AOS PROTOCOL AND PROCEDURE: REAERATION IN STREAMS

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See configuration management system for approval history.

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	10/31/2012	ECO-00680	Initial draft release
B	04/23/2014	ECO-01123	Initial production release
C	08/29/2014	ECO-02233	Minor updates based on feedback from the field
D	11/07/2014	ECO-02438	Migration to new protocol template
E	11/07/2014	ECO-02456	Minor changes based on field training
F	03/26/2015	ECO-02646	Minor changes to shipping and labeling
G	01/21/2016	ECO-03547	Minor changes following FOPS input, title change from 'AOS Protocol and Procedure: Reaeration Measuring Diffusion of O2 Across the Water-Air Interface' to 'AOS Protocol and Procedure: Reaeration in Streams'
H	02/06/2017	ECO-04431	Updated Template to RevG, updates from FOPs training; HOBOs should be logging temp in Celsius, updated battery specs to 6 or 8 volt, extended shipping time requirements, record salt slug mass, directions for sites that will model reaeration added to Appendix.

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1 OVERVIEW

1.1 Background

Stream metabolism measurements quantify the amount of primary production and respiration occurring within a reach (both benthic and water-column) by measuring changes in oxygen (O_2) concentration within a stream segment. Changes in oxygen concentration can occur from both biological, such as primary production (O_2 gain in the water column) and respiration (O_2 loss), and physical, such as gas exchange with the atmosphere (i.e., reaeration, the gain of O_2, or deaeration, the loss of O_2) as oxygen diffuses across the water-air interface. In order to understand the biological controls on oxygen within our systems, we must first account for the physical controls (e.g., reaeration or gas exchange).

Reaeration (i.e., gas exchange) is the movement of oxygen from the atmosphere into the water, and is measured as the net rate (i.e. gain and loss of oxygen) at which gas exchanges across the air-water interface. Stream reaeration rates are influenced by several physical characteristics of the site, such as the temperature, turbulence, wind, tributary and groundwater inputs, and oxygen concentration gradient across the interface. The reaeration rate coefficient (K_2) represents the combined effects of these physical characteristics. In turbulent and low productivity streams, reaeration rate coefficients may be the dominant term in the oxygen balance and for determining the potential for an oxygen deficit. Thus, it is imperative to quantify stream reaeration rates accurately as a small error in reaeration rates can dramatically skew stream metabolism results. Furthermore, reaeration represents the net flux of O_2 and CO_2 into and out of the atmosphere, and therefore accurately quantifying reaeration rates will be important for climate change research and carbon budget calculations.

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 field protocol used by NEON for measuring stream reaeration in small, wadeable streams follows the general requirements set forth by Lotic Intersite Nitrogen eXperiment (LINX) II (2004), the laboratories of Dr. Bob Hall, University of Wyoming, and Dr. Michelle Baker, Utah State University, as well as outlined in Hall and Tank (2003).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.004104	NEON Science Performance QA/QC Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC. 002382	Datasheets for AOS Protocol and Procedure: Reaeration Measuring Diffusion of O2 across the Water-Air Interface
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[09]	NEON.DOC.001085	AOS Protocol and Procedure: Stream Discharge
RD[10]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

2.3 Acronyms

Acronym	Definition
ASR	Analytical Services Request
cm	centimeter
GC	Gas Chromatograph
HDPE	High-density polyethylene
ln	Natural Log
m	meter

2.4 Definitions

Conservative tracer: A nonreactive chemical tracer that remains constant as it follows the flow of water. Ex) Chloride (Cl^-)

Deaeration: Loss of oxygen molecules from a liquid (or gas).

Diffusion: The movement of particles from an area of higher concentration to an area of lower concentration.

Discharge: The volume of water flowing past a point on a stream during a specified unit of time.

Ecosystem Metabolism: In streams, ecosystem metabolism is the measure of the production and respiration of organic matter. It is often used as a measure of steam function because it is a measure of the interaction between organic matter and nutrients across a stream reach.

Gas Exchange Rate*:* See reaeration rate.

Inert gas*:* A gas that does not interact with the environment thus making it a useful tracer of gas exchange across the air-water interface.

NaCl: Sodium Chloride (salt). The Cl of the NaCl compound is the conservative tracer used in this protocol.

Plateau: Time at which the stream is at steady state with the conservative tracers (i.e. stream concentration remains constant)

Reaeration: Physical movement of gas from the atmosphere to a body of water.

Reaeration rate (AKA gas exchange rate): The net rate at which gas exchanges across the air-water interface (i.e. gain and loss of oxygen).

SF_6: Sulfur Hexafluoride. The inert (non-reactive) gas in this protocol.

Travel time: The length of time it would take an average grouping of water molecules to travel from one location within a watershed to another location.

3 METHOD

This protocol outlines the procedures required to measure gas exchange (i.e., reaeration rate coefficient, K_2) from the atmosphere to the water by use of an inert (i.e., will not interact with any biogeochemical processes) gas tracer (e.g., Sulfur Hexafluoride (SF_6)), as well as a conservative tracer (e.g., NaCl) to account for groundwater inputs to the system. Stream reaeration is often measured by injecting an inert gas (e.g., Propane or SF_6) into the stream water at the top of a study reach. Because the inert gas concentration is higher in the stream than the atmosphere, the gas diffuses out of the stream (Figure 13). The diffusion rate of the inert gas is proportional to the O_2 gas exchange rate (Wanninkhof 1992, Raymond et al. 2012). Thus, measurements of the concentration of the inert gas are used to calculate an O_2 reaeration (or deaeration) rate coefficient (K_2). To account for dilution due to surface or groundwater inputs, a conservative solute tracer such as chloride (Cl^-) or bromide (Br^-) is added to the stream in addition to the inert gas.

This protocol will only be implemented in small, wadeable streams. Reaeration measurements should be conducted when no other work is being conducted in the stream, as disturbance of sediments and habitat may influence reaeration results.

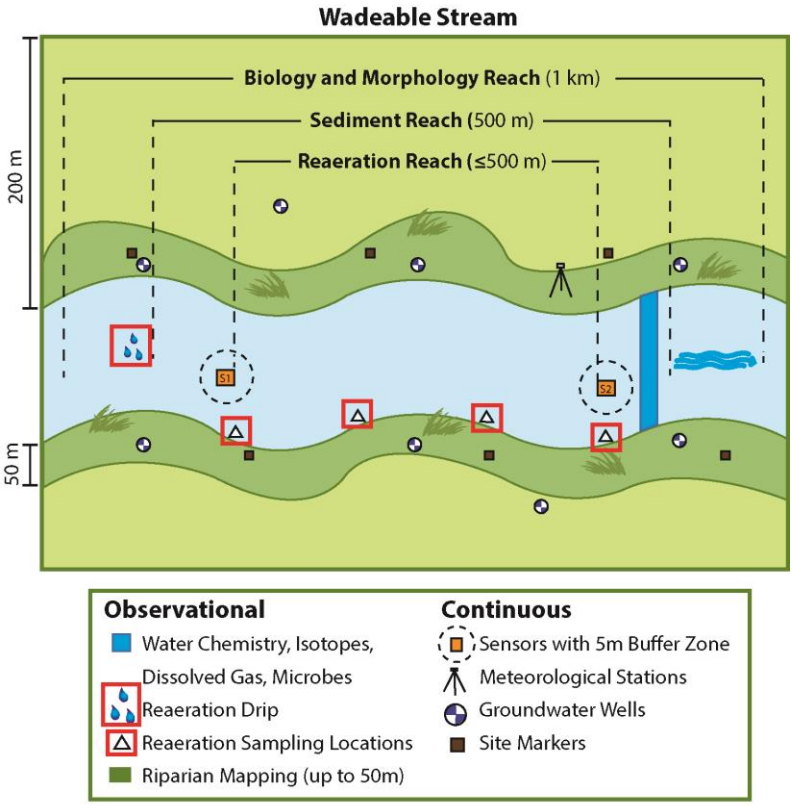


Figure 1. A generic wadeable stream site layout example with reaeration sampling stations

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

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

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

3.1 Assumptions

1. Since we are unable to account for losses from our stream to groundwater, a critical assumption of reaeration measurements is that groundwater losses are minimal.
2. Assume tracers (both the inert gas and conservative tracer) are uniformly mixed in the channel cross section.

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Reaeration measurements shall be completed up to 10 times annually during NEON Site Characterization activities and up to 6 times annually during NEON Operations in wadeable stream locations. Sites that are high risk sites for flooding resulting in changes in stream morphology may be requested to continue to collect reaeration 10 times per year. Sampling events should be spread out throughout the year so as to collect a range of flows.

Timing of sampling is site specific and determined by rules developed using historical flow regime and environmental data. For example, streams with little or no flow during the summer dry-season are sampled more intensively during wet periods. Streams with snowmelt-dominated hydrographs are sampled more intensively during spring/summer-elevated flows than during winter snow-covered months.

4.2 Criteria for Determining Onset and Cessation of Sampling

Following NEON Site Characterization, relationships will be developed between reaeration, discharge, and other physical parameters. These relationships will be checked each year during Operations to ensure the curve has not shifted over time. To establish and maintain the reaeration-discharge rating curve, simultaneous measurements of reaeration and discharge must be made over a range of discharges. Therefore, the production of a continuous record of reaeration requires periodic manual reaeration measurement checks during Operations. In the event that the curve check does not produce results similar to the original reaeration-rating curve, such as after a major flooding or scouring event, a new curve will need to be established.

In general, the reaeration curve will be checked for a high, low and average discharge event. Rating curve checks may also be completed during times selected to help fill in gaps in the measured data for the reaeration-discharge rating curve (i.e., when discharge is in a range with minimal or no reaeration measurements) in order to refine parts of the curve with little data. Stream discharge shall be measured on all reaeration measurement days RD[07].

4.3 Timing for Laboratory Processing and Analysis

Reaeration samples collected at a given site shall be processed within 3 hours of the end of sample collection at that site.

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:

Samples should be processed (shaken, filtered, and transferred to appropriate containers) as soon as possible. If necessary, stream water may be collected in syringes, kept at 4°C, and processed within 3 hours at a base camp or Domain Lab (i.e., if weather dictates the need to leave the field immediately and stream discharge is increasing). Sample collection time, processing station and processing time must be recorded on the Reaeration Data Sheet.

If weather changes during the reaeration injection making conditions unsafe or if stream discharge increases such that the physical condition of the stream has changed, stop the injection and restart on the next scheduled bout.

