AOS PROTOCOL AND PROCEDURE: REA – REAERATION IN STREAMS

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See configuration management system for approval history.

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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.
J	01/15/2018	ECO-05287	• Added barcode language • Added additional instruction on HOBO file uploading • Added SF6 canister return information • Added updates to ice-cover instructions.
K	02/28/2019	ECO-06023	• Added clarity on NaBr method in appendix • Updated shipping section • Clarity on vial taping • Added FMI pump maintenance
L	12/16/2021	ECO-06709	• Updated to new template (NEON.DOC.050006 Rev K) • Removed human-readable labels and shipping section • Added additional HOBO logger information, parallel battery assemblies • When using the ADCP to calculate discharge, use the Q-Rev discharge values.

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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 into the water, or deaeration, the loss of O_2 from the water) as oxygen diffuses across the water-air interface. To understand the biological controls on oxygen within our systems, we must first account for the physical controls (e.g., reaeration or gas exchange).

Gas exchange (i.e., reaeration) 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, tributary and groundwater inputs, turbulence driven by geomorphology, wind, and stream flow, as well as the oxygen concentration gradient (either deficit or surplus) across the interface. The reaeration rate coefficient (K_2) represents the combined effects of these physical characteristics and is multiplied by the saturation deficit (or surplus) to calculate the rate of reaeration (or degassing). 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 estimates.

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 (presently Flathead Bio Station, University of Montana), 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 Data Quality 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 in Streams
RD[06]	NEON.DOC.003282	NEON Protocol and Procedure: Site Management and Disturbance Data Collection
RD[07]	NEON.DOC.001646	NEON General AQU Field Metadata Sheet
RD[08]	NEON.DOC.001152	NEON Aquatic Sample Strategy
RD[09]	NEON.DOC.004257	NEON Standard Operating Procedure (SOP): Decontamination of Sensors, Field Equipment and Field Vehicles
RD[10]	NEON.DOC.001085	AOS Protocol and Procedure: DSC – Stream Discharge
RD[11]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[12]	NEON.DOC.005224	AOS Protocol and Procedure: Shipping Ecological Samples and Equipment

2.3 Acronyms

Acronym	Definition
ASR	Analytical Services Request
cm	centimeter
GC	Gas Chromatograph
HDPE	High-density polyethylene
ln	Natural Log
m	meter
µm	micrometer
µS/cm	microSiemens per centimeter
Psi	Pounds per square inch

2.4 Definitions

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

Deaeration: Loss of gas 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 carbon. 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.

Fulcrum: Software tool used to create NEON electronic data entry applications.

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.

Logger: Device used for making systematic recordings of measurements or observations.

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).

ServiceNow: Software tool used for problem/incident tracking and resolution.

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 the variables needed to calculate 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)), alongside a conservative tracer (e.g., NaCl or NaBr) to account for any hydrologic inputs to the ecosystem. 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. 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 can be 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. Gas exchange measurements should be conducted when no other work is being conducted in the stream, as disturbance of sediments and habitat may influence gas exchange.

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 ecologists **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 sampling at the site. It is therefore essential that field personnel carry out this protocol as outlined in this document. If local conditions create uncertainty about carrying out these steps, it is critical that field ecologists document the problem and enter it into NEON's problem tracking system.

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[05]).

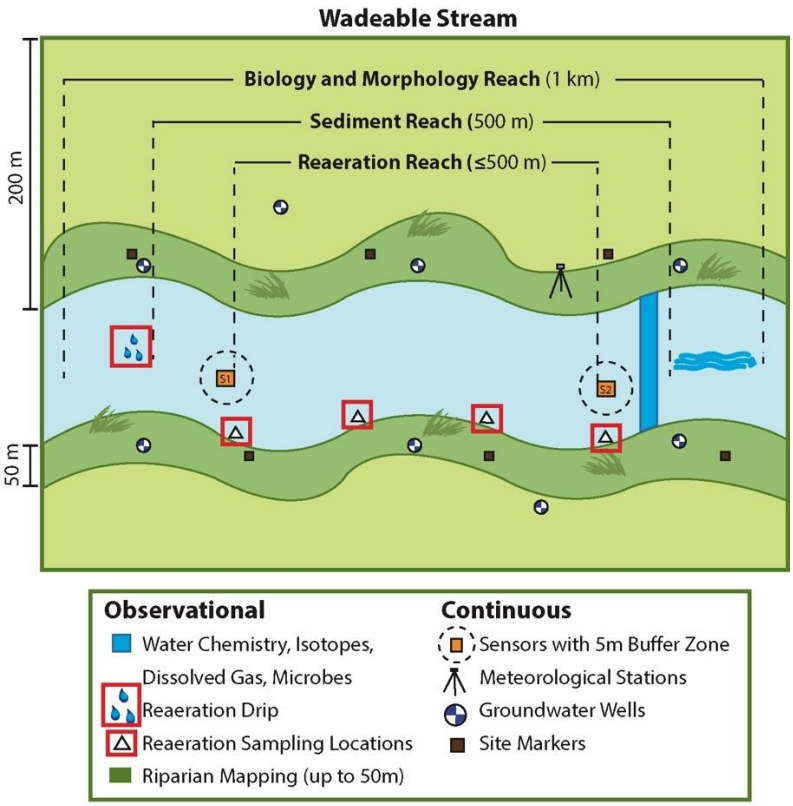


Figure 1. A generic wadeable stream site layout example with reaeration sampling stations.

3.1 Assumptions

1. Since we are unable to account for losses from the stream to the groundwater, a critical assumption of reaeration measurements is that groundwater losses are minimal.
2. Downstream tracer monitoring locations have been selected that will result in negligible influence of localized mixing effects on inert gas and conservative tracer concentrations (e.g., incomplete mixing of sampled thalweg water with either groundwater inflows or surface waters).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Gas Exchange (i.e., 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 sites. Initially, sampling events should be scheduled to capture a range of flows. It is anticipated that once gas exchange curves are established and accepted by NEON Science, this protocol will be discontinued or targeted to capture specific flows. Sites that have a high risk for major changes in stream channel structure or that have had observable changes in channel structure (e.g., sediment aggradation, channel avulsions, bank failure) may be requested to continue to collect measurements up to 6 times per year to establish a reliable strategy for estimating changes in gas exchange rates across time.

Table 1. Sampling frequency for Reaeration procedures on a per SOP and per site type basis.

SOP	Site Type	Bout Duration	Bouts Per Year	Remarks
SOP A-D	Stream	1 day	Up to 6 complete bouts	Sampling dates are synchronized with the Stream Discharge protocol. Sampling dates should be scheduled to capture a range of flows or to target certain flows once initial rating curves are established.

Scheduling Considerations

- Coordinating with stream discharge protocol implementation.

4.2 Criteria for Determining Onset and Cessation of Sampling

Gas exchange measurements, discharge, and other physical parameters are used to develop a reaeration-discharge curve. Reaeration should be scheduled to catch a range of flows and thus site-specific hydrographs should be considered during annual scheduling. This will allow the development of robust rating curves which can reasonably estimate gas exchange across a broad range of flow conditions. Once the rating curve is established as determined by the NEON Science Team in coordination with external community members, salt slug releases should continue to be conducted over a range of discharges (high, median, and low flows), to ensure that a major shift in reach morphology has not occurred by evaluating changes in wetted widths, travel time, breakthrough curves, reach velocity distributions, and mean depth curves at a given flow state. If the curve check does not produce results similar to the original reaeration-rating curve, such as after channel modification, a new curve or novel strategy for estimating changes in gas exchange over time will need to be established, potentially perpetuating the need for more frequent gas exchange measurements.

4.3 Timing for Laboratory Processing and Analysis

Samples should be processed (i.e., head space equilibration completed, filtered, and transferred to appropriate containers) as soon as possible and no longer than 3 hours after the end of sample collection.

Table 2. Timing for field and laboratory processing and handling of reaeration samples.

Sample type	Activity	Maximum Holding Time
Unprocessed syringe samples	Shake to equilibrate	3 hours before headspace equilibration
Gas (headspace) samples	Store in evacuated vials and ship to external lab	One month after headspace sampling
Conservative tracer samples	Filter, store, and ship to external lab	One month

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 3**).

Reaeration should always occur with discharge. If sampling must be rescheduled, reschedule as soon as possible. If sampling is:

- Rescheduled within 28 days of the scheduled sampling date, proceed with the reschedule and no additional action is necessary for schedule change approval.
    - If you need vial delivery dates modified, submit a problem tracking system request.
- Rescheduled > 28 days from the scheduled sampling date, submit an IS/OS Schedule Change Request. If vial delivery dates need to be updated, be sure to include that in the request.
- Cancelled completely, submit an incident ticket.

Table 3. Contingency decisions for Reaeration.

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions are unsafe for sampling, stop sampling and reschedule bout for a later date.	No adverse outcome.
	If weather conditions are unsafe for field processing, stream water may be collected in syringes, stored in containers of station water at plateau, kept at 4°C, and processed within 3 hours at a base camp or Support Facility.	Potentially reduced data quality
	If sampling stirred up sediments or added chemical constituents to the stream (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.
	If a sampling location is inaccessible, select a new location and enter a new sampling distance record in mobile app. Be sure the distance is measured as the thalweg distance downstream from drip.	No adverse outcome.
	If injection equipment malfunctions and can be fixed immediately, do so and continue the experiment, making note of the malfunction on the mobile application.	Potentially reduced data quality
Days-Weeks	If injection equipment malfunctions and cannot be fixed immediately, the rate of gas or salt addition during the injection will be altered and result in inaccurate data (i.e., if the rate changes, it is no longer a constant-rate experiment and data will not accurately represent stream conditions). Continue with a slug-only experiment to capture some travel time information. Rescheduled the activity since salt slugs do not count as a completed experiment.	Potentially reduced data quality
	If it begins raining during the injection enough to change the discharge of the stream, stop the injection and reschedule.	No adverse outcome.
	If sampling location is dry, a series of disconnected pools, or frozen, reschedule bout when stream is flowing.	No adverse outcome.
	If stream flow is too low to ensure a travel time of <3 hours, reschedule bout when conditions are appropriate for protocol implementation.	No adverse outcome.
	If sampling location is >20% ice-covered, deploy hobo loggers and complete a salt slug, if safe. This will allow us to still calculate salt-based discharge and travel time. Reschedule bout for a later date.	No adverse outcome.
	If flows are occasionally > 2000 L/s, reschedule for when flows are lower.	No adverse outcome.
Cancelled	If sampling is cancelled completely, submit an incident ticket.	Reduced data availability
	If flows are consistently >2000 L/s, submit an incident ticket.	Reduced data availability

4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more sampling locations in a given bout. For example:

1. Logistics – e.g., insufficient staff or equipment
2. Environment – e.g., deep snow, flooding, inclement weather, or
3. Management activities – e.g., controlled burns, pesticide application

Instances such as those listed above must be documented for scheduling, tracking long-term sampling location suitability, and informing end users of NEON data availability. Some types of missed sampling bouts are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[06]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

a. **Protocol Sampling Dates**: Bout-specific sampling dates (**Appendix C**, **Table 9**).
b. **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
c. **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
d. **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
e. **Rescheduled**: Missed Sampling is rescheduled for another time within the *protocol sampling dates*, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 2**).

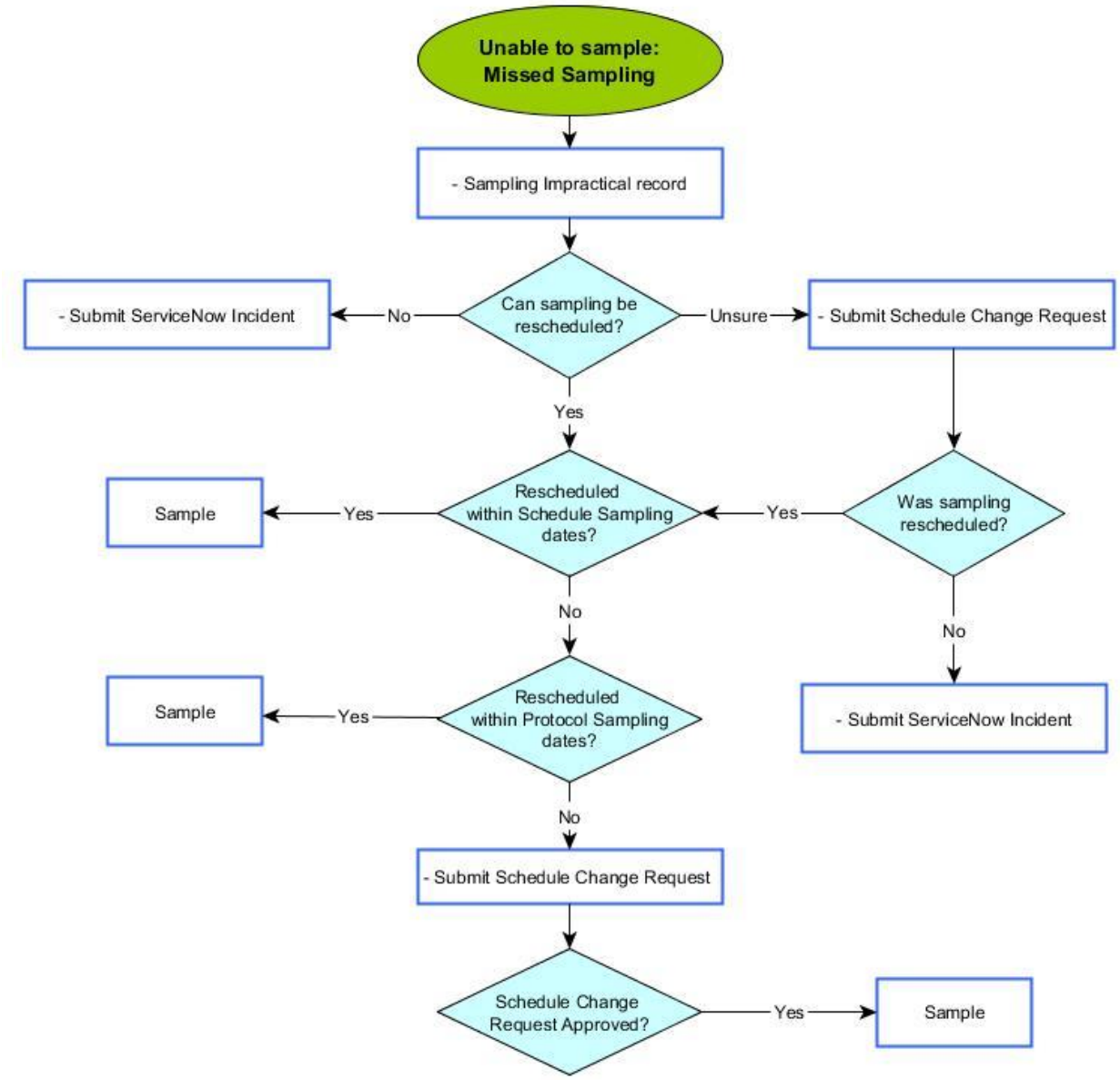


Figure 2. The documentation to account for a Missed Sampling event. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a problem tracking system incident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management record, or e) some combination of (a) – (d).

