship water chemistry samples **overnight** to the external laboratory within 24 hours from sample collection in order to minimize chemical speciation and sample degradation. Ship only samples that will be analyzed by an outside laboratory. Make sure ALK and ANC samples remain at the Domain Support Facility.
2. Ship samples "**Priority Overnight**."
   a. **DO NOT** send them "FedEX First Overnight."
   b. **DO NOT ship samples on Friday.**

F.4 Conditions

Keep samples at 0.5˚C – 6˚C. DO NOT FREEZE.

F.5 Grouping/Splitting Samples

N/A

F.6 Return of Materials or Containers

1. Include a return shipping label to your address with account information so the analyzing laboratory can return the cooler to you.
2. Place return shipping label and the AOS sample shipping inventory (RD[11]) in a resealable plastic bag and securely tape the bag to the inside cooler lid to help keep the forms dry.

F.7 Shipping Inventory

Fill out the AOS Sample Shipping Inventory (RD[11]). Each box sent should have a copy of the 'per sample' tab of the shipping inventory of its contents. The 'Shipment ID' does not need to be filled out on the hardcopy. The electronic shipping inventory that includes ShipmentIDs and IDs of all samples shipped should be emailed to the appropriate contact at the receiving analytical laboratory as well as the NEON CLA contact on the day that samples ship. Include shipping IDs and estimated arrival date(s)/time(s) in the email as well.

F.8 Laboratory Contact Information and Shipping/Receipt Days

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

8 REFERENCES

LTER Sampling protocols: http://ecosystems.mbl.edu/arc/streams/protocol2.html#WaterChemistry

Nevers, M.B., and R.L. Whitman, 2007. United States Geological Survey, USGS Lake Monitoring Field Manual.

U.S. EPA, 2004. Summary of Sample Depth Collected as part of the WQS for all Five Lakes. Appendix P.

Puls, R.W. and Barcelona, M.J. 1996. Low-Flow (Minimal Drawdown) Ground-Water Sampling Procedures: EPA Ground Water Issue. Report EPA/540/S-95/504.

U.S.G.S. 2006. National Field Manual for the Collection of Water-Quality Data. U.S. Geological Survey, Water Resources Office of Water Quality. Chapter A4. Collection of Water Samples. (Version 2.0). http://pubs.water.usgs.gov/twri9A/

Rounds, S.A., 2012, Alkalinity and acid neutralizing capacity (ver. 4.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6., sec. 6.6, September 2012, accessed December 2, 2012, from http://pubs.water.usgs.gov/twri9A6/.

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YSI Model 85 Calibration Instructions. http://www.aquaticeco.com/YSIManuals/85.pdf

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 12. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002906	Datasheets for AOS Protocol and Procedure: Water Chemistry Sampling in Surface Waters and Groundwater
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

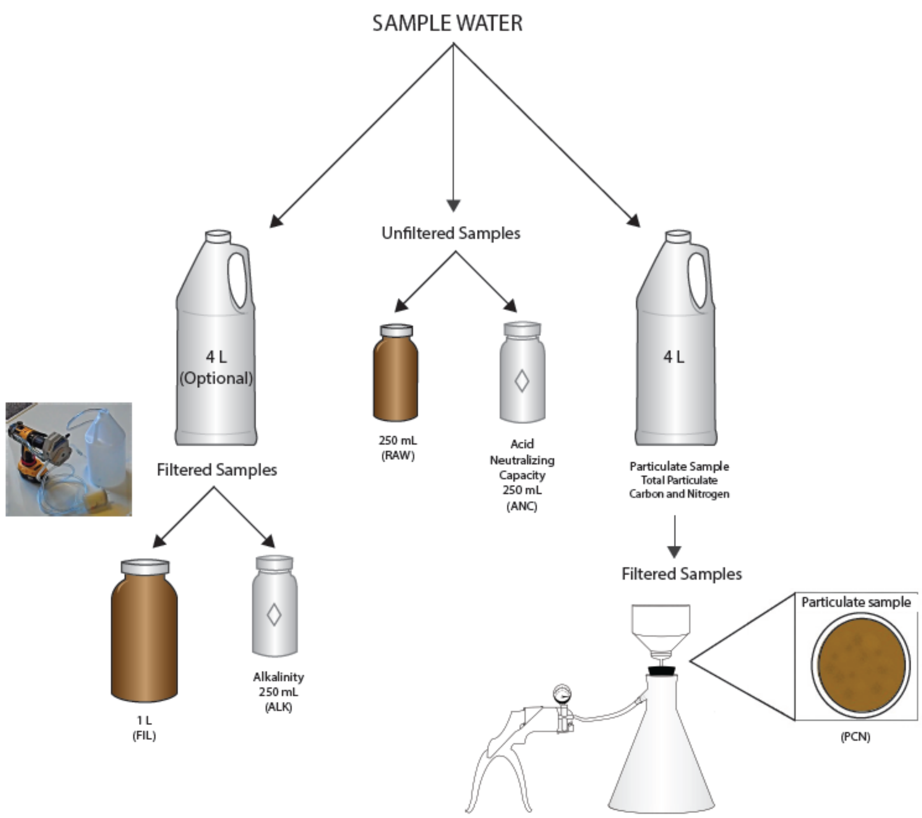
These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