Monitor the injection equipment during the injection to ensure proper functioning of the salt and gas injection equipment. If equipment malfunctions and can be fixed immediately, do so and continue the experiment, making note of the malfunction on the data sheet.

Reaeration measurements should not be conducted during or immediately after any fieldwork disrupting the stream bottom (i.e., morphology mapping, invertebrate collection, macrophytes collection, etc.). In streams with a shallow water column, samplers must be cautious not to stir up the benthic sediments when sampling the stream water. Disruption of the sediments by walking or by sampling too close to the stream bottom can contaminate your sample. 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.

The weather should be checked the day prior to the scheduled field sampling and adjusted to avoid any major storms. Should it begin raining during the reaeration injection enough to change the flow of the stream, stop the injection and restart at a later date. Reaeration measurements should not be made when the water level and discharge are changing rapidly. If the stream is exhibiting very low flow rates, is disconnected such that it is a series of pools not connected by surface water, or is dry, do not conduct an injection.

Make a note of any weather or stream conditions that could influence reaeration, including but not limited to wind, channel alterations, activities in the surrounding watershed, prior flood or rain events.

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions are unsafe for sampling (i.e. lightning, hail or flooding), stop sampling and resume work at a later time or date when conditions are appropriate for protocol implementation	No adverse outcome.
	If sampling stirred up sediments or added chemical constituents to the stream/lake (i.e., gas additions) within the past hour, allow the water to clear and disturbance to pass, sample upstream/upwind of the disturbance.	No adverse outcome.
Days	If sampling location is dry, frozen, or frozen over, resume work at a later date when stream is flowing.	No adverse outcome.
	If stream flow is too low to ensure a travel time of <3 hours, resume work at a later date when conditions are appropriate for protocol implementation.	No adverse outcome.
	If sampling location is >20% ice-covered, resume work at a later date when stream is not ice-covered.	No adverse outcome.

4.5 Criteria for Permanent Reallocation of Sampling Within a Site

Reaeration sampling will occur on the schedule described above at 4 sampling stations within *wadeable stream sites*. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (relocatable sites). However, circumstances may arise requiring that sampling within a site be shifted from one particular location to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given plot becomes compromised, a problem ticket should be submitted by Field Operations to Science.

There are two main pathways by which sampling can be compromised. Sampling locations can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot becomes permanently flooded or a stream moves after a flood and the location is no longer within the stream channel). Alternatively, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

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.

Gas cylinders must be secured during transport (i.e., the regulator should not be attached to the tank, the safety cap should be screwed on and the tank should be secured upright during transport so as not to roll around, with the bottom of the tank pointed towards the floor). Never pick up a gas cylinder by the cap. See the Compressed Gas Safety training powerpoint on the NEON Safety Sharepoint Page.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 2. Equipment list – Initial trip (**Note: This step is completed by NEON HQ staff**)

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
	R	Container for salt solution	Conservative tracer container	All	2	N
MX100514	R	Conductivity probes, handheld, calibrated	Measuring and viewing conductivity	All	2	N
	R	Stopwatch	Measuring and calculating streamflow	All	1	N
	R	Neutrally buoyant object (i.e., orange)	Measuring and calculating streamflow	All	1	N
	R	Meter tape, 50-100 m metric	Measuring and stream length	All	1	N
Consumable items						
	R	Conservative tracer: Sodium Chloride (NaCl) or Sodium Bromide (NaBr)	Conservative tracer injection	Habitat specific	1	N

R/S=Required/Suggested

Table 3. Equipment list – Gas injection

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
MX105847	R	Gas Tank (e.g., Sulfur Hexafluoride, SF_6 or propane – site specific) 5-10 lbs (10 lb tank contains sufficient gas for about ~30 reaeration measurements)	Gas injection	1	N
MX110050	R	Gas Tank Regulator	Regulating gas flow	1	N
MX100510	R	Gas Flowmeter: - Gas flowmeter, able to regulate flow from 0 – 60 psi; variable area flow meter with needle valve tube with glass float; NPT threaded barbed fittings for tubing - 1/8” MNPT to 1/4” hose barb fittings (MX100551) - Small metal hose clamps	Regulating gas flow	1 2	N
MX100552	R	Gas-impermeable tubing: ¼ inch ID (inner diameter), (e.g., Tygon)	Gas injection	1	N
	R	1-1/8 inch wrench	Connecting regulator to SF_6 tank	1	N
	R	11/16 inch wrench	Connecting tubing to gas flow regulator	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX100905	R	Fine-pore diffuser (1 per stream SITE)	Gas injection	1	N
	R	Small 125 mL squirt bottle (for soapy water)	Testing for gas leaks	1	N
MX106027	R	Plastic tote	Safe transfer of equipment	1	N
	S	Hook	Hanging gas flowmeter	1	N
Consumable Items					
	R	Teflon tape	Creating a seal on the flowmeter tube fittings	1	N

R/S=Required/Suggested

Table 4. Equipment list – Conservative tracer injection

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	5 gallon bucket/carboy (may need to be larger, depending on size of stream)	Conservative tracer container	1	N
	S	Lid for bucket/carboy	Keeping debris from falling into solution and damaging pump	1	N
	S	Extra bucket	Filling conservative tracer container or transporting pre-made conservative tracer solution	1	N
	S	4 L Jug	Filling conservative tracer container or transporting pre-made conservative tracer solution	2	N
HB07770000	R	Fluid Metering (FMI) Pump: - QB metering pump, - Stainless Steel pump head with ceramic piston - Hose barb adapter (¼" Barb * ¼" MIP) - Feed-thru cord switch	Conservative tracer injection	1	N
	R	Gel cell batteries (6 (ex: MX100217) or 8 Volt)	Powering the FMI pump	2	N
	R	¼ inch I.D. tubing – cut to approximately 10 and 1-2 foot long	Conservative tracer injection	2	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	S	3/8 inch ID or ½ inch ID tubing required on inlet side of pump instead of ¼ inch ID, if pump rates are faster than 500 ml/min	3/8" I.D. tubing or greater is required for pump rates higher than 500 ml/min and 1/2" I.D. tubing or greater is required for flows higher than 1200 ml/min.	1	
	S	3/8 ID to ¼ hose ID hose barb converter for FMI inlet (only inlet side needs larger tubing)	Connect Larger hose to FMI inlet	1	
	R	Binder clips	Weighing down or clipping the tubing to the bucket		
	R	Plastic 250 mL graduated cylinder	Calibrating the FMI pump	1	N
	R	Stopwatch	Calibrating the FMI pump	1	N
	R	Battery charger	Charging the batteries	1	N
MX106028	R	Plastic tote for FMI pump	Safe transfer of equipment	1	N
Consumable Items					
	R	Conservative tracer: Sodium Chloride (NaCl) or Sodium Bromide (NaBr) – Site Specific	Conservative tracer injection	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX105455	R	Grease (high grade machine oil)	Greasing the drive pin head on the FMI pump	1	N

R/S=Required/Suggested

Table 5. Equipment list – Sampling

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
MX100514	R	Field thermometer and conductivity meter, handheld - calibrated	Measuring and viewing conductivity	1	N
MX102546	R	Logging Conductivity probes – factory calibrated	Measuring and storing conductivity data	2	N
MX102548	S	Logging Conductivity Probe shuttle	Connecting the conductivity probes to computer	1	N
	R	60-mL syringes, with luer-lok tip, 1 mL graduations (individually numbered and covered with clear packing tape to protect syringe labeling)	Sample collection	25	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	1-way male stopcocks, one per syringe	Sample collection	24	N
MX100548	S	Gas vial rack	Sample storage in the field	1	N
	R	Sharps container	Needle disposal	1	N
	R	Meter-tape, 50- metric field tape	Measuring stream width	1	N
MX104742	S	Rangefinder	Optional measuring of stream width for streams with average width >2m	1	N
Consumable Items					
	R	Conservative tracer: Chloride (NaCl) or Bromide (NaBr)	Conservative salt tracer injection	1	N
	S	Flagging, roll	Marking each sampling station	1	N
	R	60-mL HDPE sample bottles (e.g., Nalgene), pre-labeled. Plus extras	Conservative tracer sample container	25	N
	R	12 mL Exetainer gas vials with Double Wadded White Caps, pre-evacuated and pre-labeled – from external lab	Gas sample container	20	N
	R	Labels for gas vials: 1 inch * 2-$^{5}/_{8}$ inches (e.g., Avery 5661)	Labeling samples	20*	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX106200	R	Needles, 27 gauge, disposable 0.5 inches in length	Transferring gas from the syringe to the sample vial	20	N
MX108794	R	30 mm/ 0.7 µm pore size syringe filters (more filters may be required in colored or turbid water)	Filtering and transferring injectate sample from the syringe to the 60 mL bottle	25	N

R/S=Required/Suggested

Table 6. Equipment list – Site-specific supplies

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
	S	Infrastructure, such as stakes, rebar, or ring stand	Stabilizing tracer injection tubing	As needed	N
	S	Bridge/plank	Walking in designated areas	As needed	N
	S	Small cooler (~9 qt)	Transporting samples	As needed	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Consumable Items					
	S	Zip ties/Cable ties	Injection setup	As needed	N
	S	Ice or ice packs	Keeping gas samples cold	As needed	N
	S	Conductivity Standards	Calibrating equipment	As needed	N

R/S=Required/Suggested

Table 7. Equipment list – Shipping

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
	R	Lock and Lock box, ~2qt	Sample storage container	1	N
	R	Shipping cooler and boxes	Shipping container	1	N
Consumable Items					
	R	Frozen ice packs	Gas sample shipping	1	N
RD[10]	R	Shipping inventory	Providing sample information	1	N
	R	Laboratory data sheets	Providing sample information	As needed	N
	R	Pencils	Filling out data sheets	1	N
	R	Permanent markers	Filling out data sheets and labels	1	N
	R	Clear Packing	Securing labels to vials	As needed	N

R/S=Required/Suggested

6.2 Training Requirements

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

See the Compressed Gas Safety training powerpoint on the NEON Safety Sharepoint Page..

6.3 Specialized Skills

Personnel are required to have working knowledge of gas tank handling and usage.

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.

This protocol requires approximately 1-2 hours of pre-field work activities, such as tracer injection calculations, charging batteries, weighing salt, and labeling bottles. We estimate field sampling and processing requires 2 technicians for 4 hours each sampling day plus travel to and from site. More time (additional 1-2 hours) may be needed in low flows.

7 STANDARD OPERATING PROCEDURES

SOP A Initial Trip (Note: This step is completed by NEON HQ staff to determine sensor locations.)