To Report Missed or Incomplete Sampling:

1. Missed or Incomplete Sampling must be communicated to Science by a problem tracking system Incident if sampling cannot be rescheduled within 28 days of the scheduled sampling date (**Figure 2**).

    a. For Missed Sampling that is rescheduled, there are some cases that require approval by Science and Operations (**Figure 2**).

2. Create a record in the Reaeration mobile application for each Missed Sampling event in the field.

3. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the parent Water Chemistry mobile collection device (**Table 5**).

Table 4. Guidance for responding to delays and cancellations encountered during implementation of the Reaeration protocol.

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
Reaeration Sampling	> 28 days, or < 28 days if vials needed	IS/OS Schedule Change Request	Submit incident ticket
Shipping (all samples)	> 45 days	Notify ^List-CLA	Notify ^List-CLA

Table 5. Protocol-specific Sampling Impractical reasons entered in the Reaeration mobile application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
High discharge	Discharge >2000 L/s
Location dry	Location dry
Location frozen	Location frozen (ice cover >20%)
Logistical	Sampling impractical due to equipment failure/Scheduling conflicts

Reaeration sampling will occur on the schedule described above at 4 sampling stations within wadeable stream sites (**Figure 1**). Ideally, sampling will always occur at these sampling locations. However, circumstances may arise requiring that sampling within a site be shifted from one particular location to another. The exact thalweg distance downstream of the injection location for each station is critical for calculating reaeration rates. Thus, when a location is moved **a new location must be created in the app and the thalweg distance downstream from the drip measured and recorded**. Even if a station does not move to a new location, but the stream channel changes length (i.e., increases or decreases in sinuosity), a new distance from the injection location must be recorded. 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 stream becomes compromised, a problem ticket should be submitted by Field Operations to Science.

4.6 Estimated Time

The time required to implement a protocol will vary depending on several factors, such as skill level, system diversity, environmental conditions, and distance between sampling locations. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, an incident ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Table 6. Estimated staff and labor hours required for implementation of reaeration sampling. More time may be required at sites with multiple sampling stations, such as a stratified lake or a lake with inflows and outflows.

SOP	Estimated time	Suggested staff	Total person hours
SOP A.1: Preparing for sampling	1 h	1	1 h
SOP A.2: Labels and Identifiers	0.5 h	1	0.5 h
SOP B.2-B.3: Collecting samples	0.3 h per station	2	0.6 h per station
SOP B.4: Processing and transporting samples	0.5 h	2	1 h
SOP D: Sample shipment	0.5 h	1	0.5 h

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. Anyone can stop work, and you are expected to use that authority when there is uncertainty about the safe conduct of work.

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 Training in the Training Center.

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

1. Field ecologists should be aware of any site-specific water hazards of that particular location (i.e., current status, tidal charts, etc.).
2. Access to Safety Data Sheet shall be available for work with chemicals associated with this protocol.

6 PERSONNEL

6.1 Training Requirements

All field ecologists must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, field ecologists must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]). Personnel are to be trained in reaeration measurements and safe working practices for stream fieldwork.

See the Compressed Gas Safety Training PowerPoint on the NEON Safety Training in the Training Center.

6.2 Specialized Skills

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

7 STANDARD OPERATING PROCEDURES

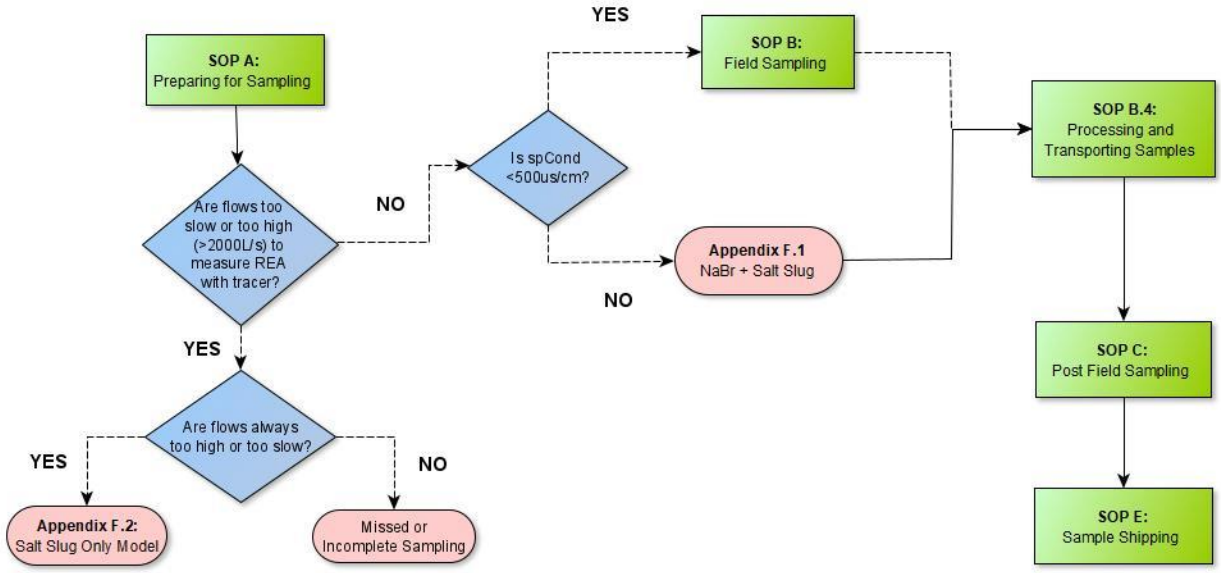


Figure 3. High level workflow diagram of how the included SOPs are sequentially connected. Green boxes represent SOPs and red nodes represent parts of SOPs, with dashed lines between SOP sections. Diamonds represent a decision that must be addressed.

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged and synced at the beginning of each field day, whenever possible. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL).

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 always be carried along with the mobile devices to sampling locations. 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.

A.2 Preparing for Field Sampling

1. Check the weather the day prior to the scheduled field sampling and adjust the schedule to avoid any major storms.
2. Syringes should be pre-labeled (1-20) to align with vial sample collection numbers. Cover the syringe graduations and pre-labeled number with clear packing tape to prevent markings from wearing off during field sampling and ensure accurate volume collections.
3. Prepare pump before going to the field.
    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 4**).
    b. Ensure pump has been assembled correctly (See Appendix E.1 for more details):
        1) If piston has been withdrawn more than 2 inches from the cylinder (**Figure 4a**) or removed completely from the pump head (**Figure 4b**), 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 4a**) to hold drive carrier in place.

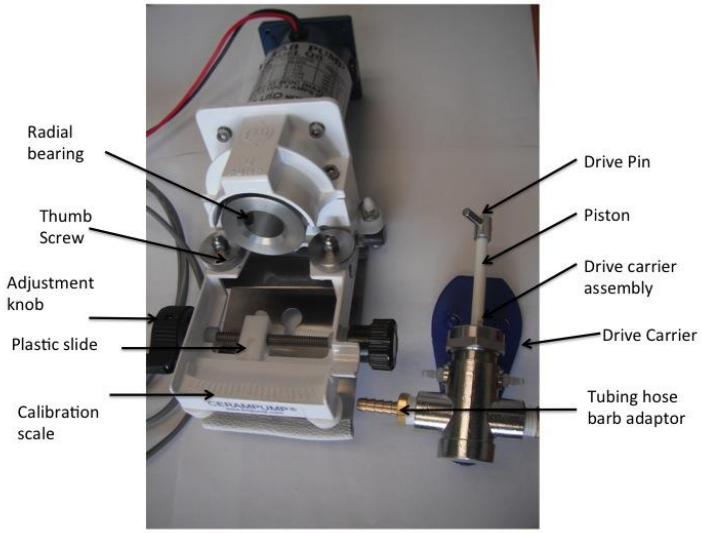


Figure 4. Fluid metering pump a) Components of pump drive (left) and b) pump head (right)

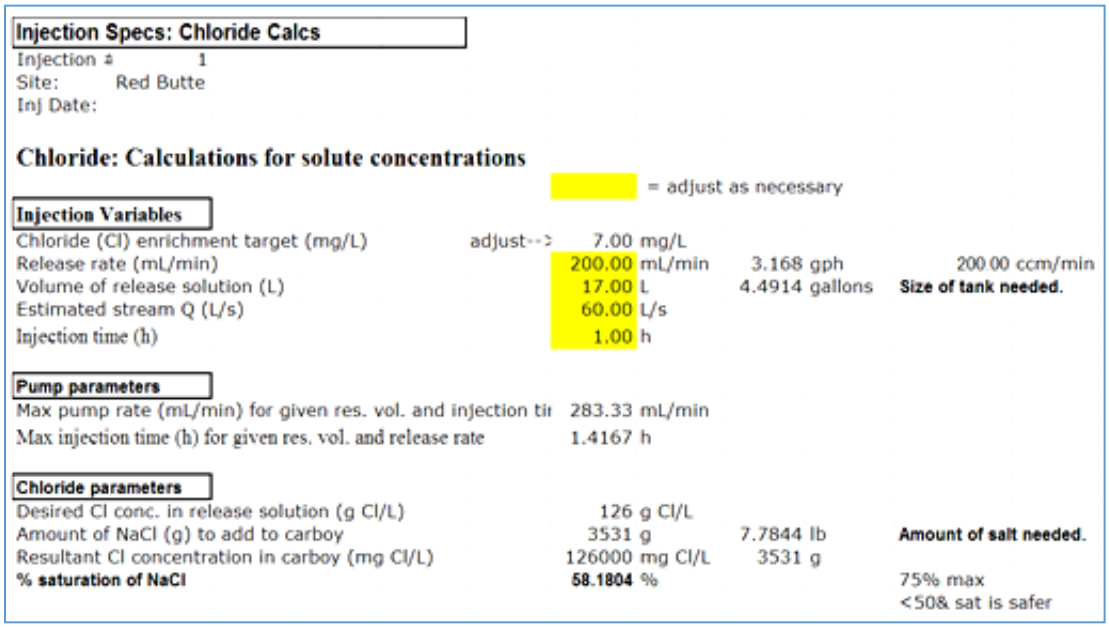
c. Test Pump: Place pump on the rim of a 5-gallon bucket with inlet and outlet tubing ends placed in the bucket containing tap water. Connect the pump to an 8V battery (or several 8V batteries in series. See Appendix SOP EE.2) and run the pump to make sure everything is working properly.
d. Look for leaks and replace tubing as necessary.
e. 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. For example, if the cylinder is pointed to the 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) Flow rate on these pumps can vary depending on the amount of lift needed to get the injectate from the reservoir to the pump head. Set-up pump so that the reservoir is similar to the elevation gradient it will experience at the stream site. You will still need to calibrate and verify flow rates after it is set up at stream. This step is to get you close to the desired pump rate so there is less adjustments in field.
    3) If you need to adjust flow rate, loosen the two thumbscrews slightly and use the adjustment knob as necessary. Remember that the pump flow rates are very sensitive,

thus small adjustments may be all that is required. Retighten thumbscrews when adjustment is complete.

4. Use the injection calculation spreadsheet (**Figure 5**; Reaeration Data sheet excel file (RD[05]) to calculate the quantity of the conservative tracer (NaCl) you will need to add to the bucket/carboy. If there are observed or expected inflows over the reach, be sure to use the discharge at the downstream end of the reach (not the injection site) for calculations.
    a. **NOTE:** If you are not able to use NaCl as a continuous tracer at a site, see Appendix G.
    b. The load of tracer added to the stream needs to be large enough to allow for a measurable concentration after dilution in the stream before 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 NaCl (10 – 30 µS/cm increase in conductivity), with less than 40 - 50% saturation of the conservative tracer (absolute maximum 75% saturation for which you will need to allow a substantial amount of time for dissolution in unheated water). Keep saturations as low as possible to reduce the time it takes to dissolve salt into solution and to reduce the effect of propagation of error in pump rate. In low conductivity streams (<100 µS/cm), an increase of 5- 10 µS/cm will be sufficient to quantify a change.
    c. If the spreadsheet returns an error, you need to adjust the parameters in the yellow squares, because the error indicates that you cannot dissolve enough salt to make the concentration solution you are suggesting. To fix the error, use a larger volume bucket so you can dissolve more salt and/or increase the pump rate (the faster you pump, the less salt you need because you are creating a higher load from the same concentration by pumping at a higher rate).
    d. Ensure you do not pump so fast that you run out of solution before the end of the experiment. Use the spreadsheet to determine the 'Max injection time' and aim to end the experiments 15 minutes before so you do not run the pump dry.



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 5. Example of injection calculation worksheet for NaCl. Modified from LINX II. The squares in yellow are the ones that you are free to adjust based on your stream conditions. Start by entering your estimated stream Q. In this example, we are targeting to increase the Chloride by 7 mg/L. Our stream flow is 60 L/s and we want to release for an hour and mix our salt into 17 L of water. If we have a release rate of 200 mL/min, the spreadsheet calculates that we can actually pump for 1.4 hours before we are out of solution. In order to make this salt solution, we need to weigh out 3531 g of NaCl and dissolved in 17 L of water.

5. The amount of salt required is a product of the target enrichment, the estimated stream flow, and the injection rate.
   a. Once stream discharge rating curves are available for a site, you may use the current pressure transducer data and discharge rating curve to estimate current discharge used in the calculation spreadsheet.
   b. The below equation is used in the spreadsheet to calculate the amount of NaCl needed for the continuous injection.

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

6. 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). Concentrated solutions will need larger volumes or to be mixed in field.
   a. Ensure the tracer is completely dissolved.
   b. If you estimate your discharge for calculations, take extra, pre-weighed salt into the field with you in case your estimates were low, and you need more salt.

1) Scales used in the field are considerably less accurate than those used in the lab. Rather than weigh salt in the field, pre-weigh discrete amounts of the conservative tracer in the lab so that you can adjust conservative tracer mass in the field, as needed. Scales should be accurate to nearest mg.

7. **Record** the amount of salt added in the Reaeration mobile application.
8. Confirm that you have evacuated gas vials from a gas laboratory in good condition. Vials do not have an expiration date, but should not have any water in them. Water in the vials indicates that vacuum has been compromised and the vial should not be used.
    a. Older vials are more likely to have compromised vacuums, so be sure to use oldest vials first and check vials for signs of leakage carefully prior to use.
9. Ensure stopcocks for syringes are in good condition. These can develop hairline fractures over time.
10. Charge 8 V batteries OVERNIGHT. The pump manufacturer suggests using 8V batteries with the pump.
    a. You may need to wire four 8 V batteries in parallel to maintain pump speeds throughout the experiment (Appendix SOP EE.2). Batteries wired in parallel should ideally be of the same make and charge state.
    b. 12 V batteries are not suggested because they can decrease the life of the pump.
11. Check the battery life of the handheld meter and logging probes.
    a. Since you will always use a computer with the shuttle, the shuttle does not require batteries. Remove the batteries from the shuttle to reduce risk of the shuttle batteries corroding the circuit board and damaging the shuttle.
12. Calibrate the conductivity handheld meter (make sure entire probe and the two black holes at the top are completely immersed and the holes have not captured any bubbles).
    a. Best practice is to be sure you get the same number twice after completely removing and replacing the probe in the solution.
    b. Check loggers against calibrated conductivity meter. Logging conductivity probes do not need to be calibrated but should be validated against calibrated hand-held measurements.
    c. Loggers should be within 3% or 5 µS/cm specific conductance (low-range) or 3% or 20 µS/cm (for full-range) of each other and the calibrated hand-held.
        1) Submit a trouble ticket if probes or loggers are not reading correctly and postpone sampling until issue is resolved. This step can be completed up to one week prior to the experiment.
13. Set logging probes to begin logging (10 second intervals) on the reaeration field day. If you are completing an injection at a site with very fast travel times (<10 min), set the HOBOs to log every second.
    a. Be sure to set the HOBOs to begin logging just before placing into the stream so that you don't use up the storage prior to the injection. You can set the loggers to start on a delay but be sure they are logging before the injection starts so that we can get a good background reading.

b. Every time you relaunch a HOBO data logger, the device overwrites the old data.