B.1 Considerations for Implementation

Samples must be kept cool at all times (4°C ± 2°C). Samples should be processed (filtered and chilled) within 3 hours, but if logistical constraints make it difficult, processing samples within 6 hours is acceptable. The sooner the samples are processed, the better the data quality. If there is a problem with sample filtration and particulates can still be seen in the filtered samples, samples can be re-filtered, ONLY if it is within the 6-hour time window from collection.

B.2 Flowchart of Sample Collection and Filtration



◊ Indicates sample bottles that will remain at the Domain Support Facility for analysis. Letters in parenthesis indicate the codes that correspond to the chemistry labels.

B.3 Steps for Water Chemistry Sampling

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

Step 2 – Prepare labels (2" * 4").

a) b)

Blank NEON Chemistry Labels for a) the External Analytical Laboratory and b) Internal NEON Domain Support Facility Measurements.

Step 3 – Ensure the General AQU Field Metadata Sheet (RD[07]) is completed per field site visit.

Step 4 – Navigate to the sampling location or groundwater well. In lakes take a Secchi depth reading and determine thermal stratification.

Step 5 – Rinse the collection bottles and caps with the appropriate sample water (i.e., use filtered water to rinse filtered samples).

Step 6 – Collect samples

1. Filtered Samples: Fill glass bottle to the bottom of bottle neck, and fill ALK completely to reduce any changes in CO_2 concentrations due to headspace:
   a. 1-L burned amber glass bottle for external lab – Filled to neck (Code FIL)
   b. ANC - 250 mL wide-mouth, HDPE – FILLED (Code ALK) *to be analyzed at the Domain Support Facility.
2. Unfiltered Samples: Fill glass bottle to the bottom of bottle neck, and fill ANC completely to reduce any changes in CO_2 concentrations due to headspace:
   a. 250 mL burned glass amber bottle – Filled to neck (Code RAW)
   b. Acid Neutralizing Capacity: 250 mL wide mouth HDPE – FILLED (Code ANC) *to be analyzed at the Domain Support Facility.
   c. 4 L jug (for PCN and/or Filtered Sample)

Step 7 – Filter for PCN particulate samples.

Step 8 – Ship samples overnight with ice packs within 24 hours of collection.

Step 9 – Complete ALK and ANC titrations in Domain Facility.

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare all equipment including labels and filters.
- ☑ Pre-print labels on waterproof paper.
- ☑ Fill out the labels before they get wet.

Sample collection: Be sure to…

- ☑ Do not sample anywhere you or other field technicians have walked, or locations that appear recently disturbed. Wait for disturbance to pass.
- ☑ Use caution when sampling as items can easily fall into water while sampling.
- ☑ Fill ALK and ANC bottles completely (no headspace), and fill all other sample bottles to the bottom of the neck.
- ☑ DO NOT FREEZE samples.

Sample filtering: Be sure to…

- ☑ Keep track of the volume of sample water filtered for PCN.
- ☑ Once poured, filter all of the water in the tower because particles will start to settle.
- ☑ DO NOT add more water into the filter tower than you can filter.

Sample titrations: Be sure to…

- ☑ Add titrant in smaller increments around equivalence points (pH~5)
- ☑ After each addition of titrant, allow the stirrer to homogenize the sample for 15-30 s. In low conductivity samples, stir manually.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

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

APPENDIX E SITE-SPECIFIC INFORMATION

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