A.1 Locate Reaeration Reach (AKA Sensor Reach)

1. Determine Reaeration Reach by determining where in-stream sensors will be placed (completed by HQ), which is representative of the stream and has an injection site (i.e. the station at the top of the reach where we will add the SF_6 gas and NaCl solution) with a good mixing zone and minimizing the inclusion of large pools or dead zones (which increase travel time and water storage).
   a. There will be 4 sampling stations within the reach, located downstream of the injection site (Figure 4).
      1) The 1st sampling station (Station 1 located at Sensor Set 1) should be just downstream of the distance it takes the conservative tracer to completely mix with the streamwater (25 - 100 m; higher flow streams often need more length to completely mix). During higher flows, mixing lengths may increase and injection sites may need to be moved upstream.
      2) The last sampling station (Station 4) will be located at the bottom of the reaeration reach, located at Sensor Set 2.
2. Locate Reaeration Reach (completed by FOPS). The reaeration is collocated with the sensor reach, where reaeration station 1 is collocated with sensor set 1 and reaeration station 4 is collocated with sensor set 2. The reaeration drip station should be upstream of sensor set 1. One sensors are installed, be sure to adjust your reaeration stations accordingly to ensure colocation with sensors.

A.2 Determine Reach Length

1. The top of the reach must start with a good mixing zone to completely mix tracer with stream water. Best mixing zones are upstream of shallow pools with converging and diverging flows (Figure 5). This should allow for complete mixing before the first sampling station (Station 1), often occurring over a distance of 25 – 100 m. Wide, slow moving streams may have difficulties mixing. To ensure complete mixing at Station 1, during the continuous NaCl injection measure conductivity across main flowing section of the stream after steady state has been reached (i.e., the conductivity is no longer increasing). Depending on flows this could take 20 minutes to several hours to attain. If the site is well mixed, conductivity should be similar across this main flow.
2. Measure travel time between Station 1 and Station 4 - The best reach lengths are those that take ~40-45 minutes, during baseflow, for water to travel from Station 1 to Station 4. A simple way to estimate water travel time is to place an orange in the stream at Station 1 and follow the orange as it moves downstream for ~40-45 minutes to Station 4. Salt-pulse additions may be necessary to estimate travel time in small streams (<25-30 L/s), where travel time between

Stations 1 and 4 is the difference in the timing of the salt profile half-height as it passes station 1 and 4. Adjust Station 4 upstream or downstream to obtain the appropriate travel time.

SOP B Outline of Major Steps on All Reaeration Days

1. Measure Discharge
2. Measure background conductivity and collect a background Cl^- sample at each of the four reaeration stations.
3. Continuous injection of inert gas (e.g., SF_6) and conservative tracer (e.g., NaCl or NaBr) – At the same time and location, add inert gas (to account for diffusion) and conservative tracer (to account for groundwater inputs) to the stream. Rate of addition depends on stream flow.
   a. SF_6 Addition:
      1) Streams with flows of <200 L/s (0.05 – 0.2 m^3/s), receive approximately 100 mL/min.
      2) Most streams <1000 L/s (<1 m^3/s) can also receive 100 mL/min. Increase gas flow rate, as necessary (i.e., if lab analysis can't detect gas at the bottom of the reach)
      3) In a bigger stream (>1000 L/s), increase gas flow to 200-300 ml/min.
   b. Salt Addition:
      1) 5-15 mg Cl^-/L NaCl above background (~10-30 µS/cm) or 0.025 – 0.05 mg Br/L of stream discharge.
      2) If pump rates > 500 ml/min you will need to adjust tubing to accommodate higher flows rates. 3/8" I.D. tubing or greater is required for flows higher than 500 ml/min and 1/2" I.D. tubing or greater is required for flows higher than 1200 ml/min.
4. Collect Plateau Samples – While injection continues, take samples starting at the most upstream station (Station 1) after the furthest downstream station (Station 4) reaches plateau of conservative tracer (e.g., NaCl).
   a. Collect five 40 mL water samples at each of the 4 sampling stations into pre-labeled syringes – each syringe will provide a gas and water sample. Sample from **upstream to downstream**.
   b. Record stream temperature, conductivity, and time when samples are taken at each of the 4 sampling stations.
5. Process samples (pull in air and shake for 5 minutes) at a base camp away from stream and upwind of the injection site to limit potential gas contamination. From each syringe collect a gas and water sample.
6. Store samples appropriately.
   a. Gas samples → Sealed in gas vials.
   b. Water tracer samples → Tightly sealed in labeled 60-mL HDPE bottle.
7. Measure wetted widths at 30 evenly spaced locations along the stream reach (between Station 1 and Station 4).

SOP C Preparation

C.1 The Day Before

1. Test pump before going to field (Pump should be connected to a 6 or 8 Volt battery).
   a. Lubricate pump prior to use by placing a small dab of high-grade machine oil on the piston drive pin immediately before inserting into the radial bearing (Figure 2b).
   b. Ensure pump has been assembled correctly:
      1) If piston has been withdrawn more than 2 inches from the cylinder (Figure 2a), or removed completely from the pump head (Figure 2b), you MUST take special precautions before reassembling pump or **Lip Seals will be damaged**. **See Appendix E for correct assembly instructions.**
      2) Tighten thumbscrew (Figure 2a) to hold drive carrier in place.

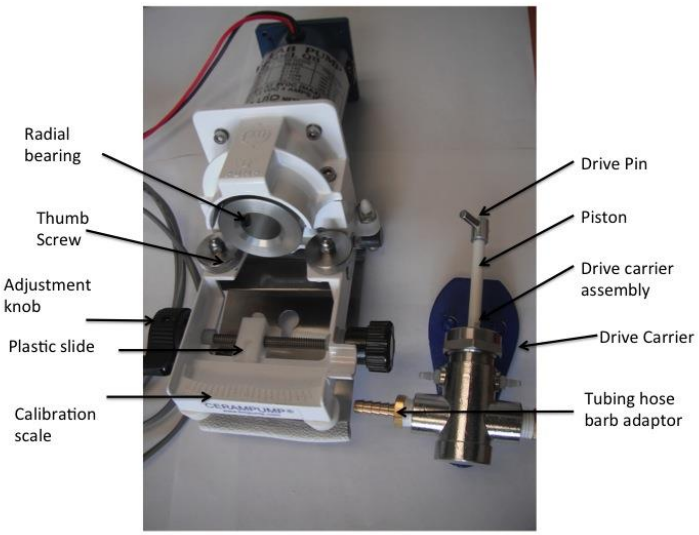


Figure 2. Fluid metering pump a) Components of pump drive (left) and b) pump head (right)

2. Test Pump: Test pump by placing it on the rim of a 5-gallon bucket with inlet and outlet tubing ends placed in the bucket containing tap water. Connect the pump to a battery and run the pump to make sure everything is connected and working properly. Look for leaks.
   a. Using the adjustment knob and the calibration scale, adjust rate to achieve the desired flow rate. If the carrier does not move when adjustment knob is turned, the knob is not properly attached to the plastic slide.
      1) The angle of the drive shaft cylinder with respect to zero (center) on the calibration scale controls the magnitude and direction of flow. Ex) If cylinder is pointed to left at 10 on the scale, the fluid will be passed from the right port to the left port at 100% of the

maximum rated volume. If the cylinder is pointed to the 5 on the right calibration scale, the fluid will be passed from the left to the right port at 50% of the maximum flow rate.

- **NOTE:** 200 mL/min is a very common flow rate for most NEON streams. The maximum pump flow rates will be 1296 ml/min (Q2 piston code). Minimum pump rate for this pump is 129.6 ml/min.

2) If you need to adjust flow rate, loosen the two thumbscrews slightly and use the adjustment knob as necessary. You can change the flow rate while the pump is still pumping. Remember that the pump flow rates are very sensitive, thus small adjustments may be all that is required. Retighten thumbscrews when adjustment is complete.

3. Use the injection calculation spreadsheet (Figure 3) to calculate the quantity of the conservative tracer (NaCl) you will need to add to the bucket/carboy. Note: If not able to use salt as a continuous tracer, see Site-Specific Information (Appendix G). Tracer addition needs to be large enough to detect the tracer at the most downstream sampling station and will vary by location and time as discharge and background conductivity values change. Aim for a 5-15 mg/L increase in Cl^- (10 – 30 µS /cm), with <50% saturation of the conservative tracer. In low conductivity streams (<100 µS /cm), an increase of 5- 10 µS/cm will be sufficient to detect a change. The calculation spreadsheet (Figure 3) will be provided by NEON.

Injection Specs: Chloride Calcs					
Injection #	1				
Site:	Red Butte				
Inj Date:					
Chloride: Calculations for solute concentrations					
			= adjust as necessary		
Injection Variables					
Chloride (Cl) enrichment target (mg/L)	adjust-->	7.00	mg/L		
Release rate (mL/min)		200.00	mL/min	3.168 gph	200.00 ccm/min
Volume of release solution (L)		17.00	L	4.4914 gallons	**Size of tank needed.**
Estimated stream Q (L/s)		60.00	L/s		
Injection time (h)		1.00	h		
Pump parameters					
Max pump rate (mL/min) for given res. vol. and injection ti		283.33	mL/min		
Max injection time (h) for given res. vol. and release rate		1.4167	h		
Chloride parameters					
Desired Cl conc. in release solution (g Cl/L)		126	g Cl/L		
Amount of NaCl (g) to add to carboy		3531	g	7.7844 lb	**Amount of salt needed.**
Resultant Cl concentration in carboy (mg Cl/L)		126000	mg Cl/L	3531 g	
% saturation of NaCl		**58.1804**	%		75% max
					<50& sat is safer

Figure 3. Example of injection calculation worksheet. Modified from LINX II.