14. Launch Conductivity Meters. **NOTE:** Take precautions if launching these loggers in the field to minimize exposure to direct sunlight when communicating (Keep the logger and shuttle in their shadow at a minimum).
    a. Ensure HOBOWare Pro software is installed on your laptop and launch the program. Each Domain should have a license for this software. Update firmware as needed.
    b. Connect shuttle to laptop using USB-mini cable.
    c. Attach HOBO to shuttle and press power bar so that the green light shows.
    d. Green light must be on for shuttle to be connected to HOBO. NOTE: shuttle will turn off after disconnecting a device. You will need to turn it back on before connecting the second HOBO.
        - NOTE: If you hit launch on your computer screen when the shuttle is off your screen may still show "launch successful" even though the HOBO is not connected since the shuttle is off.
    e. Ensure Name is in the correct format.
        1) In the top box "Name" in the Launch window and "Description" in the Plot Setup window (**Figure 6**), follow the below format: **SITE_S#_YYYYMMDD,** where S# is either S1 or S4 for reaeration station 1 or station 4.

            ex) LEWI_S1_20170816 OR LEWI_S4_20170816

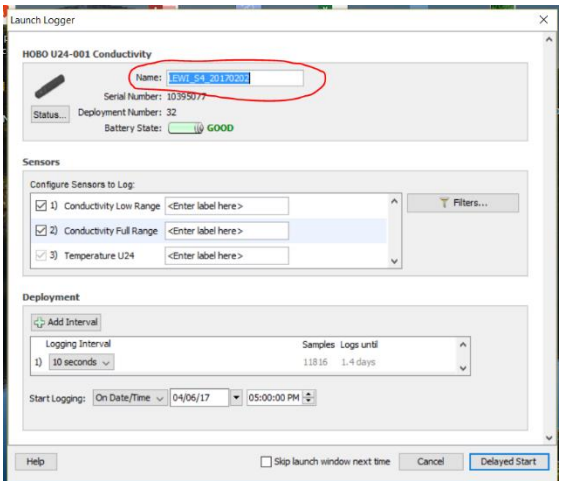


Figure 6. Examples of entering the correct file name structure into HOBOware.

    f. Ensure temperature is set to Celsius.
    g. Sync HOBO clock. The clocks drift over time and should be synced each time.
        1) If launching with the shuttle connected to the computer (recommended), then it will automatically sync.

2) If launching with the shuttle not connected to the computer (not recommended), then you must push the sync shuttle clock button. More details can be found in the HOBO Waterproof shuttle manual (U-DTW-1) found on the Onset website.

h. Before disconnecting HOBO check that shuttle was powered on while launching.

i. Check the HOBO status in HOBOware Pro before disconnecting to verify the HOBO is logging or waiting for a delayed start. You can do this several ways: by clicking status button on the toolbar, click status from the device menu, or click 'Ctl+I'.

j. Ensure you check both status and see the "Shuttle Launched Successfully" (**Figure 7**).

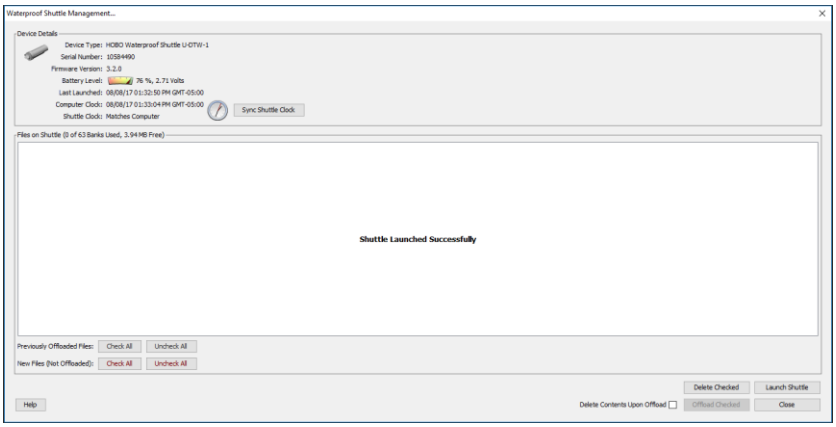


Figure 7. Examples of successful launch notification in HOBOware.

A.3 Labels and Identifiers

Barcodes are useful for minimizing transcription errors and tracking samples from the domain support facility to external locations. **All field samples must use a barcode and all samples being shipped from the DSF must have a barcode.**

Note: All barcodes need to be applied to dry containers for 30 mins before use. Type I (prefix A, plus 11 numbers) are for all field samples and any non-cryo applications; they have a tolerance from 4C to 105C and still scan.

1. Prepare sample bottles and vials with labels before going in the field. You should have 20 gas sample vials and 25 tracer bottles.
2. Remove submerged gas vials from storage container and dry before applying labels.

3. Attach a Type 1 barcode label (**Figure 8**).

Figure 8. NEON Example of Type I barcode

a. Add adhesive barcode labels to the sample containers along the length of the sample container (**Figure 9**).

Figure 9. Example of appropriate attachment of reaeration gas vial labels.

b. Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database.
c. Allow the adhesive to cure for 30 minutes.
d. Cover the barcode with clear packing tape.
    1) You may write the syringe number on the vial (not the barcode) prior to taping, if that assists your workflow.
    2) Wrap the tape completely around the vial, perpendicular to the label.
    3) Ensure the ends of the tape overlap slightly to keep the tape from coming unstuck. You may need two rows of tape to completely cover barcode.
    4) Use as LITTLE tape as possible.
    5) DO NOT use heavy duty clear packing tape.

4. Write the ID on the sample bottles to help you quickly locate the correct sample during processing.
    a. Write the tracer sampleID on the top of the 60 mL Nalgene bottles with permanent marker (B1-B4, 01-20, INJ).
    b. Write the vialID number (1-20) on the top of each vial cap with permanent maker (**Figure 11**). Labeling the vial holder rack will also help keep vials organized.

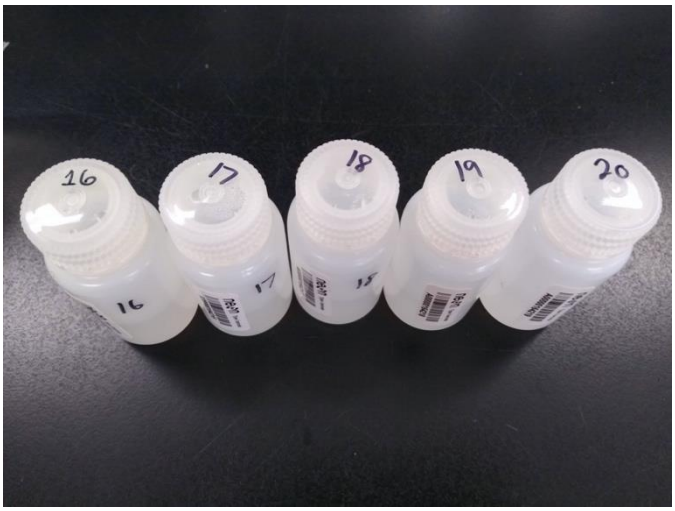


Figure 10. SampleID number written on the top of each tracer sample container.

Table 7. SampleID codes format.

SampleID		
Sample Type	Location	ID
Tracer sample only	Background: B Station # (1 – 4, where 1 is Station 1).	B1-B4
	Injectate (make red dot on lid with permanent marker and write INJ on the lid)	00
Tracer and Gas samples	Station 1	01-05
	Station 2	06-10
	Station 3	11-15
	Station 4	16-20

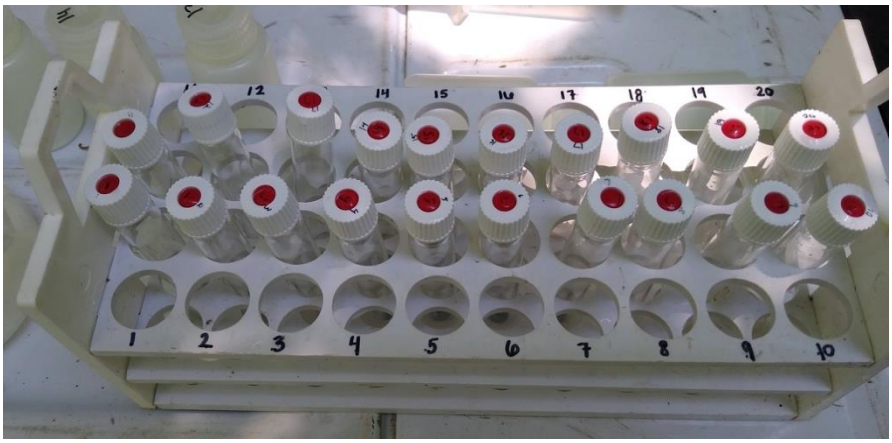


Figure 11. VialID number written on the top of each vial to help locate vials quickly.

5. Return gas vials upside down to the storage container so the cap is submerged for transport to field site. This helps buffer vials from changes in atmospheric pressure, which can impact vial integrity.

SOP B Field Sampling

B.1 Spatially and Temporally Linked Protocols

Synchronized protocols and SOPs include:

AOS Protocol and Procedure: DSC – Stream Discharge (RD[10])

Stream discharge should be collected immediately before completing reaeration. **NOTE:** You should still conduct a discharge measurement even if the staff gauge is damaged or missing.

1. At stream or lake inflow/outflow sites, if discharge was measured using the flowmeter method during a REA bout while the staff gauge was displaced or not present at the site:
   a. When creating the discharge file in the field or post-field using a text editor (such as NotePad ++, not Microsoft Excel) add an extension of "_REA" to the four digit site code in the Profile Name (i.e. "BLUE_REA").
   b. The file should successfully load in SOM despite having no characters or values following the "Stage Reference:" field in version 1 formats and either "0" or "-" characters contained under the "Stage Reference" column in version 2 formats (these values are automatically populated by the flowmeter software if no stage value is entered).
   c. In the Field Metadata and Gauge Height Fulcrum app, create a Sampling Impractical record and choose the dropdown option that best describes why a gauge height measurement could not be obtained.
2. At stream or lake inflow/outflow sites, if discharge was measured using the ADCP method during a REA bout while the staff gauge was displaced or not present at the site:
   a. Add a note in the remarks section of the WinRiver II .mmt file associated with the measurement explaining that the staff gauge was not present during the REA bout and leave the "Inside Gauge Height" box blank (within the rating information tab where gauge height is usually entered).
   b. In the Field Metadata and Gauge Height Fulcrum app, create a Sampling Impractical record and choose the dropdown option that best describes why a gauge height measurement could not be obtained.
3. When using the ADCP to calculate discharge in the field, use the Q-Rev discharge values.

B.2 Background Sampling – Complete BEFORE setting up the injection to avoid contamination of background samples.

1. Ensure the General AQU Field Metadata information is completed (RD[06]).
2. Place logging conductivity probes in the thalweg at Station 1 and Station 4 (for WALK modifications, see Appendix F). Since we are measuring gas exchange rates between the sensor sets, **conductivity probes must be placed at the same transect as the sensors sets (within 0.1 m of main infrastructure)**.
   a. Probes should already be logging at 10 second (or 1 second if travel times are fast) intervals to ensure ample background readings prior to the injection.

b. Probes must be suspended in water column if water depth allows. Probes should be at approximately the same location as the in-stream sensors. You can suspend them from the stream infrastructure, temporarily installed rebar, etc.
c. Remember that the conductivity sensor is located at the opposite end of the probe's removable cap (**Figure 12**), thus the entire sensor must be submerged.

3. Prior to setting up the injection, visit each of the four sampling stations (**Figure 13**), and at each station record four specific conductance measurements within the stream thalweg (i.e., the main flow of the stream).
   a. Make sure the hand-held conductivity meter is set on the temperature-corrected setting (i.e., specific conductance) with units **SPC,** µS/cm. Wait for the meter to adjust to the stream temperature for more accurate conductivity measurements.
      1) **NOTE:** The conductivity sensor is located at the top of the probe where the two black holes are located, so the **ENTIRE** probe must be underwater to get the measurements (**Figure 12**). Ensure the water is deep enough to cover the entire probe.
   b. The handheld sensor should be at the same depth in the stream from which the samples will be collected. DO NOT set the sensor on the stream bed, as specific conductance can be different near the sediment-water interface than in the stream column.
   c. Jiggle the probe to make sure no air bubbles are next to the conductivity sensor (2 black holes) so the conductivity measures correctly.
   d. Make sure there are no air bubbles in the handheld port by taking the probe completely out of the water and reinserting it. You should have the same specific conductance reading after reinserting it as before.

Figure 12. Conductivity sensor is located at the top of the handheld probe and HOBO logger. Ensure the entire probe is in the water and no air bubbles are around the sensors for accurate readings.

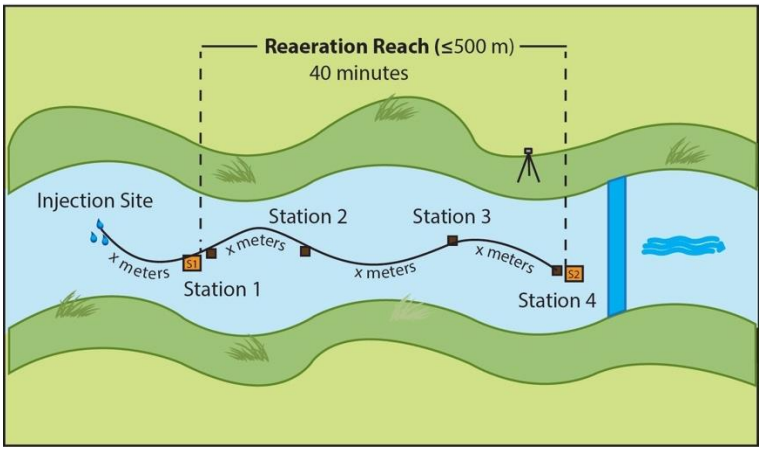


Figure 13. Schematic of stream reach with injection site and four downstream sampling stations. Travel time between Station 1 and 4 should be ~40 minutes. Note that Reaeration station 1 is co-located with Sensor set 1 and Reaeration station 4 is co-located with sensor set 2.

4. Make sure your hands are clean and not contaminated with injection solution. A very small amount of concentrated injection solution can easily contaminate sampling equipment. Rinse and clean hands immediately before sampling.
5. Rinse the appropriate background collection syringe and stopcock three times with the sample water at each of the four stations, being sure to collect from the thalweg.
    a. Place the syringe tip (with 2-way stopcock attached and turned to open (**Figure 17**) into the stream so that the water is sampled ~10 cm under the surface. Stopcocks should always remain on the syringe.
    b. Pull in ~20-30 mL of water and remove syringe from stream.
    c. Pull syringe plunger back to 60 mL to draw in air, close stopcock, and shake syringe vigorously for ~5-10 seconds. Make sure you pull plunger back to at least 60 mL to rinse the entire syringe.
    d. Expel air and water and repeat rinsing steps one more time.
    e. On the third rinse:
        1) Place the syringe under the water and draw in ~40 mL of water, followed by removing syringe from the water and pull 20 mL of air. These volumes do not have to be exact.
        2) Roll the air slowly around the syringe and plunger tip to collect air bubbles.
        3) Turn syringe tip-upward and tap the side of the syringe to release any trapped air bubbles. Large air bubbles affect volume readings so they should be removed. Tap hard to remove air bubbles. Removing small air bubbles may not be possible.
        4) Holding the syringe upright, expel the air and all but 3-5 mL of the water sample.
6. Place the syringe tip back into the water so that it is ~10 cm below the surface, and expel the remaining water into the stream, which will help reduce the collection of air bubbles when sampling.

a. Removing air bubbles is not as important for the background samples as it is for plateau sampling since we are not collecting gas samples. However, too many air bubbles can damage the filter.

7. **SLOWLY** pull the plunger to draw a water sample until the syringe is completely full and the plunger is at the **60-mL mark** (**Figure 18**). 60 mL is more than needed but collecting more will make the processing step easier.
8. **BEFORE** removing the syringe from the stream, immediately turn the 2-way stopcock to the closed position (**Figure 17**).
9. Remove the syringe from the stream and attach a 30 mm diameter, 0.7 µm pore size filter to syringe.
10. Triple rinse, with a ~5 mLs of filtered water from the syringe, the corresponding pre-labeled 60 mL bottle with filtered stream water. Discard rinse water downstream or on the bank.
11. Collect a minimum of 30 mL of sample, as you would all other salt samples.
12. Mark and label each station with flagging tape, if necessary, to help find the sampling stations during the injection.

B.3 Field Set-up and Sampling

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.
   a. Rinse the pre-filled salt container into the injection carboy to ensure all salt is transferred to the injection solution.
2. **Record** the volume of the saltwater solution on the tablet or injection data sheet to the nearest 0.5 L (RD[05]). Mark on the bucket the volume line you will be using to make filling the bucket easier. Measure out 2-5 L graduations on a dedicated reaeration bucket ahead of time.
3. Set up the bucket/carboy, tubing, battery, and pump on a level surface at the injection site (**Figure 14**). If a level surface on the bank is not adequate, place pump set-up on a bridge or plank laid across the stream.

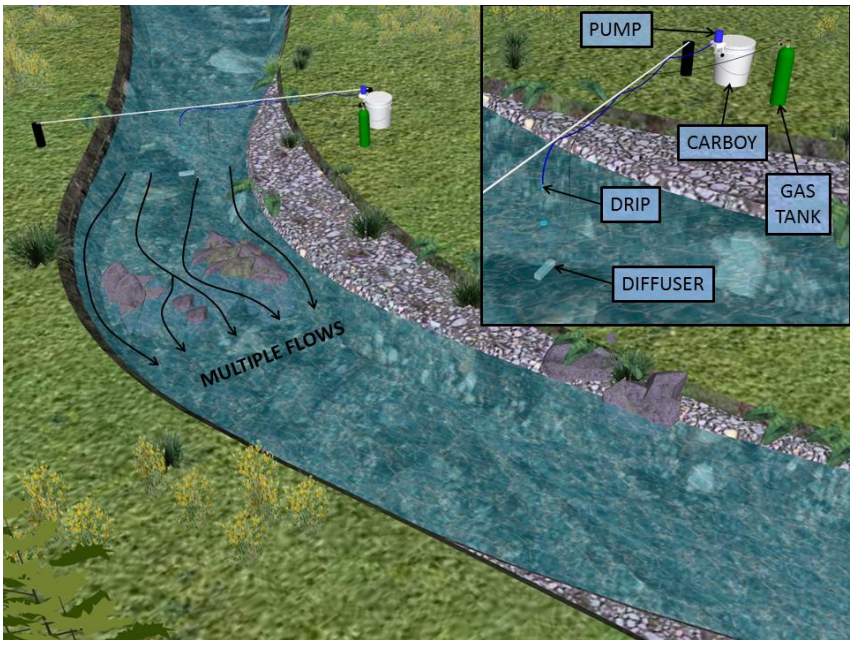


Figure 14. Field setup of the injection site with conservative tracer and inert gas.

4. Pump setup:
   a. Make sure you have collected background samples before setting up the pump to avoid contamination. Salt solution is easily transferred from the injection equipment to the sampling equipment and samples. Extreme care should be taken to not contaminate samples.
   b. Mount pump upright for best performance. Pump can easily be mounted on the wall of a 5-gallon bucket (**Figure 15**). Note that as saltwater level lowers in the bucket it can get top heavy with the pump and fall over, take precautions to prevent this. (Hanging the pump INSIDE a second, empty bucket is a good option).
   c. 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).
      1) 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.
      2) If the stream water has a lot of particulates, you may want to screen the inlet end of your tubing or bring additional water with you from the lab. You do not want to run particulates through the pump.
   d. Attach the pump electrical wires to the battery (Red to Red and Black to Black).

Figure 15. Mounting of the pump on the conservative tracer bucket

5. Ensure you have a way to set-up pump tubing so that the end of the tubing is located a few inches above the water surface. Attach the tubing to something stable, such as a piece of rebar, temporarily pounded into the stream bed or a tree above the stream.
   a. **DO NOT** put the tubing into the stream because it will change the pump rate.
   b. Make sure the tubing will INJECT (i.e., Drip) INTO THE THALWEG so the solution will mix as quickly as possible with the stream water.
6. **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 5**). 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. For example, if during the injection the tubing will be placed 4 inches above the stream surface, then hold the tubing 4 inches above the stream surface during calibration. It is best to calibrate the pump with the salt solution at the exact location.

   - **NOTE**: This step may take several minutes, but it is extremely important to get the correct pump rate.

   a. Flow rates should be measured at 30 seconds or 1-minute intervals. Measuring flow for 1 minute will be more accurate than measuring for 30 seconds.
   b. Every time you test the rate, pour the injectate back into the bucket/carboy and return the outlet tubing to the bucket. 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).
   d. Place the outlet end of the tubing back into the bucket. Keep the pump running while you continue setting up the experiment.

7. Collect a 60-mL bottle of conservative tracer injection solution into the pre-labeled injectate bottle (Bottle ID – '00'). This is an **EXTREMELY IMPORTANT** step so that we know the exact concentration of the solution we added to the stream.
   a. Triple rinse corresponding syringe with conservative tracer injection solution.
   b. Attach 30 mm diameter, 0.7 µm pore size to syringe and triple rinse (~ 5 mL per rinse) corresponding 60 mL pre-labeled sample bottle with filtered tracer solution to remove any particulates that could clog the analyzer. Collect a minimum of 30 mL of sample, as you would all other salt samples.
   c. Make sure the cap is labeled with "INJ" and there is a red dot on the lid to indicate to the lab this is an injectate sample.
   d. Enter data into the mobile application and scan sample.
   e. 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.
8. **Gas Injection Set-up:**
   a. At the injection site, set-up the SF_6 gas tank, regulator, and flow meter (**Figure 16**).
      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. Tubing should be cut to site-specific lengths.
      3) Use gas-impermeable (e.g., Tygon) tubing to connect the TOP of the flowmeter to the diffuser (air stone). The tank can lie horizontal on the ground.
      4) Place diffuser in water at the same location in the stream as the conservative tracer injection (**Figure 14**). Make sure diffuser is completed submerged and secured so that it will remain underwater and in the same location. Add weights tied to tubing and diffuser during high flows, if necessary.
      5) Ensure the gas flowmeter is **vertical and secure**. The flowmeter must remain upright for the best performance. Do NOT lay it on the ground.

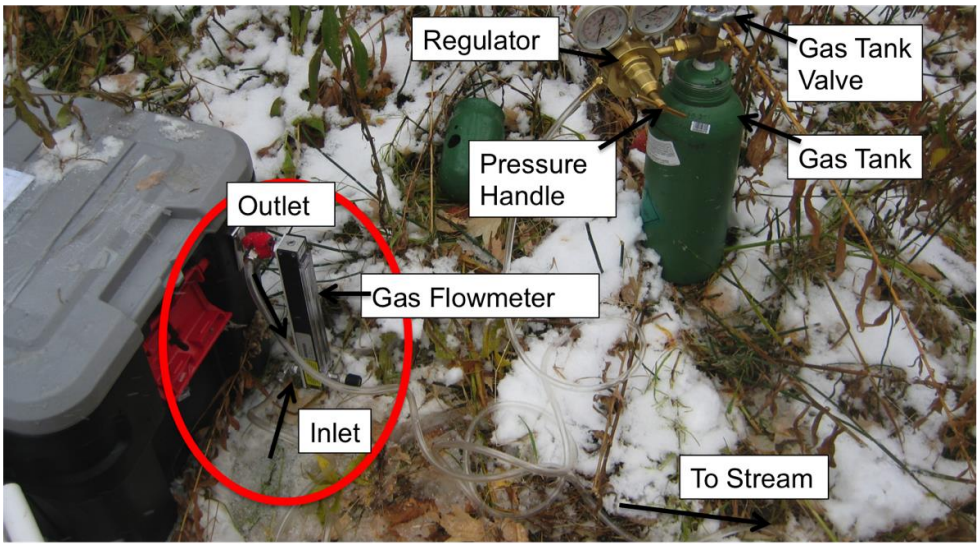


Figure 16. Gas tank, regulator, and flowmeter field setup.

6) Connect all parts of the gas set-up before opening the main valve 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 gas tank regulator at 12 psi.
11) On the flowmeter, turn the valve so that it reads approximately 35 psi. Only adjust the psi settings if your stream flows are
    (a) <25 L/s – set flowmeter to 20 psi
    (b) >1000 L/s and your stream is very high-gradient and turbulent (i.e., lots of reaeration) – set flowmeter to 40 psi.
12) Make sure you see little bubbles coming out of the diffuser (air stone) while the diffuser is under water. If the bubbles are not visible due to reflections or ripples on the surface of the water, verify bubbles in a bucket of still water.
13) Troubleshooting diffuser:
    (a) If the glass ball is stuck, try blowing the gas in the opposite direction (top to bottom) to get it unstuck. Reassemble in correct orientation once unstuck.
    (b) 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.
    (c) Ensure the connection between the tubing and the air-stone is secure so gas isn't escaping before entering the diffuser.

(d) If pores seem clogged or filled with algae, clean diffuser using hard-bristled brush or 10% bleach solution.

9. Once you have calibrated the pump and made sure the gas set-up is working and there are no leaks, start the stream injection by securing the pump tubing above the stream surface at the **same time** as you turn on the gas injection.
   a. The conservative tracer tubing should be just above the water surface. Do not put the tubing in the water, as flow rates can be impacted if tubing is placed in the water.
   b. If using NaBr, you will also need to do a salt slug so that you will know when to sample and we can estimate travel times. See Appendix G.

NOTE: After injection into stream begins, **do NOT change the flow rate of the gas or the conservative tracer.**

10. Record the start time on the tablet or Reaeration Field Data Sheet (RD[05]).
11. Spend a few more minutes at the injection site making sure the salt and gas injections are working properly.
12. **Monitor for plateau:** 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.
    a. **DO NOT WALK IN THE STREAM**.
13. Once at the most downstream station, place the handheld conductivity meter in the stream, just downstream of the Station 4 logger. 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 at 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. Meter should be placed at the same depth in the stream column from where you would take your sample. Do not lay the probe on the streambed.
    c. Jiggle the probe to make sure no air bubbles are next to the conductivity sensor (2 black holes), so the water makes proper contact with the electrodes.
14. Continue to observe specific conductance until the stream has reached plateau (when salt concentration/specific conductance measurement is no longer rising during continuous injection or when stream specific conductance has returned to near background conditions if using salt slug). Sampling must not begin until the stream has reached plateau at the MOST DOWNSTREAM station, usually 30 minutes to an hour during low flows and less time during high flows.
15. Once the most DOWNSTREAM station has reached plateau (or the specific conductance has returned to near background conditions with a salt slug), start sampling at the most UPSTREAM station (i.e., Station 1, the station closest to the injection site) and work downstream (**Figure 13**). The idea is that you are following a parcel of water as it moves downstream.

a. When travel times are very slow, you will sample from Station 1 to station 4, but at a faster rate than the travel time. That's ok, as long as the stream is at plateau. For example, if the travel time of your stream is 40 minutes and you sample station 1 through station 4 in 10 minutes, as long as the stream is at plateau, the data quality will not be impacted.

16. At Station 1, record 5 specific conductance measurements at four different locations 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).
    a. If stream is not mixed by station 1, you should move your injection station further upstream to allow for more stream length for mixing to occur. **NOTE:** During higher flows, mixing lengths may increase and injection sites may need to be moved upstream.
    b. For NaBr additions, you only need to record 1 conductivity measurement in the thalweg.
17. Prepare for sampling. 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 from where you are standing.

NOTE: If multiple people are simultaneously collecting samples at the same station, make sure they are standing at the same distance from the injection site (i.e., side by side, not upstream or downstream from one another).

18. Rinse the collection syringe and stopcock three times with the sample water.
    a. Place the syringe tip (with 2-way stopcock attached and turned to open (**Figure 17**) into the stream so that the water is sampled ~10 cm under the surface. Stopcock should remain on the syringe at ALL times.

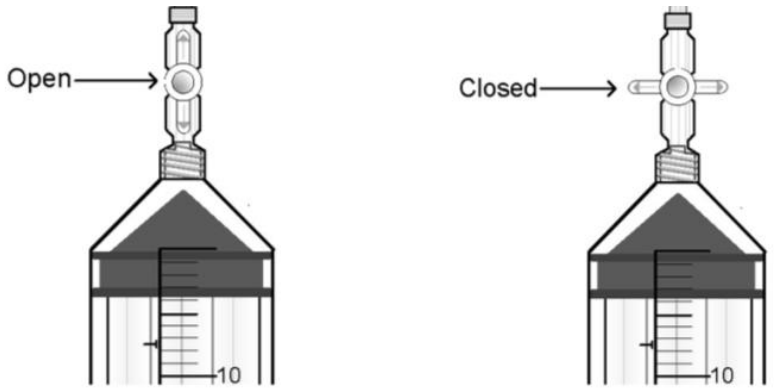


Figure 17. Example of a syringe and stopcock in the "open" and "closed" positions.

    b. Pull in ~20-30 mL of water and remove syringe from stream.
    c. Draw in air to the 60 mL mark, close stopcock, and shake syringe vigorously for ~5-10 s.
    d. Expel air and water and repeat the rinsing steps one more time.
    e. On the third rinse:

1) Place the syringe under the water and draw in ~40 mL of water, then remove the syringe from the water and pull approximately 20 mL of air. These volumes do not have to be exact.
2) Roll the air slowly around the syringe and plunger tip to collect air bubbles.
3) Turn syringe tip-upward and tap the side of the syringe to release any trapped air bubbles. Large air bubbles affect volume readings so they should be removed. Tap syringe hard to remove air bubbles. Removing small air bubbles may not be possible.
4) Holding the syringe upright, expel the air and all but 3-5 mL of the water sample.