4. The amount of salt required is a product of the target enrichment, the estimated stream flow and the injection rate. Use current pressure transducer data and discharge rating curve, if available.

$$Amt\ of\ NaCl\ (g) = ((((Cl^{-}\ target\ enrichment\ (mg/L) * Stream\ Q\ (L/s) * 60)/(injection\ rate\ (mL/\min)\ ) * 58.44247/35.4527) * Volume\ of\ Tracer\ Solution\ (L)$$

5. Weigh out the amount calculated and dissolve the salt in tap water in a small container, as needed (i.e., a liter bottle or a 4-L jug). Ensure the tracer is completely dissolved.
6. **Record** the amount of salt added on the Injection Field Data Sheet (RD[05], Field Data Sheets).
7. Confirm that you have newly evacuated gas vials from a gas laboratory. Vials should be < 2 months old and should not have any water in them.
8. Charge two gel-cell batteries OVERNIGHT.
9. Check the battery life of the handheld and logging probes. Calibrate the conductivity handheld meter (make sure entire probe, including the 2 black holes at the top, is completely immersed). Logging conductivity probes do not need to be calibrated, but should be validated against hand held measurements occasionally. Submit a trouble ticket if probes are not reading correctly.
    a. Set logging probes to begin logging (10 second intervals) on the reaeration field day. Ensure temperature is set to Celsius. Note: Probes should be logging prior to placing them in the stream. See user manual for calibration and usage instructions. Update firmware, as needed.
10. Label (1 * 2-$^{5}/_{8}$) all gas vials and conservative tracer bottles (60 mL HDPE).
    a. Gas:

    1) Sample IDs: **SITE.vialID.YYYYMMDD.GAS**

    where site is 4 letter SITE code, vialID corresponds to 2-digit syringe number (01-20), and date (YYYYMMDD).

    b. Conservative Tracer (TCR): Label 25 sixty (60) mL plastic bottles.

    1) SampleIDs: **SITE.bottleID.YYYYMMDD.TCR**

(Ex. Red Butte Creek, Station 2, Sample 06 on May 14 2014 is REDB.06.20140514.TCR).

Bottle IDs are **2-digit codes**: B1-B4 – Background Station 1 – Station 4, 00- Injectate, 01-05 indicates the five samples taken at Station 1, 06-10 indicates the five samples taken at Station 2, 11-15 samples at Station 3, 16-20 samples at Station 4 (Table 8). The bottle IDs 01-20 correspond to the field syringe IDs.

Table 8. List of bottle ID numbers for the conservative tracer

Sample Station	Bottle ID
Background	B1-B4: B Station # (1 – 4, where 1 is Station 1).
Injectate	00
Station 1	01-05

Sample Station	Bottle ID
Station 2	06-10
Station 3	11-15
Station 4	16-20

SOP D Sample Collection in the Field

In the field, fill out the General AQU Field Metadata Sheet (RD [08]) before collecting samples.

D.1 Background Sampling

1. First, visit each of the four sampling stations (Figure 4), and at each station record four conductivity measurements from the stream thalweg (i.e. the main flow of the stream), for a total of 16 conductivity measurements (See RD[05], Field Data Sheets). Make sure the hand-held conductivity meter is set on the temperature-corrected setting and units (SPC, uS/cm). If the setting is not correct, press the mode button until you enter the temperature-corrected mode. Wait for the meter to adjust to the stream temperature for more accurate conductivity measurements.

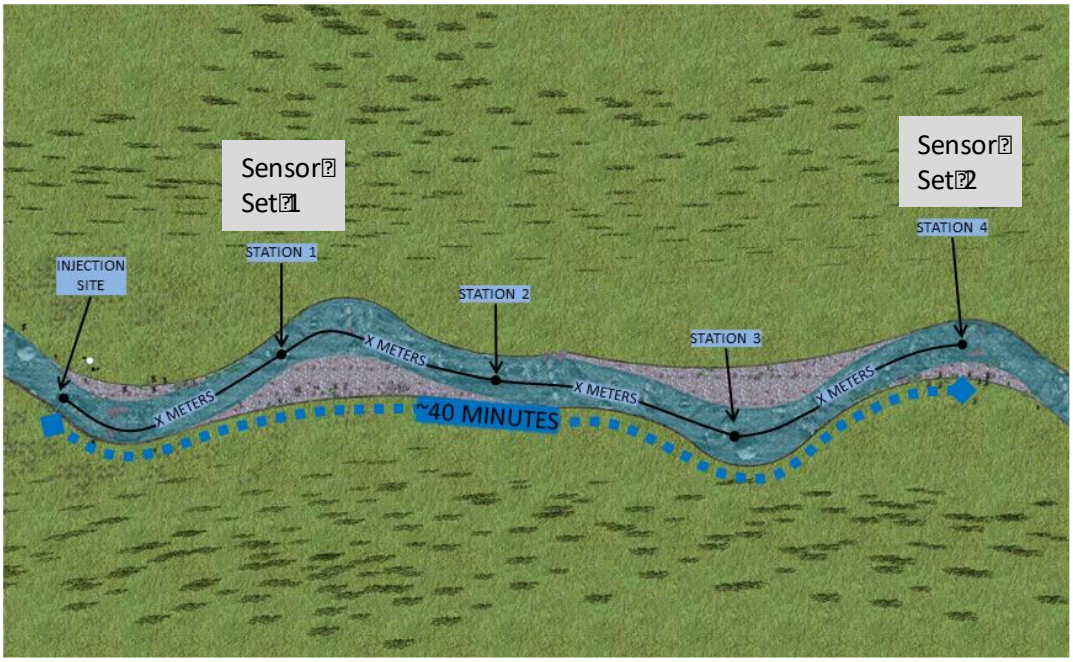


Figure 4. Schematic of stream reach with injection site and four downstream sampling stations. Travel time between Station 1 and 4 should be ~40 minutes. Note that Reaeration station 1 is co-located with Sensor set 1 and Reaeration station 4 is co-located with sensor set 2.

2. At each station, collect 60 mLs of stream water from the thalweg using the corresponding background syringes. Using a new syringe filter at each station, rinse and filter the sample into a 60 mL bottle for a background Cl^- sample. Label as Site ID, B (background), Station # (S1 – S4, where S1 is Station 1).
3. Mark and label each station with flagging tape, if necessary, to help find the sampling stations during the injection.
4. Place logging conductivity probes in the thalweg at Station 1 and Station 4. Probes should already be logging at 10 second intervals. Probes should be suspended in water column if water depth allows. Remember that the conductivity sensor is located and the opposite end of the probe removable cap, thus the entire sensor must be submerged.

D.2 In Field (plan 3-4 hours)

1. Fill a 5-gallon bucket/carboy with stream water and stir in the prepared concentrated salt solution. This will allow some time for the water to warm while you get the rest of the set-up ready and will allow any undissolved salt to dissolve more easily. You may need to increase the size of your bucket if the stream is large or flows are very high. **Record** the volume of the bucket/carboy on the injection data sheet (RD[05]). You may want to mark on the bucket the volume line you will be using to make filling the bucket easier.
2. Set up the bucket/carboy, tubing, battery, and pump on a level surface at the injection site (Figure 5). If a level surface on the bank is not adequate, place pump set-up on a bridge or plank laid across the stream.

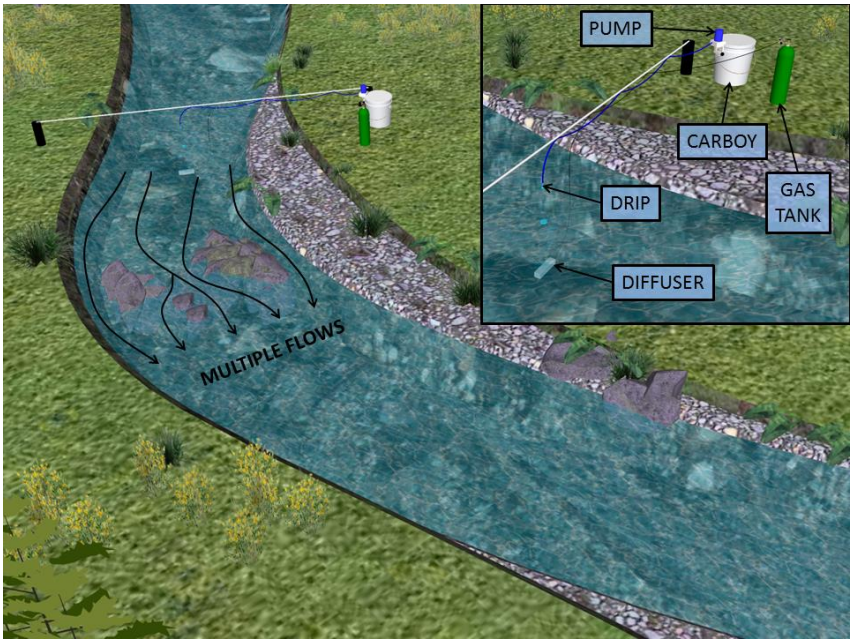


Figure 5. Field setup of the injection site with conservative tracer and inert gas

3. Pump setup:
   a. Mount pump upright for best performance. Pump can easily be mounted on the wall of a 5-gallon bucket (Figure 6).
   b. Place the pump inlet tube into the container with the saltwater solution. Ensure the end of the tubing remains in the solution (e.g., weight the end of the tube or use a binder clip to secure the tube to the side of the 5-gallon bucket so that the tubing end remains in the solution). The end of the tube should be near the bottom of the bucket so that as the tracer level draws down throughout the injection, the tubing will remain in the solution. Pumping air through the pump will damage it.
   c. Attach the pump electrical wires to the battery (Red to Red and Black to Black).

Figure 6. Mounting of the pump on the conservative tracer bucket

4. Pump Calibration: Allow the pump to run, with the end of the outlet tube feeding back into the bucket/carboy for several minutes to allow for the tubing to fill with the injection solution. Calibrate the pump using a stopwatch and graduated cylinder to the desired pump rate from the injection spreadsheet (Figure 3). To ensure a more accurate calibration, make sure to test the injection at the stream and at the same height that the tubing will be placed during the injection.
   a. NOTE: This step may take several minutes, but it is extremely important to get the correct pump rate.
   b. Every time you test the rate, pour the injectate back into the bucket/carboy and return the outlet tubing to the pump. DO NOT dispose of injectate solution.
   c. Record the actual (what was measured in the cylinder) pump rate on the data sheet as "Start Pump Rate" (See RD[05], Field Data Sheets).

5. Pump Set-up: The end of the tubing should be placed a few inches above the water surface. **DO NOT** put the tubing in the stream because it will change the pump rate. Attach the tubing to something stable, such as a piece of rebar pounded into the streambed or a tree above the stream. Make sure to INJECT (i.e., Drip) INTO THE THALWEG so the solution will mix as quickly as possible with the stream water (Figure 5).
6. Collect a 60 mL bottle of conservative tracer injection solution into the bottle labeled with 'SampleID 0'. This is an **EXTREMELY IMPORTANT** step so that we know the exact concentration of the solution we added to the stream.
    a. Filter the injectate sample to remove any particulates that could clog the analyzer.
7. **Gas Injection Set-up:**
    a. At the injection site, set-up the SF_6 gas tank, regulator and flow meter (Figure 7).
        1) Attach the regulator to the gas tank using the appropriate size wrench (1-1/8 inch wrench). Do NOT use Teflon Tape on the fitting that goes into the tank. (Note: Teflon can be used on all fittings associated with the regulator, except the one that goes into the tank.)
        2) Use gas-impermeable (e.g., Tygon) tubing to connect the SF_6 gas tank and regulator to the gas flow meter. Make sure that the tube running from the gas tank is connected to the BOTTOM connector of the flowmeter.
        3) Use gas-impermeable (e.g., Tygon) tubing to connect the TOP of the flowmeter to the diffuser (air stone). The tank can lie on the ground.
        4) Make sure diffuser remains underwater and is at the same location in the stream as the conservative tracer injection (Figure 5).
        5) Ensure the gas flowmeter is **vertical**. The flowmeter must remain upright for the best performance. Do NOT lay it on the ground.

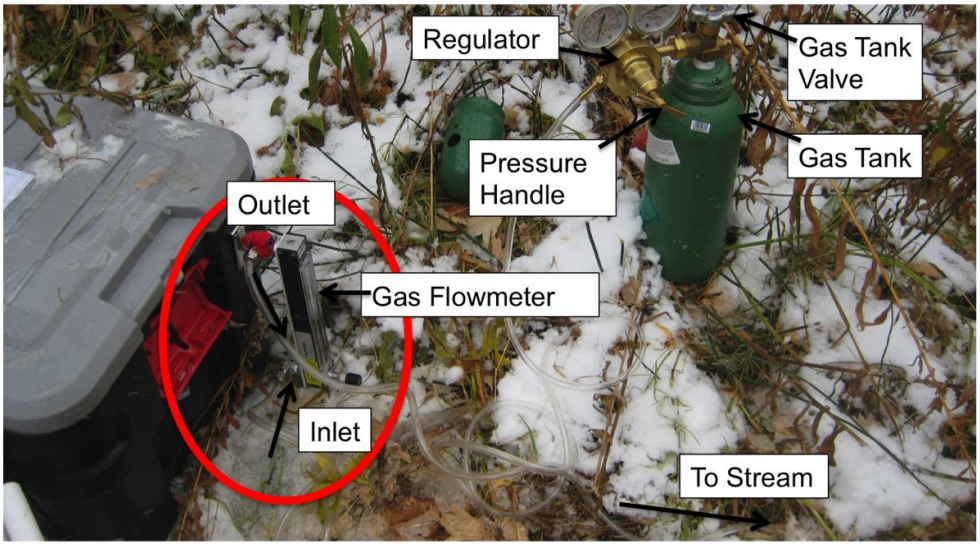


Figure 7. Gas tank, regulator, and flowmeter field setup

6) Connect all parts of the gas set-up before opening the main value on the tank.
7) Turn regulator pressure handle completely to the closed position on the regulator. This will close the regulator valve and keep your gas from blowing through before you are ready.
8) Open the gas tank valve.
9) Slowly turn the regulator pressure handle to the left to allow gas to flow to the flowmeter.
10) Set the regulator at 12 psi.
11) On the flowmeter, turn the valve so that it reads approximately 35 psi. If stream flows are low, you will need to increase the gas pressure to ensure enough gas makes it to the downstream sampling stations. Make sure you see little bubbles coming out of the diffuser (air stone).

8. Start the pump for the salt injection at the **same time** as the gas injection. NOTE: If using NaBr, you will also need to do a salt slug so that you can know when to sample and we can estimate travel times. See Appendix G: Site-Specific Information.
9. Note the start time on the Reaeration Field Data Sheet (RD[05]).
10. Spend a few more minutes at the injection site making sure the salt and gas injections are working properly.
11. Next, walk to the MOST DOWNSTREAM station (with your travel time in mind, make sure you arrive in time to take rising limb conductivity readings). You will need a hand-held conductivity meter.
12. Once at the most downstream station, place the handheld conductivity meter in the stream. Make sure the probe is fully submerged in the main flow. Do not put the probe in a side pool.
    a. **NOTE:** The conductivity sensor is located a the top of the probe where the two black holes are located, so the **ENTIRE** probe must be underwater to get the measurements. Ensure the water is deep enough to cover the entire probe.
    b. Jiggle the probe to make sure no air bubbles are next to the conductivity sensor (2 black holes) so the conductivity measures correctly.
13. Continue to observe conductivity until the stream has reached plateau (when salt concentration/conductivity measurement is no longer rising). Sampling should not begin until the stream has reached plateau at the MOST DOWNSTREAM station, usually 30 minutes to an hour during low flows and will be faster during high flows.
14. Once the most DOWNSTREAM station has reached plateau, start sampling at the most UPSTREAM station (i.e., Station 1, the station closest to the injection site) and work downstream (Figure 4). The idea is that you are following a parcel of water as it moves downstream.
15. At Station 1, record 5 temperature-corrected conductivity measurements across the main flowing section of the stream. You only need to do this step at Station 1 to ensure the NaCl tracer is mixed across the stream (See RD[05], Field Data Sheets). For NaBr additions, you only need to 1 conductivity measurementin the thalweg.

16. Take 5 syringe samples in the thalweg at each sampling stations. You may need to get into the stream to do this. Only step into the stream at designated sampling stations and do your best to not disturb the sediment as you walk. If you do kick up benthic sediments, wait for the area to clear before sampling. ALWAYS take samples upstream of where you are standing.
17. RINSE: Place the syringe tip (with 1-way stopper attached and turned to open; Figure 8) into the stream so that you are sampling the water ~10 cm under the surface of the water. Pull in ~20 mL water and remove syringe from stream. Rinse the syringe by pulling the stopper all the way back (without removing it) and shake. Expel the rinse water downstream or onto the bank.

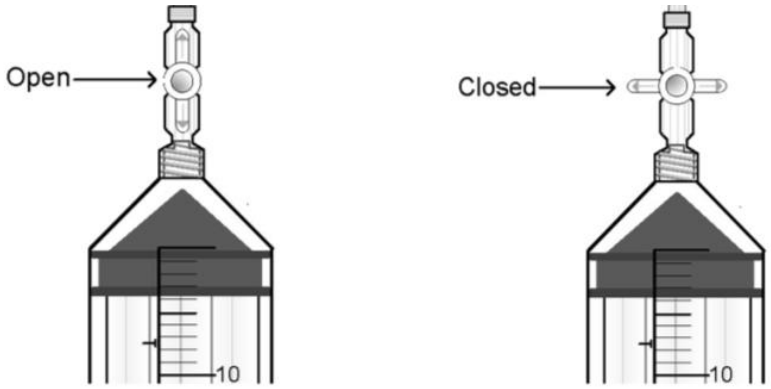


Figure 8. Example of a syringe and stopcock in the "open" and "closed" positions

18. SAMPLE: Put the syringe tip back into the water, below the surface, and pull the plunger until the syringe is completely full. Remove the syringe and tap the sides of the syringe firmly with your hand/fingers to remove the air bubbles. EXPEL water, leaving approximately 1 mL in the syringe, which will help reduce air intake on the next sampling. Put the syringe back in the water, expel the final 1 mL of aerated water below the stream surface, and slowly fill to the 40 mL mark, being careful not to entrain any air bubbles (Figure 9). Tip: You can put the entire syringe in the stream, horizontal to stream bed, to reduce air intact. Immediately turn the 1-way stopper to the closed position before removing the syringe from the stream (Figure 8). Take 5 separate syringe samples all within the main flow of water. Place the syringe in a small cooler to help maintain stream temperature at time of collection.

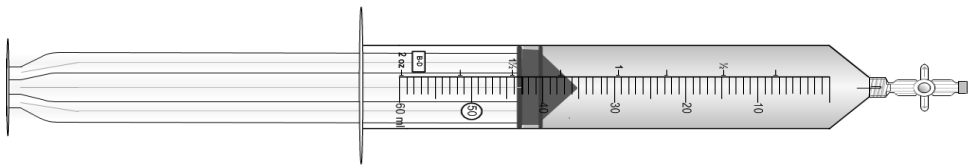


Figure 9. A syringe with a 40 mL water sample

19. At each station (for all injection types), note the time, 5 syringe IDs, water temperature, and conductivity on the reaeration spreadsheet (See RD[05], Field Data Sheets).

20. REPEAT (Steps 17-19) for each sampling station, always working from upstream to downstream. When walking between stations, do not walk in the stream.
21. Return to the Injection Site. Test and Record the 'End Pump Rate' and time on the field data collection sheet (See RD[05], Field Data Sheets).
22. END INJECTION: Turn off Gas and Pump.
23. If you did not already, SAVE some conservative tracer solution in a 60 mL bottle, labeled with Sample ID (SITE.00.YYYYMMDD.TCR),
    a. Make a dot on the label with a red permanent marker to indicate to the lab this is an injectate sample.
    b. Store sample in its own resealable plastic bag to reduce contamination. This will also ensure the lab knows it is an injectate sample, and will need to be diluted.
24. RINSE INJECTION EQUIPMENT:
    a. Return any remaining conservative tracer solution to the lab, via the bucket or the plastic jugs used to transport the concentrated solution to the field. The salt solution will need to be disposed of in the lab, so as not to add too much salt to the stream.
    b. Fill a bucket/carboy with fresh stream water and run the pump for a minimum of 30 minutes to flush the NaCl from the pump equipment.
    c. Rinse the outside of the pump in stream water to remove all salt from the pump. Ensure any parts that were touching the salt solution are thoroughly rinsed.
25. COLLECT logging conductivity meters. See Additional Directions/Notes for additional information on downloading HOBO data files.
26. BREAK DOWN the gas and NaCl injection set-ups.

D.3 Sample Processing

1. Away from stream, where there will be no contamination in the air from the injection, such as upstream and upwind, open stopcock and draw the plunger to the 60 mL, so you have 40 mL of water and 20 mL of air (Figure 10). Close stopcock and leave stopcock attached to syringe at all times. VERY IMPORTANT: To be consistent, be sure to pull the plunger from the 40 to the 60 mL mark (Figure 10).

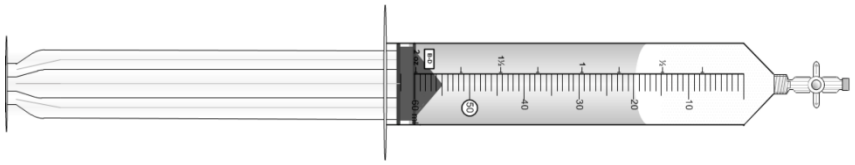


Figure 10. A syringe with 40 mL of water and 20 mL of air

2. SHAKE: Once samples have been collected from ALL stations, shake each syringe for 5 minutes to equilibrate the air and SF_6. To save time, shake multiple syringes at once.
3. SAMPLE GAS: Label the gas vials with the sampleID (SITE.syringenumber.YYYYMMDD.GAS). Syringe number should be a 2-digit number (01-20)

4. After shaking, place needle with needle cover still attached on 1-way stopcock. Once needle is attached, remove the plastic covering. Holding the syringe upright, open the stopcock (Figure **11**) and push a small amount of air (~0.5 mLs) through the needle to purge the air in needle.