19. Place the syringe tip back into the water so that it is ~10 cm below the surface, and expel the remaining water into the stream, which will help reduce the collection of air bubbles when sampling.
20. **SLOWLY** pull the plunger to draw a water sample until the syringe is completely full and the plunger at the 60-mL mark (**Figure 18**). 60 mL is more than needed but collecting more will make the processing step easier.
    a. Be careful not to entrain any air bubbles. If large air bubbles are captured in the syringe, remove bubbles, and resample.
21. **BEFORE** removing the syringe from the stream, immediately turn the 2-way stopcock to the closed position (**Figure 17**).
22. Collect 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.

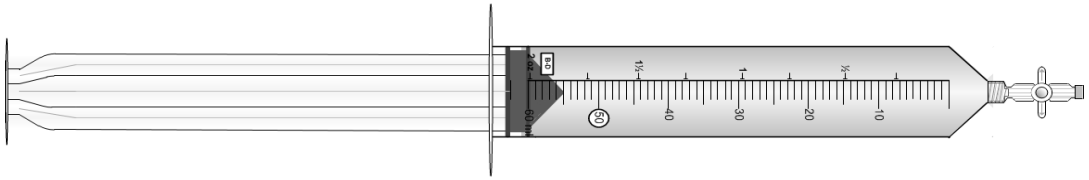


Figure 18. A full 60 mL syringe with a closed 2-way stopcock.

23. At each station (for all injection types), record the time, 5 syringe IDs, water temperature, and specific conductance (5 readings at Station 1) in the mobile application.
24. REPEAT (Steps 18-23) for each sampling station, always working from upstream to downstream. When walking between stations, do not walk in the stream.
25. Return to the Injection Site. Measure and record the 'End Pump Rate' in the mobile application.
26. END INJECTION: Turn off Gas and Pump.
27. If you did not already (SOP B.3, Step 7), collect a sample of the conservative tracer solution in a 60-mL bottle, labeled with 'INJ' on cap.
    a. Make sure the cap has a red dot and is labeled with 'INJ' 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.

B.4 Processing and Transporting Samples

NOTE: Samples should be processed immediately, if possible, and no longer than 3 hours after sample collection. If you are not able to process the samples immediately, place the syringe in a storage container (i.e., small cooler, pitcher, or bucket) with station-specific water at time of collection to help maintain temperature and to decrease degassing across the syringe walls. You will need a storage container for each sampling station, with water at plateau from each station. Samples should be processed well away from the stream. When adding air to syringe for headspace equilibration, move at least 5 m from the water's edge to minimize the potential for contamination by SF_6 evasion from the tank, diffuser, or the stream.

Vial condition should be evaluated before going in the field. Properly evacuated vials will often result in atmospheric pressure moving the syringe plunger and pushing gas from the syringe into the vial; however, this is not always the case for stickier plungers and should not be used as an indicator of a properly evacuated vial. If liquid water is observed in the evacuated gas vial prior to sampling or you are unable to push the typical volume of gas into the vial with the plunger, the vial was likely compromised and should not be used. If it is evident that a gas vial is not fully evacuated at time of processing (i.e., water in the vial), use another evacuated gas vial. If multiple vials have been compromised, drop replicates per site as needed and submit an incident ticket before sending the samples for analysis. Label all improperly evacuated vials as such and return to the gas lab. While properly evacuated gas vials will often result in some volume of the sample being pushed into the vial by atmospheric pressure, the collector must push on the syringe plunger to finish collecting the equilibrated headspace sample. Always attempt to transfer the same amount of gas/sample to each vial (~18 mL) for all samples.

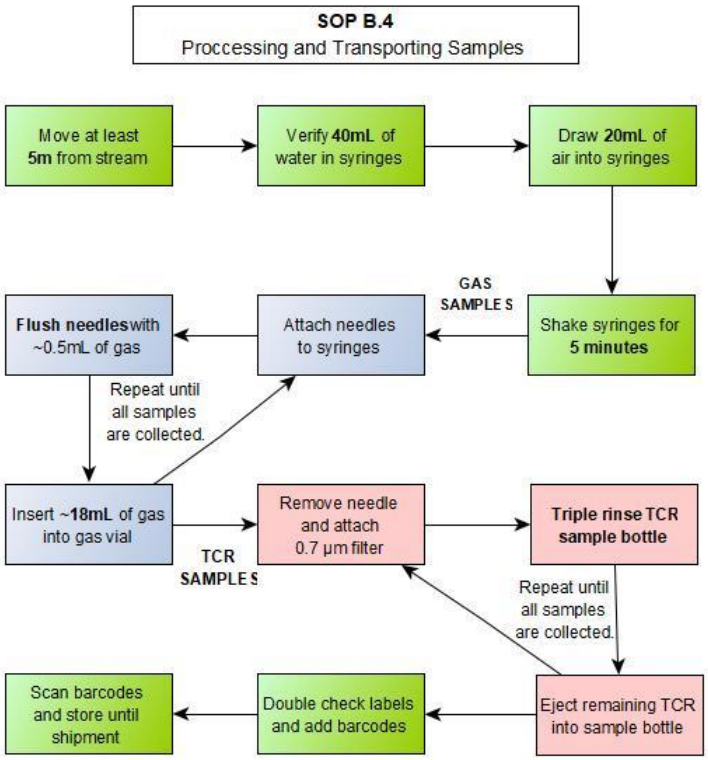


Figure 19. An expanded of the workflow for SOP B.4 Processing and Transportation Samples.

1. Away from stream (at least 5 m), where there will be no contamination in the air from the injection, such as upstream and upwind, open the stopcock and push plunger of the full syringe from 60 mL mark to 40 mL mark.
2. Draw the plunger back to the 60 mL mark, so the syringe contains 40 mL of water and 20 mL of air (**Figure 20**). Close stopcock and leave stopcock attached to syringe at all times. VERY IMPORTANT: To be consistent, read the volumes from the plunger location, not from the air-water line.

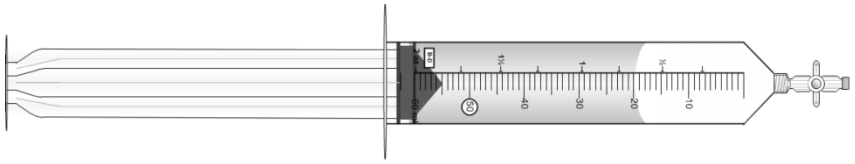


Figure 20. A syringe with 40 mL of water and 20 mL of air

3. SHAKE: Once samples have been collected and headspace air added for ALL stations, shake each syringe for 5 minutes to equilibrate SF_6 in solution with the partial pressure of SF_6 in the headspace of the syringe.
   a. Hold the syringe at the base, just above where the plunger enters the syringe below the 60 mL mark to avoid changing syringe water temperature.
   b. To save time, shake multiple syringes at once.

1) TIP: Using large resealable bags or rubber bands helps shake multiple syringes at once but be sure the stopcocks stay closed during shaking.

4. After shaking, place needle with cover still attached (avoiding accidental pricks) on the tip of the stopcock.
   a. Slightly twist needle to ensure a good friction seal with the tip of the stopcock.
   b. Once needle is attached, remove the needle cover.
5. Holding the syringe with the needle pointed upwards, open the stopcock (**Figure 21a**) and push a small amount of equilibrated headspace air (~0.5 mL) through the needle to purge the ambient air and any water in the needle.

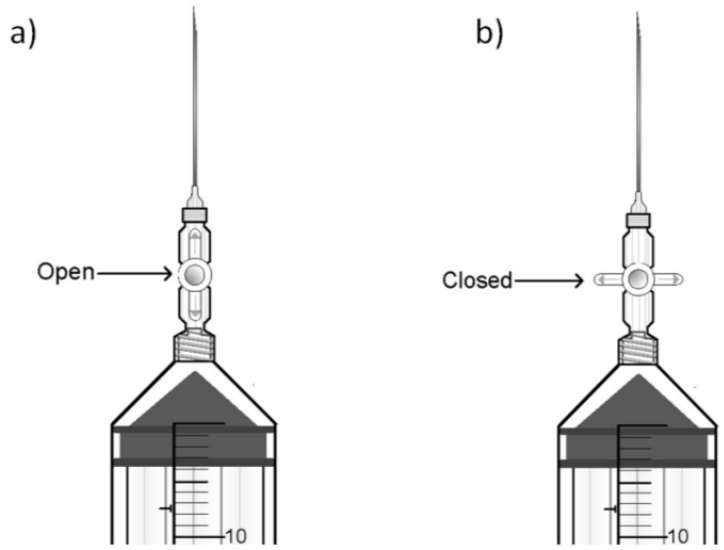


Figure 21. Example of a syringe with a stopcock with attached needles in the a) "open" and b) "closed" positions

6. With stopcock still 'open' and syringe held upright, insert the needle through the rubber septum of the gas vial (**Figure 22**) and push gas into the vial.
   a. Do not hold the plunger. Most properly evacuated vials will pull gas from the syringe into the vial as a result in the higher atmospheric pressure pushing in the syringe plunger and pushing gas into the vial, but this is not always the case if the plunger is sticky. Do not assume a vial is bad unless you can see water in the vial or it is not possible to push a full sample of headspace gas into the vial with an unclogged needle (see next step).
   b. If you cannot push gas into the vial, the needle may also be clogged by material from the septum. Remove the needle from the vial, turn the stopcock off, attach a new needle, purge the new needle, and insert into the vial described above.
7. Push as much gas into the vial as possible without injecting the water sample in the vial (~18 mLs of gas).
   a. Make sure to OVER-PRESSURIZE THE VIALS to minimize the potential for ambient air from leaking into the vial and dilute the headspace gas sample. Gas moves from high to low

pressure, so by over-pressurizing the vials you reduce change of ambient air moving into the vial as pressure changes.

b. You do not need to record the volume of gas injected into the vial, as long as the vial is over-pressurized.

8. While continuing to press up on the plunger, remove the syringe needle from the vial, without closing the stopcock. Closing the one-way stopcock will cause sample to be sucked out of the vial, resulting in gas sample loss.
9. You may re-use the same needle across a single station per SITE on a sampling day if 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 22. Insertion of the needle into a gas vial through the rubber septum.

10. SAMPLE CONSERVATIVE TRACER: Cover needle and remove but leave stopcock in place. 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. Repeat for all syringes.
11. Scan barcodes in the mobile app as each sample is collected.
    a. If available, scan the barcode label with the tablet (**Figure 23**).
12. DISCARD filter. 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.

Figure 23. Barcode label scanning.

SOP C Field - Post Sample Collection Tasks

C.1 AFTER SAMPLING FIELD TASKS

1. Break down the gas and conservative tracer injection set-ups.
2. Rinse Injection Equipment as soon as possible (The FMI pump should be rinsed in the field if possible, but can be done back at the DSF immediately upon return if necessary). **This step is important because it lessens the potential for the salt to dry in the pump head and damage the pump, as well as decreases potential for contamination on the next use**.
    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. This can be done while you are processing samples.
    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.
        1) You can disassemble the piston assembly immediately after use to fully rinse and dry. Be sure you read pump manual to avoid damage during disassembling and reassembling pump.
3. Collect wetted widths after samples have been collected and processed so as not to stir up samples and impact measurements
    a. 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).
        1) Measure the full wetted width of the stream, including undercut banks.
        2) When measuring wetted widths, the meter tape should be parallel to the ground, perpendicular to the banks, and as close to the stream as possible to get accurate wetted width measurements.
        3) 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. Note, that the laser range finder is not as accurate as a meter tape, thus the meter tape is the preferred measuring tool.
4. Pull the loggers from the stream as the very last thing you do before leaving for the day. Conductivity values should have returned to background (+/- 5% of starting value) levels before pulling loggers. This allows users to calculate transient storage times.
    a. In streams with slower travel times and more storage, loggers may need to be left in the stream overnight.

1) If left overnight, ensure loggers are securely attached in case of high flow events, and the loggers are clearly marked to allow them to be located
2) When logistics do not allow retrieval the next day, loggers may be picked up within 2-3 days after deployment.

5. Check HOBO loggers to ensure data was logged, and then upload exported .csv file to NEON spreadsheet uploader. See SOP D for more detailed information.
6. Upload HOBO logger CSVs to AOS Dropbox as back-up (See SOP D).

C.2 EQUIPMENT REFRESH AND MAINTENANCE

1. Refreshing the sampling kit
   a. Restock the sampling kit 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) Upon returning to Domain support facility:
         a) Soak piston in warm DI water for a few hours.
         b) Ensure no salt remains on the FMI pump.
         c) 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.
3. Track SF6 tank pressure and ensure you get extra SF6 on hand when needed. Record cylinder PSI on cylinder before and after bouts, so you know how much you use per bout and can order more/refill tank, when necessary.
4. Refill SF6 canister when empty: SF6 canisters are re-usable and can be refilled. Follow instructions in Shipping Ecological Samples and Equipment (RD[12]).

SOP D Additional Conductivity Logger Information

D.1 Saving and Uploading HOBO files

1. After the reaeration experiment, check HOBO files to make sure the HOBOs logged during the experiment. If the HOBOs did not launch, do not ship samples and submit an incident ticket to discuss with Science how to move forward with data collected.
2. Ensure you are using HoboWare Pro version of the software. Each Domain office should have a product key. You need the Pro version to evaluate measurements as specific conductance. Evaluate your HOBO data as specific conductance to account for the influence of temperature on electrical conductivity.
3. Ensure the file Description is in the correct format:
   a. Open the Plot Setup Window. In the top box "Name" in the Launch window and "Description" in the Plot Setup window, follow the below format: **SITE_S#_YYYYMMDD**, where S# is either S1 or S4 for reaeration station 1 or station 4

   ex) LEWI_S1_20170816 OR LEWI_S4_20170816

   b. Once the description is set, it will automatically save this description as the file name when you export as a .csv file.

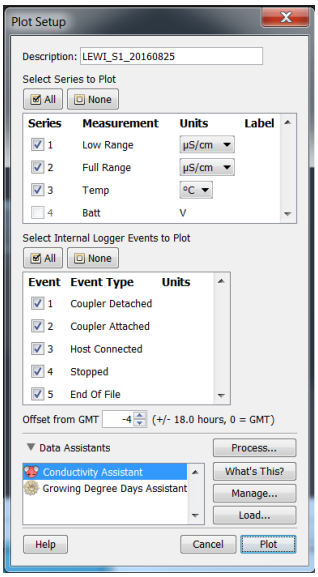


Figure 24. Example of plot setup window with correct description label format and appropriate boxes checked.

   c. Ensure temperature units are in Celsius.
   d. Select "Low Range" if Conductivity is <1000 µS/cm.
   e. Double click on the Conductivity Assistant in the *Data Assistants* section at the bottom of the Plot Setup window (**Figure 24**).
4. In the Conductivity Assistant window, select **Conductivity Low Range** from the dropdown if your conductivity values are ≤ 1000 µS/cm. Select **Full Range** if conductivity values are >1000 µS/cm.

a. Select both 'Low Range' and 'Full Range.' If you aren't sure which conductivity range to select or if your tracer experiment takes it from low to high range.
b. Leave all other default settings and click Create New Series.

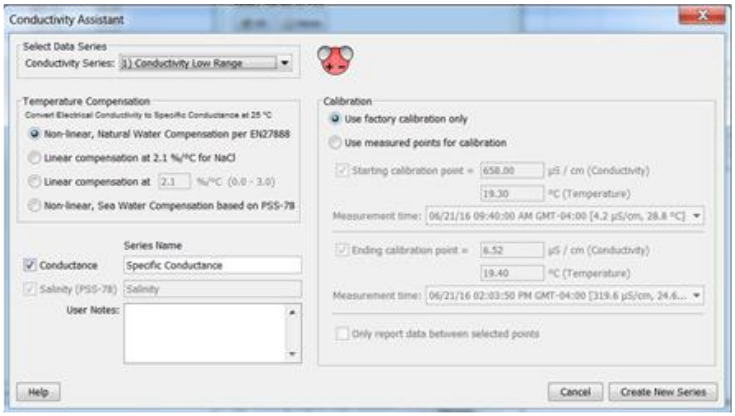


Figure 25. Example of the Conductivity Assistant window with appropriate boxes checked.

5. **Specific Conductance** series should now be listed in the Plot Setup Window (**Figure 26**).
   a. NOTE: You can still upload your data if you do not have specific conductance, as it can be calculated after ingest using the temperature and conductivity data. However, you will be unable to evaluate your HOBO data with confidence if it is not calculated as specific conductance.

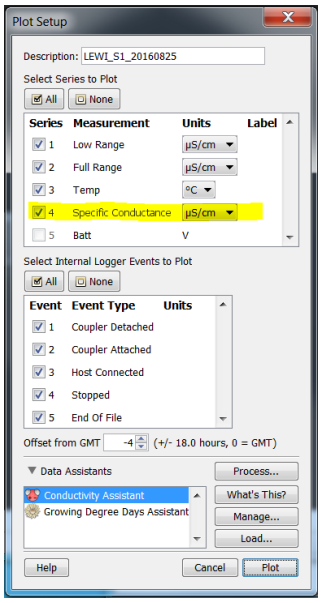


Figure 26. Example of plot setup window with specific conductance included.

6. Click Plot to view your data.
   a. Ensure you can see the reaeration peaks or plateaus (depending on type of salt release).

7. Export your data by clicking on the export button  in the toolbar.
8. Save your file as a .csv with the same name as the description (the "Name" you entered when launching the HOBO) (**Figure 26**).
    a. File -> Export Data Table. (TIP: you can also use the shortcut "Ctrl+E")
    b. If these names are not the same and are not in the correct format, the spreadsheet uploader will reject the file.
9. Leave all the boxes selected and click Export.

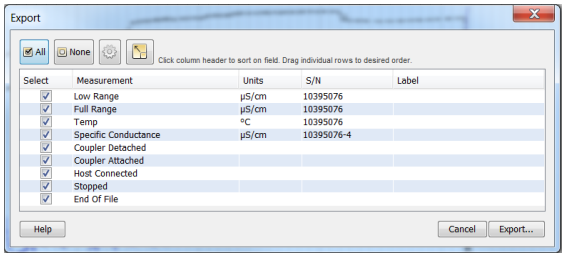


Figure 27. Example of export window with correct description label format and all boxes checked.

10. Save the exported .csv HOBO files to the AOS box folder.
    a. Note: If HOBOs were left overnight, open the .csv file in Excel and delete data rows past 36 hours after injection start time prior to data upload.
11. Upload files to NEON SOM portal (max 36 hours of data)
    a. Select 'File Data Ingest'
    b. Lab Name: NEON Aquatic Observation System
    c. Type of Data to Upload: Reaeration_Logger_File
    d. Choose File – navigate to the file you wish to upload and select file for upload
    e. Click "Upload File for Ingest"
    f. Look for "Your request completed successfully" message.
    g. If your upload fails, read the message in the "File Upload Failure" box.
        1) Common errors include:
            a) **File with identical checksum (likely identical content)** This means the file has already been uploaded. If the data is not in the L0 database or on the portal after 2 months submit a problem tracking incident to AOS science support.
            b) **[The file may not contain Fahrenheit values.]** This means the file needs to be re-exported in Celsius.
            c) **File was processed for DP ID 'NEON.DOM.SITE.DP0.20190.001' and table 'rea_conductivityFieldData_in'.** This means the file has the wrong name in the description field and does not match the actual file name of the .csv file.

D.2 Troubleshooting HOBO files:

1. Missing data files: If you are unable to find your data on the HOBO after sampling, it may have downloaded to the shuttle. Connect the shuttle and readout the device.
   a. Unable to communicate with logger: If you are having trouble communicating with the loggers/shuttle, ensure you are using the most updated version of HOBOware Pro.
   b. Corrupt HOBO file headers:
      1) Corrupt headers are caused by a communications error during the launch sequence. This can be caused by:
         a) Prematurely removing the logger from the coupler.
         b) Dirt, scratches, or other physical deformities on the optic window or (in the case of the Pendant) the housing that attenuates the low-level IR transmission. If the cases get dirty or scratched, the signal will be interrupted, and these errors can happen.
         c) High levels of ambient IR radiation (i.e., bright sunlight). The IR signal used to communicate between logger and shuttle/base station is very low in power level and can be overpowered by bright sunlight. Take precautions when launching these loggers in the field to minimize exposure to direct sunlight when communicating (Keep the logger and shuttle in their shadow at a minimum).
      2) If a logger is found to have a corrupt header the only way to repair it is to relaunch the logger directly from a computer, not with just the shuttle. NOTE: The shuttle has no capacity to rewrite a header so the error will be perpetuated in subsequent deployments (meaning you will keep getting corrupt headers continuously until the logger is relaunched from a computer).

SOP E Sample Shipment

For detailed shipping information see Shipping Ecological Samples and Equipment (RD[12]).

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APPENDIX A QUICK REFERENCES

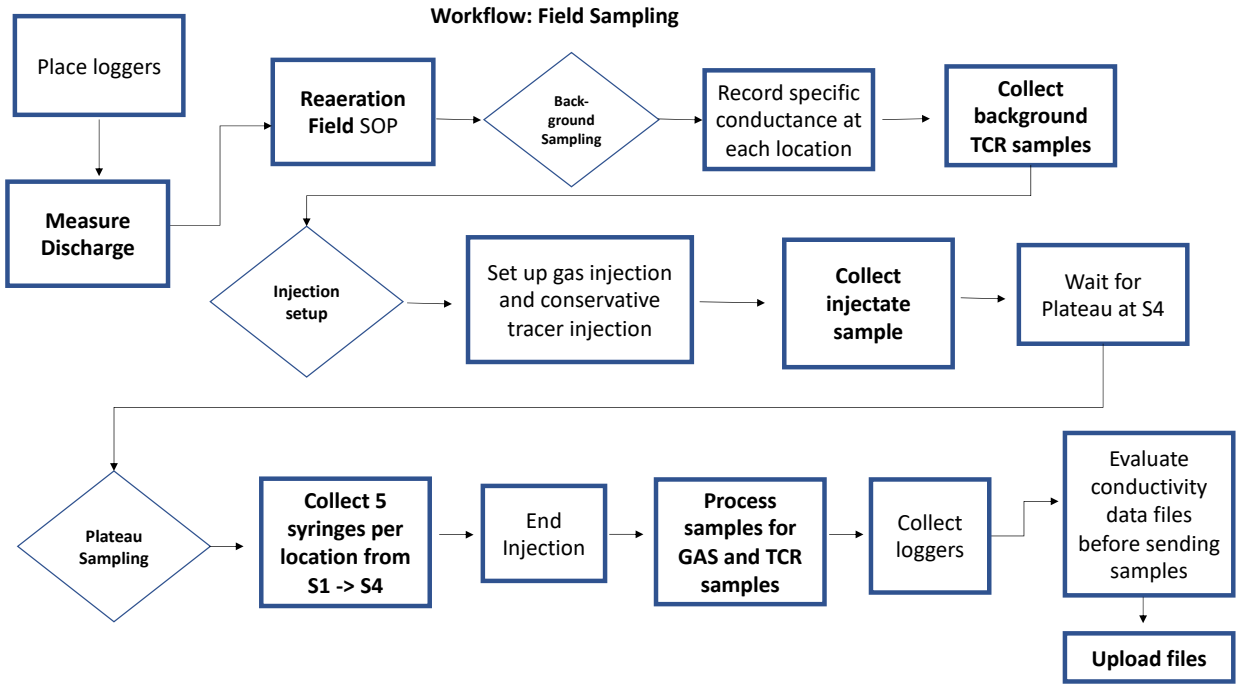


Figure 28. Workflow: Field Sampling.

1. Deploy conductivity loggers at sampling stations 1 and 4 within 0.1 m of the sensor set main support. Suspend the loggers in the water column using the sensor set infrastructure or other temporary feature (i.e., rebar) rather than placing them on the streambed. Sensors should be at the approximate height of where you will collect the sample.
2. Measure Discharge (It is OK to deploy HOBO loggers first).
3. Measure background conductivity and collect a background Cl^-/Br^- sample at each of the four reaeration stations. Be sure you hands have been cleaned prior to collecting samples so you do not contaminate samples. Small amounts of salt from the injection set up can easily be transferred, and extreme caution should be used to avoid contaminating samples.
4. 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 either tracer addition depends on stream flow but must NOT change once the experiment begins or the loss rate calculations will be inaccurate.
    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. In more turbulent streams you may need to Increase gas flow rate, as necessary (Note, if lab analysis is unable to detect gas at the bottom of the reach, Science will notify domains).

3) In a larger (>1000 L/s), steep, and turbulent streams, you may need increase gas flow to 248 ml/min (scale reading 40), so that the SF_6 is still detectable at station 4. Please contact Science if you have questions.

Table 8. Psi to mL/min conversion chart.

Scale Reading	Glass Flow mL/min
50	308
40	248
30	181
20	118
10	70

b. Salt Addition:
   1) 5-15 mg Cl^-/L NaCl above background (~10-30 µS/cm) or 0.05 – 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.
   3) If stream flows are >2000 L/s, discuss with NEON Science, as it may be too challenging to get enough conservative tracer into the stream. Bouts may need to be rescheduled for when flows are <2000 L/s.

5. Collect conservative tracer injectate sample.
6. Collect Plateau Samples – After the furthest downstream station (Station 4) reaches plateau of conservative tracer (e.g., NaCl) or the salt slug has returned to background levels and while injection continues at a constant flow rate, take samples starting at the most upstream station (Station 1)
   a. Collect five 60 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. Ensure syringes prior to collection and samples have minimal air bubbles.
   c. Record stream temperature, specific conductivity, and time when samples are taken at each of the 4 sampling stations.
7. Stop tracer injections.
8. Process samples – Move at least 5 m away from stream. Push plunger from 60 mL to 40 mL, pull in 20 mL of ambient air and shake for 5 minutes. From each syringe collect a gas and water sample.
9. Store samples appropriately.
   a. Gas samples → Sealed in gas vials.
   b. Water tracer samples → Tightly sealed in labeled 60-mL HDPE bottles.

10. Measure wetted widths at 30 evenly spaced locations along the stream reach (between Station 1 and Station 4). It is recommended this be completed at the end of the experiment, so as not to stir up stream bottom.
11. Clean FMI pump.
12. Collect loggers from station 1 and 4 after conductivity returns to background levels (+/- 5% of starting value).
13. Check conductivity files to ensure appropriate salt curves are captured on both HOBOs by evaluating the specific conductance data. Submit incident if any problems with the files exist. If problems do exist, do not send samples until Science has approved shipment.
    a. If pump speeds have decreased during the experiment, check the hobo data to make sure the stream was still at plateau when you collected the samples. Submit an incident ticket.

APPENDIX B REMINDERS

Before heading into the field: Make sure you…

Collect and prepare all equipment.
Attach barcode labels
Ensure all syringes are labeled with 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 three times with stream water.
Remove large air bubbles 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.
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 tablet or data sheet.
Pull HOBOs only after conductivity has returned to near background levels.
Clean FMI pump immediately.

Sample preservation: Be sure to…

Keep the gas sample vials cool to limit gas expansion.

APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Each domain has site specific guidelines for timing of sample collection and can be found in Domain Specific Sampling Designs (**Table 9**). The dates in the Sampling Design documents are estimated from historical hydrologic data. Dates presented are only a guide and are derived according to the logic presented in Section 4.3. Because individual years may vary widely from the average dates provided below, it is essential that domain staff monitor real-time conditions to determine when to start (and stop) sampling per environmental conditions, as described in Section 4 of this protocol.

Table 9. Aquatic Site Sampling Design documents.

Domain Number	Document Number	Document Name
01	NEON.DOC.003600	Aquatic Site Sampling Design – NEON Domain 01
02	NEON.DOC.003601	Aquatic Site Sampling Design – NEON Domain 02
03	NEON.DOC.003602	Aquatic Site Sampling Design – NEON Domain 03
04	NEON.DOC.003603	Aquatic Site Sampling Design – NEON Domain 04
05	NEON.DOC.003604	Aquatic Site Sampling Design – NEON Domain 05
06	NEON.DOC.003605	Aquatic Site Sampling Design – NEON Domain 06
07	NEON.DOC.003606	Aquatic Site Sampling Design – NEON Domain 07
08	NEON.DOC.003607	Aquatic Site Sampling Design – NEON Domain 08
09	NEON.DOC.003608	Aquatic Site Sampling Design – NEON Domain 09
10	NEON.DOC.003609	Aquatic Site Sampling Design – NEON Domain 10
11	NEON.DOC.003610	Aquatic Site Sampling Design – NEON Domain 11
12	NEON.DOC.003611	Aquatic Site Sampling Design – NEON Domain 12
13	NEON.DOC.003612	Aquatic Site Sampling Design – NEON Domain 13
14	NEON.DOC.003613	Aquatic Site Sampling Design – NEON Domain 14
15	NEON.DOC.003614	Aquatic Site Sampling Design – NEON Domain 15
16	NEON.DOC.003615	Aquatic Site Sampling Design – NEON Domain 16
17	NEON.DOC.003616	Aquatic Site Sampling Design – NEON Domain 17
18	NEON.DOC.003617	Aquatic Site Sampling Design – NEON Domain 18
19	NEON.DOC.003618	Aquatic Site Sampling Design – NEON Domain 19

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. Make sure to keep vials in water when traveling to and from the site to reduce impact of elevation changes on vial pressure.

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.

Concentrated injection solution can easily contaminate samples via your hands. Be sure to collect background samples before setting up the injection and rinse hands thoroughly before collecting all background and plateau samples.

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.
- Not sampling at the exact sample location in the stream (i.e., someone sampling 2 m upstream from where you are sampling for the same station)
- Forgetting to shake syringes prior to gas sampling.