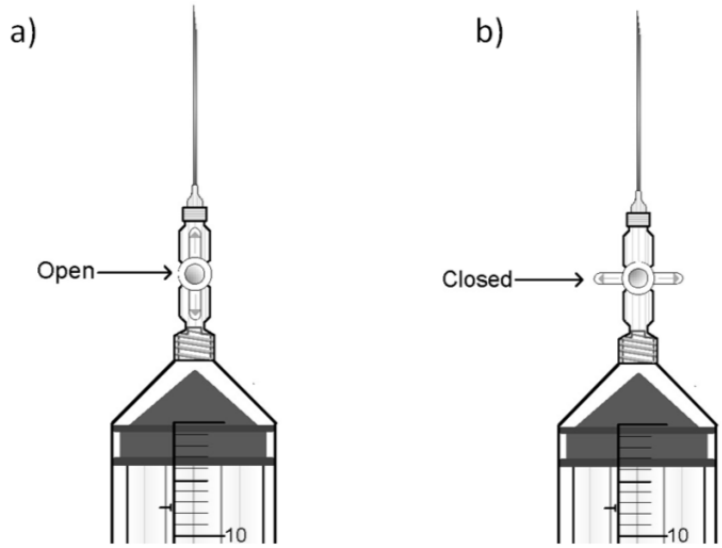


Figure 11. Example of a syringe with a stopcock with attached needles in the a) "open" and b) "closed" positions

5. With stopcock still 'open' and syringe held upright, insert the needle through the rubber septum of the gas vial (Figure 11 and **Figure 12**) and push gas into the vial. Properly evacuated vials will automatically suck in the gas.
    a. If vial does not suck up air it is not properly evacuated. Remove the needle from the syringe and use one of your backup vials.
6. Push as much gas in as possible without injecting the water sample in the vial.
7. Make sure to OVER-PRESSURIZE THE VIALS to prevent gas from leaking into the vials. Pull the syringe needle out quickly without closing the 1-way stopper. This makes loss of gas less likely.
8. You may re-use the same needle across a single station per SITE on a sampling day as long as the needle remains structurally sound and the needle is purged between samples to remove any non-sampled gas. If needle bends or breaks, use a new needle. Discard needles in a sharps container.

Figure 12. Insertion of the needle into a gas vial through the rubber septum.

9. SAMPLE CONSERVATIVE TRACER: Remove the 1-way stopcock. Hold syringe upright and expel remaining air from the syringe (Pushing air through the filter can cause the filter to rupture). Attach a 30 mm/ 0.7 µm filter capsule onto the syringe. Rinse the 60 mL bottle with 5 mLs of filtered sample twice. Filter the remaining syringe sample (~30 mLs) into the bottle. Be sure the bottle is labeled with sampleID (site ID.bottleID.date (YYYYMMDD).TCR). BottleID should match the syringe IDs. Repeat for all syringes.
10. DISCARD filter and replace stopcock on each syringe. Note: you may re-use the same filter across a single station per SITE on a sampling day, as long as the filter is not clogged and the filter is rinsed between samples with the new sample water.
11. Additional data to collect: 30 stream wetted width measurements evenly spaced throughout the reach, between Station 1 and Station 4 (e.g., 10 wetted width measurements between station 1 and 2, 10 measurements between station 2 and 3 and 10 measurements between station 3 and 4) completed at the end of each injection.
    a. If available, a laser rangefinder can be used to measure wetted widths provided stream is greater than 2 m wide, on average. This can be done while waiting for the tracer to plateau, as long as you are not getting in the stream during the injection.

D.4 Troubleshooting

1. Diffuser:
   a. If no bubbles are coming out of diffuser plate, then check 1) tank is turned on and regulator is open and 2) all tubing connections. Apply SNOOP (or soapy water) to the tube connections and watch for bubbles, which indicate a gas leak.
   b. Diffuser: No gas-bubbles coming out of air-stone.
      1) Ensure the connection between the tubing and the air-stone is secure so gas isn't escaping before entering the diffuser.
      2) If pores seem clogged or filled with algae, clean diffuser using hard-bristled brush or 10% bleach solution.
2. Pump: If the pump is not working or is not working correctly:
   a. Check battery charge and try another battery. It is always a good precaution to bring an extra battery with you to the field with you even if it is not fully charged. If changing the battery changes the pump rate, it is a battery problem. If changing the battery doesn't affect the rate, check to make sure all wires are well connected.
   b. Ensure pump thumbscrews are securely tightened.
   c. Check pump connection to battery to make sure they are secure.
   d. Refer to pump user's manual.
3. Conductivity probe: If hand-held conductivity probe is not measuring properly:
   a. Jiggle the probe to make sure no air bubbles are next to the conductivity sensor (2 black holes).
   b. Make sure entire probe, including the 2 black holes at the top, is completely immersed.

D.5 Sample Preservation

1. Gas Samples:

   a. Gas samples should upside down in containers with water so the vial caps and submerged in water. This reduces the risk of sample loss. **VERY IMPORTANT:** Gas samples should not be warmed, (i.e., room temperature is fine), and away from a light and heat source, such as in a cooler.
   b. Secure labels to vials with clear packing tape prior to shipping. Labels should be attached along the horizontal axis of the tube (**Figure 12**) with clear tape wrapped completely around the vial, perpendicular to the label. The ends of the tape should overlap to keep the tape from coming unstuck. You may need two rows of tape.
2. Chloride Samples:
   a. There are no preservation requirements for chloride samples. Store injectate sample (labeled with red dot) in its own resealable plastic bag.

D.6 Ending the Sampling Day

1. Refreshing the sampling kit
   a. Restock the sampling kit (shipping coolers) with newly evacuated gas vials, new chloride sampling bottles (with new labels), syringe filters, needles, etc.
2. Equipment maintenance, cleaning and storage
   a. Pump:
      1) Run clean water (this can be stream water) through the pump and tubing for 30-60 minutes to rinse salt water from equipment. You can do this in the field while you complete sample processing, or in the lab later that day.
         a) Fill bucket approximately half full with clean water.
         b) Place the intake and outtake tubing into the clean water and then you can run the pump without fear of the reservoir going dry.
      2) Rinse of external parts of the pump with freshwater to remove any salt solution.
      3) Upon returning to Domain support facility, allow pump to air dry before storage
   b. Place clean and dried pump in plastic storage bag, before placing in plastic storage tote with foam. Empty all water from tubing before storage.
   c. Charge batteries.

SOP E Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

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

E.1 Calculations and Data Entry

Note, calculations are for informational purposes only. They will be completed at NEON HQ.

1. Discharge: Discharge can be calculated from the conductivity measurements at Station 1 and Station 4.
   a. Background-correct conductivity measurements at Station 1 and Station 4.
   b. Calculate Discharge as: (a) $Q=(C_{salt} * Q_{salt})/ C_{station}$, Where C_{salt} is the conservative tracer concentration (Cl^-) in the injection solution, Q_{salt} is the injection rate (in L/s) and $C_{station}$ is the background-corrected conservative tracer concentration at the Station 4.
   c. Station concentration can be calculated from conductivity measurements where 1 µS/cm = 0.5 ppm Cl^- (mg Cl^-/L).
2. Reach Velocity: Calculate Average Velocity of the Reach: V=X/Travel Time, Where X is the distance between Station 1 and Station 4, and Travel Time is the time it takes from to reach ½ half height at Station 4 minus the time to reach ½ height at Station 1.
3. Reaeration Coefficient
   a. Plot the natural log (LN) of the RATIO of the tracer gas concentration (or GC peak area) to the background-corrected Cl concentration (Y-axis) by Stream Distance (X-axis) (Figure 13).

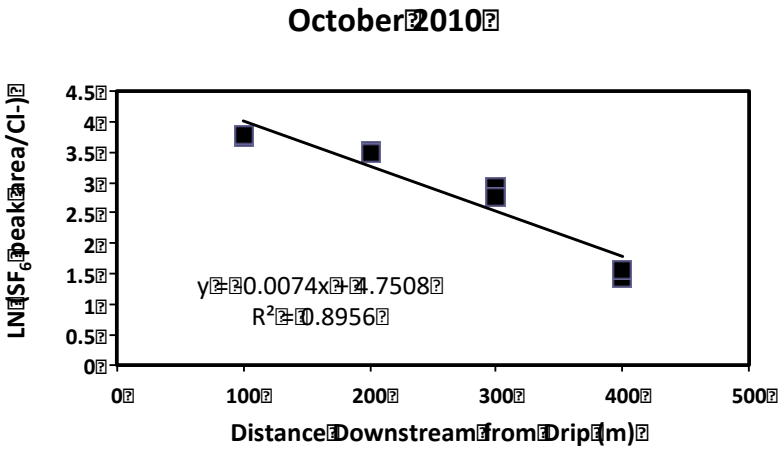


Figure 13. Example SF6 loss rate data calculation.

b. The slope of the line is the SF_6 loss rate (m^{-1}).
c. Calculate Reaeration Coefficient for SF_6: Multiply SF_6 loss rate by the reach velocity (m min^{-1}) to get reaeration rate coefficient for SF_6 (K_{SF6}; min^{-1}). Reach velocity is the distance between station 1 and 4 divided by the difference in timing of the maximum slope of the conservative tracer ascending limb at station 1 and 4.
d. Convert Reaeration Coefficient for SF_6 to O_2 ($K_{O2} = K_{SF6} * 1.34$).
e. Convert K_2 values from ambient stream Temp to standard temperature (T=20C)
    1) $K_2(T=20C) = K_2(T) * (1.0241^{(20-(Upstream(T)+Downstream(T)/2))})$

4. Develop a relationship between discharge and K_{O2}.
5. Enter data into excel file named "Reaeration Field Data." Save file in format:
    a. Reaeration_SITE_YYYYMMDD.xls
    b. Station ID will be 1 (top of reaeration sampling reach) or 4 (bottom of reaeration sampling reach)
6. Stop conductivity probes from logging and download conductivity probe data. Save files as:
    a. Reaeration Conductivity logger_SITE_StationID_YYYYMMDD
        1) Station ID will be 1 (top of reaeration sampling reach) or 4 (bottom of reaeration sampling reach)

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 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

1. Gas Samples
    a. Use clear packing tape to secure labels to vials prior to shipping, if you have not done so already. Wipe excess water off vial prior to adding packing tape, and fully wrap tape around vial and label.
    b. Ship upside down in water in a watertight container, wrapped with electrical tape and placed in a 9-qt cooler (for 1 or 2 sites). Containers should be 2/3 full with water. This will allow for water expansion if freezing occurs, without breaking the gas vial. Container should be kept upright to ensure gas vial lids stay submerged in water.
    c. Pack liquid absorbent material around container tubes.
    d. Fill any remaining space with regular packing material.
    e. Place 'per sample' tab of AOS shipping inventory (RD[10]) in a resealable plastic bag and tape to the inside top of cooler.
    f. Prepare a shipping inventory detailing the contents of the shipment, using the protocol-specific templates found on CLA's NEON intranet site. Include a printed copy of the inventory in the shipment box.
    g. Save the inventory with the following naming convention: "**DXX_MOD_ShippingInventory_YYYYMMDD**"
    h. Complete packing slip, address shipment, and ship ground to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.
    i. Email an electronic copy of the shipping manifest and tracking number to the email addresses listed in the CLA "Shipping Information for External Facilities" document.
        1) Include the shipment tracking # (Shipment ID) in the email body, as well as the electronic copy of shipping manifest.