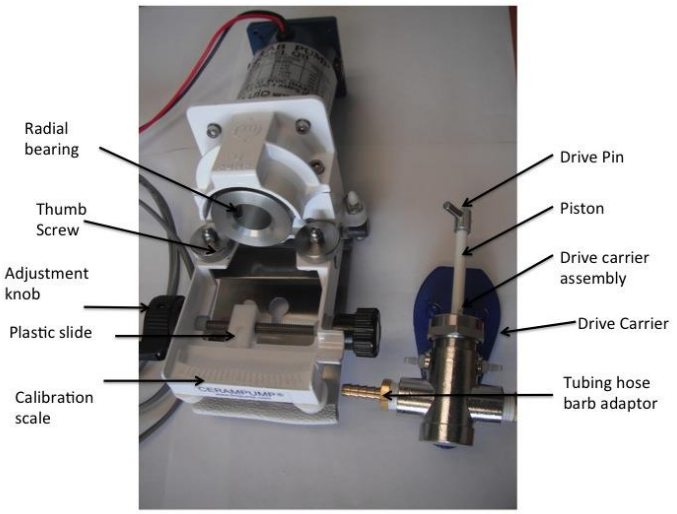
APPENDIX E PUMP AND BATTERY ASSEMBLY

E.1 Pump Assembly

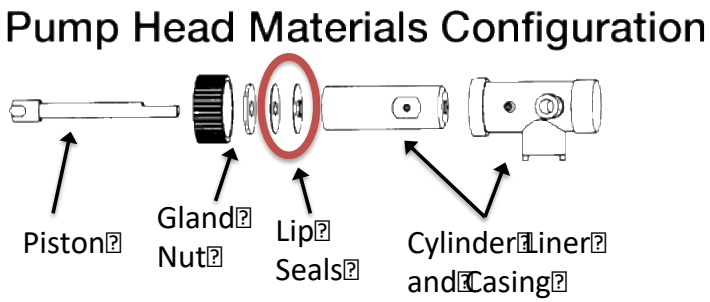
1. If piston has been withdrawn more than 2 inches from the cylinder (**Figure 29a**) or removed completely from the pump head (**Figure 29b**), you MUST take special precautions before reassembling pump or **Lip Seals will be damaged**.
    - Remove Gland Nut and install Lip Seals one at a time (**Figure 29b**) 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.

- Add a small drop of grease (high grade machine oil) to the drive pin head (**Figure 29a**) just before it is inserted into the radial bearing.
- Insert piston drive pin into the radial bearing in the spindle assembly **Figure 29c**). You can pull the piston out ~1 inch to make the insertion easier. Do not pull the pin out more than 2 inches.
- At the same time as you insert the drive pin into the bearing, slide the drive carrier into the pump base assembly (**Figure 29b**), 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 29a**) at the same time.
- 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.
    - 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.
    - Minimum Pump rates are 10% of the maximum rated flow rate.
    - **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.
- Tighten thumbscrew (**Figure 29a**) to hold drive carrier in place.
- 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 29. 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.

E.2 Parallel Battery Assembly

It is a good idea to hook up several 8-volt batteries in parallel to ensure the battery will last throughout the injection (See Equipment List (**Table 11**) for more details). Batteries should be of the same make and charge state to get full life out of the battery bank.

1. First, connect all of the positive terminals together
2. Second, connect all the negative terminals together.
3. Use terminal blocks to make connections easier.
4. Connect the positive lead of the pump to the positive terminal block and the negative pump lead to the negative block.
    a. TIP: Small alligator clips added to the ends of the pump leads make it easy to clip on and off.
5. Store the batteries in a plastic bin so they are protected.

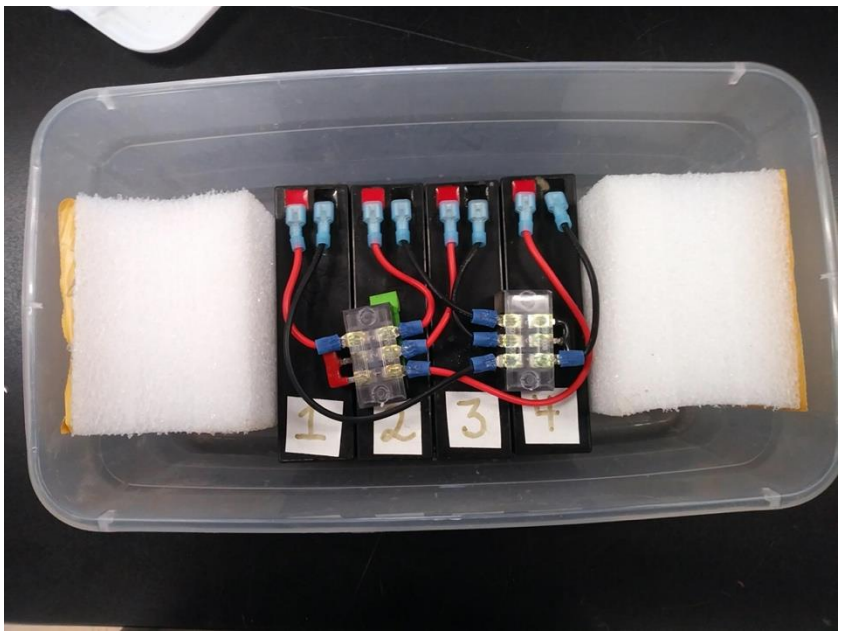


Figure 30. Image of batteries connected in parallel.

E.3 Water-resistant Parallel Battery Assembly

Another option for a multi-battery assembly is water resistant set-up, which is similar to the above design, but uses a sealed toolbox (**Figure 31**) and bulkhead connectors (**Figure 32**) instead of alligator clips. See Equipment List (**Table 11**) for more details. Additionally, you can adapt the alligator clips on the 8V battery tender to a bulkhead fitting, allowing you to plug in after a day of REA and charge all three batteries at once.

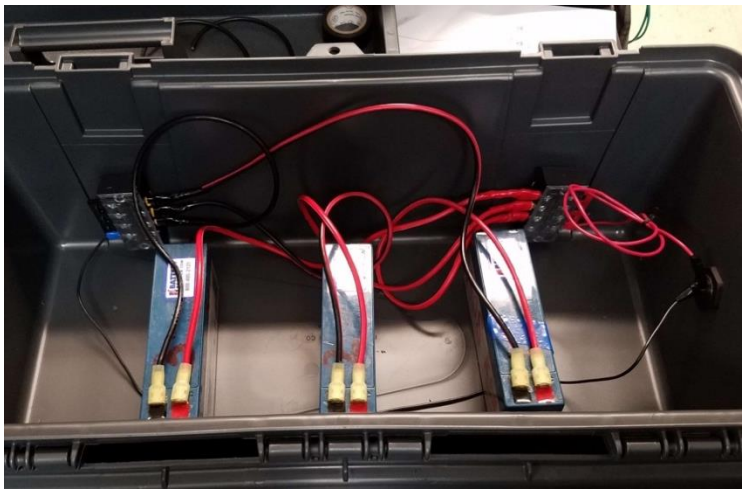


Figure 31. Batteries connected in parallel in toolbox.

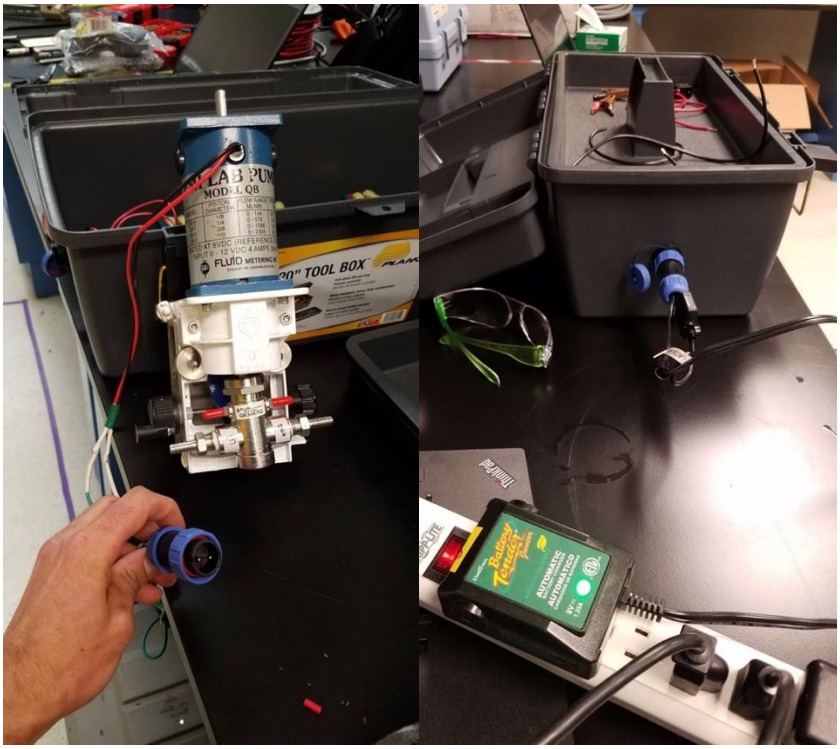


Figure 32. Example of bulkhead connector on pump and battery charger.

APPENDIX F SITE-SPECIFIC INFORMATION

See the Site-Specific Sampling Strategy Documents (Appendix C).

F.1 Injection Modifications during high or low flow:

1. Increasing flow rates: Some sites will need to increase concentration of injectate or increase gas flow rate during higher flows, such as during snowmelt or for sites that have very high reaeration rates. These modifications are site-specific.
   - D01 HOPB: Minimum of 300 g of NaBr during low flows.
   - D07 LECO: Gas flowmeter set to 140 for any flows >400 L/s, set to 70 for any flows <400 L/s.
   - D13 WLOU: Increase NaBr concentration to 0.4 mg/L during snowmelt or other high flow events.
   - D13 COMO: Increase NaBr concentration to 0.4 mg/L during snowmelt or other high flow events. Minimum of 20 g of NaBr.
   - D18 OKSR: Increase NaBr concentration to 0.4 mg/L during snowmelt or other high flow events.
2. Decreasing flow rates: Other sites can decrease the flow of gas tracer at low flows.
   - Decrease SF_6 flowmeter to 20 if flows <15 L/s:
     - D07 WALK
     - D13 COMO
   - Decrease SF_6 flowmeter to 20 if flows < 25L/s for:
     - D02 POSE
     - D13 WLOU
     - D15 REDB
     - D17 TECR
3. D07 WALK - At WALK, during high flows, there is an ephemeral channel that joins the main one just above S1. S1 is not uniformly mixed when this happens, but all other REA stations are well mixed. When there is an ephemeral channel during high flows, place HOBO 1 at REA Station 2 (it can still be called HOBO 1). You will only sample REA plateau samples at stations 2, 3, and 4. Continue to collect background samples at Stations 1-4. In the mobile application, in the parent record under 'Logger information', select "No" for the question: "**Hobos at sensor sets?"**. Then enter the measured thalweg distance between HOBO 1 (at a new location) and HOBO 2 (at sensor set 2/REA station 4).

APPENDIX G ALTERNATIVE INJECTION INFORMATION

G.1 NaBr plus Salt SLUG Method

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

For stream sites with high conductivity water (>500 µS/cm), using NaCl as a constant rate tracer can prove difficult because you cannot dissolve enough salt to detect the increase and/or there are concerns about adding too much salt to a stream. In other situations, logistics dictate the need to use a different tracer than NaCl to reduce weight of supplies carried into a field site. In such cases, Sodium Bromide (NaBr) is used as the conservative tracer. Salt slugs will need to be conducted at the same time, so that you can determine when to sample by monitoring conductivity with the hand-held conductivity meter at station 4.

Follow the above protocol for the continuous injection, but instead of using NaCl as your continuous tracer, you will use NaBr and a salt slug. Instructions for that are below.

1. Place the HOBO loggers in the stream at station 1 and station 4. Hobos should be at the same transect as the sensor sets and suspended in water. You can suspend them off the sensor infrastructure. Probes should be at approximately the same location as the in-stream sensors.
2. Mix NaBr constant rate solution:
    a. Weigh out NaBr to accommodate 0.05-0.5 milligrams Bromide for each L of stream discharge (0.064-0.64 mg NaBr/L). The spreadsheet will calculate the appropriate mass of NaBr to add (Line 21) to achieve the desired increase in Br. If you are unsure what to use for your site, please add 0.4 mg Br/L (515 mg NaBr/L).
        1) For a stream with a discharge of 50 L/s, you would mix 23.2 - 232 milligrams of NaBr into your continuous injection solution, depending on site-specific needs.
        2) See the 'Bromide Injection Prep Sheet' tab on the Reaeration Datasheet to help calculate the amount of NaBr added to your stream (**Figure 5**).
        3) 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. **We suggest you add on the higher range initially (0.4 mg Br/L) to ensure it can be detected at the external facility.**
    b. Wear gloves while mixing the solution and be careful not to get any NaBr on your clothes so as not to contaminate samples during collection and processing. Be sure to remove gloves prior to background sampling.
    c. Rinse bromide container, such as plastic bags or containers used for weighing portions in the lab, with tracer solution water in the bucket to ensure all Bromide has been added to the bucket
3. **Record the mass of NaBr (to the nearest 0.001 mg) and the volume of water (to the nearest 0.5 L) used for the constant rate tracer.**

4. Weigh a salt slug of 2 kg NaCl per each m^3/s of stream flow or 2 g of NaCl per each L/s of stream flow (minimum). This should be enough to see on the conductivity meter, but the actual increase will depend as much on longitudinal dispersion as it does on the mass of tracer used.
   a. Increase salt concentrations, as needed, if you do not observe a conductivity increase.
   b. Example: In a stream with a flow of 50 L/s, you would add a minimum of 100 g of salt. Be sure to use **non-iodized** salt. You may need to add more salt in high conductivity streams (>500 µS/cm) or streams with lots of dispersion and storage.
   c. You can use fine grain feed salt from a farm and ranch store.
5. **Record the mass of NaCl added** (to the nearest 0.5 gram).
6. Completely dissolve the salt in water (1-2 L). You may need a larger bucket depending on the amount of salt added to completely dissolve the salt. Use PVC to stir and dissolve salt, as needed.
7. At the same time as the NaBr is injection is started, dump the salt slug into the stream as quickly as possible at the drip station.
   a. Do NOT pour out of a narrow mouth bottle (i.e., 4L jug), as those pour too slowly. If your concentrated solution is in 4L jug, transfer to a bucket first to add the slug in one quick pulse.
   b. Rinse bucket with stream water and pour rinse water into stream **immediately** to ensure all salt added to the bucket made it into the stream.
8. Once the peak has passed and conductivity has returned to near background levels at station 4 (+/- 5% of starting value), you may begin your sampling at reaeration station 1.
9. Pull the loggers as the very last thing you do before leaving for the day.

G.2 SALT SLUG ONLY - MODEL

For sites where gas exchange rates will be inferred from variation in oxygen (ARIK and BLUE), no continuous injections will be completed, but several physical parameters still need to be measured. This will be completed 5X/year.

Note: For sites with extremely low flows during a scheduled reaeration experiment, a limited number of these types of injections can be performed with HQ approval. However, higher flows should be targeted for future experiments and no more than 2 experiments should be performed at low flows each year for a single site. The constant rate tracer injection ***cannot*** be used in lieu of a slug release.