2. Conservative Tracer Samples
    a. Ship 60-mL Nalgene bottles, organized in plastic, resealable bags, in a cardboard box at room temperature.
        1) Ensure lab knows which sample is the injectate by:
            a) Labeling with a red dot/permanent marker
            b) Placing injectate sample in a separate resealable bag.
    b. Place in a properly sized box (e.g., 9*8*4), lined with a garbage bag. Surround with absorbent packaging materials.
    c. Place 'per sample' tab of AOS shipping inventory (RD[10]) in a resealable plastic bag in the box.
    d. Prepare a shipping inventory detailing the contents of the shipment, using the protocol-specific templates found on CLA's NEON intranet site. Include a printed copy of the inventory in the shipment box.
    e. Save the inventory with the following naming convention: "**DXX_MOD_ShippingInventory_YYYYMMDD**"
    f. Complete packing slip, address shipment, and ship ground to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.
    g. Email an electronic copy of the shipping manifest and tracking number to the email addresses listed in the CLA "Shipping Information for External Facilities" document.
        1) Include the shipment tracking # (Shipment ID) in the email body, as well as the electronic copy of shipping manifest.

F.3 Timelines

1. Gas Samples
    a. Ship Ground within 1 month to gas analysis laboratory. Changes in temperature and elevation can alter the gas pressure in the gas vials.
2. Conservative Tracer Samples
    a. Ship Ground, within 1 month, the injectate sample, the 4 background samples, and the 20 plateau conservative tracer sample bottles for analysis.

F.4 Conditions

1. Gas Samples
    a. Shipped Ground in a cooler to maintain room temperature.
2. Conservative Tracer Samples
    a. Cl^- samples can be shipped at ambient temperature.

F.5 Grouping/Splitting Samples

1. Conservative Tracer Samples
   a. Organized by station in plastic, resealable bags.
   b. Ensure each injectate sample is in a separate resealable bag.

F.6 Return of Materials or Containers

1. Gas Samples
   a. The external gas analysis lab will return the cooler with new evacuated vials.

F.7 Shipping Inventory

Fill out the AOS Sample Shipping Inventory (RD[10]). 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

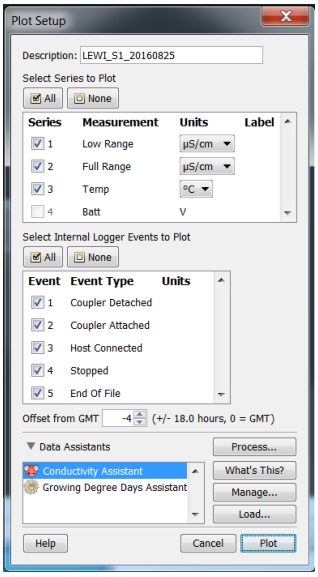
See the CLA shipping document on CLA's NEON intranet site.

F.9 Additional Directions/Notes

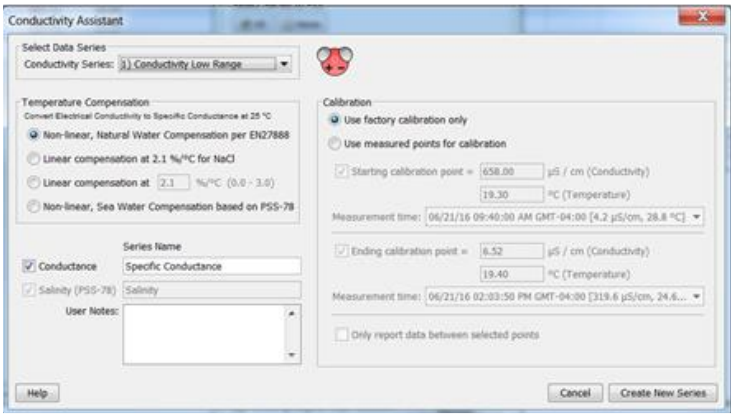
Make sure to note what the expected concentration of the injectate is in the “comments to lab” section for the appropriate sampleID (RD[10]); they will need to dilute the sample before analysis. The expected concentration can be taken from the injection spreadsheet used to calculate the concentration and injection rate of the conservative tracer (Figure 3).

Save HOBO files with Specific Conductance/Create a new specific conductance time series:

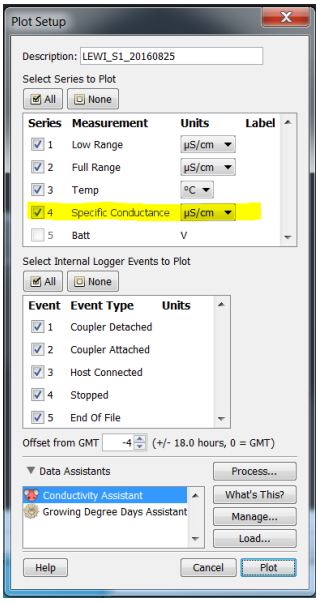
1) Ensure temperature units are in Celsius.
2) After reading out the HOBO, you will see the Plot Setup window below. In the Data Assistants section at the bottom, double click on Conductivity Assistant.



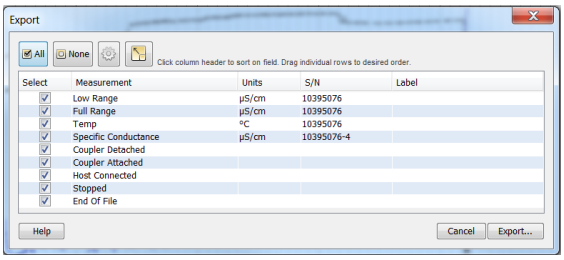
3) In the Conductivity Assistant window, select Conductivity Low Range from the dropdown if your conductivity values are ≤ 1000 us/cm. Select Full rang if our conductivity values are > 1000 us/cm. Leave all other default settings and click Create New Series.



4) Now you should have a Specific Conductance series listed in your Plot Window. Click Plot to view your data.



5) Export your data by cliking on the export button  in the toolbar. Leave all of the series selected and click Export. Then save your file as a CSV with the default file name (the name you entered when launching the HOBO).



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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 9. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC. 002382	Datasheets for AOS Protocol and Procedure: Reaeration Measuring Diffusion of O2 across the Water-Air Interface
NEON.DOC.001646	General AQU Field Metadata Sheet

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

APPENDIX B QUICK REFERENCES: OUTLINE OF MAJOR STEPS ON ALL REAERATION DAYS

Step 1 – Measure background conductivity and collect a background Cl^- sample at each of the four reaeration stations.

Step 2 – Continuous injection of inert gas (e.g., SF_6) and conservative tracer (e.g., NaCl) – At the same time and location, add inert gas (to account for diffusion) and conservative tracer (to account for groundwater inputs) to the stream. Rate of addition depends on stream flow.

1. SF_6 Addition:
   a. Streams with flows of <200 L/s (0.05 – 0.2 m^3/s), receive approximately 100 mL/min.
   b. Most streams <1000 L/s (<1 m^3/s) can also receive 100 mL/min. Increase gas flow rate, as necessary.
   c. In a bigger stream (>1000 L/s), increase gas flow to 200-300 ml/min.
2. Salt Addition:
   a. 5-15 mg Cl^-/L NaCl above background (~10-30 µS/cm)

Step 3 – Collect Plateau Samples – While injection continues, take samples starting at the most upstream station (Station 1) after the furthest downstream station (Station 4) reaches plateau of conservative tracer (e.g., NaCl).

1. Collect five 40 mL water samples at each of the 4 sampling stations into pre-labeled syringes – each syringe will provide a gas and water sample. Sample from upstream to downstream.
2. Record stream temperature, conductivity and time when samples are taken at each of the 4 sampling stations.

Step 4 – Process samples (pull in air and shake for 5 minutes) at a base camp away from stream and upwind of the injection site to limit potential gas contamination. From each syringe collect a gas and water sample.

Step 5 – Store samples appropriately.

1. Gas samples → Sealed in Gas Vials.
2. Water tracer samples → Tightly sealed in labeled 60-mL HDPE bottle.

Step 6 – Measure wetted widths at 30 evenly spaced locations along the stream reach (between Station 1 and Station 4).

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare all equipment.
- ☑ Pre-print labels on waterproof paper.
- ☑ Ensure all syringes are labeled with sample ID.
- ☑ Assemble and test pump.

Sample collection: Be sure to…

- ☑ Once the conservative tracer plateau is reached at Station 4, start sampling from the most UPSTREAM (Station 1) station to the most DOWNSTREAM (Station 4) station.
- ☑ Do not walk in the channel when moving between stations.
- ☑ Rinse the sample syringe twice with stream water.
- ☑ Remove large air bubbles from gas sample syringes.
- ☑ Use stopcock to ensure no sample is lost during storage or shaking.
- ☑ Shake for the full **5 minutes**.
- ☑ Over-pressurize the gas sample vials.
- ☑ Collect 5 samples at each station.
- ☑ Cover the needle holes in the sample vial lids and store in water filled, water tight containers to limit gas leakage.
- ☑ Collect 30 wetted width measurements between Station 1 and Station 4.
- ☑ Carefully record all metadata, measurements, and observations on data sheet.

Sample preservation: Be sure to…

- ☑ Keep the gas sample vials cool to limit gas expansion.