1. Measure Discharge
2. 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). Hobos should be at the same transect as the Sensor sets and suspended in water. You can suspend off the sensor infrastructure. Loggers should be set to log every 10 s and should be set to the appropriate units (see SOP D).

a. Make salt slug solution with a minimum of 2 kg of NaCl for each m^3/s of stream flow or 2 g of NaCl for each L/s of stream flow.
    1) Ex, in a stream with a flow of 50 L/s, you would add 100 g of salt. Be sure to use **non-iodized** salt. You may need to add more salt in high conductivity streams (>500 µS/cm).
b. **Record the mass of NaCl added** (to the nearest 0.5 gram)
c. Completely dissolve the salt in water (1-2 L).
d. Dump the fully dissolved salt slug into the stream as quickly as possible at the drip station.
    1) Do NOT pour out of a narrow mouth bottle (i.e., 4L jug), as those pour too slowly. If your concentrated solution is in 4L jug, transfer to a bucket first to add the slug in one quick pulse.
    2) Rinse bucket with stream water and pour rinse water into stream immediately to ensure all salt added to the bucket made it into the stream.

3. 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). Measure the full wetted width of the stream, including undercut banks.
    a. When measuring wetted widths, the meter tape should be parallel to the ground, perpendicular to the banks, and as close to the stream as possible to get accurate wetted width measurements.
    b. 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. Note that the laser range finder is not as accurate as a meter tape, thus the meter tape is the preferred measuring tool.
4. Pull the loggers as the very last thing you do before leaving for the day. Conductivity values should have returned to background levels (+/- 5% of starting value) before pulling loggers. This allows users to calculate transient storage times.
    a. In streams with slower travel times and more storage, loggers may need to be left in the stream overnight.
        1) If left overnight, ensure loggers are securely attached in case of high flow events.
        2) When logistics do not allow retrieval the next day, loggers may be picked up within 2-3 days after deployment.
        3) Delete data rows past 36 hours after injection start time prior to data upload.

APPENDIX H VIAL EVACUATION PROCEDURE – D18/19 ONLY

H.1 Background

Shipments of evacuated vials from the external lab require transport on a plane to arrive in Alaska. Due to pressure differences that the vials may undergo in transit, Domain 18/19 must use a vacuum pump to evacuate vials that are used in the Reaeration and Dissolved Gas protocols.

H.2 Summary

The procedure for evacuating vials at the Domain 18/19 domain support facility is simple but needs to be outlined for future use of the vacuum pump. The pump is currently set up to evacuate one vial at a time but can be improved to evacuate 3 - 5 vials at once (similar to how the external lab evacuates vials).

H.3 Details

Equipment:

· Fisher Scientific Maxima C Plus Model M4C Vacuum Pump
· Fisherbrand Maxima C Plus Pump Oil
· CVT-275-101 Convection Gauge Sensor Tube, 1/8" NPT Port
· Terranova Convection Gauge Controller Model 906A
· K7160 Polyspring hose, 3/8" ID, 3/5" OD
· Tygon S3 E-3603 tubing, 1/4" ID, 1/2" OD
· Swagelok parts (Note: this includes additional components for a future build to evacuate multiple vials at once)
· SS-6-CS Cross (QTY 1)
· SS-600-4 3/8" Union Cross (QTY 1)
· SS-600-3 3/8" Union Tee (QTY 1)
· SS-6-HRN-4 Hex Reducing Nipple 3/8" MNPT x 1/4" MNPT (QTY 2)
· SS-4-HC-A-601 Hose Connector Adapter (QTY 7)
· SS-604-1 3/8" Back Ferrule (QTY 7)
· SS-603-1 3/8" Front Ferrule (QTY 7)
· SS-6-HC-1-6 Hose Connector (QTY 3)
· SS-602-1 3/8" Swagelok Nut (QTY 3)
· One-way Male Stopcock
· BD PrecisionGlide Needle, 25G x 1"
· 50mL Falcon Centrifuge Tube
· Lock&Lock Food Container, Tall, HPL808, 3.5 Cup, 29oz
· Exetainer 12mL Soda Glass Vials, Flat Bottomed (Labco Limited Part #736W)
· Spare Double Wadded Caps, White (Labco Limited Part #VC329)

H.4 Procedure:

1. Verify that the vacuum pump has sufficient oil for operation. Use the "Oil Level" arrows on the front of the unit to determine oil level.
2. Ensure that the pump and gauge controller are powered on.

Figure 33. Front face of the Maxima C Plus Vacuum Pump.

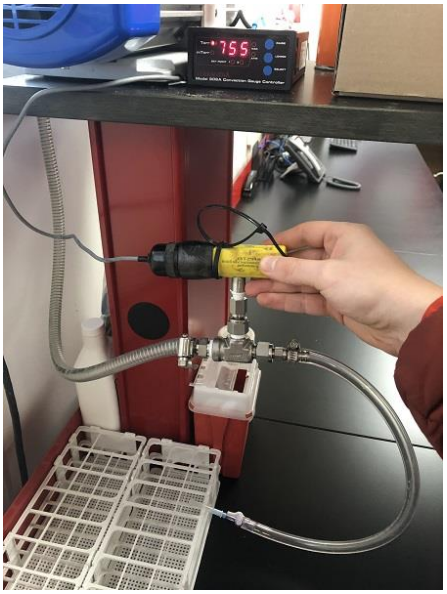


Figure 34. The vacuum pump system for evacuating one vial at a time.

2. Ensure that the pump and gauge controller are powered on.
3. Attach a new, unused double-wadded cap to an un-evacuated glass vial.

4. Fill a falcon tube (or Lock&Lock container) with tap water to ¾ capacity.
5. Attach the glass vial to the needle.

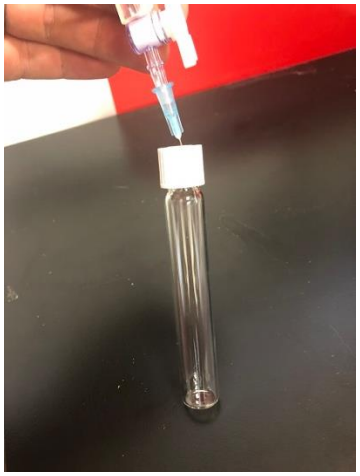


Figure 35. Vial attached to needle end of vacuum pump system.

6. Turn on the vacuum pump with the switch located on the side opposite the oil level markers.
7. The pump will start to evacuate the vial and the gauge controller will read the pressure of the system.

Figure 36. Power switch of vacuum pump and gauge controller reading <200 mTorr.

8. Once the gauge controller reads 200 mTorr or lower, remove the vial from the needle and quickly turn off the pump.
    a. Running the pump while "dry" for excessive amounts of time can damage the pump.
    b. Replace the needle every 2-3 vials or as necessary (dispose of used needles in a designated sharps container).
9. Place the evacuated vial in the water-filled falcon tube (or Lock&Lock container) and note the date that the vial was evacuated.
    a. Evacuated vials are good for two months following evacuation.

Figure 37. Evacuated vial placed upside down in water with date of evacuation noted.

APPENDIX I ESTABLISH REAERATION REACH

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

I.1 Locate Reaeration Reach (AKA Sensor Reach)

1. Determine the study reach by determining where the in-stream sensors will be placed (completed by HQ). This reach should be representative of the stream and have room upstream (within permitted reach) for an injection site (i.e., where we will add the SF_6 gas and NaCl solution). A good injection sites has a good mixing zone (with diverging and converging flows) and minimizes the inclusion of large pools or dead zones, which can increase water storage and travel time.
    b. There will be 4 sampling stations, located downstream of the injection site (**Figure 13**).
        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 stream water (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). Samples are collocated within the sensor reach, where reaeration station 1 is collocated at sensor set 1 and reaeration station 4 is collocated at sensor set 2. The drip station is located upstream of sensor set 1. Once sensors are installed, be sure to adjust your reaeration stations accordingly to ensure colocation with sensors.

I.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. This should allow for complete mixing before the first sampling station (Station 1), thus the drip station is often 25 – 100 m upstream of station 1. Wide and shallow streams may have difficulties mixing. To ensure complete mixing has occurred by 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 will be similar across this main flow. If it is not mixed, moved the injection site further upstream.
2. Measure travel time between Station 1 and Station 4 - The best reach lengths are those that take ~40-45 minutes, during median flow, 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 1 and/or 4 upstream or downstream to obtain the appropriate travel time. Once station 1 and 4 are set, spread out station 2 and 3 between them as equally as possible (this does not have to be exact).

APPENDIX J 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 10. Equipment list – Gas Injection.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Gas (e.g., Sulfur Hexafluoride, SF_6 or propane – site specific) 10 lbs. (10 lb. tank contains sufficient gas for about ~30 reaeration measurements)	Gas injection	1
	N	Gas Tank, re-usable canister	Gas injection	1
	N	Gas Tank Regulator	Regulating gas flow	1
	N	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	Gas-impermeable tubing: ¼ inch ID (inner diameter), (e.g., Tygon)	Gas injection	1
	N	1-1/8-inch wrench	Connecting regulator to SF_6 tank	1
	N	11/16-inch wrench	Connecting tubing to gas flow regulator	1
	N	Fine-pore diffuser (1 per stream SITE)	Gas injection	1

Supplier/Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Small 125 mL squirt bottle (for soapy water)	Testing for gas leaks	1
	N	Plastic tote	Safe transfer of equipment	1
Consumable items				
	N	Teflon tape	Creating a seal on the flowmeter tube fittings	1

Table 11. Equipment list – Conservative tracer injection.

Supplier/Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
	N	5-gallon bucket/carboy (may need to be larger, depending on size of stream)	Conservative tracer container	1
	N	Lid for bucket/carboy	Keeping debris from falling into solution and damaging pump	1
	N	Extra bucket	Filling conservative tracer container or transporting pre-made conservative tracer solution	1
	N	PVC stirrer	Stirring salt in bucket to ensure it is dissolved before injection start	1
	N	4 L Jug	Filling conservative tracer container or transporting pre-made conservative tracer solution	2
Fluid Metering, Inc: QB Metering pump with stainless steel pump head and ceramic piston (MX103139)	Y	Fluid Metering (FMI) Pump: - QB metering pump, - Stainless Steel pump head with ceramic piston - Hose barb adapter (¼" Barb * ¼" MIP)	Conservative tracer injection	1

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
		- Feed-thru cord switch		
Fluid Metering, Inc	Y	Piston Linear Assembly, QA31111C Port Seals, FMI Part # R419 (Quantity 2, plus extra 2) Lip Seals, FMI Part # R408-1A (Quantity 2, plus extra 2) Gland Washer, FMI Part # R409-1 (Quantity 1) Lip Seal installation tool, FMI part # 500071	REPAIR: Replace Piston liner assembly. Only needed when repairs are needed	As needed
	N	Gel cell batteries (8 Volt)	Powering the FMI pump, may need up to 4 wired in parallel	2
Amazon B00VGO28CW	N	Battery Charger, 8V battery tender jr., 1.25 Amp Battery Charger	Charging 8 Volt batter	1
Amazon B010GWZOUW	N	Yueton 100pcs Female Fully Insulated Wire Crimp Terminal Nylon Quick Connectors Wiring Spade	Batteries connected in parallel	1
Amazon B07Y21YYVD	N	Glarks 70Pcs(5Sets) Terminal Block Set, 5Pcs 3 Positions 600V 15A Dual Row Screw Terminals Strip + 5Pcs Pre-Insulated Barrier Strips + 60Pcs Insulated Fork Wire Connector (3P+Fork Connector)	Batteries connected in parallel	1
Amazon B01C5CANVG	N	BNTECHGO 18 Gauge Silicone wire spool red and black each 25ft Flexible 18 AWG Stranded Copper Wire	Batteries connected in parallel	1
Amazon **B07B7FHTN5**	N	Bulkhead fitting (1 for battery to pump, 1 for battery to charger)	Water-resistant parallel battery set-up.	2
Amazon **B08GCF5GHM**	N	Terminal blocks + fork connector kit	Water-resistant parallel battery set-up.	1

Supplier/Item No.	Exact Brand	Description	Purpose	Quan-tity
Amazon **B0032Y8RIS**	N	Toolbox	Water-resistant parallel battery set-up.	1
Amazon **B018RQJJUA**	N	Toggle switch	Water-resistant parallel battery set-up.	1
	N	16 or 18 gauge wire for connecting	Water-resistant parallel battery set-up.	1
	N	¼ inch I.D. tubing – cut to approximately 10 and 1-2 foot long	Conservative tracer injection	2
	N	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
	N	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
	N	Binder clips	Weighing down or clipping the tubing to the bucket	
	N	Plastic 250 mL graduated cylinder	Calibrating the FMI pump	1
	N	Stopwatch	Calibrating the FMI pump	1
	N	Battery charger	Charging the batteries	1
	N	Plastic tote for FMI pump	Safe transfer of equipment	1
Consumable items				
	N	Conservative tracer: Sodium Chloride (NaCl) non-iodized (fine grain salt from feed store works well) or Sodium Bromide (NaBr) – Site Specific	Conservative tracer injection	1
	N	Grease (high grade machine oil)	Greasing the drive pin head on the FMI pump	1

Table 12. Equipment list – Sampling.

Supplier/Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
	N	Mobile data entry tablet, fully charged and synced before field work	Field data entry	1
YSI Pro2030	Y	Field thermometer and conductivity meter, handheld - calibrated	Measuring and viewing conductivity	1
Onset U24-001	Y	Logging Conductivity probes – factory calibrated	Measuring and storing conductivity data	2
Onset U-DTW-1	Y	Logging Conductivity Probe shuttle	Connecting the conductivity probes to computer	1
Onset BHW-PRO-CD	Y	HoboWare Pro (must be Pro Version)	Computer software for conductivity probes.	1
	N	60-mL syringes, with luer-lock tip, 1 mL graduations (individually numbered and covered with clear packing tape to protect syringe labeling)	Sample collection	25
	N	1-way male stopcocks, one per syringe	Sample collection	24
	N	Gas vial rack	Sample storage in the field	1
	N	Sharps container	Needle disposal	1
	N	Meter-tape, 50- metric field tape	Measuring stream width	1
	N	Rangefinder	Optional measuring of stream width for streams with average width >2m	1
	N	Two-way Radios	Communication between samplers	2
Consumable items				
	N	Flagging, roll	Marking each sampling station	1

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
External lab will clean and return to you periodically (at least annually).	N	60-mL HDPE sample bottles (e.g., Nalgene), pre-labeled. Plus extras	Conservative tracer sample container	25
From external lab – ordered and delivered based on sampling schedule.	Y	12 mL Exetainer gas vials with Double Wadded White Caps, pre-evacuated and pre-labeled – from external lab	Gas sample container	20 (plus spares)
	N	Weatherproof Labels for gas vials: 1 inch * 2-$^{5}/_{8}$ inches	Labeling samples	20
	N	Adhesive barcode labels	Labeling sample bottles with barcode-readable	1 sheet
	N	Needles, 27-gauge, disposable 0.5 inches in length	Transferring gas from the syringe to the sample vial	20
	N	30 mm/ 0.7 □m 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

Table 13. Equipment list – Site-specific supplies.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Infrastructure, such as stakes, rebar, or ring stand	Stabilizing tracer injection tubing	As needed
	N	Bridge/plank	Walking in designated areas	As needed
	N	Small cooler	Transporting samples	As needed
Consumable items				
	N	Zip ties/Cable ties	Injection setup	As needed

Supplier/Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Ice or ice packs	Keeping gas samples cool	As needed
	N	Conductivity Standards	Calibrating Hand-held conductivity meter	As needed