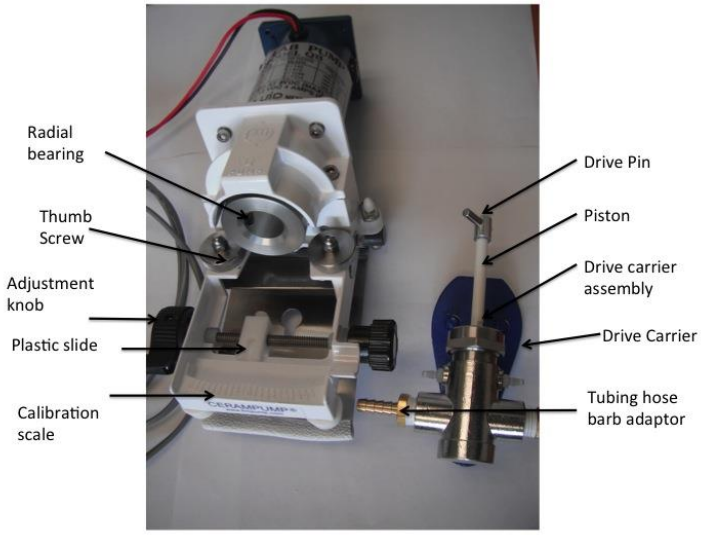
APPENDIX D CONSIDERATIONS FOR IMPLEMENTATION

- Exposing gas vials to heat should be avoided as increases in temperature can influence gas vial storage and increase the risk of gas lost. Changes in pressure (e.g., elevation changes) can increase the risk of gas lost and samples should be shipped ground.
- It is extremely important that good travel time estimates be calculated. Ensure you are adding enough salt to detect and the HOBO loggers are programmed and launched correctly.
- Ensure conservative tracer reservoir does not run dry. Adjust pump rate to ensure reservoir is not depleted prior to reaching plateau and sampling. Pump rate should also be measured at the start and just before stopping the injection.
- Extremely saturated salt solution can clog the filter in the filter housing and influence the pump rate.
- Try to keep salt solutions to <50% NaCl.
- Samples must be processed away from stream to avoid contamination. Common errors include:
  - Sampling from downstream (Station 4) to upstream (Station 1), rather than the appropriate upstream to downstream sampling. Imagine that you are sampling from upstream to downstream so that you can sample the same parcel of water as it moves in the downstream direction.
  - Not sampling in thalweg.
  - Forgetting to shake syringes prior to gas sampling.

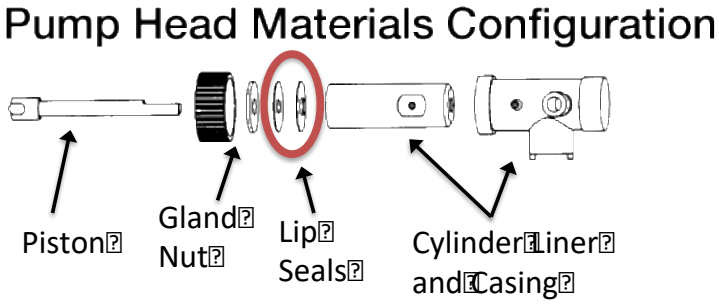
APPENDIX E ASSEMBLE AND TEST PUMP BEFORE GOING OUT

1. If piston has been withdrawn more than 2 inches from the cylinder (Figure 2a), or removed completely from the pump head (Figure 14b), you MUST take special precautions before reassembling pump or **Lip Seals will be damaged**.
    a. Remove Gland Nut and install Lip Seals one at a time (Figure 2b), and ensure you do not damage the seal or the 'lips' (See Section 18 Piston Seal Replacement in FMI pump manual for more detail). Note, top Lip Seal should have 'lip' facing up while bottom Lip Seal should have 'lip' facing down.
2. Add a small drop of grease (high grade machine oil) to the drive pin head (Figure 14a) just before it is inserted into the radial bearing.
3. Insert piston drive pin into the radial bearing in the spindle assembly (Figure 14c). You can pull the piston out ~1 inch to make the insertion easier. Do not pull the pin out more than 2 inches.
4. At the same time as you insert the drive pin into the bearing, slide the drive carrier into the pump base assembly (Figure 14b), which will slide the ceramic piston completely into the cylinder. **IMPORTANT:** As you slide the drive carrier into the assembly, you must ensure that the knob on the underside of the drive carrier slides into the plastic slide (Figure 14a) at the same time.
5. Using the adjustment knob and the calibration scale, adjust rate to achieve the desired flow rate. If the carrier does not move when adjustment knob is turned, the knob is not properly attached to the plastic slide.
    a. The angle of the cylinder with respect to zero (center) on the calibration scale controls the magnitude and direction of flow. Ex) If cylinder is pointed to left at 10 on the scale, the fluid will be passed from the right port to the left port at 100% of the maximum rated volume. If the cylinder is pointed to the 5 on the right calibration scale, the fluid will be passed from the left to the right port at 50% of the maximum flow rate.
    b. Minimum Pump rates are 10% of the maximum rated flow rate.
    c. **NOTE:** The majority of NEON maximum pump flow rates will be 1296 ml/min (Q2 piston code). Minimum pump rate for this pump is 129.6 ml/min. A Q1 piston code maximum pump rate is 576 ml/min.
6. Tighten thumbscrew (Figure 14a) to hold drive carrier in place.
7. If you need to adjust flow rate, loosen the two thumbscrews slightly and use the adjustment knob as necessary. You can change the flow rate while the pump is still pumping. Remember that the pump flow rates are very sensitive, thus small adjustments may be all that is required. Retighten thumbscrews when adjustment is complete.

a)



b)



c)

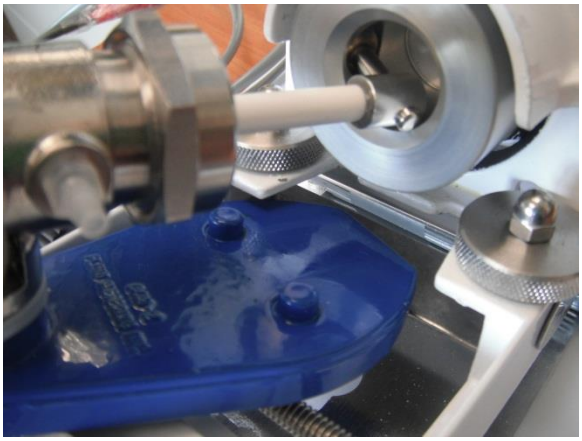


Figure 14. Fluid metering pump a) Components of pump drive (left) and pump head (right), b) Configuration of pump head (modified from fluidmetering.com/materials-construction.html), and c) Assembly of pump head into pump drive

APPENDIX F ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

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

APPENDIX G SITE-SPECIFIC INFORMATION

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

1) For Sites with specific conductivity values >500 uS/cm or at sites where using NaCl as a continuous tracer is not feasible:

For stream sites with high conductivity water (>500 us/cm), using NaCl as a tracer can prove difficult because you cannot dissolve enough salt to see the increase and/or if there are concerns about adding so much salt to a stream. In such cases, Sodium Bromide (NaBr) may be used as the conservative tracer (MX110110 for 500 g of NaBr or MX110105 for 100 g of NaBr).

NaBr can be added at 0.025-0.5 milligrams Bromide/L of stream discharge (0.032-0.64 milligrams NaBr/L). For a stream with a discharge of 50 L/s, you would mix 11.6 - 232 milligrams of NaBr into you continuous injection solution, depending on site-specific needs. See the 'Bromide Injection Prep Sheet' tab on the Reaeration Datasheet to help calculate the amount of NaBr added to your stream. Note: NaBr may need to be added in higher amounts depending on stream chemistry. Thus, site specific needs will need to be worked out between FOPS, the external facility, and NEON HQ.

Salt slugs will need to be done at the same time, so that you will be able to determine travel time, and so that you can accurately determine when to sample by using the hand-held conductivity meter at station 4 to monitor the conductivity and know when the salt has traveled from the top of the reach to the bottom. You place the HOBO loggers in the stream at station 1 and station 4 just as you would if you were using a continuous salt injection. See protocol for details. For a salt slug, make a solution of 2 kg NaCl/m3/s or 2 g of NaCl/L/s. So, in a stream with a flow of 50 L/s, you would add 100 g of salt. **Record the mass of salt added** for the salt slug on the injection field sheet. Be sure to use **non-iodized** salt. You may need to add more salt in high conductivity streams (>500 microS/cm). Dissolve the salt in water (1-2 L) and dump the salt in one quick pulse into the stream at the drip station. Once the peak of the slug has been observed at station 4, you may begin your sampling at reaeration station 1. If you have trouble detecting the pulse at the most downstream station, follow the pulse as it goes downstream at each station to ensure it arrives at the downstream end before sampling.

2) For sites where reaeration will be modeled (ARIK and BLUE), no continuous injections will be completed, but several physical parameters will still need to be measured.

    a. Measure Discharge

    b. Complete a salt slug: Salt slugs will need to be completed to determine travel time. Place the HOBO loggers in the stream at reaeration station 1 (sensor set 1) and reaeration station 4 (Sensor Set 2). Loggers should be set to log every 10 s and should be set to the appropriate units (see SOP 7F.9).
        i. Make a solution of 2 kg NaCl/m3/s or 2 g of NaCl/L/s using **non-iodized** salt. Ex. In a stream with a flow of 50 L/s, you would add 100 g of salt.
            1. **Record the mass of salt added** for the salt slug on the injection field sheet. Be sure to use **non-iodized** salt.
            2. You may need to add more salt in high conductivity streams (>500 microS/cm).
            3. Dissolve the salt in water (1-2 L) and dump the salt in one quick pulse into the stream at the drip station.
                a. Note, in very large streams/rivers (Ex, Blue River), you will need to increase the volume of slug and release the solution into the stream at multiple locations to ensure mixing.
        ii. Release the solution into the stream, as quickly as possible.
        iii. Rinse bucket with stream water and pour rinse water into stream to ensure all salt added to the bucket made it into the stream.
        iv. Allow the peak to pass and conductivity to return to near background levels before pulling the loggers from the stream.

    c. Collect wetted widths: 30 stream wetted width measurements approximately evenly spaced throughout the reach, between Station 1 (Sensor Set 1) and Station 4 (Sensor set 2) (e.g., 10 wetted width measurements between station 1 and 2, 10 measurements between station 2 and 3 and 10 measurements between station 3 and 4).
        i. If available, a laser rangefinder can be used to measure wetted widths provided stream is greater than 2 m wide, on average. This can be done during the salt slug, as long as you are not getting in the stream.